Understanding Synapses in the Brain

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5 April 2019 NSF Large Facilities Workshop – Austin
Envisioning the future of facility science and cyberinfrastructure
NeuroNex Award No.1707356
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Goals for Today

• What is a Synapse?
• How does coordination with a cyberinfrastructure facility help us to understand synapses.

2013 Kuwajima, Spacek, Harris
Neuroscience 251:75
Synapses are the sites of communication between neurons.

**Diagram:**
- Neuron
  - Cell Body
  - Dendrites
  - Axon
  - Axonal Bouton
  - Dendritic Spine
- Dendrites
- Neuropil
- Axons

*Adult Rat Light Microscopy – Golgi preparation (Harris, 1980)*
Diseases of Dendrites and Spines

Normal 6 months
Mentally Retarded 12 months
Alzheimer’s Adult
Epilepsy Adult

Purpura 1974
Schiebel 1983, 1986

Light Microscopy Provides

- Large fields
- Visualization of living cells over time
- But, not enough resolution to study synapses.
- Need Electron Microscopy (EM)
Synapses on Dendritic Spines in EM
Synapses on Dendritic Spines in EM

Dendrite
Spine
Mitochondrion
Glia
Axon
PSD
vesicles

Spine Apparatus
10% of spines

1 micron

Adult Rat
3D of these Synapses

1 cubic micron
3DEM reveals subcellular resources in context of synapses
Cruise Through Hippocampal Neuropil

Credits

University of Texas at Austin
Josef Spacek, Kristen Harris, Larry Lindsey, Patrick Parker
Chandra Bajaj, Jarred Bowden

The Salk Institute
Justin Kinney*, Tom Bartol, Terry Sejnowski
Dan Keller, Varum Chaturvedi

3D – Blender
Music by: Camille Saint Saëns, Carnival of the Animals, Aquarium

Available: YouTube – 2014 New York World Science Festival with narration
https://www.youtube.com/watch?v=hpxHSISSUes&feature=youtu.be
Or on SynapseWeb with other tutorials:
https://synapseweb.clm.utexas.edu/tutorials - bottom of the page.
First fully reconstructed volume.

(\sim 180 \, \mu m^3)
~500 (498) synapses in volume = 1 RBC!!

Repeat ~8 trillion times for 1 human brain (1500 cm$^3$)
Current methods, > 8 trillion years of human labor!

Many papers have resulted from sharing this dataset.

NSF NeuroNex Hub

Motivation:

• Variance in synapses is not known.
  • dimensions, connectivity, and content across cell types, brain regions, species….

• Knowledge needed to assess if model systems represent human brain synaptic functions.

• Current approaches are limited by
  • resolution
  • inefficient and insufficient data collection
  • analysis bottlenecks
  • use and dissemination of data and knowledge
NSF NeuroNex Hub
Aims:

1) Collect, compare, and share nanoscale volumes of synaptic neuropil across brain regions and species.

2) Improve axial resolution with tilt tomography on the scanning electron microscope.

3) Integrate and test software tools to enhance analysis of synaptic neuropil.

4) Integrate and disseminate enhanced imaging output and tools with high performance computing.
NSF NeuroNex Hub
Progress so far

- First Human tSEM series posted
- Designed and implemented “tomoSEM”
- Collected 1st conical tilt images
- Launched Portal at TACC
- Procured new tSEM and ultramicrotome
- Refined en bloc staining
- Refined Neuropil Tools and Virtual Ultramicrotome
- Initiated ReconstructJAVA with SWiFT-IR alignment
- Created Github sites for software development
- Created Github testbeds for data sharing
- Held first community workshop at UT-Austin
- Initiated multiple collaborations
- Attended Brain Initiative meeting
- Publishing papers, talks, and conferences

(Details on pages 2-3/17 annual report and 2-4/18 interim report.)
Scanning Electron Microscopy in the Transmission Mode (tSEM)

Kuwajima, Mendenhall, Lindsey, Harris (2013) PLOS 1
Maintains resolution needed to identify and measure organelles.
Human Neocortex Location: Epitumorous Temporal Lobe

Josef Špaček – MRI of tumor

Yellow dot, location

Woman 56, anaplastic astrocytoma
Human Neocortex
Series Location: Layer II
Aligned and cropped using TrakEM2
Human Neocortex – Layer II
Great tissue preservation
Human Neocortex – Layer II
Great tissue preservation
Human Neocortex – Layer II
Great tissue preservation
Integrate Cell Biology in Understanding Neural Circuits

SER+ PR+

PR- SER-
Need EM Tomography
E.g. to know the spine apparatus
EM Tomography needed even for thin sections – 50 nm:

Thin cut sections ~50 nm: Fragile and still obscure
TEM Tomography Reveals obscured ultrastructure.

- 150 nm thick section
- 15 nm virtual sections
- Limitation:
  - small field size ($\sim 1 \, \mu m^2$)
tomoSEM (Tomography in tSEM)

- Large field
  - 2,500 -10,000 µm²
- Fewer, thicker sections (250 nm)
  - 40 instead of 200 serial sections
  - Less human cut time
  - Stronger sections
  - En bloc stain homogeneity
  - Few or no flaws
- 10-15 nm virtual z
  - Reveals buried structures
  - Better auto-segmentation
- Total Images (no big deal):
  - 40 sections * 25 image / section = 1000
  - Automatable for multi-image,
  - Multi-section, multi-grid
  - Human time minimal – setup
tomoSEM  
Conical Tomography:

• Single tilt Angle  
• One dynamic focus transform  
• Conventional stage and detector  
• Rotation to 220 degrees.  
• Rotation 360 degrees if flip the grid.
Equal time and advantages

- Tilt tomography proposes 5x fewer sections cut for same total tissue volume
- Requires 5x more images for total volume than conventional ssTEM
- Results in thinner virtual sections
  - More accurate identifications and measurements
  - Improved auto-segmentation
Conical tomography acquisition:

Sequential tSEM images acquired at single tilted plane by rotation around ROI.
Conical tomography acquisition:

Sequential tSEM images acquired at single tilted plane by rotation around ROI.
First Conical Tilt images: Maintained ROI
tSEM of 250 nm thick sections

• 15 kV – good contrast, signal throughout section depth.
• Can recognize many structures in regions of interest.
tSEM of 250 nm thick section

- Can even recognize spine apparatuses (SA)
Next Goal for tomoSEM: (Techniques in hand)

Resolution (pixel size):
\[ X-Y = 2 \text{ nm}, \ Z = 10-15 \text{ nm} \]

X-Y field:
\[ 2,500 \text{ -} 10,000 \ \mu\text{m}^2 \]

Total extent in Z:
\[ 200 \ \text{@} 250\text{nm} = 50 \ \mu\text{m} \]

Total tomoSEM Volume:
\[ 500,000 \text{ –} 1,000,000 \ \mu\text{m}^3 \]
Aim 3:

• Integrate and test software tools to enhance analysis of synaptic neuropil.

• Developed in Sejnowski Lab
• Tested in Harris Lab
• To be Shared on TACC - Carson
Neuropil Tools for Accurate Surface Areas – Axon example:

Presynaptic ultrastructure changes in response to LTP stimulation in stratum radiatum of hippocampal neurons

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Abstract

Long-term potentiation (LTP) is the fundamental mechanism involved in synaptic plasticity. LTP is a form of synaptic strengthening that occurs as a result of high-frequency stimulation. This process is important for learning and memory. LTP is thought to involve the activation of specific ion channels, such as NMDA receptors, leading to an enhancement of postsynaptic potential. The changes in synaptic strength are thought to be mediated by changes in the number and/or density of synaptic vesicles. However, the mechanisms underlying these changes are not fully understood. In this study, we investigated the effects of LTP on presynaptic ultrastructure using 3D electron microscopy. We found that LTP leads to an increase in the number and size of synaptic vesicles, as well as an increase in the surface area of the presynaptic terminal. These findings provide new insights into the molecular events underlying LTP and suggest novel targets for therapeutic intervention.

Background and Significance

1. Presynaptic and spine growth 3 hours after LTP is balanced with these changes compared to control.
2. Vesicle dynamics and spine dynamics are altered following LTP.
3. LTP-induced changes in surface area and vesicle density are correlated with increased LTP.

Methods: tools coming soon to https://3DEM.org

Results: Vesicle drop enlarges boutons

1. Sustained drop in reserve pool after LTP
   - Boutons exhibit increased surface area and vesicle density. There is a significant decrease in reserve pool size.
2. Bouton Surface Area increases in response to vesicle number
   - Boutons from both control and LTP conditions display larger surface area when compared to control.
3. The summed surface area from the vesicle drop can account for the added surface area of boutons in LTP condition
   - Bouton size increase due to LTP.

Future Directions

1. Determine if there are other covariates that predict pre-synaptic bouton growth (e.g., neuron size, mitochondria volume).
2. Density analysis of vesicles.
3. Analyze boutons lacking mitochondria.

References


5 April 2019 NSF Workshop
NeuroNex Award No. 1707356
Expanded Analyses of Storage Capacity of Hippocampal Synapses:

A Tight Lower Bound on the Storage Capacity of Synapses in the Middle of Stratum Radiatum in Hippocampal Area CA1 in Rat

ABSTRACT

A detailed computational analysis of a dense three-dimensional reconstruction of serial section electron microscopy from the middle of stratum radiatum in hippocampal area CA1 of rat has been analyzed to determine how much information can be stored at a synapse through synaptic plasticity. In a previous study (TM Baudet, & Eich, 2013) the authors measured the coefficient of variation of spine head volumes of 10 nanodots sodium amine (SDNA) spots from the data set and applied a good guess of signal-to-noise ratio (SNR) with a value of 1.1. Then, with a simulation analysis approach, it was found that the SNR Gaussian distributions could span the range of spine sizes of the SDNA spots, implying that the storage capacity of the input ensembles for this region is 4.7 bits of information. We have expanded the analysis data set of 287 spine head volumes using novel clustering approaches and applied information theory and found that there are 42 distinguishable synaptic stochastic equivalent in storing 5-30 bits of information at each synapse. This is a new tight lower bound on the storage capacity of synapses in various subregions in the hippocampal area CA1. Moreover, we determined the SNR of the unique sizes to be 0.10 by analyzing the spine head volume clusters with fitted normal distributions and overlaps of consecutive clusters. Lastly, we have calculated the exact amount of overlap between the consecutive Gaussian distributions (Percentage overlap=6%) by varying 42 normal distributions spanning the range of 287 spine head volumes, which ranged in size over a factor of 16.

Background

The Precision of Synaptic Strength (Calculating the coefficient of variation)

The precision of the coefficient of variation of spine head volume differences between pairs (three-dimensional variance) SDNA spots, CV = 0.065 and was as precise for small synapses as it was for large ones.

Calculating the SNR

We calculated the SNR for synapse sizes in CA1 (SNR = 0.10), assuming the normal distributions spanning the range of 287 spine head volumes (a factor of 16). This means that the area of overlap between two consecutive clusters is 44%. The following graphs show the relationship between the SNR and the amount of overlap for overlapped normal distributions. (Two figures from Schulz, S. 2007. 1. SNR = K × (exp(−k(p, q)2) − 1)/2

Non Overlapping Clustering

We sorted the spine head volumes in decreasing order, chose the first value and then calculated its CV with all other values to cluster those with which the CV is less than 0.063.

Maximum Storage Capacity

Can each synapse adopt all 42 distinguishable sizes?

CONCLUSION

The information stored at a single synapse is encoded in the form of synaptic strength, which reflects the pre- and postsynaptic history experienced by the synapses. The storage capacity of synapses in the middle of stratum radiatum in hippocampal area CA1 of rat is calculated with values of 5.30 bits of information. And the overlap between consecutive clusters of synapses with CV less than or equal to 0.063 is 44% equivalent to SNR 0.10.

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Modeling Synaptic Activity in Sleep sharp wave (REM-memory)

Bartol and Sejnowski, Salk Blender and MCell modeling
Aim 4:

- Interactive Portal (3DEM.org)
- Live link to EM images
- Incorporate 3DEM tools
- Add community tools
- Workshops and Hackathons
- Disseminate content
- Online tutorials
- Broaden access to 3DEM.org
- Now for James Carson and the portal.