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Transient increases in dendritic spine density contribute to dentate gyrus long-term potentiation

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Abstract

Dendritic spines are the primary sites for excitatory neurotransmission in the adult brain and exhibit changes in their number and morphology with experience. The relationship between spine formation and synaptic activity has been best characterized along the apical dendrites of pyramidal neurons in the hippocampal CA1 subfield. However, less is known about the structural mechanisms at the spine that mediate plasticity in other hippocampal subfields. The dentate gyrus is the predominant point of entry for synaptic input to the hippocampus, and dentate granule cells differ from CA1 pyramidal neurons in terms of their morphology and biophysical properties. In order to understand the structural mechanisms for plasticity in the dentate gyrus, we measured dendritic spine density in hippocampal slice preparations at different intervals following synaptic stimulation. We observed that transient increases in dendritic spine density are detectable thirty minutes after induction of long-term potentiation (LTP). By sixty minutes post-stimulation, dendritic spine density has returned to basal levels. Both early LTP and enhancements in dendritic spine density could be blocked by destabilizing actin filaments, but not by inhibitors of transcription or protein synthesis. These results indicate that spine formation is a transient event that is required for dentate gyrus LTP.

Keywords

medial perforant path; synaptic plasticity; cytoskeleton; dentate gyrus

Dendritic spines are motile protrusions that exhibit dynamic changes in response to neuronal stimulation. Spines expand and contract (Kovalchuk et al., 2002), and can be formed de novo following paired pre- and post-synaptic firing (Kwon and Sabatini, 2011). The mechanisms underlying spine formation and disappearance of spines remains an active area of investigation, with most studies focusing on the dendrites of neurons in the hippocampal CA1 subfield. Pyramidal neurons in CA1 exhibit turnover among spines and synapses following stimulation patterns that resemble those that occur during learning (Bourne and Harris, 2011). Learning itself enhances dendritic spine density among CA1 pyramidal neurons (Leuner et al., 2003), and the mechanisms for new synapse formation likely involve actin polymerization (Kramár et al., 2006).

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Despite the wealth of mechanistic information surrounding activity-dependent plasticity among spines in CA1, less is known about the structural mechanisms underlying plasticity in the dentate gyrus. Early work focused on this hippocampal subfield, with classical studies demonstrating spine formation in response to the induction of long-term potentiation at medial perforant path synapses on dentate gyrus granule cells (Trommald et al., 1996). This line of research further suggests that spine formation may be transient, with early LTP accompanied by increases (Trommald et al., 1996) and later time points characterized by a return to basal levels (Popov et al., 2004). Ultrastructural studies also identified morphological plasticity among dendritic spines in the dentate gyrus following perforant path stimulation (Fifková and Van Harrevel, 1977). Although these elegant studies were able to characterize alterations in spines and synapses using an ultrastructural approach, the molecular mechanisms underlying activity-dependent spine formation among dentate gyrus granule cells largely remain to be elucidated.

In order to probe the structural mechanisms underlying plasticity in the dentate gyrus, we have visualized spines in slice preparations at different intervals following induction of LTP. Slice preparation and recording followed previously published methods (Stranahan et al., 2010). All procedures followed NIH guidelines and were approved by the Animal Care and Use Committee of Georgia Health Sciences University. In brief, six-week-old male C57Bl6/J mice (Jackson Laboratories) were decapitated within three minutes of cage disturbance in order to limit stress-induced changes in dendritically relevant hormones. The brain was immersed in artificial cerebrospinal fluid (ACSF) and sectioned at four hundred micron thickness on a vibrating tissue slicer (Leica). Afterwards slices were allowed to recover for one to four hours at 37°C before recording. Control conditions consisted of sixty minutes of recording at 0.05Hz; LTP was induced with a one-second train delivered at 100Hz in the presence of 100µM picrotoxin to disinhibit mature granule cells and suppress GABAergic excitation among immature granule neurons as described (Stranahan et al., 2008). Stimulation at 100Hz for 1 second (in the presence of picrotoxin) evokes LTP that is dependent on the contributions of mature neurons (Stranahan et al., 2008; Saxe et al., 2006). Because dentate granule neurons selected for analysis in the current study represent a likely population of mature neurons, with a well developed dendritic arbor extending up into the molecular layer and cell bodies located in the mid- to upper portion of the granule cell layer, this stimulation protocol was selected for LTP induction to evoke maximal changes among the selected cell population. For some experiments, the actin destabilizing agent latrunculin A (0.1µM, Sigma-Aldrich), the protein synthesis inhibitor anisomycin (25µM, Sigma-Aldrich), or the transcriptional suppressor actinomycin D (25µM, Sigma-Aldrich) were added to the perfusion medium. In these experiments, slices were exposed to the drug for thirty minutes before beginning recording, as described (Gelinis et al., 2008; Sajikumar et al., 2008; Ramachandran and Frey, 2009).

Thirty or sixty minutes post-tetanzation, dendritic spines were visualized using the lipophilic membrane tracer 1,1'-dioctadecyl-3,3,3',3'-tetramethyl-indocarbocyanine perchlorate (DiI) (Molecular Probes), as described (Stranahan et al., 2007), with minor modifications. Slices were fixed in 4% paraformaldehyde in phosphate buffer after recording, and DiI crystals were placed in the dentate hilus proximal to the recording site. Nuclei were counterstained with Hoechst (1:1,000 for 5 min; Sigma-Aldrich). This method

labeled a cluster of fluorescent granule neurons at the same site where LTP was induced. In order to be selected for analysis, dendritic segments had to be smooth, with no beading, which would indicate poor slice health. Segments were also traced back to the cell body, which had to be positioned in the mid- to superficial portions of the dentate granule cell layer. Inspection of Hoechst counterstaining revealed that cell nuclei appeared healthy without any chromatin condensation. We sampled three segments per cell from the secondary or tertiary dendrites of five cells per slice, and the average spine density across those cells was used for statistical analysis. In order to be counted as a spine, protrusions were required to be greater than 0.5 microns in length and exhibit a clearly resolvable head and neck (Supplementary Figure 1). Length criteria were based on an earlier study that measured the length of dendritic spines within this region of the dendritic arbor on DiI-labeled dentate gyrus granule cells (Stranahan et al., 2007). Spines protruding along the axial plane were not counted. Dendritic spine density and the amount of potentiation were compared across post-stimulation time points and pharmacological conditions using one-way ANOVA with Tukey's post hoc and significance at $p < 0.05$.

Time points were selected based on previous work on the time course for synapse formation in the hippocampal CA1 subfield (Bourne and Harris, 2011). Comparison of dendritic spine densities between control slices, slices fixed thirty minutes after LTP induction, and slices fixed sixty minutes after LTP induction revealed that early LTP is accompanied by increased dendritic spine density among dentate gyrus granule neurons (Figure 1, $F_{3,14} = 4.86$, $p = 0.02$). Importantly, slices that received tetanic stimulation but did not show LTP did not exhibit changes in dendritic spine density (spines/10 micron, Tet-noLTP = 10.56 ± 0.82 , control = 10.50 ± 0.69).

In the current experiment, slice recordings were conducted a minimum of one hour and a maximum of four hours after slice preparation. Previous studies in hippocampal area CA1 suggest that slices require three hours of recovery in order to regain morphological and functional characteristics similar to those in the intact brain (Fiala et al., 2003). In order to determine whether the duration of slice recovery was associated with functional and structural parameters in the dentate gyrus, we conducted a correlational analysis. There was no significant association between slice recovery time and LTP magnitude at either 30 or 60 minutes post-tetanzation ($p > 0.10$ following Pearson's correlation). To further assess whether dendritic spine density was related to the duration of slice recovery, we correlated spine density among control slices with varying recovery times, beginning at one hour after slicing and extending to four hours after slicing. There was no correlation between dendritic spine density and duration of recovery in control slices ($p > 0.10$ following Pearson's correlation). Finally, to determine whether enhanced spine densities observed 30 minutes after tetanzation might be sensitive to the duration of prior slice recovery, we again performed correlational analysis. There was no significant correlation between the duration of slice recovery and dendritic spine densities at 30 minutes post-tetanzation, the time point when we observed enhanced dendritic spine density in potentiated slices ($p > 0.10$ following Pearson's correlation).

Next we performed pharmacological manipulations to determine whether enhancements in dendritic spine density following early LTP were dependent on protein synthesis, gene

transcription, or actin motility. In these experiments, destabilization of actin filaments, but not inhibition of protein synthesis or gene transcription, prevented both early LTP (Figure 2A; $F_{2,22}=3.98$, $p=0.03$) and enhanced dendritic spine density (Figure 2B–C, $F_{2,22}=5.43$, $p=0.01$). The insensitivity of early LTP to inhibitors of protein synthesis and transcription is consistent with early studies on the molecular mechanisms underlying dentate gyrus LTP (Nguyen and Kandel, 1996). These observations indicate that dynamic actin filaments are essential for both spine formation during early LTP and synaptic potentiation.

These studies suggest that transient increases in dendritic spine density contribute to the induction of early LTP in the dentate gyrus. Our detection of enhanced dendritic spine density at thirty minutes post-stimulation is in agreement with early work using in vivo LTP induction in rats (Trommald et al., 1996). Previous work using in vivo LTP recordings in the rat dentate gyrus suggests that six hours poststimulation, there is no change in synapse density (Popov et al., 2004), which is consistent with our observation of a return to basal spine density values at sixty minutes after stimulation. Other previous studies suggest that twenty-four hours maintenance of LTP is accompanied by new synapse formation (Stewart et al., 2000), opening the possibility that synaptic structural adaptations during LTP occur in waves over time. However, the possibility that spine formation occurs in waves over time requires generalization between in vivo and in vitro studies, and the consistency of in vivo and in vitro results presents some difficulties. It is noteworthy that the basal, unstimulated spine densities observed along the secondary and tertiary dendrites of dentate granule neurons in the current in vitro study are comparable to previous work using DiI labeling to visualize dentate gyrus granule cells in perfusion-fixed hippocampus (Stranahan et al., 2007). Future studies will address the possibility that LTP and learning may indeed be accompanied by waves of spine and synapse formation among dentate granule cells. Moreover, as light microscopy methods are not sufficient to address whether spines indeed form synapses, ongoing experiments combine confocal microscopy with analysis of serial section electron microscopy images to address ultrastructural indices of synaptic connectivity following perforant path stimulation.

The studies in the current report have identified actin motility as a critical element for early LTP and spine formation. By contrast, early LTP and increased spine density were not affected by protein synthesis or transcriptional inhibitors. Previous studies have observed a distinct role for protein synthesis in the enlargement of the spine head that occurs following LTP induction at the perforant path-dentate gyrus synapse (Fifková et al., 1982). These early studies reported that spine head enlargement is a dynamic event that is, at certain post-stimulation time points, dependent upon protein synthesis. The observations of Fifková and colleagues (1982) are not inconsistent with the current report, as the time course and protein synthesis-dependence of morphological plasticity among individual spines, as assessed by Fifková and colleagues (1982), might be different from the time course and protein synthesis-dependence of alterations in spine density, as evaluated in the current study. Our observations underscore the importance of the actin cytoskeleton for changes in spine density with successful induction of medial perforant path LTP. Much work remains to be done to fully elucidate the molecular cascades involved in transient spinogenesis among dentate gyrus granule neurons. This report introduces a tractable system in which to

understand the mechanisms underlying synaptic plasticity at this essential relay for information processing in the hippocampus.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Transient enhancement of dendritic spine density accompanies early LTP in the dentate gyrus

(A), Experimental design. (B), Low-magnification confocal image of a dentate gyrus granule cell labeled with DiI (green) and counterstained with Hoechst (blue). Scalebar = 50 microns. (C) shows a dendritic segment from the secondary and tertiary regions of the dendritic arbor of a dentate gyrus granule cell from a control slice, a slice fixed thirty minutes after induction of LTP, and a slice fixed sixty minutes after LTP induction. Arrows indicate dendritic spines. Scalebar = 5.0 microns and applies to all dendritic segments. (D), Robust LTP among dentate gyrus granule cells can be detected following medial perforant path stimulation with a 1 second train of pulses delivered at 100Hz. Inset traces depict pre-stimulation response (1) and post-stimulation response (2) at the indicated time points; scale bar for traces is 1.0mV (y-axis) by 10msec (x-axis). (D), Comparison of the amount of LTP observed between minutes 20–30, versus the amount of potentiation detected at minutes 50–60, reveals comparable enhancement of fEPSP slopes at both time points. (E), Quantification of dendritic spine density along the secondary and tertiary dendrites of granule cells suggests that transient increases in spine density accompany early LTP. Asterisk (*) indicates significance relative to control at $p < 0.05$ following ANOVA with Tukey's post hoc. Abbreviations: fEPSP, field excitatory postsynaptic potential.

Figure 2. Pharmacological destabilization of actin filaments prevents both spinogenesis and early LTP among dentate gyrus granule neurons

(A), Early LTP is blocked by the actin destabilizing agent latrunculin A, but not by protein synthesis (anisomycin) or transcriptional inhibitors (actinomycin D). (B), Dendritic segments from dentate gyrus granule neurons visualized with DiI following pharmacological treatments. Scale bar = 5 microns and arrows indicate dendritic spines. (C), LTP-evoked spine formation is also blocked by destabilization of actin filaments. Asterisk (*) indicates significance relative to control at $p < 0.05$ following ANOVA with Tukey's post hoc. Abbreviations: fEPSP, field excitatory postsynaptic potential.