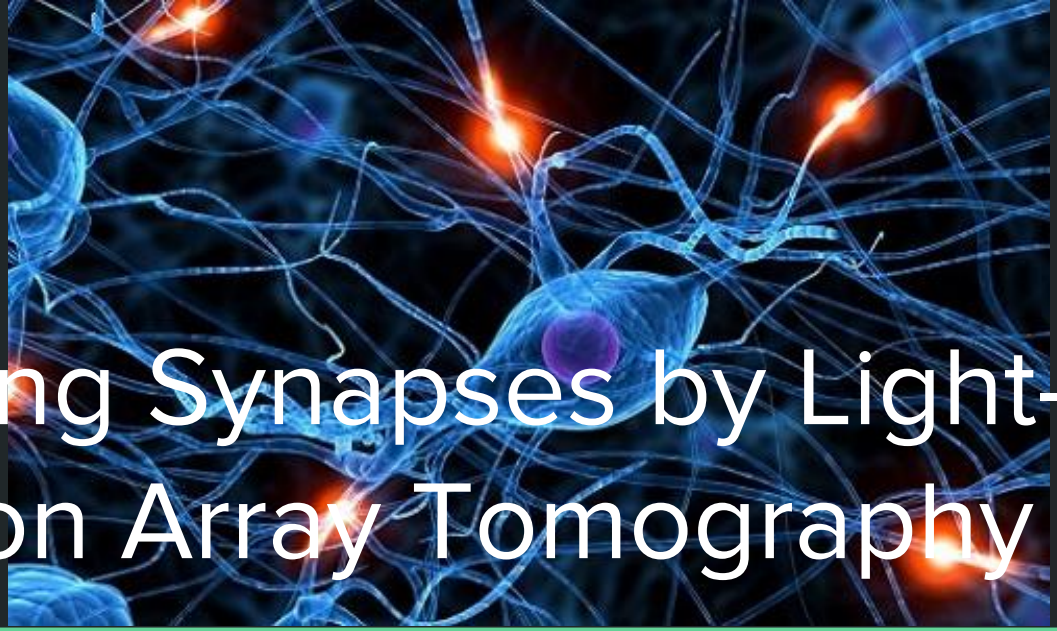


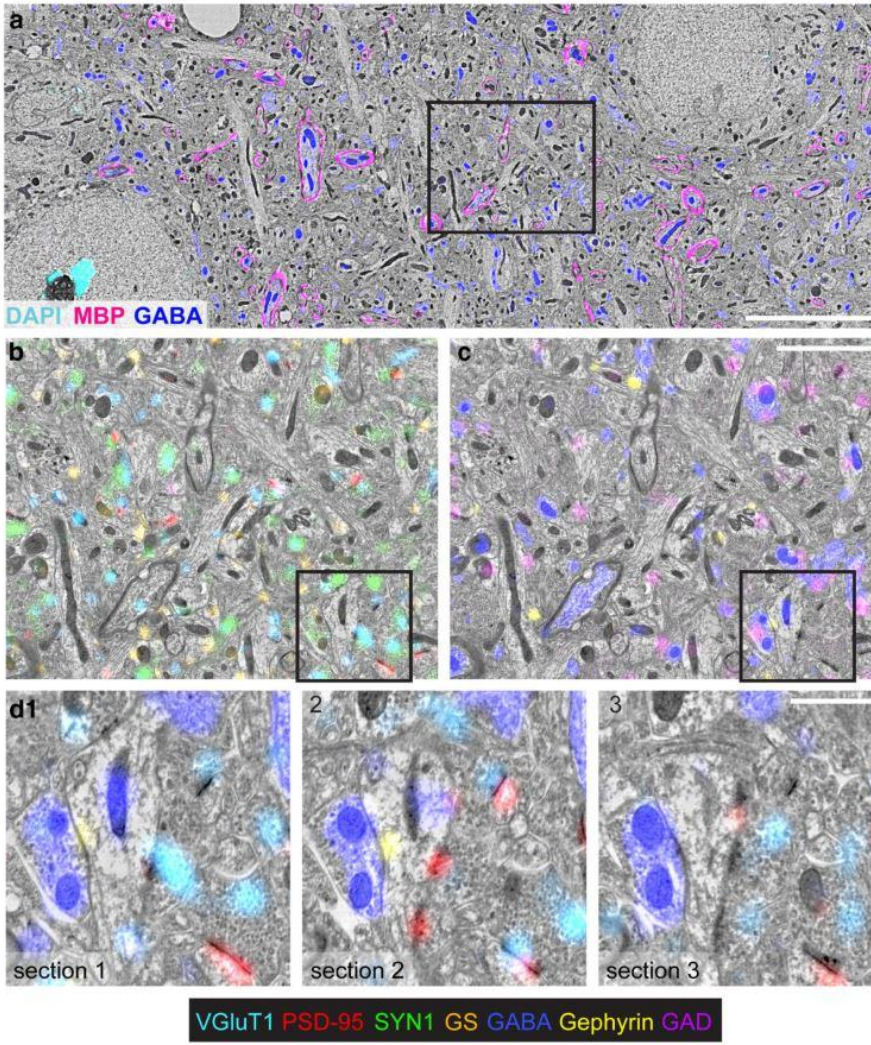
Mapping Synapses by Light-Electron Array Tomography

Forrest Collman, JoAnn Buchanan, Kristen D. Phend, Kristina D. Micheva, Richard J. Weinberg, and Stephen J Smith

Presented by Elizabeth Morgan

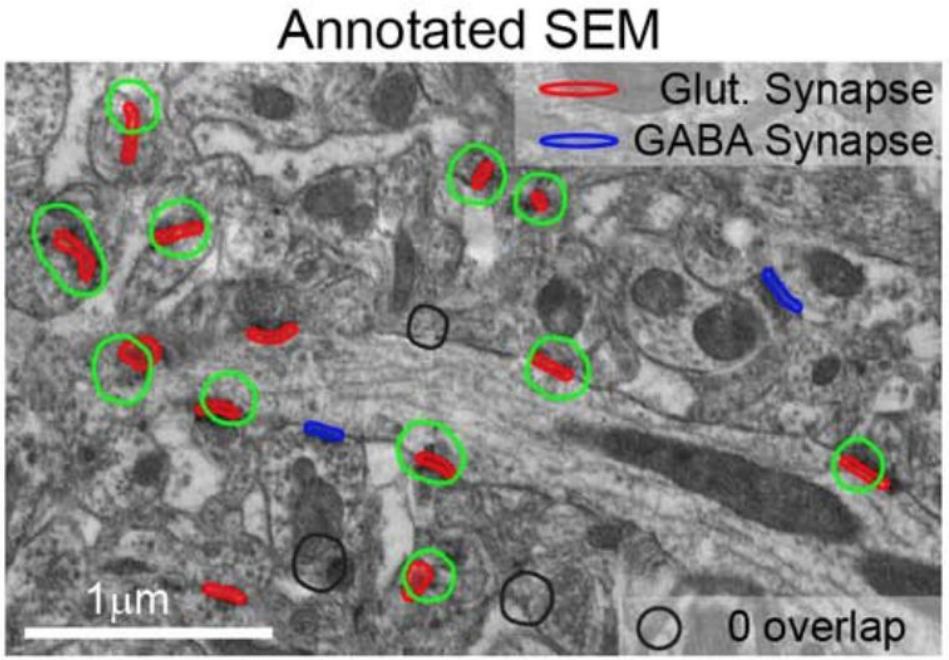
Summary

- Conjugate array tomography
  - Molecular survey of synapses from a region in the mouse neocortex
- Combined immunofluorescence and scanning electron microscopy array imaging with computational image reconstruction, visualization and analysis methods



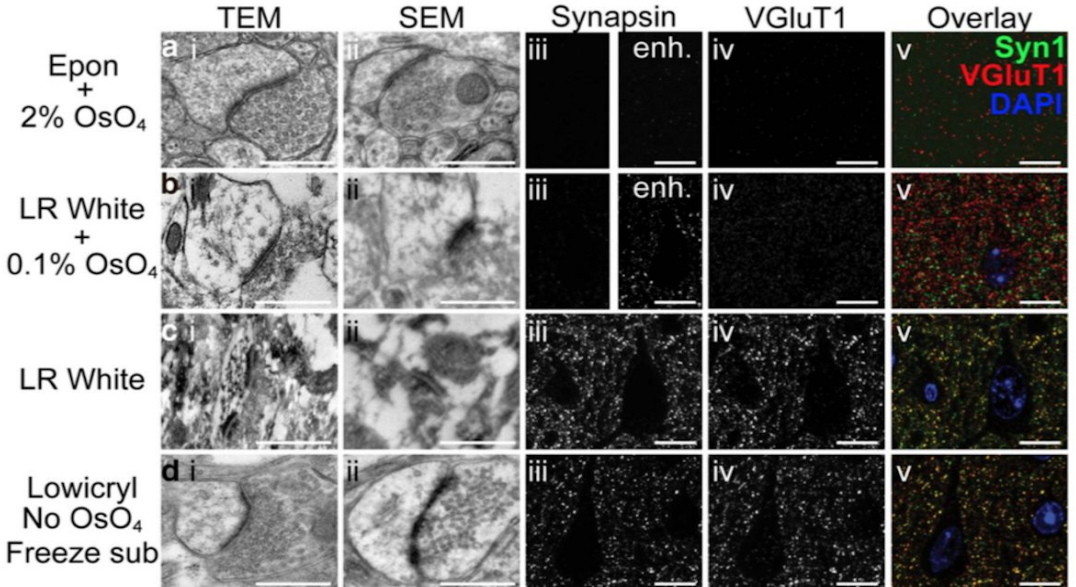
Opportunity

- Synapses have many variations in structure
  - Understanding development, plasticity, storage, etc.
- Quantitative analysis and mapping of synapses have been limited by single-synapse measurement methods

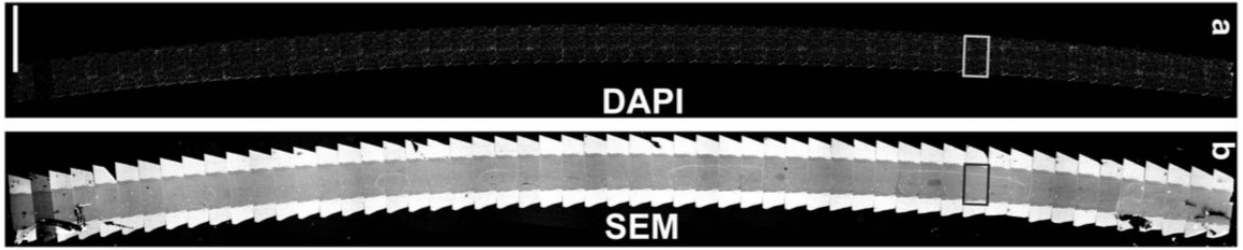


Challenge

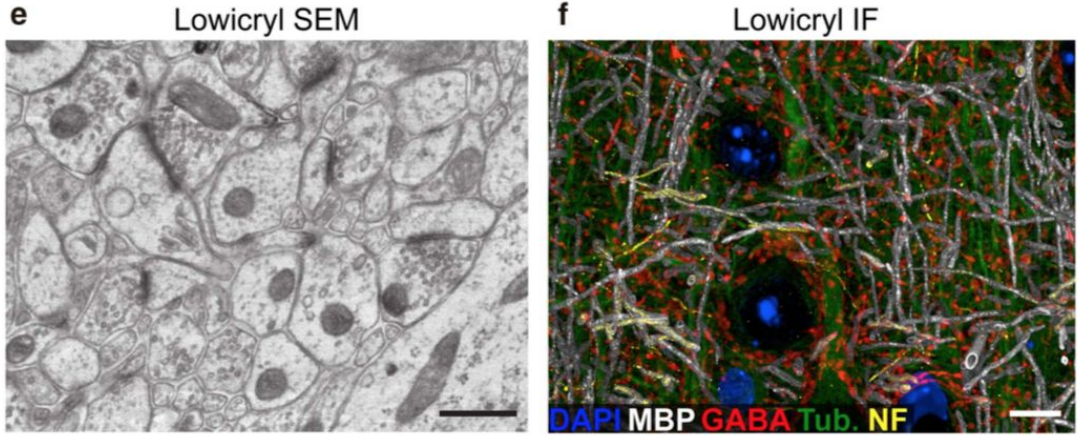
- Array tomography (AT) - reconstruction from ultrathin sections
  - Immunofluorescence (IF) - molecular information and large synapse populations
  - Scanning electron microscopy (SEM) - detailed synapse recognition
  - Different requirements for preservation of immunoreactivity and ultrastructure limit the use of IF-AT and SEM-AT on the same specimens



Action



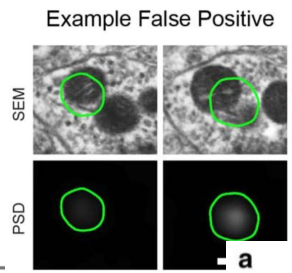
- Optimization of freeze-substitution methods
  - Lowicryl HM-20
- Used both presynaptic and postsynaptic markers
- Identified synapses belonging to 4 basic subtypes
- Methods for computational alignment and registration of different types of images

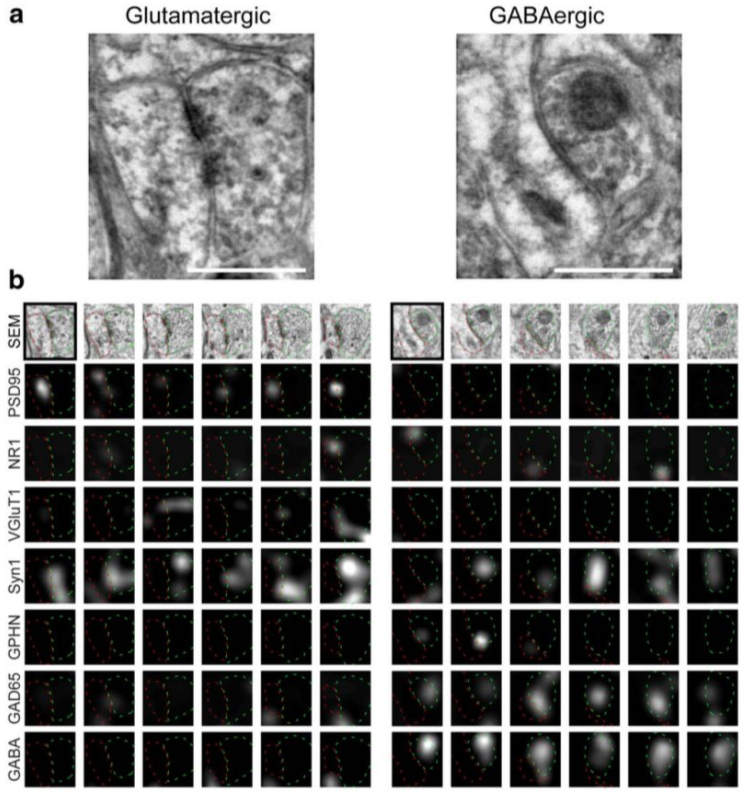


Action

Table 1. List of antibodies, sources, and dilutions used in this study

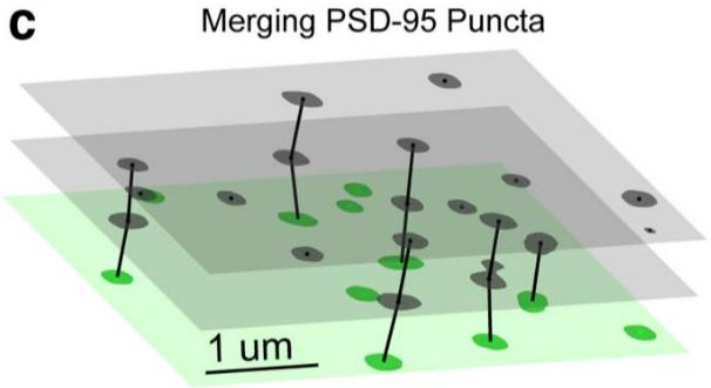
Antigen	Host	Antibody source	Dilution
Synapsin 1	Rabbit	Cell Signaling Technology 5297S	1:200
Synaptophysin	Mouse	Abcam ab8049	1:15
VGluT1	Guinea pig	Millipore AB5905	1:5000
PSD-95	Rabbit	Cell Signaling Technology 3450S	1:200
GluN1	Mouse	Millipore MAB363	1:200
GABA	Guinea pig	Millipore AB175	1:10000
GAD2	Rabbit	Cell Signaling Technology 5843	1:200
Gephyrin	Mouse	Biosciences Pharmingen 612632	1:100
α-Tubulin	Rabbit	Abcam ab18251	1:100
Acetylated α-tubulin	Mouse	Sigma-Aldrich T6793	1:100
βIII-tubulin	Chicken	Abcam 41489	1:200
γ-Actin	Mouse	Sigma-Aldrich A8481	1:100
Glutamine synthetase	Mouse	BD Biosciences 610517	1:25
Prohibitin	Rabbit	Abcam ab28172	1:100
GFP	Chicken	GeneTex GTX13970	1:100
MBP	Chicken	AVES MBP	1:100

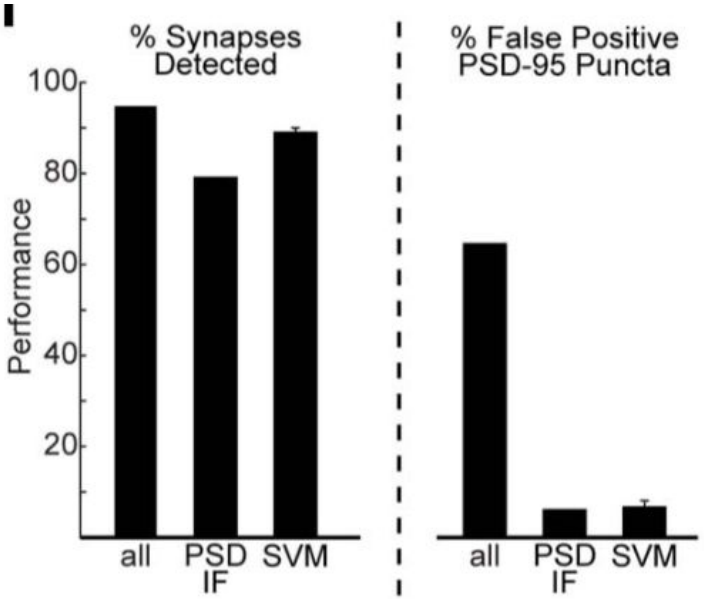




Resolution

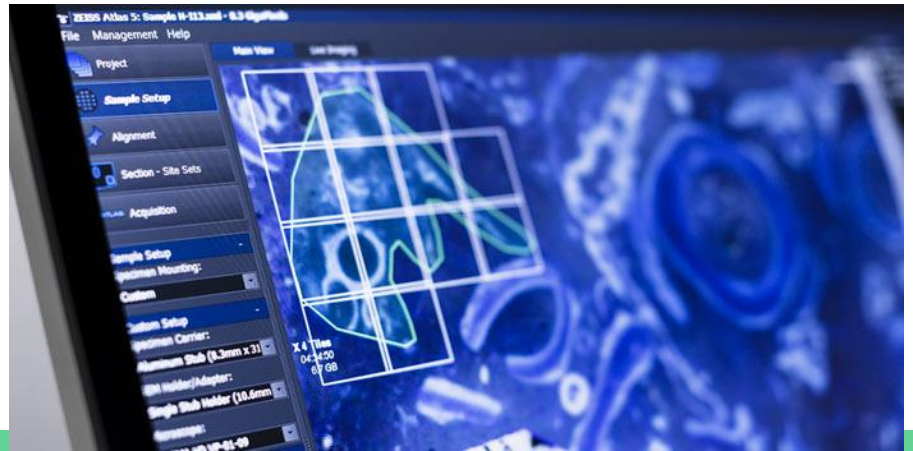
- Conjugate AT provides high-resolution structural and molecular imaging
- Alignment and tracing accurate within 1 pixel
- Found 90.9 ± 2.1% of glutamatergic synapses
- Map 3D shape of synapses





Future

- Broader efforts to map synapses and the connectome
  - can be applied in many different brain regions, tissues, and species
- Further improvements in treating the samples might allow for better EM imaging
- Automated synapse detection from EM is being developed
  - immunofluorescence channels could aid automated detection



Discussion

Pros

- Allows identification of all synapses within SEM
- Provides gold standard to compare with automated detection

Cons

- Doesn’t replace techniques optimized for EM imaging
- Detailed axonal processes can’t be reliably traced over long distances