CA1 pyramidal neurons are the major output of the hippocampus (Amaral and Witter 1989) and activity-dependent changes such as long-term potentiation (LTP) and long-term depression (LTD) at CA1 synapses are important cellular correlates of learning and memory. The strength of CA1 excitatory synapses in the hippocampus is not fixed, but can change depending upon their activation pattern (Zucker 1999; Zucker and Regehr 2002). For example, it is well documented that different stimulation protocols can induce distinct forms of plasticity in the distal part of CA1 pyramidal neurons (Sajikumar et al. 2005a). The early and late forms of LTD can be induced by weak and strong stimulation paradigms (Malenka and Bear 2004). These protocols exert their action by activating different subcellular signaling pathways. One of the most important pathways in this respect involves the activation of an atypical PKC isotype, PKMζ (PKMζ) by a strong tetanization protocol (Sacktor 2008). PKMζ is critical not only for the maintenance of late-LTP, but also for the associative processes of LTP, i.e., synaptic tagging and capture (STC) (Ling et al. 2002; Sajikumar et al. 2005b). STC is a mechanism that explains how weakly activated synapses express long-term plasticity (expression of late-LTP in a weakly tetanized synaptic input) by capturing plasticity-related proteins (PRPs) from a nearby strong synaptic input (Young and Nguyen 2005; Sajikumar et al. 2009). Recently, it has been reported that STC is compartment restricted to apical or basal dendrites of CA1 pyramidal neurons (Alarcon et al. 2006; Sajikumar et al. 2007; Sajikumar and Korte 2011). The consequence of such a model is that one distinct form of synaptic plasticity induced and expressed in a certain compartment will not affect other compartments (Alarcon et al. 2006; Sajikumar et al. 2007; Sajikumar and Korte 2011).

A typical pyramidal neuron consists of apical and basal dendrites differing in their morphology, biophysical characters, and in their LTP induction and expression mechanisms (Arai et al. 1994; Haley et al. 1996; Kramar and Lynch 2003). The neuromodulatory requirements for inducing long-lasting plasticity in basal and apical dendrites is different: for instance, in apical dendrites dopamine receptor activation by dopamine or its agonists can induce slow onset potentiation lasting 6 h (Navakkode et al. 2005, 2007), while in basal dendrites cholinergic activation in concert with cAMP is required for the initiation of long-lasting plasticity (Navakkode and Korte 2011). How different forms of synaptic plasticity differ in the proximal and distal parts of CA1 apical dendrites is a crucial question to address, since possible variation might have functional consequences.

In this study, we show that the plasticity thresholds for inducing LTP are different in proximal and distal compartments of apical dendrites. In addition, we show another important aspect of associative interactions between the proximal and distal compartments of apical dendrites by providing evidence that even a subthreshold stimulus in the distal compartment (a stimulus incapable of activating PRPs, for example, a 100 Hz, 21-pulse stimulation in distal region results only in a protein-synthesis-independent early-LTP) can activate PRPs such as PKMζ in the proximal dendrites, which enables associativity between two distinct dendritic compartments in apical dendrites to occur.

A total of 49 hippocampal slices prepared from 49 male Wistar rats (6–7 wk old) were used for this study. All procedures were approved by the guidelines from the Animal Committee on Ethics in the Care and Use of Laboratory animals of the TU-Braunschweig. Briefly, after anaesthetization using CO2, the rats were decapitated and the brains were quickly removed to 4°C artificial cerebrospinal fluid (ACSF). Transverse hippocampal slices of 400 μm were prepared from the right hippocampus using a manual tissue chopper (Stoelting) and incubated at 32°C in an interface chamber (Scientific System Design). Slice preparation, incubation, and maintenance were conducted as previously described (Sajikumar et al. 2005a). Two monopolar lacquer-coated, stainless-steel electrodes (5 MΩ; AM Systems USA) were positioned within the stratum radiatum of the CA1 region for stimulating two separate independent synaptic inputs in the two regions of CA1 apical dendrites, S1 (proximal, pS) and S2 (distal, dS) (Fig. 1A). Pathway independence was tested by paired-pulse facilitation (PPF) protocol with an interpulse interval of 30 msec as described in Kim and Lisman (2009) and Young and Nguyen (2005). Facilitation of fEPSPs was observed only when two consecutive pulses with an interval of 30 msec were applied to the same pathway. When two consecutive pulses were applied to different pathways within 30 msec, no facilitation was observed if compared with fEPSP of the same input (S1-S2-S1) (Fig. 1B). The following stimulation protocols were used to induce different

**Brief Communication**

**Different compartments of apical CA1 dendrites have different plasticity thresholds for expressing synaptic tagging and capture**

Sreedharan Sajikumar and Martin Korte1

Zoological Institute, Division of Cellular Neurobiology, TU, Braunschweig, D-38106 Germany

The consolidation process from short- to long-term memory depends on the type of stimulation received from a specific neuronal network and on the cooperativity and associativity between different synaptic inputs converging onto a specific neuron. We show here that the plasticity thresholds for inducing LTP are different in proximal and distal compartments of apical dendrites. In addition, we show interactions between the proximal and distal compartments of the apical dendrites by providing evidence that even a subthreshold stimulus can activate plasticity-related proteins, such as PKMζ, enabling associativity between two distinct dendritic compartments in apical dendrites to occur.
forms of plasticity in pS and dS inputs. (1) Late-LTP was induced in dS using a strong tetanization (STET) protocol consisting of three stimulus trains of 100 pulses, 100 Hz duration, 0.2 msec/polarity, and intertrain interval of 10 min. (2) Late-LTP was induced in pS using a weak tetanization protocol (WTET) consisting of one stimulus train of 21 pulses, 100 Hz, duration 0.2 msec/polarity (represented by WTET [pS-21]). (3) Early-LTP in dS was induced using WTET consisting of one stimulus train of 21 pulses, 100 Hz, duration 0.2 msec/polarity (represented by WTET [dS-21]). (4) In pS early-LTP was induced using WTET consisting of a single stimulus train of 12 pulses, 100 Hz, duration 0.2 msec/polarity (represented by WTET [pS-12]). Baseline was recorded for 60 min in all experiments. Four 0.2 Hz biphasic constant-current pulses (0.1 msec/polarity) were used for baseline recording and testing (every 5 min).

Anisomycin (25 μM) was dissolved in ACSF and 0.01% DMSO. PKMε inhibitory peptide, myristoylated pseudo substrate peptide (ZIP) (AnaSpeck, USA) was prepared in distilled water as a stock solution (5 mM). The required volume of 1 μM was dissolved in ACSF immediately before bath application. Myr-ZIP is 142-fold more potent on PKMε than on conventional PKCζ and 196-fold more potent on PKMγ than on cAMP-dependent protein kinase (Ling et al. 2002).

First we tested whether a stimulation protocol which reliably induces a protein synthesis-independent form of short-lasting LTP (early-LTP, lasting 2–3 h), in the distal region of the apical CA1 dendrite, could induce the same form of early-LTP in the proximal part of the apical CA1 dendrite. Thus, we recorded a stable baseline of 60 min in the pS and dS synaptic inputs (recordings from proximal and distal inputs are always represented by filled circles and open circles, respectively) after which WTET (pS-21) was delivered to the pS input, while dS was recorded as a control. Surprisingly, this stimulation in the pS region resulted in LTP lasting 6 h (Fig. 1C, filled circle). The control input remained stable for 6 h (Fig. 1C, open circles) (Wilcoxon U-test, \( P < 0.05 \)). Next, we addressed the question of whether these distinct forms of LTP require new protein synthesis for their persistence. As shown in Figure 1D, bath application of a reversible protein synthesis inhibitor anisomycin (ANI, 25 μM) 30 min before and after the induction of LTP in pS resulted in a transient form of LTP (Fig. 1D, filled circles). Statistically significant LTP was observed up to 130 min after LTP induction (U-test, \( P < 0.05 \)) or up to 145 min (Wilcoxon, \( P < 0.05 \)). In search of a reliable protocol for inducing early-LTP in the pS compartment of apical CA1...
dendrites, we standardized a stimulation protocol consisting of 12 biphasic constant-current pulses (WTET \[ps-12\]). This protocol resulted in a transient form of LTP in the pS region lasting nearly 2 h (Fig. 1E, filled circles). Statistically significant LTP was observed up to 95 min after LTP induction (\(U\)-test, \(P < 0.05\)) or up to 110 min (Wilcoxon, \(P < 0.05\)).

In the next series of experiments, we probed whether late-LTP in the proximal region of apical dendrites can initiate STC. To test this hypothesis, LTP was induced at the pS input by WTET (pS-21) (Fig. 2A, filled circles), then 30 min later, WTET (dS-21) was applied to the distal region of CA1 apical dendrites (dS) (Fig. 2A, open circles). The distal synaptic input (dS) under these circumstances expressed late-LTP lasting 6 h (posttetanization potentials in pS and dS showed significant potentiation compared with their prepotentiation values, Wilcoxon signed rank test; \(P < 0.05\)). Next, we investigated whether PKM\(_2\) inhibition had an effect on STC. The experimental design was similar to Figure 2A except that ZIP (1 \(\mu\)M) was bath applied starting at 60 min until the end of the experiment. As shown in Figure 2B, ZIP not only prevented the persistence of late-LTP in pS, but also interfered with the capture processes in dS, thus preventing expression of late-LTP at the distal synaptic input (significant potentiation was expressed up to 185 min and 115 min in pS and dS, respectively, Wilcoxon test; \(P < 0.05\)). Next, we studied whether a late-LTP in the distal compartment can influence an early-LTP in the proximal compartment. To test this, we induced late-LTP in dS by a STET protocol (Fig. 2C, open circles) and 30 min later, early-LTP in pS by WTET (pS-12) (Fig. 2C, filled circles). Surprisingly, the pS input expressed late-LTP showing STC. Statistically significant potentiation was observed in both pS and dS after the induction of LTP (Wilcoxon signed rank test; \(P < 0.05\)). We then studied whether PKM\(_2\) is involved in this form of STC. To this aim, we did an experiment similar to Figure 2C, but, in addition, ZIP was applied 60 min after the induction of late-LTP in dS and continued for 6 h (Fig. 2D). Potentials in dS and pS (open and filled circles) decayed to baseline within 2–3 h, thus demonstrating that PKM\(_2\) inhibition prevents STC. Significant potentiation was observed up to 195 min in pS and up to 115 min in dS (Wilcoxon test; \(P < 0.05\)).

The difference in the functionality of synapses that we observed in distinct parts of a neuron is often ignored when different forms of plasticity are induced at the apical dendrites. For instance, studying STC requires a set of independent and weakly activated synaptic populations in association with a set of strongly activated synapses (Martin and Kosik 2002). It remains unclear whether a weak stimulation used to induce a transient form of plasticity in a specific region of a neuron can be effectively used for inducing the same form of plasticity in other regions. It has been reported recently that low-frequency stimulation can induce late-LTD in distal, but not at proximal dendrites (Parvez et al. 2010). Interestingly, the investigators showed positive associative interactions between LTP and LTD (cross-capture), and they found

Figure 2. Synaptic tagging and capture between proximal and distal apical dendrites. (A) After recording a stable baseline of 60 min, late-LTP was induced in proximal part using WTET(pS-21) (filled circles); 30 min later early-LTP was induced by WTET (dS-21) in dS (open circles). dS expressed late-LTP by synaptic tagging and capture from pS to dS (\(n = 7\)). (B) Experimental design was same as in A, but in addition, the PKM\(_2\) inhibitor ZIP (1 \(\mu\)M, open rectangle) was applied 60 min after the induction of late-LTP in pS. ZIP not only prevented the maintenance of late-LTP in pS (filled circles) but also the capturing of PKM\(_2\) by dS (open circles) (\(n = 7\)). (C) Induction of late-LTP in dS by repeated tetanization (3xTET, 100 Hz, 100 pulses, open circles) in dS; 30 min later early-LTP was induced in pS by a WTET (pS-12). Here pS expressed late-LTP by synaptic tagging and capture (\(n = 7\)). Single arrows represent the time point of WTET; pS-21, dS-21, and pS-12 in the representative synaptic inputs. The triple arrows represent the time point of induction of strong late-LTP in corresponding synaptic input. Traces as in Figure 1.
that an early-LTP induced in the proximal region expressed late-LTP. They propose that this is due to newly synthesized proteins that are triggered by the induction of a late-LTD in the distal region of the apical dendrites. The stimulation protocol used for the induction of early-LTP in the proximal region was the same as that of the standard stimulation protocol used for the induction of early-LTP in a distal region (i.e., 100 Hz, 21 pulses). Our studies indicate that a standard stimulation protocol used in the distal region of CA1 pyramidial neurons cannot be used for inducing a similar form of plasticity in the proximal region. While the WTET (ps-21) protocol that we expected to produce early-LTP in the proximal region expressed a protein synthesis-dependent form of late-LTP, this is not the case for the distal regions. Thus, the expression of late-LTP in the proximal region is not due to the cross-capture of PKMζ, as previously suggested by Parvez et al. (2010). In fact, the classical early-LTP stimulation protocol they used itself resulted in the activation of PKMζ within the proximal region. We also show here that the proximal part of apical dendrites does require only threshold stimuli of 12 pulses for inducing protein synthesis-independent forms of early-LTP. These results are in line with our earlier findings showing that the induction of early-LTP in the basal dendrites requires only threshold stimuli (Sajikumar et al. 2007). Moreover, late-LTP can be induced in the apical region even without repeated stimulation (Sajikumar et al. 2008). Other examples show that although, a weak stimulus induces late-LTP in the proximal region, a strong low-frequency stimulation that generally results in late-LTD in the distal region is unable to induce late-LTD in the proximal region (Parvez et al. 2010). In short, the plasticity thresholds in the distal and the proximal part of the apical dendrites are different for both LTP and LTD induction. While the threshold for inducing LTP is lower in the proximal region and increases from the proximal to the distal part of the apical dendrites, the reverse situation is observed for LTD. Here, the threshold is lower in the distal part and increases from the distal to the proximal region of the apical dendrites. The different plasticity thresholds observed in the proximal and distal part of the apical dendrites might be due to a difference in the morphology or neuromodulatory inputs between the two regions. The hippocampus Schaffer Collateral (SC) axons extend from CA3 pyramidal cells to the CA1 region and form excitatory synapses onto mid and proximal dendrites of CA1 pyramidial cells in the stratum lacunosum–moleculare (SLM). This represents a fundamental difference in the presynaptic function of the two major inputs to CA1 neurons, which could alter the flow of information in the hippocampus as it is shown by Witter (1993) and Speed and Dobrunz (2009). How the TA pathway operates in regulating the plasticity threshold is still not well understood. In addition, the differences in the activation of voltage-dependent calcium channels (VDCC) and the higher GABAergic innervation of the proximal region compared with the distal one also contribute to the existence of different plasticity rules in the distal and proximal regions of apical dendrites (Megias et al. 2001; Golding et al. 2002). Finally, the difference in the neuromodulatory inputs to the different regions of the apical dendrites could also contribute to the different plasticity thresholds (Hasselmo and Schnell 1994; Spruston 2008). For instance, while a spatially restricted effect of the action of acetyl choline (ACh) at muscarinic acetyl choline receptor (mAChR) suppresses synaptic transmission at proximal apical dendrites, synapses that innervate apical tufts are less affected (Hasselmo and Schnell 1994; Spruston 2008).

An important aspect of our study was to investigate whether the late-LTP expressed in the proximal region by a subthreshold stimulus (ps-21) can interact with a short-lasting plasticity form in the distal region. Indeed, we found that the tag set due to an early-LTP in the distal part could capture proteins, like PKMζ, synthesized from the proximal region, and thereby expresses late-LTP.

In a similar fashion, an early-LTP induced by a subthreshold stimulus (ps-12) in the proximal region could also benefit from a late-LTP in the distal part by capturing PKMζ, because PKMζ inhibition during the tagging process prevented STC. These results are in line with our earlier findings that PKMζ is critical for synaptic tagging during LTP (Sajikumar et al. 2005b, 2009). It is well documented that PKMζ exerts its action by releasing AMPARs from an extra-synaptic pool and enhancing NSF/GluR2-mediated trafficking to maintain LTP and STC (Yao et al. 2008). Recently, it has also been reported that STC is restricted to distinct dendritic compartments, thus the functional processes in one compartment are independent of other compartments (Alarcon et al. 2006; Sajikumar et al. 2007). We report here that in certain circumstances functional interaction between different compartments can be established within apical dendrites (here ps and ds) even with modest subthreshold stimulation protocols. The plasticity in the proximal and distal part of apical dendrites differs with the type of information or stimulation that each synaptic population receives, possibly enabling the effective coding of long-term memory. These results are an important pre-requisite for investigating homosynaptic and heterosynaptic shutdown of LTP and STC in proximal and apical dendrites. It has been shown earlier that in adult neurons a homeostatic regulatory mechanism exists to regulate the overstrengthening of synapses beyond a physiological level (Young and Nguyen 2005; Roth-Alpermann et al. 2006). However, it remains unclear how the homeostatic regulatory apparatus varies in distal and proximal compartments of apical dendrites.

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Sreedharan Sajikumar and Martin Korte

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