New Tools to Study epileptogenesis

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Disclosures

Quanteon, LLC (G Gerhardt owner)
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Tools

Viral Vectors
Optogenetics
Microelectrode Arrays
Optogenetics

- A tool of exquisite temporal (msec) and spatial resolution (single cell)

- It has the power to *enhance* or *diminish* intrinsic neuronal firing patterns by controlling specific cells using light.

- Depolarize cells (+)
  - (Channelrhodopsin 2)

- Hyperpolarize cells (-)
  - (Halorhodopsin)
Channelrhodopsin 2

- ChR2 absorbs blue light
- All-trans-retinal → 13-cis-retinal → pore opens to ≥ 6Å
- Retinal relaxes back to all-trans in msecs and pore closes
- Designer channelrhodopsins
Viral Vectors

- Used to deliver genetic material into cells: transduction
- Retroviruses (Lentiviruses), Adenoviruses, Adeno-associated Viruses


Optogenetic Challenges

• Expression of vector
• Vector constructs and specificity
• Limited light penetration
  – less than 1 mm
  – good and bad!
• Power limitations
  – local heating with high power
• Anatomy of target structure
• Photovoltaic Effects
What it looks like
Translational Applications

1. Hippocampal Circuitry
   • Partial Complex Seizures
   • Epileptogenesis

2. Thalamo-cortical Circuitry
   • Absence Seizures

3. Basal ganglia circuitry
   • Parkinson’s disease
Hippocampal Pathways

Perforant Pathway (EC to DG)
Mossy Fibers (DG to CA3)
Schaeffer collaterals (CA3 to CA1)
Methodology

**Hippocampal Virus Injection:**
- AAV5-hSyn-hChR2(H134R)-EYFP (~$10^{14}$ genome/ml)
- AAV5-hSyn-eNpHR 3.0-EYFP (~$10^{14}$ genome/ml)
  - 1 µl per injection
  - DG (AP: -4.4 mm; ML: -2.2 mm; DV: -3.8)

**Laser Source and Optical Fiber Configuration:**
- Glass Fiber optic (Ø200 µm Core, 0.39 NA)
- Laser source
  - Blue (OBIS LS 488 nm, 100mW)
  - Yellow (LaserGlow 594 nm, 100mW)

**Electrochemistry:**
- Glutamate Selective Measurements
- S2 microelectrode array (MEA) configuration
- FAST16mkIII potentiostat (Quanteon LLC)
- Second-by-Second recordings (10 Hz data display)
- mPD size exclusion layer
- Photovoltaic effects are removed by self-referencing channel
- Guide cannula for optical fiber 200 µm from surface of MEA
current (pA)

frequency ≈ 26 sec.

Time

2.5 μM

60 sec.

+GluOX

−GluOX
Glutamate Release in Rat Hippocampus

- Male SD rats
- In Vivo Electrochemistry
- CA3 AAV-5-channelrhopsin 2
Distribution of ChR2 in Hippocampus After 1 Injection

A. ChR2-YFP expression in DG (10X);
B. GAD67-RFP in DG GABA neurons (10X).
C. Merge showing differential localization of markers with GAD67- RFP in GABA cell bodies and ChR2-YFP in bouton-like clumps in neuritic field (60X).
D. Yellow fluorescence protein imaging of NpHR expression in DG, note needle track (4X).
Light-induced Glutamate Release Measured in the DG

Blue laser-induced release of glutamate in rat DG using MEA + waveguide array. Pulse trains (100 pulses) with variable pulse durations and constant blue laser activations using a range of 5-50 mW laser intensities were used to produce reproducible, but light dose-dependent, increases in extracellular glutamate.
Methodology

Electrophysiology
- Whole cell patch-clamp
- Isolated and visualized Dentate Gyrus Granule Cells
- Optical activation
- Sutter Lambda XL shutter system coupled to an Olympus BX51WI microscope with fluorescent optics.

Behavioral Studies
- ChR2 animals
  - Optical fiber implanted in DG, Stimulated with blue light 2x day @ 50 mW
- NpHR animals
  - Amygdalar kindling
  - Bipolar electrode implanted in amygdala (100-800 µA. 2x day)
  - Optical fiber implanted in DG for yellow light stimulation (~40 mW) during kindling
AAV5-Syn-ChR2-EYFP resulted in predominantly direct activation responses of dentate granule cells. **A.** Representative traces of ChR2 responses before and after co-application of the ionotropic glutamate receptor antagonist Kynurenic Acid (1 mM) and the Na⁺ channel blocker TTx (1 mM). **B-D.** Peak current, charge transfer and latencies of responses during ChR2 activation. The large inward currents activated by optical stimulation of ChR2 were insensitive to Kynurenic Acid & TTx, and exhibited response latencies consistent with direct activation of dentate granule cells. Small transient inward currents during ChR2 activation were sensitive to these compounds indicating a separate glutamatergic synaptic component.
AAV5-Syn-NpHR-EYFP resulted in synaptic activation responses of dentate granule cells. E. Representative traces of NpHR responses before and after application of type A GABA receptor antagonist Bicuculline (30 mM). F-H. Peak current, charge transfer and latencies of responses during NpHR activation. The outward currents activated by optical stimulation of NpHR were abolished with Bicuculline and exhibited response latencies that are consistent with synaptic activation of dentate granule cells.
Blue Light (ChR2-animals) Induced Kindling
Using yellow light activation 5 seconds prior to electrical stimulation of amygdala and throughout the seizure, NpHR animals required more stimuli (P<0.03) to achieve stage 4/5 seizures (21.4 ± 5.1 [SD]; n=5) than shams (11.0 ± 5.4; n=4) or historical controls (8.2 ± 3.1).
Major Findings

1. AAV5-hSyn-hChR2(H134R)-EYFP and AAV5-hSyn-NpHR 3.0-EYFP distribution were not limited to the injection site suggesting that a lower titer is required for a more precise distribution.

2. Light-induced glutamate release was power dependent.

3. Glutamate dynamics obtained with optogenetics were in the same range as we have previously reported using other forms of stimulation (high potassium, drug induced or behavior).

4. Epileptogenesis can be induced or retarded with appropriate optical modulation.

5. AAV5-Syn-ChR2-EYFP resulted in predominantly direct activation responses of dentate granule cells.

6. AAV5-Syn-NpHR-EYFP resulted in synaptic activation responses of dentate granule cells.
Biochemistry: NT Vesicular Release

• The 7S SNARE complex (7SC: minimum requirement for vesicle-plasma membrane fusion at the “active zone”) and certain regulators (NSF, SV2, tomosyn/STXBP5) are altered in a proconvulsant and hippocampal subregion-specific manner during the epileptogenesis of kindling, regardless of stimulation site.

• Changes are Racine-stage-dependent and stable once a fully kindled state is achieved, suggesting permanently altered activity of the neurosecretory machinery.

• The biochemical alterations appear reversible at Racine Stage 3.

• These alterations in 7SC and SNARE regulators correlate with increased hippocampal glutamate release
Summary

These results demonstrate the feasibility of in vivo real-time measure by MEAs and exquisite control with optogenetics of neuronal/network glutamate release. Furthermore, these data suggest that AAV5-Syn-ChR2-EYFP is selectively expressed in glutamatergic neurons whereas AAV5-Syn-NpHR-EYFP is selectively expressed in GABAergic neurons, allowing selective activation to modify behavior.

Future

• Optogenetically induce epilepsy by stimulating focally discrete areas in rodents transfected with channelrhodopsin2, and evaluate modulation of potential inhibitory/excitatory network or neural changes using quantification of vesicular release, slice electrophysiology and immunohistochemistry.
• Using conscious, free-roaming rodents transfected with hyperpolarizing halorhodopsin, determine whether there are common hippocampal epileptogenic elements in certain TLE seizure models (kindling, TBI/CCI, post-SE KA) that are sensitive to modulation by light therapy.
Hippocampus

From Figure 9-9, Prentice Hall, Silverthorn 2001
Participating Scientists/Organizations

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