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Postsynaptic protein mobility in dendritic spines: Long-term regulation by synaptic NMDA receptor activation

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Reorganization of molecular components represents a cellular mechanism for synaptic plasticity. Dendritic spines, major sites for glutamatergic synapses, compartmentalize dynamic changes in molecular composition. Here, we use fluorescence recovery after photobleaching (FRAP) in cultured hippocampal neurons to show that spine proteins undergo continual exchange with extra-spine pools. Each spine component has a distinctive mobility: calcium/calmodulin activated protein kinase CaMKIIα > GluR1 AMPA glutamate receptor > PSD-95 scaffolding protein > NR1 NMDA glutamate receptor. Stimulation of synaptic NMDA receptors by a protocol that induces chemical LTP resulted in a long-lasting reduction in the mobility of spine CaMKIIα and an increased mobile fraction but slower kinetics for spine GluR1. Stimulation also increased the resistance of postsynaptic CaMKIIα to detergent extraction. These results suggest long-lasting changes in affinity of protein–protein interactions and/or ongoing alterations in exo/endocytosis. Such lasting changes in protein mobility may contribute to maintaining alterations in synaptic efficacy.

Keywords: CaMKII; GluR1; NR1; PSD-95; FRAP; LTP; Hippocampal

Introduction

Glutamatergic synapses on dendritic spines of pyramidal neurons are the major sites of excitatory synaptic transmission. The postsynaptic density (PSD) of spines is a highly organized network of interacting proteins (Kennedy, 2000; Kim and Sheng, 2004). Among the myriad spine proteins, four key players in controlling synaptic plasticity are GluR1, NR1, PSD-95, and CaMKIIα. Hippocampal NMDA-receptor-dependent long-term potentiation (LTP) is thought to be mediated by synaptic insertion of GluR1-containing AMPA receptors (Malinow and Malenka, 2002; Bredt and Nicoll, 2003). Activation of CaMKIIα by calcium entry through NMDARs is widely accepted to be a required step in LTP, perhaps via phosphorylation of GluR1 or other targets including NR2B, densin-180, and SynGAP (Hudmon and Schulman, 2002; Lisman et al., 2002). Overexpression of PSD-95 can also induce a form of LTP by increasing AMPARs at synapses through the intermediary stargazin (Bredt and Nicoll, 2003). However, the role of endogenous PSD-95 in plasticity is more complex (Migaud et al., 1998), presumably reflecting its numerous interactions with other spine proteins, including NR2A/B, neuroligin, and nNOS (Kim and Sheng, 2004). Consistent with a central role in plasticity, targeted deletion or mutagenesis of NR1, GluR1, PSD-95, or CaMKIIα alters LTP and spatial learning in vivo (Tsien et al., 1996; Migaud et al., 1998; Matynia et al., 2002; Reisel et al., 2002; Lee et al., 2003).

Thus, CaMKIIα, PSD-95, GluR1, and NR1 are key players in determining the effects of synaptic glutamate release on short- and long-term signaling. Activity-regulated changes in steady-state distributions of CaMKIIα and GluR1 have been reported by numerous laboratories (e.g. Shen and Meyer, 1999; Lu et al., 2001; Liao et al., 2001; Otmakhov et al., 2004). However, relatively little is known about the mobility of these proteins. To what extent do these proteins move in and out of spines? Is the mobility different for each spine protein, and is it modifiable by activity? Previous studies have assessed baseline mobility of PSD-95, NR1, or CaMKIIα, but limited to more immature non-spiny synapses for NR1 or involving overexpression for CaMKII (Okabe et al., 2001; Tovar and Westbrook, 2002; Groc et al., 2004; Okamoto et al., 2004). No studies have yet determined whether there are long-lasting changes in mobility of any of these proteins following synaptic activation. To address these questions, we expressed tagged forms of CaMKIIα, PSD-95, GluR1, and NR1 in cultured hippocampal neurons and performed FRAP to monitor mobility. We then determined how mobility of spine pools is affected following activation of synaptic NMDA receptors, a

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paradigm that results in chemical LTP (cLTP; Lu et al., 2001; Liao et al., 2001).

Results

Expression of tagged PSD proteins at close to endogenous levels

To image protein dynamics in spines of living neurons, we expressed postsynaptic proteins tagged with variants of green fluorescent protein: PSD-95-EYFP, EGFP-CaMKIIα, EYFP-NR1, and EYFP-GluR1. All imaging experiments were performed on mature hippocampal pyramidal neurons at 17–19 days in culture. We confirmed that the tag and expression did not interfere with appropriate localization of the recombinant proteins (Supplementary Fig. 1). Continual small scale movement of the tagged protein clusters (Supplementary Movie 1 and as in Fischer et al. (1998)) and DIC or phase contrast were used to ensure health during all imaging experiments.

The EYFP-NR1 and EYFP-GluR1 probes as used here are strongly biased towards sensing cell surface pools over intracellular pools in spines. Due to its pH sensitivity, the fluorescence of EYFP in the extracellular milieu (pH 7.2) is 10- to 20-fold greater than in late exocytic and endocytic organelles (pH 5.5–6.0) (Llopis et al., 1998; Demaurex, 2002). As expected, we observed a reversible loss of dendritic and spine EYFP-NR1 or EYFP-GluR1 fluorescence signal when neurons were placed in extracellular solution of pH 5.0, whereas the intracellular pools were also

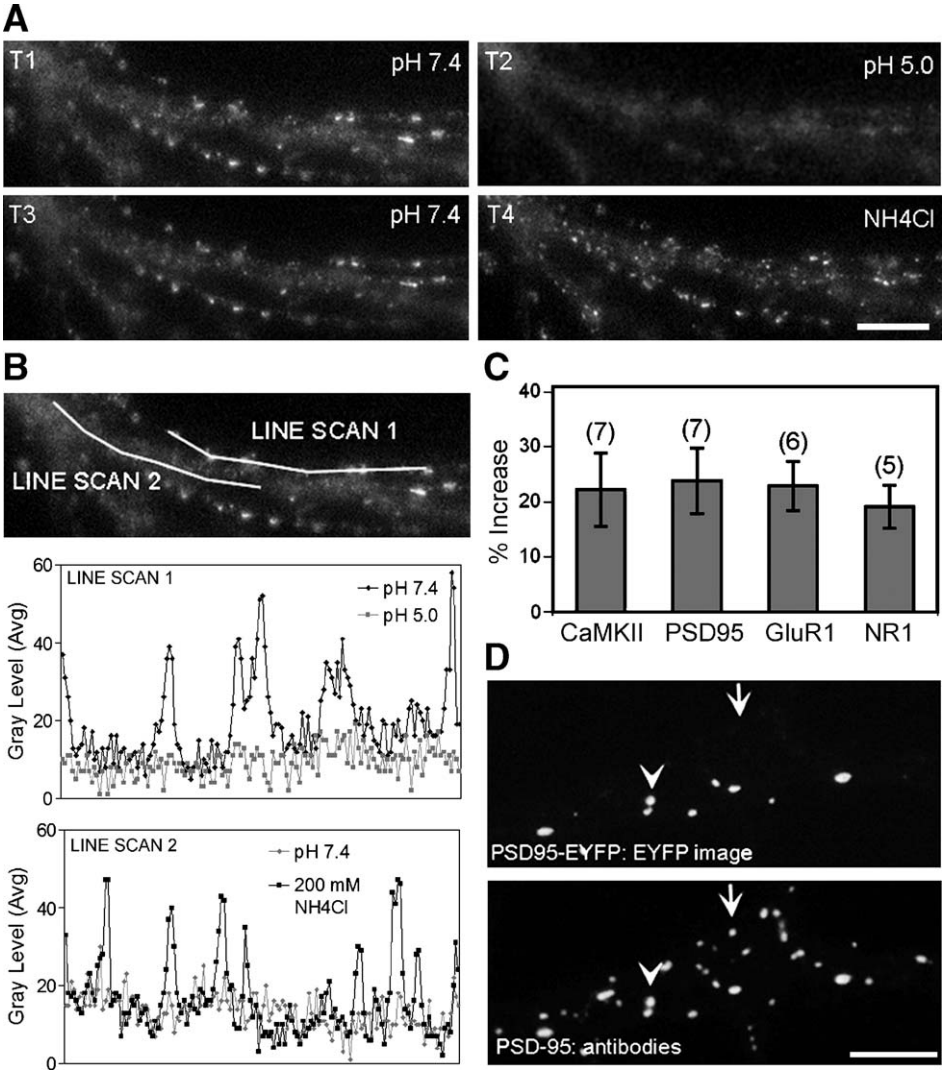

Fig. 1. Imaging of tagged surface receptors at close to endogenous expression levels. (A) EYFP-NR1 expressed in hippocampal dendrites was imaged sequentially. EYFP-NR1 spine and shaft fluorescence was reversibly quenched by low extracellular pH, indicating surface localization. Addition of NH_4Cl to neutralize intracellular pH revealed additional intracellular puncta. EYFP-GluR1 gave similar results (data not shown). (B) Linescan 1 across spine heads confirms loss of spine EYFP-NR1 fluorescence with low extracellular pH. Linescan 2 along a dendrite shaft confirms the appearance of new EYFP-NR1 fluorescent puncta upon addition of NH_4Cl. (C) Expression of tagged proteins increases the total pool by only 20–25%. These values were derived by comparing total immunofluorescence for the endogenous plus recombinant protein in dendrite regions of ~100 μm length, including shaft and spines, between transfected and non-transfected neurons in a single field of view. (D) Sample field comparing levels of PSD-95 in transfected (arrowhead) versus non-transfected (arrow) dendrites and spines. Scale bars: 10 μm.