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Clustered plasticity in Long-Term Potentiation: How strong synapses persist to maintain long-term memory

https://doi.org/10.1515/nf-2018-A006

Abstract: The storage of memory requires at least in part maintenance of long-term potentiation (LTP) in dendritic spine synapses. Neighboring synapses are frequently arranged into functional clusters. At present, it is still unclear how these clusters evolve, why they are stable for longer time periods and how spines interact within a cluster. In this review, we will provide an overview of current concepts of clustered plasticity and we will discuss cellular as well as molecular mechanisms that might be relevant for spine stability and associated functions in the context of LTP. We will propose that dynamics of initially formed clusters depend on compartmentalization of dendrites and that activity-dependent gene expression kicks in to preserve differences in synaptic weight. We will discuss how mechanisms of synaptic tagging, the presence of secretory organelles in dendrites and the incorporation of synaptic scaling factors that are encoded by immediate early genes interact to preserve clustered plasticity.

Keywords: Dendritic spines, plasticity-related products, synaptic tagging, gene expression, compartmentalization

Introduction

Specificity, capacity and duration of memory storage are believed to depend on both plasticity and stability of synaptic contacts (Pozo and Goda, 2010). In particular, dendritic spines, a specialized type of glutamatergic synapse in the forebrain, are associated with higher cognitive function. They contain an electron-dense protein meshwork called the postsynaptic density (PSD), which serves to anchor neurotransmitter receptors, ion channels and synaptic cell adhesion molecules as well as signalling components of the spine. Dendritic spines can differ in size, shape and stability over time. Mature spines frequently have a mushroom-like shape with a broad spine head (up to 0.8-1 μm in diameter) containing the PSD and a thin spine neck (0.1-0.2 μm) that connects the spine to the dendritic shaft and that serves as a diffusion barrier (Bosch and Hayashi, 2012). Actin filaments (F-actin) represent the major cytoskeletal components of spines and are involved in structural plasticity, in anchoring of mRNA granules and organelles as well as transport in and out of the spine (Konietzny et al., 2017). Dendritic spines contain highly dynamic branched F-actin in the spine head near the PSD, a stable pool of F-actin that is essential for the maintenance of spine structure is located at the spine base and straight bundles as well as a periodic actin lattice are found in the neck. Such nano-domain organization of F-actin in spines allows for rapid responses to extracellular stimuli on one side and ability to stabilise most optimal shape over extended periods of time on the other. Larger spines might in addition contain various organelles like the spine apparatus, polyribosomes and others. Synaptic transmission at excitatory synapses involves activation of N-Methyl-D-aspartate receptors (NMDARs) and α–amino-3-hydroxy-5-methyl-4-isocazolepropionic acid receptors (AMPARs). Activity-dependent changes in synaptic transmission strongly correlate with changes in receptor number, as well as shape and size of dendritic spines (Carlisle and Kennedy, 2005).

Studies utilizing time-lapse imaging of spines indicate that the lifetime of synaptic connections is strikingly different between apical and basal dendrites and also varies between brain regions. In the CA1 region of the hippocampus for instance, the population of spines at basal dendrites is highly dynamic. The average lifetime of basal CA1 spines (receiving input from primarily from CA3 cells) is estimated to be 10 days, and this makes in principal a complete remodeling of the circuitry possible within 3 to 6 weeks (Attardo et al., 2015). Interestingly, long-term imaging of apical tuft dendrites of pyramidal neurons

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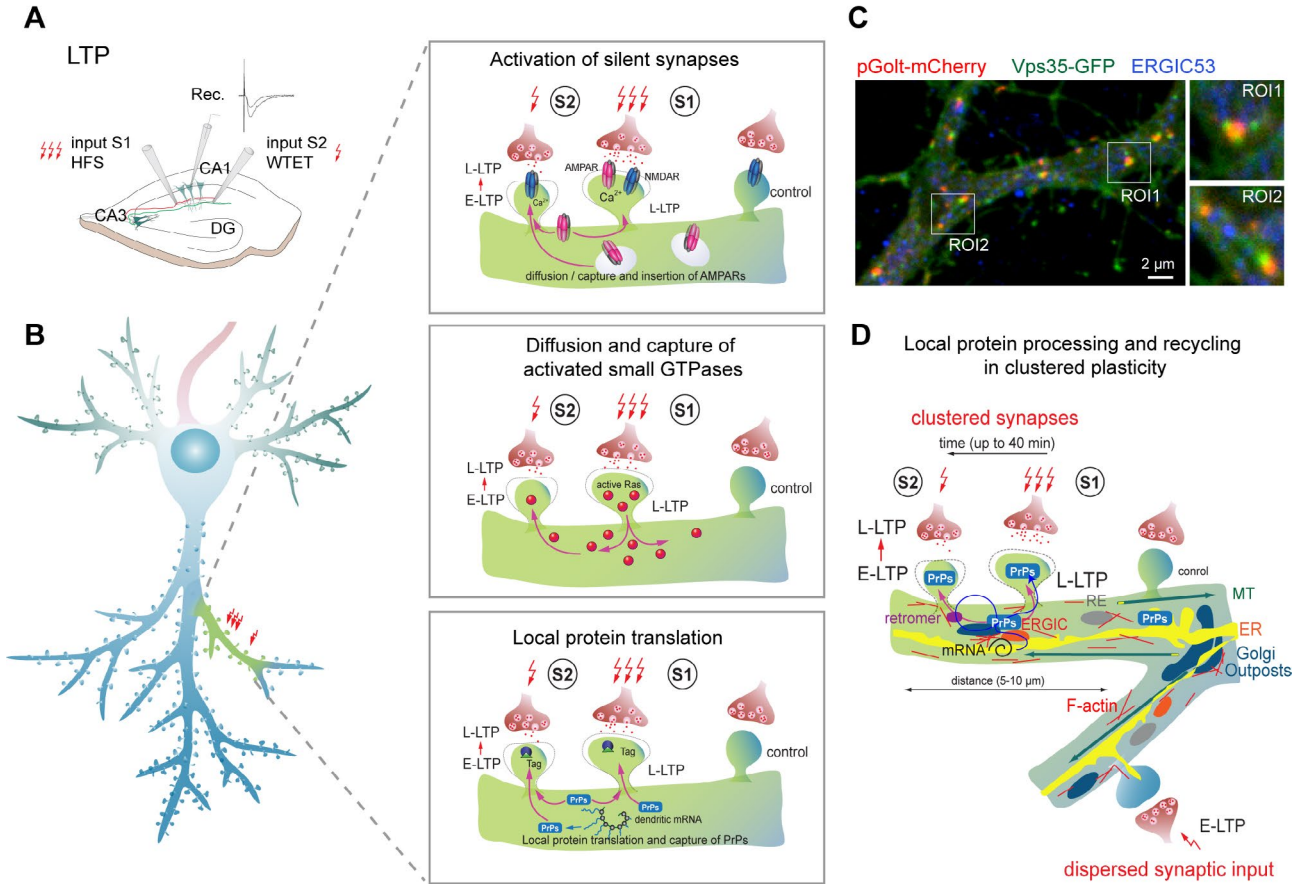

Figure 1: Molecular mechanisms of clustered plasticity.
(A) Induction of LTP and synaptic tagging in hippocampal slices in vitro. HFS – high frequency stimulation, WTET – *weak* tetanic stimulation with stimulation electrodes (Input S1 and S2), Rec. – recording electrode.
(B) Possible mechanisms acting during establishment of clustered plasticity at different time scale. The threshold for LTP induction is lower in spines located in proximity to the spine where LTP was successfully induced. Thus, stimuli that normally would produce only an early form of LTP (E-LTP) will cause transformation into a late form (L-LTP). Possible molecular mechanisms are depicted (see also the main text)
(C) Confocal image of a hippocampal neuron transfected with Golgi satellite marker (pGolt-mCherry), retromer marker (Vps35-GFP) and stained for endogenous ERGIC (ERGIC53) indicate close spatial distribution of post-ER secretory organelles. Right panel: high magnification regions of interest (ROI). Reprinted with permission from (Mikhaylova et al., 2016).
(D) Dendritic secretory organelles may contribute to dendritic compartmentalization of clustered synapses. ER – endoplasmic reticulum, MT – microtubule, RE – recycling endosome, PrPs – plasticity-related proteins, ERGIC – ER to Golgi intermediate compartment.

from CA1 (receiving input from the entorhinal cortex) or the neocortex, showed that most spines can persist over 3 months (>50 %) (Gu et al., 2014; Holtmaat et al., 2005). These spines are usually larger, which indicates that they might also be stronger, whereas small spines appear and disappear more frequently (Holtmaat et al., 2005). Experimental data also indicate that the PSD size correlates with spine stability *in vivo* (Cane et al., 2014). Differences in spine stability on one hand support the idea that the transience of hippocampal-dependent memory directly reflects the higher turnover of hippocampal synapses. However, the situation might be more complex. Stability and plasticity of spines seems to be compartmentalized not only in apical and basal dendrites but also within a given dendritic segment. Several lines of evidence support the 'clustered plasticity hypothesis' which suggests clusters, rather than single synaptic contacts, may be a fundamental unit for storage of long-term memory (Figure 1A-B). Pyramidal neurons of the cortex and hippocampus harbour up to 10.000 spines and the concurrent growth and removal of synapses must be regulated not only at each excitatory input but also at the level of functional clusters. Collectively these observations raise an important question: Given molecular turnover, how can clustered synapses that underwent LTP maintain strong for the long time periods that memories can persist?

Clustered plasticity in dendritic segments

Relatively little is known how LTP impact clustered plasticity as such and about its underlying principles. Synaptic activation of sufficient strength can induce LTP at individual spines (Harvey and Svoboda, 2007; Matsuzaki et al., 2004). However, the required strength for potentiation can be reduced when a nearby spine becomes potentiated. This phenomenon occurs during the activation of synaptic clusters (Govindarajan et al., 2011; Harvey and Svoboda, 2007). The dendritic branch forms an ideal segment for signaling molecules to pass through. Indeed, the induction of NMDAR-dependent LTP at individual dendritic spines activates signaling cascades that can spread into the parent dendrite over 5 to 10 μm. Moreover, dendritic branches contain translation machinery for the synthesis of new proteins and secretory trafficking organelles that ensure proper folding, modification and delivery of plasticity-related membrane proteins (Hanus and Ehlers, 2016; Mikhaylova et al., 2016). Therefore, dendritic compartmentalization at the level of individual branches could provide an autonomous means for the building and maintenance of clustered synapses.

Several studies have indeed demonstrated the existence of dendritic compartmentalization *in vivo* and *in vitro (Govindarajan et al., 2011; Kleindienst et al., 2011; Makino and Malinow, 2011; Takahashi et al., 2012)*. Interestingly, molecular mechanisms of synaptic clustering appear to differ in young and adult brain. Upon synaptic activation in development, calcium spreads over the dendrite and helps to strengthen other co-active spines by lowering the stimulation threshold required for potentiation. Spatially clustered and temporally correlated synaptic inputs show local cooperative plasticity and synapse maturation is spatially regulated with clustering of synaptic weights in developing dendritic arbors (Lee et al., 2016; van Bommel and Mikhaylova, 2016). In adult neurons, an increased density of synaptic clusters is observed during learning. In this case calcium elevation is mainly confined to the spine head and signaling between neighboring spines depends on local depolarization, activation and diffusion of signaling molecules as well as dendritic mRNA translation. Studies aimed to learn how synaptic clustering relates to LTP and synaptic tagging show that several key players also have a role in the induction of LTP at single synapses (van Bommel and Mikhaylova, 2016).

Currently three mechanisms acting at different time scales have been proposed (Figure 1B) (Winnubst and Lohmann, 2012):

1) Initially, an 'active' dendritic cluster is generated by activation of silent synapses. 'Silent synapses' are synapses that contain NMDARs but no AMPARs (Hanse et al., 2009). Induction of LTP induces the release of the Mg^{2+} block from NMDARs and increases exocytosis of AMPARs, a process which occurs within seconds.
2) The concurrent activation of the small GTPases Ras and RhoA during high frequency stimulation causes crosstalk with neighboring spines (Harvey et al., 2008; Murakoshi et al., 2011). Ras activity spreads over approximately 10 μm in dendrites and invades neighboring spines by diffusion (Harvey et al., 2008). Ras is then able to activate mitogen-activated protein kinase (MAPK) signaling which stimulate protein synthesis required for LTP (Kelleher et al., 2004) whereas RhoA activates the Rock pathway which is important for actin reorganization to enlarge spines (Murakoshi et al., 2011). This process occurs within minutes.
3) The 'synaptic tagging and capture' hypothesis suggests that induction of LTP 'tags' active synapses independently from activation strength (Frey and Morris, 1997). High frequency stimulation, which induces the protein synthesis-dependent late phase of LTP (L-LTP), will cause the production of plasticity-related proteins (PrPs) that will then later on be captured by any active synapse, not necessarily the one that originally received high-frequency stimulation (Frey and Morris, 1997). A plausible model combines synaptic tagging with synaptic clustering since neighboring synapses located on one branch are more likely to capture the 'tag' (Govindarajan et al., 2006). Following the induction of LTP increased expression of PrPs will promote synaptic clustering in neighboring synapses within hours.

However, other factors like localization of dendritic protein translation and processing mashineries, synapse-to-nucleus-and-back signalling, synaptic tagging and reverse tagging may play important role in compartmentalization of potentiated synapses. Below we will discuss potential contribution of these factors.

A role of dendritic micro-secretory systems in dendritic compartmentalization?

Neurons are highly polarized cells with a complex dendritic tree. This complex cytoarchitecture pose unique

challenges for proteostasis (Dieterich and Kreutz, 2016; Rosenberg et al., 2014). While the majority of protein synthesis and degradation machinery is localized in the soma, roughly 20 % of *de novo* protein synthesis occurs locally in dendrites, where the machineries for both protein synthesis and degradation are present and have been shown to regulate protein availability during synaptic transmission. In recent years it has become apparent that satellite microsecretory systems exist in neuronal processes that even allow for local synthesis and processing of synaptic transmembrane proteins. The endoplasmic reticulum in pyramidal neurons of the hippocampus is continuous between spines and the outer nuclear membrane, and dendrites contain ERGIC, Golgi satellites, retromer, dendritic mRNA and polyribosomes can be found throughout (Dieterich and Kreutz, 2016; Hanus and Schuman, 2013) (Figure 1C-D). It has been shown that synaptic plasticity depends on differential sorting, delivery and retention of neurotransmitter receptors and that NMDAR and AMPAR are processed through dendritic ER, ERGIC, GS and retromer (Mikhaylova et al., 2016). A spatial confinement for the potentiation of clustered synapses is likely based on the presence of local microsecretory machinery that serves the demand for membrane proteins and that defines the available pool. The dendritic satellite Golgi-containing microsecretory system exists throughout the dendritic tree of pyramidal neurons but it will only enable recruitment of proteins to membranes in spatially confined dendritic segments. It will be interesting to test whether the presence of microsecretory systems in dendrites contribute to clustered plasticity (Figure 1D).

Integration of local processes and activity-dependent gene expression: a balance between dispersed and clustered plasticity?

Intriguingly, the tendency to accumulate potentiated spines in one branch is counterbalanced by nuclear ERK signaling induced by spatially dispersed inputs that might be important for developing balanced spatial distribution of synaptic weights (Zhai et al., 2013). What could be an underlying mechanism for this type of dispersed plasticity? Activity-dependent gene expression has been proposed to feed back to synaptic function to maintain long-term memory (Kaushik et al., 2014; Rosenberg et al., 2014). However, the specific contribution of gene transcription to the formation of long-term memory is still to a large extent elusive. A key challenge in terms of clustered plasticity is to preserve synaptic connections that maintain upstream and downstream connectivity within the engram cell ensembles. Computational modeling suggests that a unimodal synaptic weight distribution is essential for synaptic stability (Smolen, 2015). The stability of this distribution needs resource competition between synapses organized into small clusters. With competition, these clusters are stable for years (Smolen, 2015).

An intriguing possibility that links activity-dependent gene expression to the stability of synaptic weight distribution in light of the competition for resources concerns inverse synaptic tagging (Okuno et al., 2012). In a seminal study Okuno and colleagues could show that recently inactive spines capture the immediate early gene Arc due to accumulation of inactive CaMKII-β. This then results in AMPAR-endocytosis and further weakening of synaptic responses as compared to neighboring recently active spines (Okuno et al., 2012). The idea that immediate early gene protein products, that are involved in down-scaling of synaptic weights via endocytosis of AMPAR, selectively act on a subset of inactive synapses is appealing (Figure 2). In fact, for IEGs like Arc, PLK2 or Homer 1A that are all localized at synapses, a role in down-scaling of synaptic responses has been described (Hayashi et al., 2012). A prerequisite is the presence of a tag that captures IEG proteins at inactive spines like it was shown for CaMKII-β. Since the preservation of differences in synaptic weight is crucial for clustered plasticity and accordingly stability of LTP it is tempting to speculate that inverse tagging of IEG proteins links activity-dependent gene expression to the maintenance of long-term memory. In parallel, spatio-temporal control of the local abundance of various PrPs can be achieved by targeting specific dendritic mRNAs within ribonucleoprotein particles (RNPs) to the dendritic compartments with high or low synaptic activity. This has been proposed as the 'sushi belt model' where RNPs would be captured and released by multiple synapses, thus providing further molecular means for synaptic tagging (Doyle and Kiebler, 2011).

Acknowledgement: M.M. is supported by grants from the Deutsche Forschungsgemeinschaft (DFG Emmy-Noether Programm (Ml 1923/1-1), FOR2419 (MI 1923/2-1), DFG MI1923/3-1). M.R.K is supported by grants from the DFG (Kr1879 3-1, 5-1, 6-1, SFB 779 TPB8), the Bundesministerium für Bildung und Forschung 'Energi' FKZ: 01GQ1421B, The EU Joint Programme – Neurodegenerative Disease Research (JPND) project STAD, People Programme (Marie Curie Actions) of the European Union's Seventh

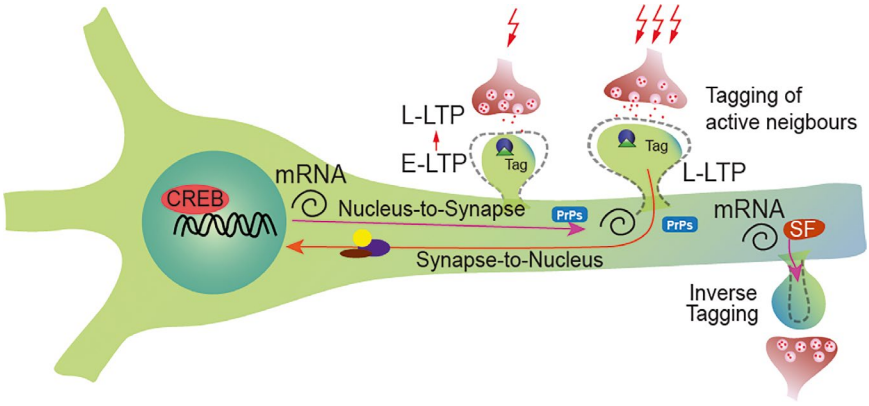

Figure 2: Synaptic tagging and capture and inverse tagging in clustered plasticity. The 'sushi belt model' allows capturing of PrPs to maintain LTP at potentiated synapses wheres incorporation of synaptic scaling factors at inactive synapses will contribute to preserve differences in synaptic weight. SF – scaling factors. CREB – cAMP response element-binding protein.

Framework Programme FP7/2007-2013/ under REA grant agreement n° [289581] and Leibniz Foundation (Pakt für Forschung 2014, 2015, 2017).

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Article note: German version available at https://doi.org/10.1515/nf-2018-0006

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