# Morphological Characteristics of the Synapse and Their Relationship to Synaptic Type: An Electron Microscopic Examination of the Neocortex and Hippocampus of the Rat

ETAN J. MARKUS, TED L. PETIT, JANELLE C. LeBOUTILLIER, AND WILLIAM J. BROOKS
*Department of Psychology and Program in Neuroscience, University of Toronto, Toronto, Ontario M1C 1A4, Canada*

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Synapses show a high degree of structural diversity and plasticity, including differences in size, curvature, and the existence of perforations. In concave (relative to the presynaptic element) synapses, the presynaptic density curves or pushes into the postsynaptic element, while in convex synapses the postsynaptic element pushes into the presynaptic terminal. Changes in synaptic curvature appear to parallel changes in the developmental or functional state of neurons although these changes appear to vary between the neocortex and the hippocampus (Calverley and Jones, 1990; Cronin et al., 1987; Desmond and Levy, 1990; Devon and Jones, 1981; Dyson and Jones, 1980; Hayes and Roberts, 1973; Markus and Petit, 1989; Petit et al., 1989; Rees et al., 1985). Perforated synapses have also been associated with neuronal activation, with an increase in perforated synapses being found following events such as environmental enrichment (Greenough et al., 1978), long-term potentiation (LTP) (Geinisman et al., 1991), kindling (Geinisman et al., 1992), and lifespan development (Jones and Calverley, 1991; Jones et al., 1992); see Jones et al. (1992) and Calverley and Jones (1990) for reviews. Given the importance of different synaptic types in neural plasticity, this research was conducted to determine if they have different morphological characteristics, and whether the characteristics are consistent in brain regions where they may play different roles, i.e., neocortex and hippocampus.

Rats were anesthetized and perfused, and tissue was prepared for electron microscopy using the ethanol phosphotungstic acid (EPTA) or osmium, uranyl acetate, lead citrate (OsUL) method (see Anthes et al. [1993] for methodological details). Following embedding and orientation, synapses were photographed in the middle third of the molecular layer of the hippocampal dentate gyrus (ventral leaf) or in layers I through IV of the dorsal occipital cortex. Photographs were taken over consecutive adjacent frames, comprising linear, non-overlapping rows. Approximately 12 synapses per animal, from one block, were examined in a given brain region and stain, for a total of 2,129 synapses: 835 OsUL and 386 EPTA synapses from the neocortex, and 800 OsUL and 108 EPTA synapses from the hippocampus (from 70, 30, 65, and 10 animals, respectively). The first 12 synapses encountered were photographed in an unbiased manner. Photographs were taken at 80,000× and enlarged to 240,000×.

Osmium stained synaptic contacts were defined as having a clear postsynaptic thickening, synaptic cleft, and a minimum of 3 adjacent synaptic vesicles. EPTA stained synapses were defined as having clear presynaptic dense projections, postsynaptic thickening (density), and cleft. Synapses were categorized according to their curvature as outlined above. Synapses which showed curvature in more than one direction were referred to as "W" shaped, and those which showed no curvature were considered flat. Synapses with breaks in their postsynaptic density were categorized as perforated. Since EPTA does not stain the adjacent membrane and nerve terminal, perforated synapses can only be determined with certainty in osmium stained material. Also, since the osmium stained material does not allow a definitive examination of the characteristics of the presynaptic dense projections (they are obscured by synaptic vesicles), measures of the presynaptic density are derived from EPTA stained material only.

An analysis of the synaptic junction was carried out using the Bioquant Image Analysis System, including quantification of the following synaptic parameters: presynaptic density length, area, and maximal dense projection height (EPTA stain only), postsynaptic density length, area, and average width (or height) (obtained by dividing the postsynaptic area by its length). Measures of synaptic length in perforated synapses included the length of the perforation.

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Address reprint requests to Dr. Ted L. Petit, Division of Life Sciences, University of Toronto, 1265 Military Trail, Scarborough, Ontario M1C 1A4, Canada.

E.J. Markus is currently at the Department of Psychology, University of Arizona.

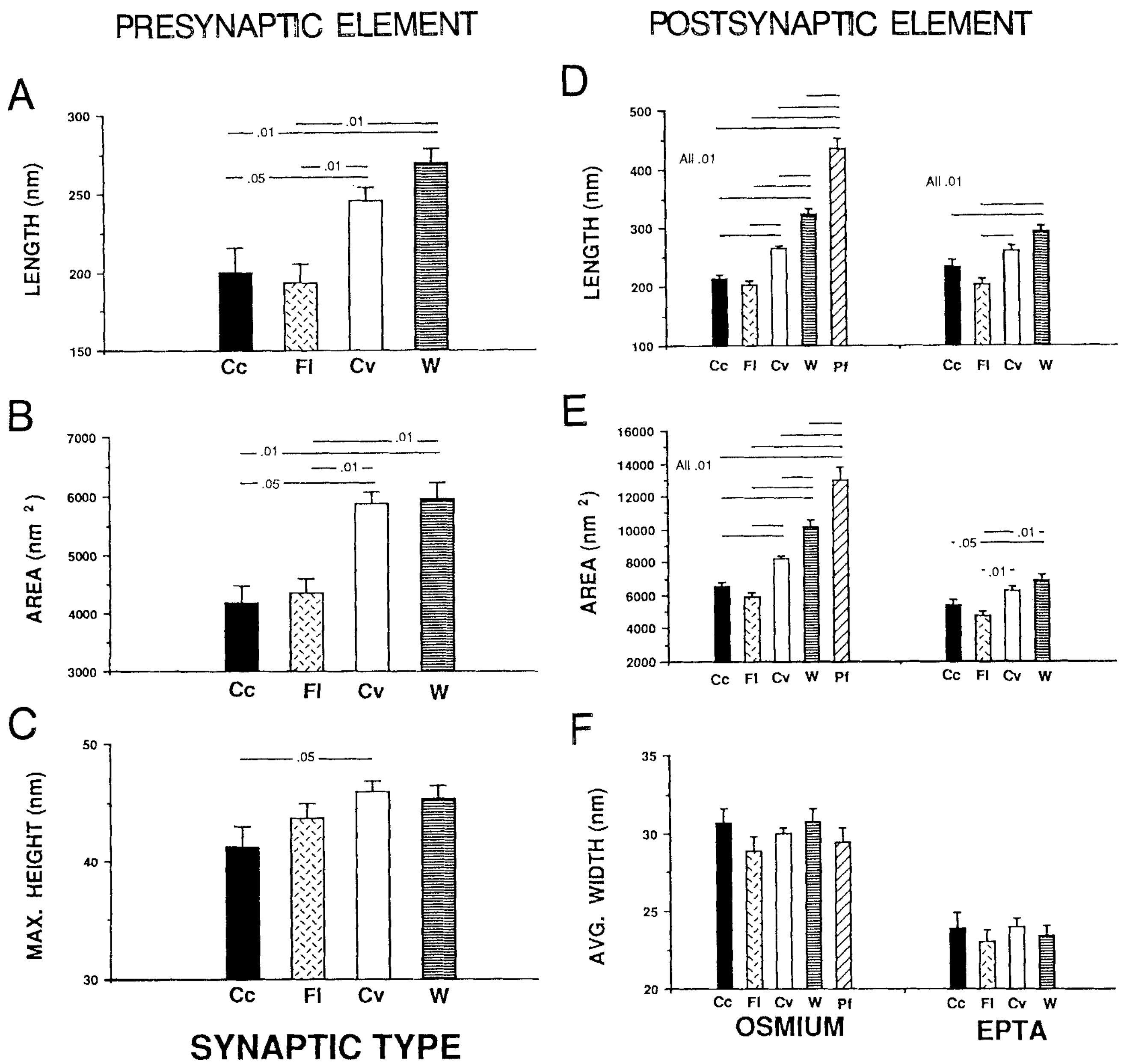


Fig. 1. Measures of the pre- and postsynaptic densities of different synaptic subtypes in the occipital cortex: A) presynaptic element length; B) presynaptic element area; C) presynaptic element height; D) postsynaptic element length; E) postsynaptic element area; and F) average postsynaptic element width. Cc = concave synapses; Fl = flat synapses; Cv = convex synapses; W = W-shaped synapses; Pf = perforated synapses. Lines indicate significant differences between groups, with $P$ values. Error bars indicate S.E.M.

All data were analyzed with the Analysis of Variance program of the BMDP Statistical Package Software. Where significant group differences were found, the Duncan post-ANOVA test was run.

In *neocortical* synapses, the *presynaptic* element is significantly longer and its dense projection area is significantly greater in convex and W-shaped synapses compared to either concave or flat synapses (Fig. 1A,B). The maximal presynaptic dense projection height is also significantly greater in convex than in concave synapses (Fig. 1C). The *postsynaptic* density of occipital cortex synapses is progressively and significantly longer in OsUL stained convex, W-shaped and perforated synapses, with all three of these synaptic types being longer than concave and flat synapses (Fig. 1D). Similar results were obtained in the EPTA stained material, where convex synapses were significantly longer than flat synapses, and W-shaped synapses were significantly longer than both flat and concave synapses. The OsUL stained postsynaptic density also has a progressively and significantly greater area in convex, W-shaped, and perforated synapses than in concave or flat synapses (Fig. 1E); similar results were again observed in the EPTA stained material where convex and W-

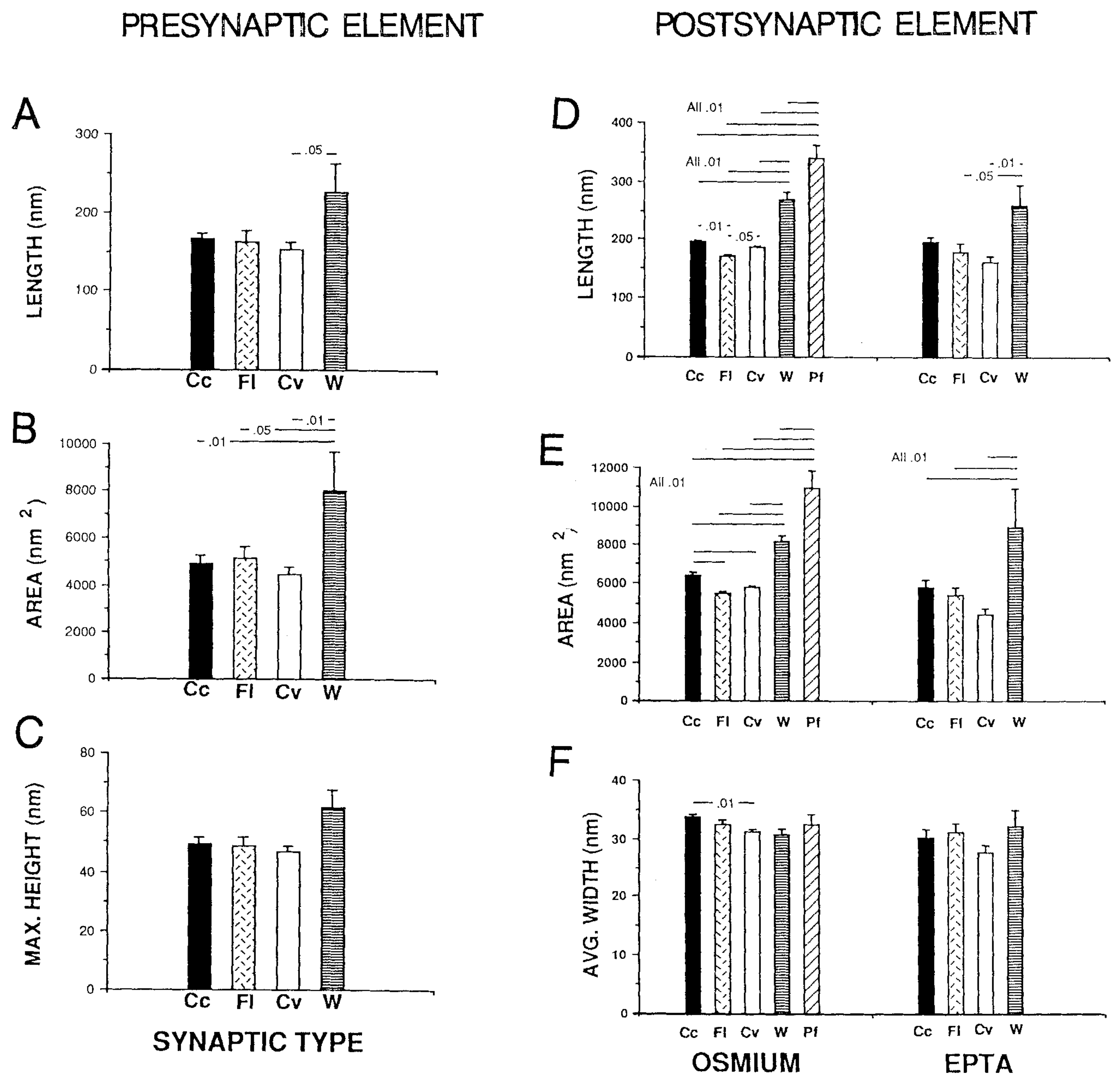


Fig. 2. Measures of the pre- and postsynaptic densities in different synaptic subtypes in the hippocampus: A) presynaptic element length; B) presynaptic element area; C) presynaptic element height; D) postsynaptic element length; E) postsynaptic element area; and F) average postsynaptic element width. Symbols are the same as in Figure 1.

shaped synapses were the largest. However, the average width of the postsynaptic density is similar for all synaptic types in both the OsUL and EPTA stained material (Fig. 1F).

In the *hippocampus*, W-shaped synapses have the greatest *presynaptic* length, which reached significance compared to convex synapses (Fig. 2A). The presynaptic area of W-shaped synapses was significantly greater than concave, flat and convex synapses (Fig. 2B). No significant differences were observed between synaptic types in maximal presynaptic height, although the trends were similar to other synaptic measures (Fig. 2C). The length of the *postsynaptic* density in hippocampal synapses is significantly greater in concave and convex synapses than in flat synapses in OsUL stained tissue (Fig. 2D); this difference was not seen in the EPTA stained material. W-shaped and perforated synapses were progressively and significantly longer than other synaptic subtypes. The postsynaptic density area was significantly greater in concave than flat or convex synapses *in osmium* stained tissue, although this difference did not reach significance in the EPTA stained tissue (Fig. 2E). Again, W and perforated synapses had significantly and progressively greater postsynaptic areas. Concave synapses have a significantly greater average postsynaptic width than convex synapses in osmium stained tissue (Fig. 2F); these differences did not reach significance in EPTA stained tissue.

The current research took an observational rather than experimental approach; however, our laboratory and others have previously related synaptic type to recent synaptic activity (see discussion below). It is not currently known what underlies synaptic curvature, e.g., the nature or shape of the postsynaptic process, transmitter type, or physiological activity; the reader is referred to Markus and Petit (1989) where such possibilities are discussed at length. In the current research we utilized both the EPTA and OsUL stains. Although the data derived from the two stains are quantitatively different due to the staining or non-staining of the membranes, they have been shown to be qualitatively similar in the current study as well as others (e.g., Markus et al., 1987), indicating the biological relevance of the two stains.

In the occipital cortex, convex synapses are larger (have a higher maximal presynaptic dense projection, are longer, and have a greater pre- and postsynaptic area) than concave synapses, while in the hippocampus, convex synapses are smaller (have a smaller postsynaptic density average width and area) than concave synapses (present study, and Desmond and Levy, 1983). Convex synapses in the neocortex are linked to neuronal activation (Devon and Jones, 1981; Benshalom, 1987), while in the hippocampus, neuronal activation has been related to a decrease in the proportion of convex synapses (Cronin et al., 1987; Desmond and Levy, 1983, 1986a,b; Petit et al., 1989; for a review, see Calverley and Jones, 1990, and Markus and Petit, 1989).

Independent of the brain region examined, irregular-shaped (W) and perforated synapses are much larger than other synaptic shapes, consistent with previous research (Jones and Calverley, 1991; Peters and Kaiserman-Abramov, 1969). Perforated synapses have been related to development (Jones and Calverley, 1991), experience or learning (Greenough et al., 1978), kindling (Geinisman et al., 1991, 1992), LTP (Geinisman et al., 1991), and cognitive functioning. Although perforated synapses are longer and therefore have greater pre- and postsynaptic areas, they do not have greater presynaptic dense projection maximal height or postsynaptic average width, i.e., the primary difference in perforated synapses appears to be increased synaptic length (diameter or contact area).

The current findings indicate that those synaptic types positively associated with neuronal activation such as development, learning, or LTP are also larger in size. This suggests that alterations in the ratios of these different synaptic types following neuronal activation is a probable mechanism by which the efficacy of synaptic populations can be altered.

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