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Balance and Stability of Synaptic Structures during Synaptic Plasticity

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SUMMARY

Subsynaptic structures such as bouton, active zone, postsynaptic density (PSD) and dendritic spine, are highly correlated in their dimensions and also correlate with synapse strength. Why this is so and how such correlations are maintained during synaptic plasticity remains poorly understood. We induced spine enlargement by two-photon glutamate uncaging and examined the relationship between spine, PSD, and bouton size by two-photon time-lapse imaging and electron microscopy. In enlarged spines the PSD-associated protein Homer1c increased rapidly, whereas the PSD protein PSD-95 increased with a delay and only in cases of persistent spine enlargement. In the case of nonpersistent spine enlargement, the PSD proteins remained unchanged or returned to their original level. The ultrastructure at persistently enlarged spines displayed matching dimensions of spine, PSD, and bouton, indicating their correlated enlargement. This supports a model in which balancing of synaptic structures is a hallmark for the stabilization of structural modifications during synaptic plasticity.

INTRODUCTION

The remarkable competence of the nervous system to adapt, learn, and form memories is considered to be based on activity-dependent modifications of synaptic connections. It is by now well established that functional activity-dependent changes are paralleled by structural alterations (Engert and Bonhoeffer, 1999; Maletic-Savatic et al., 1999; Matsuzaki et al., 2004), but it remains incompletely understood how these modifications are interrelated and how the long-term preservation of memories is accomplished in an inherently instable and constantly changing biological structure like the nervous system.

In the cortex and hippocampus, the majority of synapses connecting pyramidal neurons are located on dendritic spines and synapse size is directly related to synapse strength (e.g., Matsuzaki et al., 2001; Murthy et al., 2001; Nusser et al., 1998; Takumi et al., 1999). Furthermore, the size of different structural elements of these synapses is tightly correlated: the volume of the presynaptic bouton, the pool of synaptic vesicles, the areas of active zone and postsynaptic density (PSD), and the volume of the postsynaptic spine all go hand in hand (e.g., Arellano et al., 2007; Harris and Stevens, 1989; Schikorski and Stevens, 1999). The alignment—and therefore correlated size—of active zone and postsynaptic density is thought to be important for the speed and efficacy of chemical synaptic transmission, but otherwise the reason for the tight structural correlations remains unknown.

This tight structure-function relationship implies that synaptic plasticity, such as long-term potentiation or depression of synapses, should also result in concomitant structural changes. The first studies looking into structural changes associated with long-term potentiation used electron microscopy to study synaptic changes after plasticity induction at the population level. These studies, however, yielded conflicting results (reviewed, e.g., in Yuste and Bonhoeffer, 2001): in some experiments an increase in the size of dendritic spines (e.g., Desmond and Levy, 1983; Van Harreveld and Fifkova, 1975), PSD (e.g., Desmond and Levy, 1983, 1986), and pre- and postsynaptic appositions (e.g., Desmond and Levy, 1988) was demonstrated, whereas in other experiments such changes were not observed (e.g., Sorra and Harris, 1998). The advent of new imaging techniques such as confocal and in particular two-photon microscopy then allowed for performing chronic time-lapse imaging before, during, and after plasticity induction. These studies demonstrated clearly that strengthening of synaptic connections is structurally accompanied by the enlargement of preexisting dendritic spines and/or the formation of new spines (e.g., Engert and Bonhoeffer, 1999; Hosokawa et al., 1995; Kopec et al., 2007; Maletic-Savatic et al., 1999). More recently, at the level of individual identified synapses, it has been confirmed that functional potentiation is indeed accompanied by a tightly correlated increase in spine size (e.g., Harvey and Svoboda, 2007; Matsuzaki et al., 2004).

The above-mentioned correlation of different subsynaptic components suggests that changes in spine size should furthermore be accompanied by modifications in structures, such as the PSD, the active zone, the presynaptic bouton, and alike. However, at the level of individual, stimulated synapses, there is only limited information about the plasticity and activity-dependent changes of these subsynaptic structures. For example, in contrast to the expectation of a parallel enlargement of spine and PSD, it has been reported that PSD-95, a major structural protein of the PSD, is not accumulating after induction of spine enlargement (Steiner et al., 2008).

Furthermore, whereas the mechanisms and signaling cascades underlying spine plasticity and glutamate receptor

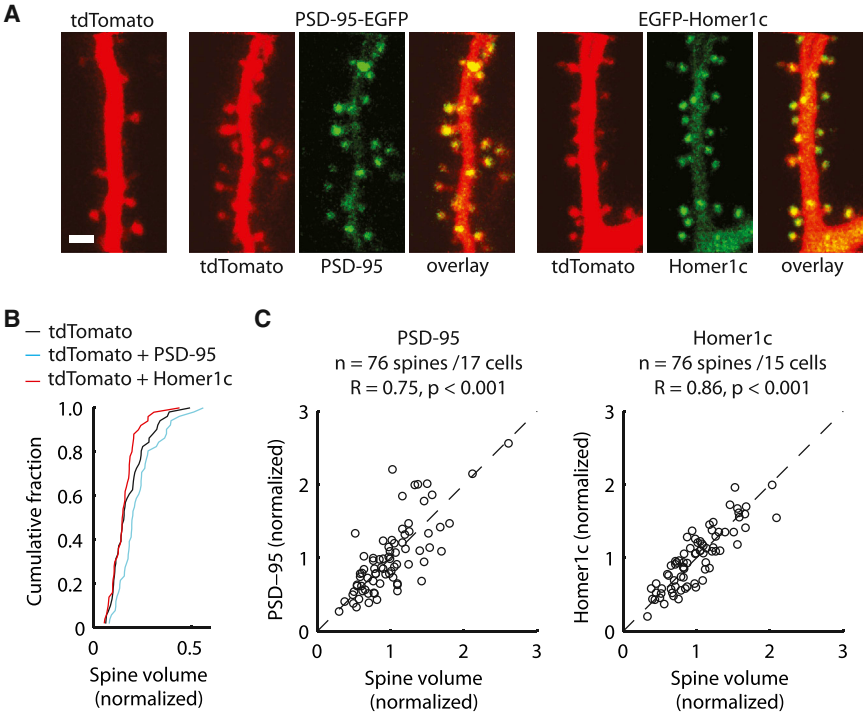

Figure 1. Labeling of Synaptic Structures and Correlation between Spine Volume and PSD Size

(A) Images of dendritic segments from pyramidal cells expressing tdTomato alone, tdTomato + PSD-95-EGFP, and tdTomato + EGFP-Homer1c. Scale bar, 2 μm.

(B) Spine volumes of cells expressing the proteins described in (A) (tdTomato, n = 51 spines/10 cells; PSD-95, n = 51 spines/13 cells; Homer1c, n = 50 spines/9 cells). Spine fluorescence data were normalized to fluorescence in a thick dendritic segment.

(C) Correlation of PSD-95 and Homer1c level with spine volume; values for individual spines were normalized to mean of all spines on dendritic segment. R = correlation coefficient, p = significance of correlation.

See also Figure S1.

insertion have been intensively studied (reviewed, e.g., in Malinow and Malenka, 2002; Murakoshi and Yasuda, 2012), the mechanisms ensuring the stabilization of structural synaptic modifications have received less attention. Studies on structural plasticity have so far mostly focused on the stability of synapses and spines in terms of their persistence versus elimination (reviewed, e.g., in Yoshihara et al., 2009), but not the stability of synapse size and strength (but see Govindarajan et al., 2011). There are models describing how synaptic strength is maintained in terms of the number of postsynaptic neurotransmitter receptors, in particular glutamate receptors, where the PSD provides slots for insertion or retention of receptors (reviewed, e.g., in Opazo et al., 2012). However, these models simply assume a fixed, stable PSD scaffold and thus do not address the structural stability of synapses.

Here, we used a combination of two-photon time-lapse imaging, two-photon glutamate uncaging, and ultrastructural reconstruction to examine whether and how—along with the spine—other subsynaptic structures, in particular the PSD and presynaptic bouton, change during synaptic potentiation. We found a close correlation between the enlargements of all synaptic components 3 hr after plasticity induction. Furthermore, we observed that the balanced enlargement of pre- and postsynaptic components was a good indicator for the stabilization and persistence of structural modifications.

RESULTS

Correlation of Spine Size and PSD Size

In our first experiments we investigated how well spine size and PSD size correlate. Historically, the PSD has been described as an electron dense darkening located just below the synaptic membrane on the postsynaptic side. It has been interpreted and shown to be due to the subsynaptic accumulation of a multitude of different proteins involved in synaptic transmission, plasticity, and scaffolding. Postsynaptic proteins like PSD-95 have always been assumed to accurately represent the location and function of the PSD. In order to get a more complete—and perhaps more refined—picture of the structure and the remodeling process of the PSD, we chose to have the PSD "represented" by two of its proteins, PSD-95 and Homer1c (e.g., Blanpied et al., 2008; Petrini et al., 2009), which play different functional roles in synaptic plasticity (e.g., Inoue et al., 2007; Steiner et al., 2008). We therefore expressed tdTomato as cytosolic marker and GFP-tagged PSD-95 or Homer1c as reporter for the PSD in CA1 pyramidal cells of cultured hippocampal slices (Figure 1A). Overexpression of both fluorescently tagged PSD-95 and Homer1c has been used already in various studies, and extensive control experiments have been performed. These showed no effects on synaptic structure, function, and plasticity or in the case of PSD-95 established the strategy to restrict the analysis to spines of normal size, which reduces the occluding effect of PSD-95 overexpression on LTP to about 30% (Okabe et al., 2001; Petrini et al., 2009; Steiner et al., 2008; Sturgill et al., 2009). We compared the size of spines overexpressing these proteins to spines that only expressed tdTomato. We only found a small increase of about 25% in average spine size for PSD-95 (tdTomato + PSD-95, volume = 23 ± 2 [a.u.], n = 51 spines/13 cells; tdTomato alone, volume = 18 ± 1 [a.u.], n = 51 spines/10 cells; p = 0.014, Wilcoxon rank-sum test; Figure 1B; compare approx. 3-fold increase in Nikonenko et al., 2008) and no increase for Homer1c (tdTomato + Homer1c, volume = 16 ± 1, n = 50 spines/9 cells; tdTomato alone, volume = 18 ± 1, n = 51 spines/10 cells; p = 0.122, two-tailed t test; Figure 1B). This indicated that the overexpression of PSD-95 should not have a dramatic effect on spine plasticity induction in our experiments, in particular because we restricted our analysis to spines in the lower range of volumes