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Ultrastructural correlates of vesicular docking in the rat dentate gyrus

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Abstract

To determine the extent to which CA1 synapses are typical of those found in other regions of the hippocampal formation, we have carried out a quantitative analysis of synapses in the middle molecular layer of the rat dentate gyrus, reconstructed from serial electron microscopy, and have compared these data with previous observations from CA1. In general, the morphology of synapses in areas CA1 and the dentate agree, other than an increased density of multisynaptic boutons. Thus, it seems that either area may form an equally effective model for the function of individual synapses in the hippocampal formation. In addition, the current study examines presynaptic curvature, which recent mathematical models have suggested may have profound effects on synaptic transmission. When synapses of distinct curvature profiles (i.e., presynaptically concave, convex, and flat) are examined, the average characteristics of these three synapse populations are distinct. In general, concave synapses have a greater number of morphologically docked vesicles, and thus, likely a greater probability of release. This, however, seems to be accounted for by the fact that these synapses are larger—the spatial density of docked vesicles remains identical across these curvature profiles. This study provides crucial data for further modeling of individual synapse function.

Keywords: Curvature; Synaptic vesicle; Active zone; Hippocampus; Dentate gyrus; Release probability

The hippocampal formation plays a critical role in understanding neural physiology due to its simple structure and its importance in learning and memory. Much of what is known about the structure of synapses in the hippocampal formation, however, is derived from extensive morphometric studies of Schaffer collateral synapses between regions CA3 and CA1 (e.g., [1,11–13,19,21]). Despite these data, several questions about synaptic physiology within the hippocampal formation remain unaddressed.

To what extent are Schaffer collateral synapses structurally like those found in other regions of the hippocampal formation? To approach this question, quantitative analysis of excitatory synapses has been undertaken in the dentate gyrus middle molecular layer (MML). This region is suitable for the physiological analysis of non-Schaffer collateral synapses since (a) it has an equally simple structure and (b) it has been implicated in many electrophysiological (e.g., [14]), anatomical (e.g., [4]), and molecular (e.g., [23]) studies of hippocampal plasticity in vivo. Thus, one of the main goals of the current study is to determine the physiologically relevant morphological properties of synapses of the dentate gyrus and compare this to the currently available information on synapses from the hippocampus proper.

A second issue to be dealt with is the relationship between several morphological parameters, such as terminal and postsynaptic density (PSD) size, and synaptic curvature. Mathematical models have suggested that compartmentalization (see [16]) may have profound effects on the probability of transmitter release (PTR). Transmitter release has long been known to depend on intracellular calcium concentrations ($[Ca^{2+}]_i$), but the exact mechanism by which $[Ca^{2+}]_i$ facilitates synaptic transmission is unclear [9,10,16,24]. Although it is not yet possible to image these phenomena in vivo, modeling local microgeometry has suggested that the smaller the compartment surrounding the release site, the higher the local $[Ca^{2+}]_i$ (since it is less able to diffuse), increasing PTR [9,10]. Initial modeling research has parsed the

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critical features contributing to compartmentalization: (a) the width of the terminal; (b) the distance from the site of vesicular release to the terminal wall, and (c) the existence and size of terminal partitions. Many of these parameters, however, have yet to be explored through quantitative morphometry.

One factor critical to compartmentalization is synaptic curvature. For example, concave synapses are generally thought to be more compartmentalized than convex synapses. In fact, in subsequent modeling research [24], synaptic curvature has been found to affect PTR in two distinct ways. Increasing a terminal's concavity (i.e., the degree to which the presynaptic terminal protrudes into the postsynaptic element) enhances PTR. In addition, PTR is enhanced by increasing the proximity of a docked vesicle to the concave inflection point (i.e., the point of maximal concave curvature). Given that curvature is an established plastic feature of the synapse (see [16] for a review) and that it may have profound impact on synaptic function [9,10], exploratory spatial characterization of these synapse types are required.

These data allow for the correlation of curvature with other morphological measures of PTR. Arguably, the best established of these correlates is the presence of morphologically docked vesicles [17], which are vesicles that appear in electron micrographs immediately adjacent to the active zone membrane. The readily releasable pool, which refers to quanta available for immediate release upon high frequency stimulation, determines PTR, and evidence suggests this pool coincides with the morphologically defined docked vesicle pool [17,19,22]. Thus, simultaneously quantification and correlation of the size, curvature, and the number of morphologically docked vesicles in individual synapses may address how they mediate PTR.

Six male Long–Evans rats aged 45–60 days were anesthetized with 2-ml/kg sodium pentobarbital (Nembutal), and perfused with 10 ml physiological saline followed by fixative [2% paraformaldahyde, 2% glutaraldehyde in 0.1 M phosphate buffer (PB), pH 7.2]. Brains were post-fixed for 24 h and given three 1 h washes in PB. Four 0.5 mm mid-dorsal sections were then randomly dissected from the ventral dentate gyrus, placed in 1% OsO_4 (4 °C, 1 h), dehydrated in a graded series of ethanol, and embedded in Spurr's (Ladd Research Industries, Burlington, Vermont) medium.

Embedded sections included the granule cell layer and the entire extent of the dentate gyrus. The MML was identified as the middle third of this expanse, and trimmed using Toluidine Blue stained semi-thin (l μm thick) sections for orientation. Using an Ultracut microtome with a diamond knife, a series of 22–33 (mean = 30) ultra-thin serial sections per block were mounted with Formvar onto slotted copper grids and counterstained with uranyl acetate and lead citrate. AnalySIS 3.0 (Soft Imaging Systems) software was used to digitally photograph and analyze the MML on a Hitatchi H-7500 electron microscope at 30,000× magnification.

Synapses were sampled if vesicles, as well as both pre- and postsynaptic densities were observed. All synapses within the reconstructed volume were included provided: (a) synapses were asymmetric, axospinuous, and macular; (b) boutons contained only a single synapse; (c) the PSD was cut tangentially (i.e., all sections had clear membrane outlines to permit accurate judgment of docked vesicles); and (d) the entire terminal and postsynaptic spine were within the volume reconstructed. Synaptic contacts were classified into several categories (presynaptically concave, convex, or flat) according to curvature (e.g., [15]). Vesicles were considered docked when the vesicle membrane was immediately adjacent to the active zone membrane (see Fig. 1).

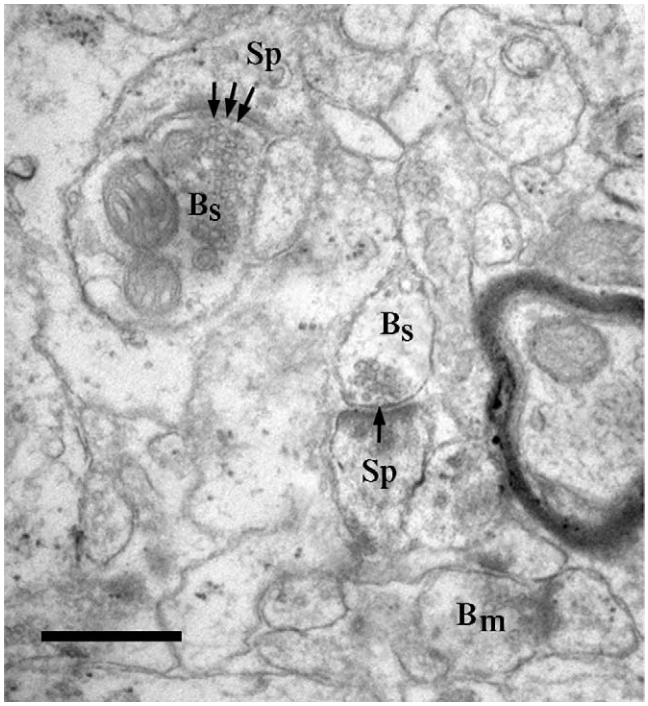

Fig. 1. Typical electron micrograph used in the current study. Depicted in the figure are two simple monosynaptic boutons (B_s) forming synapses on dendritic spines (S_p), as well as one multisynaptic bouton (B_m) excluded from the study. Docked vesicles are shown with arrows. Scale bar = 500 nm.

For each sampled synapse, a series of measurements were taken at every profile for a given synapse within the series. Section thickness was calibrated using the cylindrical diameters method [7]. Similar to previous methods [19,20], the area of the PSD equated to the length of the boundary of the PSD in each individual section times the section thickness. The sum of these products yielded the PSD area. Similarly, boutons were reconstructed by tracing their area in every section in which they appeared and multiplying this by section thickness. Curvature was measured by taking the shape of the PSD as a uniform arc (see Fig. 2). Through trigonometric identities, the angle of this arc from its origin (θ_{ARC}) can be deduced from the angle of the PSD (θ_{PSD}) by the following formula [3]:

$$\theta_{ARC} = 360 - (2\theta_{PSD})$$

To maintain direction, all concave curvatures were made positive while convex curvatures were made negative.