Light Stimulation and High Pressure Freezing

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Optogenetics
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Rationale

Many biological processes are highly dynamic (millisecond range)

Consequences for visualisation with electron microscopy:

- Chemical fixation
  → Bad temporal resolution due to slow action of fixatives

- High pressure freezing
  → Bad temporal resolution due to transfer to instrument
Optogenetics

Rationale

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Consequences for visualisation with electron microscopy:

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Trigger for precise control of events combined with instantaneous fixation
Optogenetics: light pluses can selectively activate/inactivate cellular events via

- light activated ion channels (channelrhodopsin)
- caged neurotransmitters
- caged second messengers
- photo-switchable ligands
- photoactivatable proteins
Combining Optogenetics with HPF

- HPF method of choice for fast fixation of tissues
- Combination of timed light pulses and freezing in high pressure freezers

- Neurobiology (ion channels, neurotransmitters, ...)
- Light sensitive cells (cones, rods)
- Cytoskeletal reorganization (second messengers)
- Membrane trafficking
Light Stimulation on Leica EM PACT2
Watanabe et al. (2014) Neuromethods
Modification of the Leica EM PACT2 specimen pods/bayonet
Commercially available from Marine Reef International
Light Stimulation on Leica EM PACT2

Shigeki Watanabe/Jorgensen Lab

Watanabe et al. (2014)
Light Stimulation on Leica EM PACT2
Shigeki Watanabe/Jorgensen Lab

Graph showing temperature and accelerometer outputs over time with light stimulation marked.

Watanabe et al. (2014)
Section 3

Light Stimulation on Leica EM HPM100
Light Stimulation on Leica EM HPM100

Features

- Optional accessory from Leica
- Temporal resolution of 5 ms
- Compatible with many different light sources
Light Stimulation on Leica EM HPM100

Integration into HPM100

- Optical fiber connected directly with high pressure chamber
- Transparent upper/lower halves
- Sample between sapphire discs
- Light source and control box
Optical fiber connected directly with high pressure chamber

Transparent upper/lower halves

Sample between sapphire discs

Light source and control box
Light Stimulation on Leica EM HPM100

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Leica Microsystems
Light Stimulation on Leica EM HPM100

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Light Stimulation on Leica EM HPM100

Triggering Light Pulses

- Automatically via freeze button
- Manually via control box (buttons or external, programmatic control for stimulation and freezing)
Section 4

Application Example
Ultrafast endocytosis at mouse hippocampal synapses

Shigeki Watanabe¹, Benjamin R. Rost²⁺, Marcial Camacho-Pérez⁵, M. Wayne Davis³, Berit Söhl-Kielczynski², Christian Rosenmund² & Erik M. Jorgensen¹
How are synaptic vesicles (membrane and proteins) recycled to sustain neurotransmission?

- Clathrin-mediated ($\approx 20$ s)
- Kiss-and-run ($\approx 1$ s)
- . . .
Cultured mouse hippocampal neurons expressing channelrhodopsin were stimulated optically after being kept in dark (blue light, 10 ms)

Frozen in EM HPM100 with light stimulation at different time intervals (15 ms - 10 s)

Freeze substitution, ultrathin sectioning, TEM/tomography

Statistical evaluation of vesicles at synaptic membrane
Application Example

Results

Watanabe et al. (2013)
Application Example

Results

Watanabe et al. (2013)
Application Example

Results

Watanabe et al. (2013)
Main peak of endocytosis after 100 ms in lateral regions

- Timing incompatible with clathrin mediated endocytosis
- Timing and location incompatible with kiss-and-run model
- "Ultrafast endocytosis" as primary mechanism of recycling
Section 5

Summary
Recommended Reading


Thank you for your attention.

Nexperion e.U.
Solutions for Electron Microscopy

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