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ELECTRON TOMOGRAPHIC STRUCTURE AND PROTEIN COMPOSITION OF ISOLATED RAT CEREBELLAR, HIPPOCAMPAL AND CORTICAL POSTSYNAPTIC DENSITIES

M. M. FARLEY, [a,b] **M. T. SWULIUS** [a†] **AND M. N. WAXHAM** [a*]

[a] *The Department of Neurobiology and Anatomy, University of Texas Health Science Center at Houston, 6431 Fannin Street, Houston, TX 77030, United States*

[b] *Graduate School of Biomedical Science, University of Texas Health Science Center at Houston, Houston, TX 77030, United States*

Abstract—Electron tomography and immunogold labeling were used to analyze similarities and differences in the morphology and protein composition of postsynaptic densities (PSDs) isolated from adult rat cerebella, hippocampi, and cortices. There were similarities in physical dimensions and gross morphology between cortical, hippocampal and most cerebellar PSDs, although the morphology among cerebellar PSDs could be categorized into three distinct groups. The majority of cerebellar PSDs were composed of dense regions of protein, similar to cortical and hippocampal PSDs, while others were either composed of granular or lattice-like protein regions. Significant differences were found in protein composition and organization across PSDs from the different brain regions. The signaling protein, βCaMKII, was found to be a major component of each PSD type and was more abundant than αCaMKII in both hippocampal and cerebellar PSDs. The scaffold molecule PSD-95, a major component of cortical PSDs, was found absent in a fraction of cerebellar PSDs and when present was clustered in its distribution. In contrast, immunogold labeling for the proteasome was significantly more abundant in cerebellar and hippocampal PSDs than cortical PSDs. Together, these results indicate that PSDs exhibit remarkable diversity in their composition and morphology, presumably as a reflection of the unique functional demands placed on different synapses.
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Key words: postsynaptic density, electron tomography, electron cryotomography, immunogold labeling, receptors, scaffold proteins.

*Corresponding author. Tel: +1-713-500-5621.
E-mail addresses: Madeline.M.Burgoyne@uth.tmc.edu (M. M. Farley), mswulius@caltech.edu (M. T. Swulius), M.N.Waxham@uth.tmc.edu (M. N. Waxham).
† Present address: Department of Biology, California Institute of Technology, 1200 E. California Boulevard, Mail Code 114-96, Pasadena, CA 91125, United States.
Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BSA, bovine serum albumin; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase; CaM, calmodulin; EM, electron microscopy; ECT, electron cryotomography; GABA, gamma-aminobutyric acid; GKAP, guanylate kinase-associated protein; GluR, glutamate receptor; HBS, HEPES buffered saline; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NMDA, N-methyl-D-aspartate; NP40, tergitol-type NP-40; PSD-95, postsynaptic density protein 95; PSD, postsynaptic density; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SV2, synaptic vesicle glycoprotein 2A.

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INTRODUCTION

Composed of signaling, scaffold and cytoskeletal proteins, the postsynaptic density (PSD) is an enormous ~1 GDa (Chen et al., 2005) macromolecular protein complex positioned just beneath the postsynaptic membrane (Gray, 1959; Cotman et al., 1974; Blomberg et al., 1977; Cohen et al., 1977; Sheng and Hoogenraad, 2007). The PSD regulates the efficiency of synaptic transmission by stabilizing neurotransmitter receptors in the postsynaptic membrane and functionally organizing signaling molecules within the postsynaptic compartment. Activity-dependent changes to the molecular composition and organization of the PSD are believed to underlie the synaptic modifications necessary for plasticity, and learning and memory (for reviews see (Okabe, 2007; Sheng and Hoogenraad, 2007)). In support of this concept, the composition and structure of the PSD change in an activity-dependent manner (Dosemeci et al., 2001; Ehlers, 2003; Tao-Cheng et al., 2010; Yang et al., 2011) and through development (Harris et al., 1992; Petralia et al., 2005; Swulius et al., 2010, 2012). Significant work has been done to identify the PSD proteome (Jordan et al., 2004; Li et al., 2004; Peng et al., 2004; Yoshimura et al., 2004; Dosemeci et al., 2006) and these efforts have led to a converging list of approximately 300 proteins. Additional effort has been made in mapping the spatial organization of a subset of individual proteins within the PSD (Dosemeci et al., 2001; Valtschanoff and Weinberg, 2001; Petersen et al., 2003; DeGiorgis et al., 2006; Swulius et al., 2010) in order to better understand how proteins and protein modules are functionally organized. However the degree of complexity, coupled with a dynamic protein composition, makes the PSD a particularly challenging subject for structural analysis, leading to continuing demands for experimental data describing the morphology and spatial organization of individual proteins within the PSD.

Different neuronal subtypes populate anatomically distinct regions of the brain and synaptic connections within these distinct regions are specialized to serve the functional demands unique to each region. These differences would necessarily include unique specialization of both PSD composition and structure. Yet, there has been minimal work directly quantifying differences between PSDs from different brain regions. Gross differences in morphology have been described for forebrain and cerebellar PSDs by examining fixed, thin-sectioned and negative-stained preparations by electron microscopy (EM), revealing that forebrain PSDs were disk-like in shape, ranging from ~100 to 500 nm in diameter and ~60-nm thick, while cerebellar PSDs were approximately the same diameter but thinner (~30 nm) (Carlin et al., 1980). Western blot analysis and quantitative proteomics have also highlighted molecular differences in PSD fractions from the forebrain and cerebellum for a variety of glutamate receptors, signaling molecules and PSD scaffolds (Cheng et al., 2006). While these works provide further evidence of the unique regional differences of the PSD complex, there remains a need to build a more refined description of PSD structure and composition to understand synapse specific structure and function.

To advance this goal, we isolated PSDs from cerebella, hippocampi and cerebral cortices, three brain areas amenable to straightforward isolation that contain unique distributions of neuronal cell types. Electron tomography and immunogold labeling were then employed to assess how the structure, protein composition and protein spatial organization differ in individual PSDs from these unique brain regions. We chose to employ electron tomography because of its unique capability to produce 3D structural information of the PSD at the molecular level and because it has been productively employed to visualize PSD structure (Chen et al., 2008; Swulius et al., 2010, 2012; Fera et al., 2012). 3D structures were produced of cryo-preserved PSD specimens, that avoid artifacts of fixation and staining, providing novel views of the isolated PSD as it exists in a ‘‘frozen-hydrated’’ state. Immunogold labeling was employed for a set of some of the most abundant and well-known PSD-associated proteins to map their 2D spatial distribution within PSDs isolated from each brain region.

EXPERIMENTAL PROCEDURES

PSD isolation

PSDs were isolated following a previously reported protocol (Swulius et al., 2010, 2012), which was adapted from a widely used PSD enrichment procedure (Cohen et al., 1977). For a single preparation, brains were removed within 30 s of decapitation from adult male Sprague–Dawley rats (176–200 g) and placed in an ice-cold isotonic sucrose solution of 0.5 mM HEPES/KOH pH 7.4, 0.32 M sucrose, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$. The cerebella, hippocampi and cortices were immediately dissected and separately homogenized in a sucrose solution (0.5 mM HEPES/KOH pH 7.4, 0.32 M sucrose, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 1 μg/ml leupeptin) with a motor-driven glass/Teflon homogenizer (0.2 mm clearance). All steps of the following protocol were accomplished at 4 °C. For each region, homogenates were spun at 1400*g* for 10 min, supernatants saved and pellets resuspended and spun again at 1400*g* for 10 min. The supernatants were combined and pelleted at 13,800*g* for 10 min. The resulting pellets were resuspended and hand homogenized in a second sucrose solution (0.5 mM HEPES/KOH pH 7.4, 0.32 M sucrose, 1 μg/ml leupeptin), applied to sucrose gradients (13 ml 1.4 M sucrose, 12 ml 1.0 M sucrose) and spun at 112,000*g* for 120 min. The synaptosomal fraction, at the 1.0/1.4 M interface, was diluted in an equal volume of triton extraction buffer (5 mM HEPES/KOH pH 7.4, 0.32 M sucrose, 1% TX-100), homogenized and rotated for 15 min before being applied to a second sucrose gradient (2 ml 2.1 M sucrose, 4 ml 1.5 M sucrose, 2 ml 1.0 M sucrose) and spun for 120 min at 271,000*g*. The synaptic junction fraction, the interface between the 1.5 M and 2.1 M sucrose, was then resuspended in an equal volume of a second triton extraction buffer (5.0 mM HEPES/KOH pH 7.4, 1% TX-100) and rotated for 30 min. To produce the PSD fraction, the material was then added to the final sucrose gradient (2 ml 2.1 M sucrose, 4 ml 1.5 M sucrose) and spun at 210,000*g* for 20 min. The material at the 1.5/2.1 M interface was then diluted in 5 mM HEPES/KOH pH 7.4, pelleted, resuspended in 20% glycerol in 5 mM HEPES/KOH pH 7.4, and stored as aliquots at −80 °C.

The data described in this report were produced from two independent PSD preparations that each contained the three isolated brain regions from nine rats. It is important to acknowledge that the process of isolating the PSD from the brain has the potential to alter its structure and composition. This limitation should be kept in mind when attempting to place the findings in this report in the context of PSD structure and function *in vivo*.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and Western blotting

For Western blotting, 10 μg of total protein from homogenate, synaptosome, synaptic junction or PSD fractions from cerebella, hippocampi and cortices were separated by SDS–PAGE with 10% polyacrylamide gels. Separated proteins were transferred to nitrocellulose membranes at 4 °C for 2 h at 80 volts and membranes were then incubated in blocking buffer (5% dry milk in wash buffer (10 mM Tris, pH 8.0, 150 mM NaCl, and 0.05% tergitol-type NP-40 (NP40)). Membranes were then incubated in primary antibodies to synaptic vesicle glycoprotein 2A (SV2) (Developmental Studies Hybridoma Bank) or postsynaptic density protein 95 (PSD-95) (Thermo Scientific, Waltham, MA, USA, MA1-046), diluted 1:1000 in blocking buffer, for 1 h, rinsed twice in wash buffer, and incubated in secondary antibody Alexa 488 goat anti-mouse (Molecular Probes, Eugene, OR, USA, A-11029) diluted 1:5000 in blocking buffer for 1 h. Membranes were washed twice prior to imaging on a Typhoon Trio$^+$ scanner (GE Healthcare Life Sciences, Pittsburgh, PA, USA). For protein staining, 3 μg of total protein from cerebellar, hippocampal and cortical PSD fractions were separated by SDS–PAGE with 10% polyacrylamide gels. Gels were incubated for 1 h in excess fixation solution (30% methanol, 7.5%

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