PROJECT SUMMARY (See instructions):

The long-term goal of this research is to establish a center for electron tomography (ET), supported by the BRAIN Initiative, where structural biologists and neuroscientists will collaborate to study synaptic connections in the central nervous system. ET is well-suited to analyze synapses because the tomographic reconstruction can be rotated in 3 dimensions to obtain the orientation required for precise measurements. It is particularly important for rare, but functionally-important, synapses that are unlikely to be found in the optimal orientation by chance using conventional transmission electron microscopy (EM). The experiments proposed here all deal with synapses that are relatively small, sparsely-distributed or both, and they would be very difficult to carry out without ET. They utilize mammalian retinas as model systems, but they are typical of collaborative research projects planned for the future.

Specific Aim 1: Characterize the post-synaptic densities at synapses made by amacrine cells containing vesicular glutamate transporter 3 (vGluT3). The working hypothesis, based on preliminary results using EM immunolabeling, is that these neurons make excitatory synapses onto some neurons and inhibitory synapses onto others. Fixed retinas from a transgenic mouse expressing a fluorescent protein in vGluT3 cells will be irradiated in diaminobenzidine to generate an electron-dense reaction product, and sections of the retina will be studied by ET. The postsynaptic density at inhibitory synapses would be relatively thin, only an increase in electron density of the plasma membrane. The postsynaptic density at excitatory synapses would extend 30 nm or more into the cytoplasm.

Specific Aim 2: Identify the synapses made by axon collaterals of intrinsically-photosensitive retinal ganglion cells in primate retinas. The working hypothesis, based on electrophysiology and light microscopic immunolabeling results, is that dopaminergic amacrine cells receive excitatory inputs at these synapses. The axons will be labeled with antibodies to Pituitary Adenylate Cyclase Activating Polypeptide (PACAP), and tangential sections through the distal inner plexiform layer will be analyzed by ET. Labeled synapses onto perikarya and primary dendrites of large amacrine cells are expected.

Specific Aim 3: Identify the connexin used by rods in their gap junctions with cones, and describe the structure of these junctions using ET. The working hypothesis, based on electrophysiological results, is that they use connexin 36, as the cones do, and that the junctions will appear symmetrical. Mouse and baboon retinas will be labeled with antibody to connexin 36 and the outer plexiform layer will be studied using ET. Baboon retinas will also be fixed to optimize preservation of the ultrastructure, and these unlabeled retinas will be studied using ET.

RELEVANCE (See instructions):

This collaboration will provide insight into the first stages of visual information processing, and it will also serve as an example of the benefits of pursuing connectomics and electron tomography in parallel, a novel approach. Funds are requested to support the Contact PI, Dr. David W. Marshak and a Research Associate, Ms. Andrea Bordt; both are members of the Department of Neurobiology and Anatomy at UT Medical School-Houston and have extensive experience studying the retina by electron microscopy. The subcontract will support PI Dr. Michael B. Sherman, an Assistant Professor in the Department of Biochemistry and Molecular Biology at the University of Texas Medical Branch and an expert on electron microscopic tomography. The proposed experiments on the retina are responsive to three goals of the BRAIN initiative. Describing the synaptic connections of retinal neurons is the first step in the effort to “characterize all cell types in the nervous system” and to “map connected neurons in local circuits…enabling the relationship between neuronal structure and function.” Some of the proposed experiments will be done using primate tissue, a third goal of the BRAIN initiative. The Contact PI narrowly missed funding from the BRAIN Initiative last year. The summary statement described, “the review panel’s high level of enthusiasm for this outstanding application from a superb investigative team,” and this bodes well for future submissions.