Collaborative conjugate array tomography to characterize synapses in mouse neocortex

328.08

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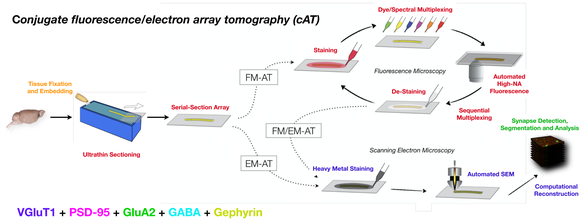
Introduction

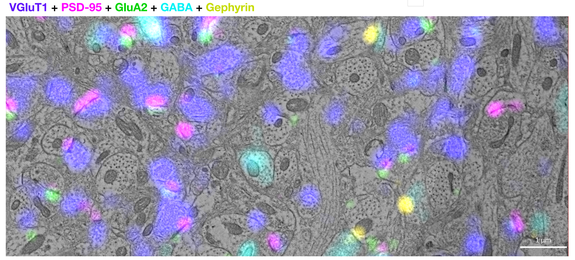
By enabling imaging of individual synaptic compartments with both highly multiplexed immunofluorescence and high-resolution electron microscopy, ***conjugate fluorescence/electron array tomography (cAT)*** offers unique advantages for the study of synaptic molecular composition and structure[1-3]. In these two posters, we describe early results from a multi-institution collaboration aiming to explore cohorts of six different postsynaptic glutamate-gated ion channel subunits present in synapses from mouse neocortex. We have begun the work of generating systematic synapse taxonomies to quantitatively summarize the distinct molecular compositions, ultrastructure and tissue context of diverse synaptic receptor cohorts present at synapses connecting diverse cell types.

1. Smith SJ: **Q&A: Array tomography**. *BMC Biol* 2018, **16**:98. PMID: **30189863**
2. Micheva KD, Busse B, Weiler NC, O'Rourke N, Smith SJ: **Single-synapse analysis of a diverse synapse population: proteomic imaging methods and markers**. *Neuron* 2010, **68**:639-653. PMID: **21092855**
3. Micheva KD, Smith SJ: **Array tomography: a new tool for imaging the molecular architecture and ultrastructure of neural circuits**. *Neuron* 2007, **55**:25-36. PMID: **17610815**

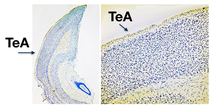
Conjugate fluorescence/electron array tomography (cAT)



VGluT1 + PSD-95 + GluA2 + GABA + Gephyrin



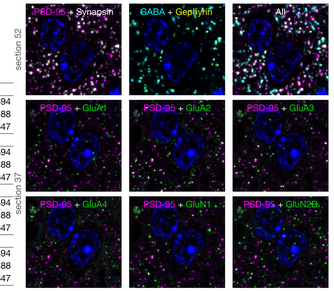
Dataset 201228: adult mouse temporal association cortex (TeA)



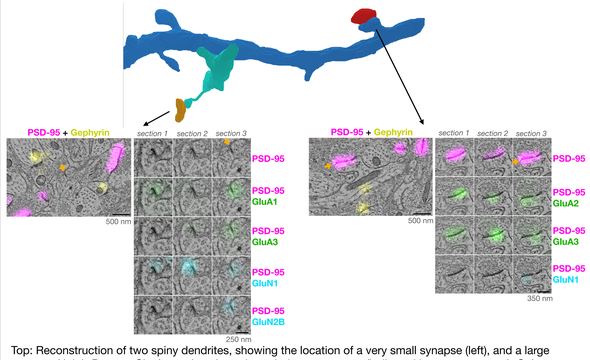
Nissl stain of adjacent vibratome section

74 serial sections, 70 nm each
12 immunofluorescent channels + DAPI + SEM
Immunofluorescence from a single ultrathin section:

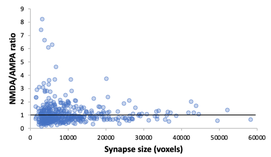
Cycle 1			
GluN1	mouse, SySy 114 011	1:500	594
GluA1	rabbit, Millipore #AB1504	1:100	488
VGluT1	guinea pig, Millipore AB5905	1:5000	647
Cycle 2			
GluA3	mouse, Millipore MAB5416	1:1000	594
GluA2	rabbit, Abcam ab206293	1:50	488
Synapsin	chicken, SySy 106 006	1:100	647
Cycle 3			
GluN2B	mouse, NeuroMab N59/36	1:500	594
GluA4	rabbit monoclonal, Cell	1:50	488
MBP	chicken, AVES MBP	1:100	647
Cycle 4			
Gephyrin	mouse, NeuroMab L106/93	1:100	594
PSD-95	rabbit monoclonal, Cell	1:100	488
GABA	guinea pig, Millipore AB175	1:5000	647



Small vs. large synapses

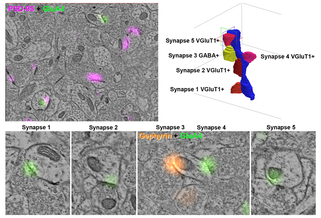


Top: Reconstruction of two spiny dendrites, showing the location of a very small synapse (left), and a large synapse (right). Bottom: Single section views through these synapses (indicated by orange arrows). Columns show different immunolabels; rows show serial 70 nm sections through the same synapse. The small synapse on the left has no detectable PSD-95 immunofluorescence.

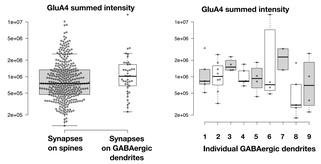


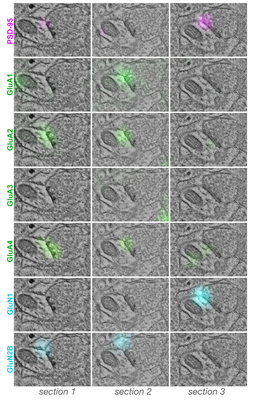
- About 4% of excitatory synapses have no detectable PSD-95 IF, though they contain glutamate receptors.
- Synapses with no or weak PSD-95 IF tend to have brighter NMDA receptor immunolabeling and weaker AMPA receptor immunolabeling.
- Synapses with bright PSD-95 IF tend to have weaker NMDA receptor immunolabeling and brighter AMPA receptor immunolabeling.

Excitatory synapses on GABAergic dendrites have more GluA4



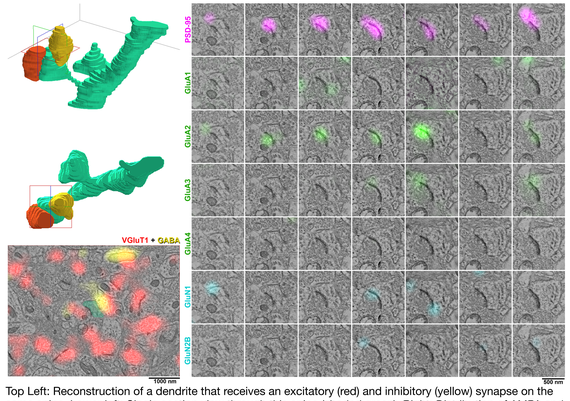
Top Left: Bright GluA4 IF is seen at synapses on GABAergic dendrites. Top Right: Reconstruction of a GABAergic dendrite and its synapses. Below: Single sections through each of the five synapses onto the reconstructed dendrite.



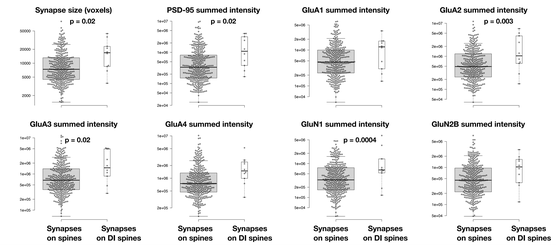


Distribution of AMPA and NMDA receptors at synapse #4. Rows show different immunolabels; columns show serial sections through the same synapse.

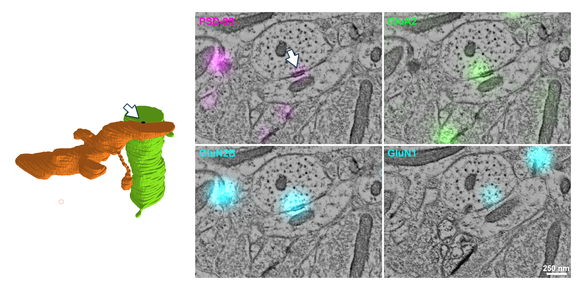
Synapses on dually innervated spines are larger and have more receptors



Top Left: Reconstruction of a dendrite that receives an excitatory (red) and inhibitory (yellow) synapse on the same spine. Lower left: Single section view through this spine (shaded green). Right: Distribution of AMPA and NMDA receptors at the excitatory synapse on the spine. Rows represent different immunolabels, and columns - serial sections through the same synapse.



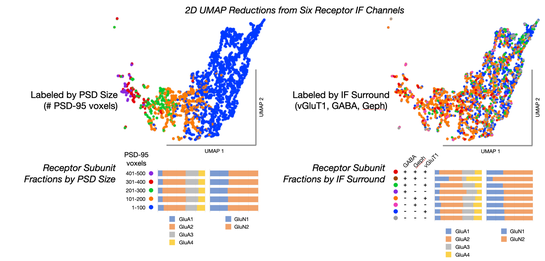
Dendro-dendritic contact



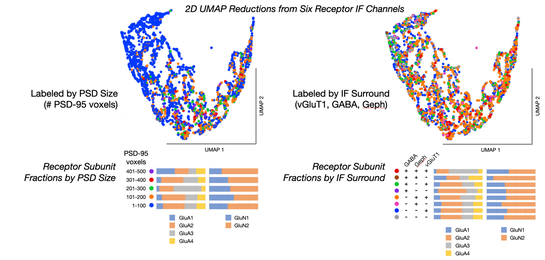
Left: Reconstruction of two dendrites that form an electron-dense contact resembling a *punctum adherens* (arrow). Right: Views from single sections through the contact, showing the presence of PSD-95 immunofluorescence, as well as AMPA (GluA2) and NMDA (GluN1, GluN2B) receptors. 4 such contacts were found within the analyzed volume.

AT Immunofluorescence targeting six postsynaptic glutamate receptor subunits: Four AMPARs (GluA1, GluA2, GluA3, GluA4) and two NMDARs (GluN1, GluN2B)

Sums of voxels segmented by single-synapse PSD-95 contours



Averages of voxels segmented by single-synapse PSD-95 contours



Conclusions

Synapses – even within constrained neurotransmitter classes such as "glutamateric" – are highly diverse in molecular, structural and functional characteristics[4-6]. Here we demonstrate the collaborative use of conjugate fluorescence/electron array tomography (cAT) to quantify synapse-by-synapse variations in the relative abundance of six different glutamate receptor subunits in postsynaptic compartments. Through these pilot studies we have shown single-synapse detections and receptor antigen measurements made by high-throughput immunofluorescence-microscopy-only imaging give results closely comparable to those validated by electron microscopy. Building upon this validation, we have begun the work of generating systematic synapse taxonomies to quantitatively summarize the distinct molecular compositions, ultrastructure and tissue context of diverse synaptic receptor cohorts present at synapses connecting diverse cell type in mouse cortex.

4. Bulovaite E, Qiu Z, Kratschke M, Zgraj A, Fricker DG, Tuck EJ, Gokhale R, Koniaris B, Jami SA, Merino-Serrais P, et al.: **A brain atlas of synapse protein lifetime across the mouse lifespan**. *Neuron* 2022. PMID: **36202095**
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6. O'Rourke NA, Weiler NC, Micheva KD, Smith SJ: **Deep molecular diversity of mammalian synapses: why it matters and how to measure it**. *Nat Rev Neurosci* 2012, **13**:365-379. PMID: **22573027**

Acknowledgements

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