

# Learning-Facilitated Synaptic Plasticity at CA3 Mossy Fiber and Commissural–Associational Synapses Reveals Different Roles in Information Processing

Hardy Hagena<sup>1,2</sup> and Denise Manahan-Vaughan<sup>1,2</sup>

<sup>1</sup>Department of Neurophysiology, Medical Faculty and <sup>2</sup>International Graduate School for Neuroscience, Ruhr University Bochum, 44780 Bochum, Germany

Address correspondence to Denise Manahan-Vaughan, Department of Neurophysiology, Medical Faculty, Ruhr University Bochum, MA 4/149, Universitaetsstrasse 150, 44780 Bochum, Germany. Email: dmv-igsn@rub.de.

**Subregion-dependent differences in the role of the hippocampus in information processing exist. Recently, it has emerged that a special relationship exists between the expression of persistent forms of synaptic plasticity in hippocampal subregions and the encoding of different types of spatial information. Little is known about this type of information processing at CA3 synapses. We report that in freely behaving rats, long-term potentiation (LTP) is facilitated at both mossy fiber (mf)–CA3 and commissural–associational (AC)–CA3 synapses by exploration of a novel (empty) environment. Exploration of large spatial landmarks facilitates long-term depression (LTD) at mf-CA3 synapses and impairs synaptic depression at AC-CA3 synapses. Novel exploration of small environmental features does not facilitate LTD at mf synapses but facilitates persistent LTD at AC synapses. Thus, depending on the quality of the information synaptic plasticity at AC-CA3 and mf-CA3 synapses is differentially modulated. These data suggest that expression of LTP as a result of environmental change is a common property of hippocampal synapses. However, LTD at mf synapses or AC synapses may subservise distinct and separate functions within the CA3 region.**

**Keywords:** CA3, commissural-associational fiber, in vivo, long-term depression, long-term potentiation, mossy fiber

## Introduction

Learning-facilitated synaptic plasticity refers to the ability of hippocampal synapses to respond with persistent synaptic plasticity to the coupling of weak afferent stimulation, which is subthreshold for the induction of plasticity, with a spatial learning experience (Manahan-Vaughan and Braunewell 1999; Kemp and Manahan-Vaughan 2007). Persistent synaptic plasticity, in the form of long-term potentiation (LTP) and long-term depression (LTD) may comprise the cellular mechanisms underlying long-term memory (Braunewell and Manahan-Vaughan 2001; Morris et al. 2003; Kemp and Manahan-Vaughan 2007). Studies conducted over the last decade, support a tight relationship between the exploration of novel space and the facilitation of hippocampal LTP (Straube et al. 2003; Davis et al. 2004; Kemp and Manahan-Vaughan 2004, 2008). Typically, only a change in the ambient environment is required to facilitate short-term potentiation into LTP that lasts for days in freely behaving rodents. This suggests that LTP may comprise a fundamental encoding response of hippocampal synapses that occurs as soon as novel spatial information processing is required.

LTD is also associated with the acquisition of spatial memory (Manahan-Vaughan and Braunewell 1999; Nakao et al. 2002; Kemp and Manahan-Vaughan 2004, 2007). Manipulations

that prevent spatial learning also prevent learning facilitation of LTD (Kemp and Manahan-Vaughan 2005; Lemon and Manahan-Vaughan 2006). Here, it is not the change of the environment *per se* that facilitates LTD—rather the novel features of the environment or more specifically object–place configurations that facilitate this form of synaptic plasticity (Kemp and Manahan-Vaughan 2004). LTD also appears to be more discriminating than LTP: Whereas prominent landmark features of a spatial environment facilitate LTD in the dentate gyrus, small and more discrete features of the environment facilitate LTD in the CA1 region (Kemp and Manahan-Vaughan 2008). This sensitivity is not interchangeable: CA1 LTD is not facilitated by landmarks, and dentate gyrus LTD is not facilitated by small contextual features (Kemp and Manahan-Vaughan 2008), suggesting that the facilitation of LTD does not simply relate to whether large objects are perceived more easily than small ones. A further striking feature of this relationship between the facilitation of LTP or LTD by distinct aspects of spatial learning is that the conditions that facilitate LTD impair LTP and vice versa (Kemp and Manahan-Vaughan 2004, 2008). This suggests a distinct functional categorization with regard to the aspects of spatial memory that may be related to LTP and LTD.

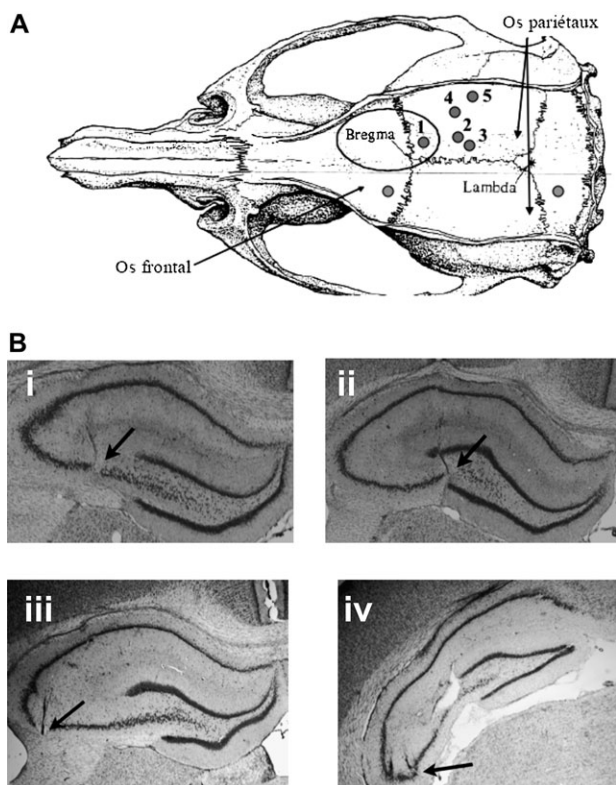
But one other key subregion of the hippocampus has remained unexplored on this level: the CA3 region. This region is distinct in that it processes information by means of 2 quite distinct synapses. The mossy fiber (mf) afferents from the dentate gyrus, synapse on the stratum lucidum and mediate kainate-dependent fast excitatory transmission (Acsády et al. 1998; Contractor et al. 2001; Kamiya et al. 2002; Bortolotto et al. 2003). These synapses express presynaptic forms of synaptic plasticity that do not depend on *N*-methyl-D-aspartate (NMDA) receptor activation (Harris and Cotman 1986). Commissural–associational (AC) fibers project onto the stratum radiatum of CA3 pyramidal cells and enable  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA)-mediated fast excitatory transmission and NMDA receptor-dependent forms of synaptic plasticity (Harris and Cotman 1986; Kakegawa et al. 2004). Furthermore, the CA3 region also receives direct input from neurons in layer II of the entorhinal cortex (Amaral and Witter 1989).

Here, we investigated mf-CA3 and AC-CA3 synaptic plasticity in freely moving rats, and examined if learning-facilitated plasticity is expressed at these synapses. We report that these synapses indeed express this type of plasticity but reveal that they do not respond identically to spatial manipulations. These data offer novel insight into the role of the different hippocampal subregions in spatial information processing and particularly highlight the significance of CA3 synapses in this regard.

## Materials and Methods

All experiments were performed according to the guidelines of the German Animal Protection Law and were approved by the North Rhine-Westphalia State Authority. Male Wistar rats (Charles River, 7–8 weeks old) underwent implantation of hippocampal electrodes and a guide cannula under anesthesia, as described previously (Hagena and Manahan-Vaughan 2010). For mf-CA3 implantations, the recording electrode was placed above the CA3 pyramidal cell layer of the dorsal hippocampus at coordinates 3.2 mm posterior to bregma and 2.2 mm lateral to midline. The bipolar stimulation electrode was implanted 3.5 mm posterior to bregma and 2.0 mm lateral to the midline (based on Derrick and Martinez 1994). For AC-CA3 implantations, the recording electrode was placed 3.1 mm posterior to bregma and 3.1 mm lateral to midline, and the stimulation electrode was placed at coordinates 3.6 mm posterior to bregma and 4.2 mm lateral to midline. The correct positions of the electrodes were determined on basis of simultaneous evoked test pulses during the implantation procedure and were later verified by postmortem histological analysis (Fig. 1).

A nonpersistent and strongly decremental form of synaptic depression was induced by low-frequency stimulation (LFS) at 1 Hz and with 600 pulses. A strongly decremental and nonpersistent form of synaptic potentiation was elicited using high-frequency stimulation (HFS) consisting of 2 trains of 100 pulses at 100 Hz. In control experiments (LFS control and HFS control), animals received electrical stimulation only, without spatial manipulations.



**Figure 1.** Placement of electrodes. (A) Schematic of electrode placement in the hippocampus (redrawn from Paxinos and Watson's Atlas 1997). The dots indicate the positions for reference and ground electrodes. (1) represents the drill holes for the cannula, (2) the recording electrode in area CA3, (3) the stimulation electrode in the mf pathway for recordings at the mf-CA3 synapse, (4) the recording electrode, and (5) the stimulation electrode for recordings at the commissural-associational-CA3 synapse. (B) Microphotograph showing recording and stimulating electrode placement in the Nissl-stained hippocampus. Black arrows point to the final position of the recording electrode in area CA3 stratum lucidum (i) and the stimulation electrode in the dentate gyrus at the origin of the mf projections (ii). The bottom row shows Nissl-stained hippocampal slices with the (iii) recording electrode placed in area CA3 stratum radiatum and (iv) the stimulation electrode in the commissural-associational pathway.

To observe the effect of learning on synaptic plasticity, we adopted a protocol first described by Manahan-Vaughan and Braunewell (1999). We introduced a 39 × 39-cm gray holeboard into the recording chamber for the duration of the plasticity-inducing stimulation. The recording chamber (40 × 40 × 50 cm) consisted of gray plastic with a perspex door with no visible cues attached to the walls. The hole board had 4 holes (5.5 cm in diameter and 5 cm in deep): one in each corner. A small object of unique appearance and size (1–2 cm in diameter and height) was placed in each hole for the animal to explore. Upon first exposure, the animals were exposed to objects that they had never seen before. Reexposure (second exposure) comprises the presentation of the same objects in the same holeboard holes. In certain cases, a third exposure took place here: now the familiar objects were presented in different holeboard holes (reconfiguration). In a separate set of experiments, 1 or 3 large objects (5–10 cm in diameter and 5–12 cm in height) were inserted directly into the recording chamber for the duration of the plasticity-inducing stimulation.

A biphasic pulse (half-wave duration of 0.2 ms) was given to evoke field excitatory postsynaptic potentials (fEPSPs). In the figures, evoked traces of an individual animal are shown for time points 5 min prior to the application of either HFS or LFS, and both 5 min and 24 h after application of either HFS or LFS. For recordings, the stimulation intensity was set to produce a fEPSP, which was 40% of the maximum obtained during an initial input–output curve (maximal stimulation 900  $\mu$ A). During application of LFS, the stimulation intensity was set to 70% of the maximum values obtained during the input–output curve. During HFS, the stimulation intensity was left at 40%.

For each time point, 5 consecutively evoked responses at 40-s intervals were averaged. The first 6 time points recorded in each experiment served as baseline.

All data points were expressed as a mean percentage  $\pm$  standard error of the mean of the average baseline value. For data comparison, one-way analysis of variance (ANOVA) was used. The level of significance was set at  $P < 0.05$ .

## Results

### *Learning-Facilitated LTD at mf-CA3 Synapses is Not Elicited by Novel Exposure to Spatial Constellations of Discrete Environmental Features*

In previous studies, we showed that the coupling of weak patterned stimulation of the Schaffer collateral (SC) input to CA1, with exploration of spatial constellations of small objects positioned in holeboard holes, results in lasting LTD at stratum radiatum synapses (Manahan-Vaughan and Braunewell 1999; Kemp and Manahan-Vaughan 2004; Lemon and Manahan-Vaughan 2006). In the present study, we recorded from the stratum lucidum of the CA3 region during stimulation of mf afferents to establish if similar properties are evident (Fig. 1). In control animals, low-frequency stimulation that was submaximal for the induction of robust LTD (sLFS, 600 pulses at 1 Hz) induced short-term depression (STD < 2 h) in mf-CA3 synapses (Fig. 2A,  $n = 16$ ) in line with other reports (Klausnitzer and Manahan-Vaughan 2008; Hagena and Manahan-Vaughan 2010). Exposure to a holeboard containing small, partially concealed objects during sLFS (Fig. 2A,  $n = 5$ ) elicited no significant difference in the profile of the STD. ANOVA of the fEPSP yielded  $P = 0.4517$ .

### *At mf-CA3 Synapses, Learning-Facilitated LTD is Elicited by Exposure to Large Environmental Landmarks*

In the dentate gyrus, LTD is facilitated by novel exploration of large landmark features of an environment (Kemp and Manahan-Vaughan 2008). Therefore, we examined whether mf-CA3 synapses respond to this type of information

processing. A large cue (9 cm in diameter and 10 cm in height) that served as an orientational feature was placed in the center of the recording chamber during the application of sLFS. Under these conditions, a facilitation of synaptic depression occurred (Fig. 3A). The difference between application of sLFS during exploration of one novel cue and sLFS alone ( $n = 14$ ) was highly significant (ANOVA,  $F_{1,71} = 34.45$ ;  $P < 0.0001$ ;  $n = 8$ ).

To address whether an increase in the spatial load influences synaptic plasticity at the mf-CA3 synapse, 3 large landmark cues

were presented to the same animals in a separate study. The cues were arranged equidistant from one another and at a fixed distance to the walls of the recording chamber. Exploration of the objects during sLFS elicited a significant facilitation of LTD compared with application of sLFS alone (Fig. 3C, ANOVA,  $F_{1,78} = 44.04$ ;  $P < 0.0001$ ;  $n = 9$ ). However, there was no difference in the profile of LTD between 1 and 3 cues (ANOVA,  $F_{1,48} = 0.19$ ;  $P = 0.66$ ).

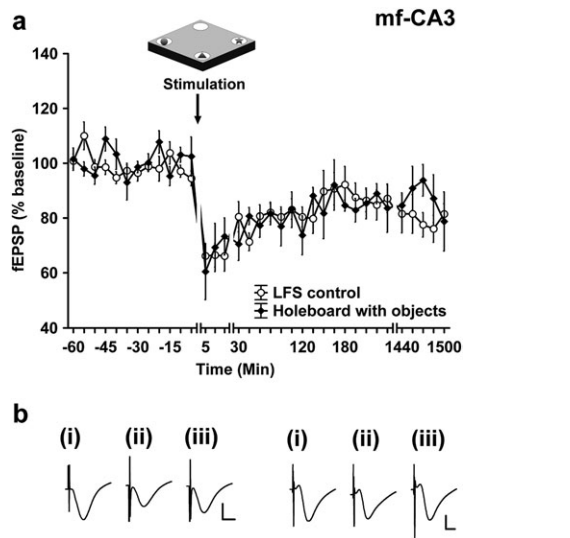
### Rearrangement of Familiar Landmark Cues Facilitates LTD at mf-CA3 Synapses

Rearrangement of landmark objects creates a novel spatial constellation, which facilitates LTD in the dentate gyrus (Kemp and Manahan-Vaughan 2008). To elucidate how information about changes in spatial features of an environment is processed at the mf-CA3 synapse, first, the experiment with a familiar constellation of cues was repeated 7 days later. Exposure to the now familiar cues did not facilitate LTD (ANOVA,  $F_{1,45} = 0.57$ ;  $P = 0.45$ ;  $n = 8$ ) (Fig. 4A), suggesting that LTD is only facilitated by new informational encoding. If the constellation of the familiar cues was changed, in a subsequent experiment conducted after a further 7 days, facilitation of LTD was observed (ANOVA,  $F_{1,4} = 145.47$ ;  $P < 0.0001$ ;  $n = 5$ ) (Fig. 4). This suggests that it is not the presence of the objects *per se*, but rather their context that facilitates the induction of robust LTD at the mf-CA3 synapses.

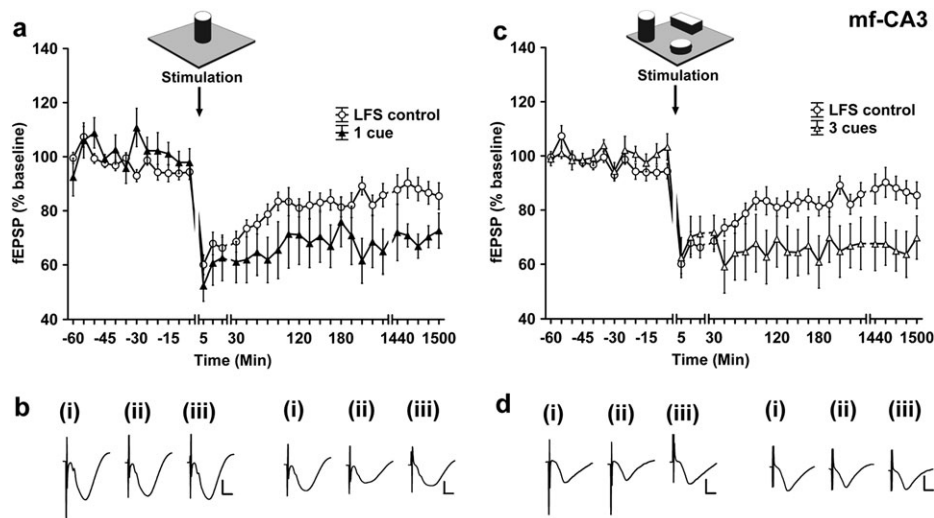
### Exposure to a Novel Empty Holeboard Facilitates LTP at mf-CA3 Synapses

In the dentate gyrus and CA1 region, exploration of a novel empty holeboard during HFS facilitates the expression of persistent LTP that lasts for several days (Kemp and Manahan-Vaughan 2004, 2007).

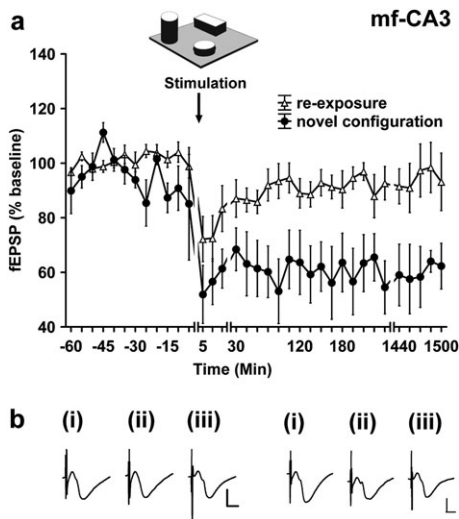
In the CA3 region, application of HFS that is subthreshold for the induction of LTP (sHFS) induced strongly decremental



**Figure 2.** Exploration of a holeboard with novel objects does not facilitate LTD of mf-CA3 synapses. (a) Transient synaptic depression occurs at mf synapses when submaximal low frequency stimulation (sLFS) is applied in either the presence ( $n = 5$ ) or the absence ( $n = 16$ ) of an object-containing holeboard. (b) Analog responses evoked during application of sLFS alone (left) and in an experiment in which the animal explored the holeboard with spatially arranged small objects (right). Traces were recorded (i) 5-min pre-sLFS, (ii) 5-min post-sLFS, and (iii) 24 h after sLFS. Vertical scale bar: 1 mV and horizontal scale bar: 4 ms.



**Figure 3.** Exploration of landmark cues facilitates LTD of mf-CA3 synapses. (a) Transient synaptic depression occurs at mossy fiber synapses when submaximal low frequency stimulation (sLFS) is applied in control animals. When sLFS is applied during exposure to one large landmark feature ( $n = 8$ ), mf LTD ( $>24$  h) occurs that is significantly different with responses evoked without exploration of an object ( $n = 14$ ). (b) Analog traces represent fEPSPs recorded in an experiment when sLFS was applied alone (left traces) and when sLFS was applied in the presence of one landmark cue (right traces). (i) 5-min pre-sLFS, (ii) 5-min post-sLFS, and (iii) 24 h after sLFS application. Horizontal scale bar: 6 ms. Vertical scale bar: 2 mV. (c) Exposure to 3 landmark objects also results in the facilitation of robust LTD ( $>24$  h) ( $n = 9$ ). However, no difference in the facilitation of LTD by exploration of 1 or 3 cues is perceptible. Changes in timescale are indicated by line breaks. (d) Left traces show analogs recorded during application of sLFS alone. Right traces show analogs recorded during the experiment when the animal explored 3 cues. Analogues represent (i) 5-min pre-sLFS, (ii) 5-min post-sLFS, and (iii) 24 h after sLFS application. Vertical scale bar: 2 mV and horizontal scale bar: 6 ms.



**Figure 4.** LTD of mf-CA3 synapses is facilitated by rearrangement of familiar landmark cues. (a) Facilitation of LTD at mf synapses does not occur when the animals are reexposed to 3 familiar cues in a familiar constellation during 1 Hz stimulation consisting of 600 pulses (sLFS,  $n = 8$ ). A novel configuration of the same objects during sLFS facilitates LTD that lasts for over 24 h ( $n = 5$ ). Changes in timescale are indicated by line breaks. (b) Left traces show analog responses evoked during the reexposure experiment and during an experiment in which the cues were arranged in a novel configuration (right traces). Analogs represent (i) 5-min pre-sLFS, (ii) 5-min post-sLFS, and (iii) 24 h after sLFS. Vertical scale bar: 1 mV and horizontal scale bar: 4 ms.

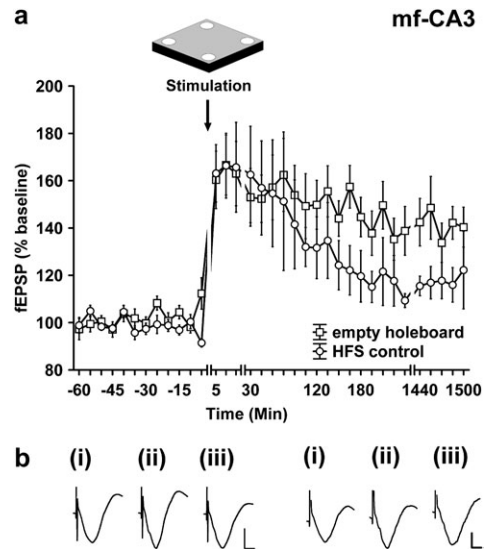
synaptic depression ( $n = 11$ ), whereas exposure to an empty novel holeboard during sHFS facilitated LTP, which lasted for over 24 h (ANOVA,  $F_{1,45} = 16.66$ ;  $P < 0.0001$ ;  $n = 10$ ) (Fig. 5).

#### Novel Exposure to Spatial Constellations of Small Environmental Features Facilitates LTD at Commissural-Associational-CA3 Synapses

Having examined the response of the mf to spatial information, we now assessed the commissural-associational (AC)-CA3 synapses. In control animals, sLFS (1 Hz, 600 pulses) induced a nonpersistent form of synaptic depression of AC-CA3 synapses (Fig. 6,  $n = 8$ ). Exploration of small, contextual features during application of sLFS significantly facilitated LTD (>24 h) at the AC-CA3 synapse (ANOVA,  $F_{1,3} = 47.1$ ;  $P < 0.001$ ;  $n = 7$ ) (Fig. 6A). Reexposing the animals 1 week later to the same objects, in the same constellation, did not alter synaptic responses compared with baseline values (ANOVA,  $F_{1,3} = 0.21$ ;  $P = 0.65$ ;  $n = 8$ ) (Fig. 6C). In contrast to effects seen in the CA1 region (Manahan-Vaughan and Braunewell 1999; Kemp and Manahan-Vaughan 2004), reconfiguration of the objects did not change the profile of synaptic responses compared with reexposure of the object-containing holeboard (ANOVA,  $F_{1,3} = 3.53$ ;  $P = 0.62$ ;  $n = 8$ ) (Fig. 6C).

#### Exploration of 3 Large Novel Cues Impairs Synaptic Depression at the Commissural-Associational-CA3 Synapse

In contrast to the mf-CA3 synapse, where spatial arrangements of landmarks facilitate STD into LTD, in AC-CA3 synapses, exploration of 3 large novel landmarks during application of sLFS (1 Hz, 600 pulses) significantly impaired the induction of synaptic depression compared with application of sLFS



**Figure 5.** Exploration of an empty holeboard facilitates LTP of mf-CA3 synapses. (a) Application of sHFS in absence of a holeboard elicits strongly decremental synaptic depression at mf synapses. Exploration of an empty holeboard during submaximal high frequency stimulation (HFS) facilitates LTP that lasts for over 24 h at the mf-CA3 synapse. Changes in timescale are indicated by line breaks. (b) Analog traces recorded during baseline (left) and during the holeboard experiment (right), 5-min pre-sHFS (i), 5 min after sHFS (ii), and 24 h after sHFS (iii). Vertical scale bar: 2 mV and horizontal scale bar: 6 ms.

alone (ANOVA,  $F_{1,43} = 12.61$ ;  $P < 0.001$ ;  $n = 11$  in both groups) (Fig. 7A).

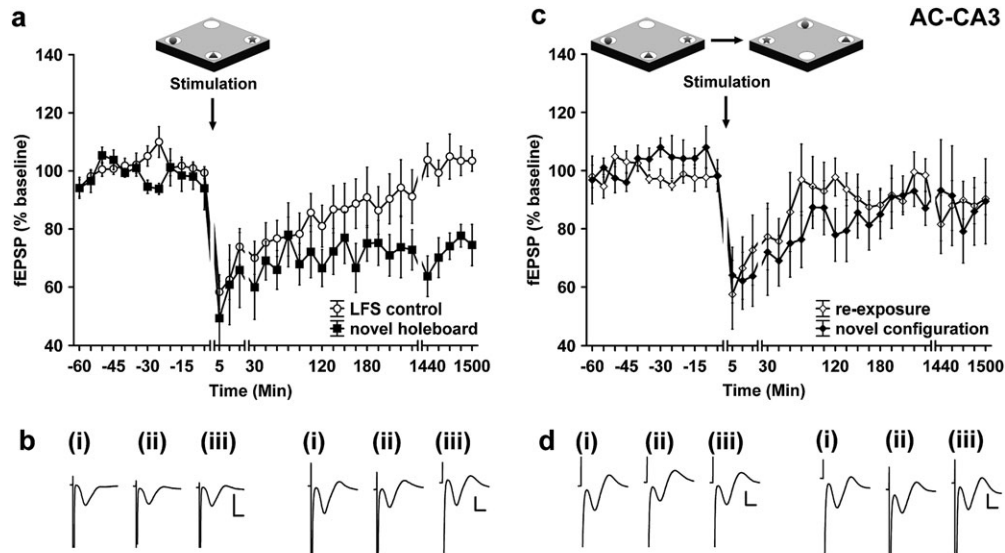
Reexposure to the same objects in the same configuration did not alter the responses compared with the sLFS control (Fig. 8A, ANOVA,  $F_{1,37} = 1.47$ ;  $P < 0.23$ ;  $n = 10$  in both groups).

#### Exploration of an Empty Holeboard Facilitates LTP in AC-CA3 Synapses

Submaximal HFS (sHFS) elicited a strongly decremental form of synaptic potentiation at AC-CA3 synapses (Fig. 9A). When an empty hole board was inserted into the recording chamber a simultaneous application of sHFS significantly facilitated LTP compared with stimulation with sHFS alone (ANOVA,  $F_{1,27} = 15.01$ ;  $P < 0.001$ ;  $n = 7$  in both groups).

#### Discussion

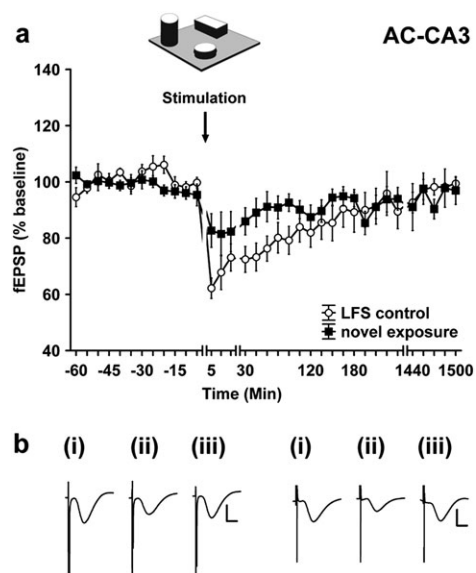
Although the encoding of spatial location through place cells has been extensively reported for the hippocampal CA3 region (Fyhn et al. 2002; Guzowski et al. 2004; Lee et al. 2004; Vazdarjanova and Guzowski 2004; Alvernhe et al. 2008; Kjelstrup et al. 2008), no study has ever addressed the relationship between the occurrence of synaptic plasticity and the encoding of spatial information at CA3 synapses. Here, we report that the coupling of patterned afferent stimulation (that normally leads to weak plasticity), with the exploration of different types of spatial environment leads to the potent strengthening of synaptic plasticity at both mf and commissural-associational (AC) synapses in the CA3 region. LTP is facilitated in both synapses by the exploration of novel space. Particularly striking however, is that the spatial conditions for enabling the facilitation of LTD are quite different depending on the synapse concerned. In line with suggestions based on neuroanatomical differences (McNaughton and Morris 1987; Amaral and Witter 1989) and their basic properties in terms of



**Figure 6.** LTD of commissural-associational-CA3 synapses is facilitated by exploration of small, spatial features. (a) STD is induced by 600 pulses at 1 Hz (sLFS), given 30 min after injection of vehicle ( $n = 8$ ). Exploration of a holeboard with objects during sLFS significantly facilitates LTD at the commissural-associational-CA3 synapse ( $n = 7$ ). Changes in timescale are indicated by line breaks. (b) Analogs showing evoked responses in an experiment where sLFS alone was applied (top row) and in an experiment where sLFS was applied during exploration of the holeboard (bottom row). The analog traces were recorded (i) 5-min pre-sLFS, (ii) 5-min post-sLFS, and (iii) 24 h after sLFS. Vertical scale bar: 1 mV and horizontal scale bar: 6 ms. (c) Reexposure of the holeboard with the same objects in the same constellation elicits synaptic depression that is not significantly different compared with synaptic depression evoked when the constellation of cues where changed in a subsequent experiment ( $n = 8$  in both groups). Line breaks indicate changes in timescale. (d) Analog traces recorded during a reexposure experiment (left) and during an experiment where the objects were reconfigured (right). Analogs were recorded (i) 5-min pre-sLFS, (ii) 5-min post-sLFS, and (iii) 24-h post-sLFS. Vertical scale bar: 1 mV and horizontal scale bar: 6 ms.

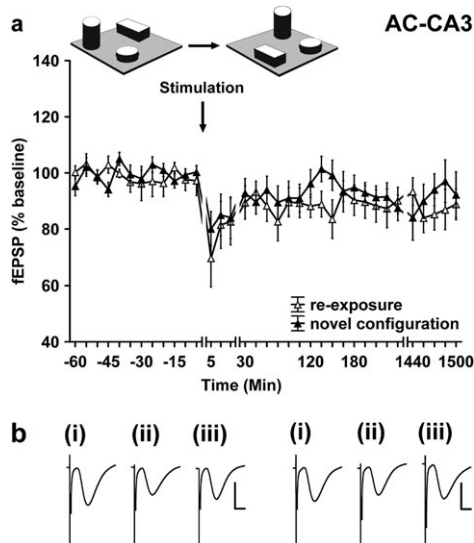
the induction of LTP (Harris and Cotman 1986; Jaffe and Johnston 1990; Zalutsky and Nicoll 1990), our findings support that mf synapses and AC synapses may subserve different roles in terms of the kind of information they process within the CA3 region.

Interpreted in light of findings with regard to the influence of spatial learning on LTP and LTD responses in other hippocampal subregions, a fascinating picture emerges. At every synapse studied to date (SC-CA1, perforant path-dentate gyrus, mf-CA3, AC-CA3), LTP is facilitated, when a new environment is explored (Manahan-Vaughan and Braunewell 1999; Kemp and Manahan-Vaughan 2004, 2008). This appears to be a universal property of hippocampal synapses and suggests that LTP may comprise a fundamental encoding response to changes in the environment. This may reflect the creation of a Hebbian network that is subsequently modified as additional and more qualitative information enters the network. This qualitative component may be conferred by LTD (Fig. 10). At perforant path-dentate gyrus synapses (Kemp and Manahan-Vaughan 2008) and at mf-CA3 synapses, polarization of an environment through the introduction of one or more large landmark components facilitates the induction of LTD. At SC-CA1 (Lemon and Manahan-Vaughan 2006) and AC-CA3 synapses, on the other hand, LTD is only facilitated by the exploration of small, spatially arranged contextual details of the environment. Thus, one cannot assume that this gradient of response derives merely from quantitative distinctions within the information perceived. Rather, this division of labor suggests a hierarchy of information encoding: LTP may subserve the encoding of any spatial change that occurs almost instantaneously once a threshold level of environmental difference is reached; whether LTD becomes involved will depend on the need to store details of this environment.

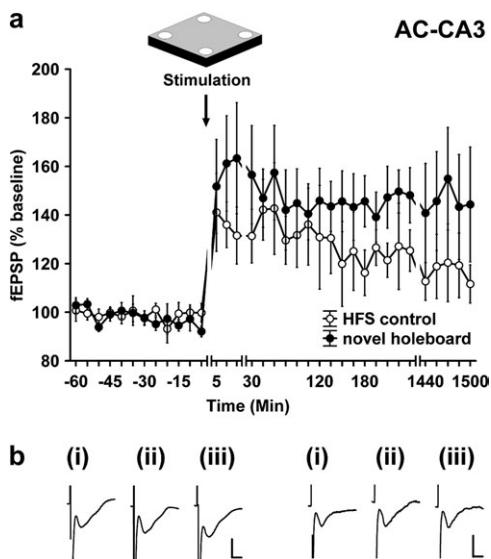


**Figure 7.** Exploration of large novel environmental cues does not facilitate LTD of commissural-associational-CA3 synapses. (a) Stimulation of commissural-associational fibers with 600 pulses at 1 Hz (sLFS) elicits strongly decremental synaptic depression in area CA3 that lasts for about 3 h, whereas exposure to 3 novel landmark cues impairs the induction of synaptic depression. Line breaks indicate change in timescale. (b) Analog traces showing a characteristic evoked field potential recorded in area CA3. Left traces show analogs recorded in an experiment where sLFS alone was applied, right traces depict analogs recorded in the novelty exploration experiment, (i) 5-min pre-stimulation, (ii) 5-min post-sLFS, and (iii) 24-h post-sLFS. Vertical scale bar: 2 mV and horizontal scale bar: 6 ms.

Landmarks that enable orientation in the environment may be enabled by LTD in the dentate gyrus and mf-CA3 synapse, whereas information about finer details of the environment

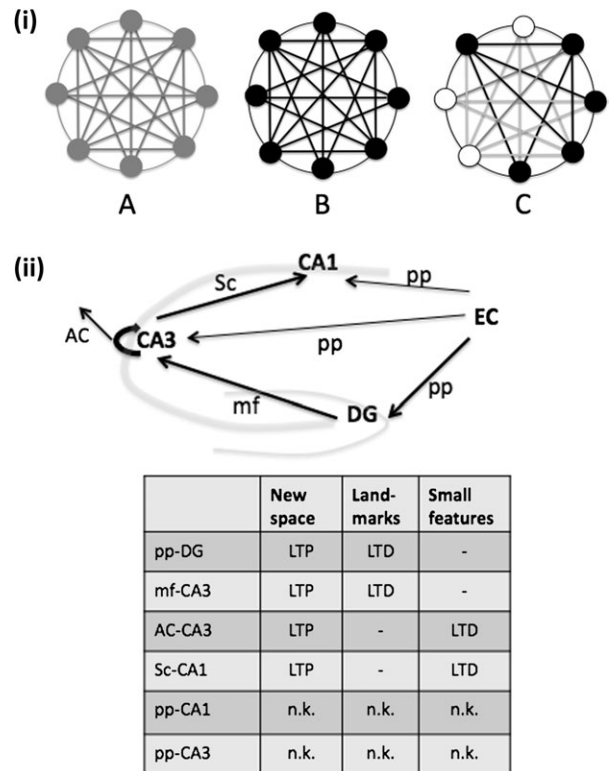


**Figure 8.** Changes in spatial constellation do not elicit LTD of commissural-associational-CA3 synapses. (a) Exploration of a familiar set and configuration of cues (re-exposure) does not facilitate LTD in commissural-associational-CA3 synapses. Changing the spatial constellation of the cues results in no significant difference in the profile of synaptic depression. Line breaks indicate changes in timescale. (b) Analog traces recorded when the animals were reexposed to the familiar objects (left) and during novel configuration of the objects (right), (i) 5-min pre-submaximal low frequency stimulation (sLFS), (ii) 5-min post-sLFS, and (iii) 24-h post-sLFS. Vertical scale bar: 2 mV and horizontal scale bar: 6 ms.



**Figure 9.** LTP of commissural-associational-CA3 synapses is facilitated by exploration of a novel empty holeboard. (a) Application of a sHFS (2 trains of 100 pulses at 100 Hz) induces decremental synaptic potentiation in commissural-associational-CA3 synapses, whereas exploration of a novel empty holeboard during application of sHFS results in enhanced LTP compared with application of sHFS alone. Changes in timescale are indicated by line breaks. (b) The left traces show field potentials obtained during an sHFS experiment and right traces are from an experiment where the animal was exposed to a novel empty holeboard, (i) 5-min pre-submaximal high frequency stimulation (sHFS), (ii) 5-min post-sHFS, and (iii) 24-h post-sHFS.

may be enabled by LTD at AC-CA3 and SC-CA1 synapses. The idea that a hierarchy occurs with regard to hippocampal information processing is in line with the parallel map theory of hippocampal function proposed by Jacobs and Schenk (2003).



**Figure 10.** Information storage through the expression of LTP and LTD. (i) In a naïve neuronal population (A), changes in synaptic weight have not yet occurred. Exposure to a novel environment (B) results in the expression of LTP (black circles) at multiple synapses, creating a stable Hebbian network, where synaptic transmission is strengthened. Acquisition of qualitative information about, for example, spatial orientation within, and spatial content of the environment (C), is enabled by means of LTD (white circles). Through the selective strengthening or weakening of synaptic transmission, a distinct network pattern is created that enables detailed and qualitative encoding of the environment. (ii) Schematic representation of the hippocampal subregions and its afferents. Information enters the hippocampus via the perforant path from the entorhinal cortex (EC). The perforant path (pp) projects strongly to the dentate gyrus but also to the CA1 and CA3 regions. The dentate gyrus transfers information to the CA3 region via the mf afferents. The CA3 region sends SC to the CA1 region and also possesses afferents that project to the both contralateral hippocampus and project back onto the CA3 pyramidal cells in the form of commissural-associational (AC)-CA3 synapses. Exposure to a novel environment facilitates LTP at the pp-dentate gyrus, mf-CA3, commissural-associational-CA3, and SC-CA1 synapses (current study, and see Kemp and Manahan-Vaughan 2004, 2008). As orientational (landmark) information is acquired, LTD at pp-DG and mf-CA3 synapses occurs, and while this is occurring synaptic depression at AC-CA3 synapses, and LTD at SC-CA1 synapses is not permitted (current study, and see Kemp and Manahan-Vaughan 2008). Acquisition of further details of the environment in the form of finer aspects of spatial context and/or spatial content, facilitates LTD at commissural-associational-CA3 and SC-CA1 synapses, and not at pp-dentate gyrus or mf-CA3 synapses (current study, and see Manahan-Vaughan and Braunewell 1999; Kemp and Manahan-Vaughan 2008). Through this hierarchy of information encoding, a finely tuned representation of the spatial environment arises (n.k.: not known).

The CA3 region may play a particular role in the consolidation of newly acquired spatial information. It is the sole origin of sharp waves (Buzsáki 1986), a distinct pattern of neuronal firing that occurs during slow wave sleep, and is believed to comprise a replay of CA1 firing sequences that occur during the awake state (Skaggs and McNaughton 1996). For example, Lee and Wilson (2002) showed that place cells recorded in rats that run repeatedly through their spatial receptive fields, showed a precise firing sequence during slow wave sleep after the experience but not before.

A striking feature of LTD at AC-CA3 synapses, is that facilitation of LTD only occurs when the new spatial constellation of novel objects is first perceived. In contrast to SC-CA1 synapses, spatial reconfigurations of known objects do not facilitate LTD. This phenomenon may reflect a specific function for AC synapses in one-trial short-term memory (Nakazawa et al. 2003) but is in contrast to theories that suggest that AC synapses are involved in pattern completion (Lee et al. 2004). This latter postulate is based on experimental evidence, showing that CA3 neurons generate consistent activity patterns following small changes in the environment (Lee et al. 2004). However, the authors did not explore how AC synapses respond when the animal is reexposed to the same environment. Our data may also suggest that AC-CA3 synapses have a much higher threshold for the facilitation of LTD-requiring not only novel object-place constellations but also novel objects themselves. It has been suggested that the recurrent collaterals may be involved in short-term or working memory (Kesner and Warthen 2009) and thus, may be more involved in processing information about novelty. Our data are in line with this postulate, as facilitation of LTD only occurred after the first presentation of a new set of spatial cues.

It has been proposed that the mf-synapse processes information about the animal's position in an environment, initiated by firing of dentate gyrus granule cells that activate downstream CA3 pyramidal cells (Henze et al. 2002; Bischofberger et al. 2006; Blaabjerg and Zimmer 2007). Along these lines, a role for mf synapses in pattern separation, in pattern completion, and in forming heteroassociative connections between discrete memory patterns has been proposed (McNaughton and Morris 1987; Treves and Rolls 1992; O'Reilly and McClelland 1994; Blum and Abbott 1996; Lee et al. 2004; Lisman et al. 2005). Our data may reflect a cellular mechanism for this, in the form of LTD.

### Concluding Comments

Our data suggest a unique and distinct function for CA3 synapses in spatial information processing that support a role for mf synapses in pattern completion and AC synapses in working memory and processing of information about novelty. Furthermore, they suggest that the CA3 region serves as an integrator of different kinds of spatial information that in turn contribute to the generation of spatial representations of the environment.

### Funding

This work was supported by a grant from the German research Foundation (Deutsche Forschungsgemeinschaft, Ma1843) to Denise Manahan-Vaughan.

### Notes

We gratefully acknowledge the assistance of Jens Klausnitzer and Beate Krenzke. *Conflict of Interest:* None declared.

### References

Acsády L, Kamondi A, Sik A, Freund T, Buzsáki G. 1998. GABAergic cells are the major postsynaptic targets of mossy fibers in the rat hippocampus. *J Neurosci.* 18:3386-3403.

Alvernhe A, Van Cauter T, Save E, Poucet B. 2008. Different CA1 and CA3 representations of novel routes in a shortcut situation. *J Neurosci.* 28:7324-7333.

Amaral DG, Witter MP. 1989. The three-dimensional organization of the hippocampal formation: a review of anatomical data. *Neuroscience.* 31:571-591.

Bischofberger J, Engel D, Frotscher M, Jonas P. 2006. Timing and efficacy of transmitter release at mossy fiber synapses in the hippocampal network. *Pflugers Arch.* 453:361-372.

Blaabjerg M, Zimmer J. 2007. The dentate mossy fibers: structural organization, development and plasticity. *Prog Brain Res.* 163: 85-107.

Blum KI, Abbott LF. 1996. A model of spatial map formation in the hippocampus of the rat. *Neural Comput.* 8:85-93.

Bortolotto Z, Lauri S, Isaac J, Collingridge G. 2003. Kainate receptors and the induction of mossy fibre long-term potentiation. *Philos Trans R Soc Lond B Biol Sci.* 358:657-666.

Braunewell KH, Manahan-Vaughan D. 2001. Long-term depression: a cellular basis for learning? *Rev Neurosci.* 12:121-140.

Buzsáki G. 1986. Hippocampal sharp waves: their origin and significance. *Brain Res.* 398:242-252.

Contractor A, Swanson G, Heinemann SF. 2001. Kainate receptors are involved in short- and long-term plasticity at mossy fiber synapses in the hippocampus. *Neuron.* 29:209-216.

Davis CD, Jones FL, Derrick BE. 2004. Novel environments enhance the induction and maintenance of long-term potentiation in the dentate gyrus. *J Neurosci.* 24:6497-6506.

Derrick BE, Martinez JL. 1994. Opioid receptor activation is one factor underlying the frequency dependence of mossy fiber LTP induction. *J Neurosci.* 14:4359-4367.

Fyhn M, Molden S, Hollup S, Moser MB, Moser E. 2002. Hippocampal neurons responding to first-time dislocation of a target object. *Neuron.* 35:555-566.

Guzowski JF, Knierim JJ, Moser EI. 2004. Ensemble dynamics of hippocampal regions CA3 and CA1. *Neuron.* 44:581-584.

Hagena H, Manahan-Vaughan D. 2010. Frequency facilitation at mossy fiber-CA3 synapses of freely behaving rats contributes to the induction of persistent LTD via an adenosine A1 receptor-regulated mechanism. *Cereb Cortex.* 20:1121-1130.

Harris EW, Cotman CW. 1986. Long-term potentiation of guinea pig mossy fiber responses is not blocked by N-methyl D-aspartate antagonists. *Neurosci Lett.* 70:132-137.

Henze D, Witter L, Buzsáki G. 2002. Single granule cells reliably discharge targets in the hippocampal CA3 network in vivo. *Nat Neurosci.* 5:790-795.

Jacobs LF, Schenk F. 2003. Unpacking the cognitive map: the parallel map theory of hippocampal function. *Psychol Rev.* 110:285-315.

Jaffe D, Johnston D. 1990. Induction of long-term potentiation at hippocampal mossy-fiber synapses follows a Hebbian rule. *J Neurophysiol.* 64:948-960.

Kakegawa W, Tsuzuki K, Yoshida Y, Kameyama K, Ozawa S. 2004. Input- and subunit-specific AMPA receptor trafficking underlying long-term potentiation at hippocampal CA3 synapses. *Eur J Neurosci.* 20:101-110.

Kamiya H, Ozawa S, Manabe T. 2002. Kainate receptor-dependent short-term plasticity of presynaptic Ca<sup>2+</sup> influx at the hippocampal mossy fiber synapses. *J Neurosci.* 22:9237-9243.

Kemp A, Manahan-Vaughan D. 2004. Hippocampal long-term depression and long-term potentiation encode different aspects of novelty acquisition. *Proc Natl Acad Sci U S A.* 101:8192-8197.

Kemp A, Manahan-Vaughan D. 2005. The 5-hydroxytryptamine<sub>4</sub> receptor exhibits frequency-dependent properties in synaptic plasticity and behavioural metaplasticity in the hippocampal CA1 region in vivo. *Cereb Cortex.* 15:1037-1043.

Kemp A, Manahan-Vaughan D. 2007. Hippocampal long-term depression: master or minion of declarative memory processes. *Trends Neurosci.* 30:111-118.

Kemp A, Manahan-Vaughan D. 2008. The hippocampal CA1 region and dentate gyrus differentiate between environmental and spatial feature encoding through long-term depression. *Cereb Cortex.* 18:968-977.

- Kesner R, Warthen DK. 2009. Implications of CA3 NMDA and opiate receptors for spatial pattern completion in rats. *Hippocampus*. doi:10.1002/hipo.20676.
- Kjelstrup KB, Solstad T, Brun VH, Hafting T, Leutgeb S, Witter MP, Moser EI, Moser MB. 2008. Finite scale of spatial representation in the hippocampus. *Science*. 321:140-143.
- Klausnitzer J, Manahan-Vaughan D. 2008. Frequency facilitation at mossy fiber-CA3 synapses of freely behaving rats is regulated by adenosine A1 receptors. *J Neurosci*. 28:4836-4840.
- Lee AK, Wilson M. 2002. Memory of sequential experience in the hippocampus during slow wave sleep. *Neuron*. 36:1183-1194.
- Lee I, Rao G, Knierim JJ. 2004. A double dissociation between hippocampal subfields: differential time course of CA3 and CA1 place cells for processing changed environments. *Neuron*. 42:803-815.
- Lee I, Yoganarasimha D, Rao G, Knierim JJ. 2004. Comparison of population coherence of place cells in hippocampal subfields CA1 and CA3. *Nature*. 430:456-459.
- Lemon N, Manahan-Vaughan D. 2006. Dopamine D1/D5 receptors gate the acquisition of novel information through hippocampal long-term potentiation and long-term depression. *J Neurosci*. 26:7723-7729.
- Lisman JE, Talamini LM, Raffone A. 2005. Recall of memory sequences by interaction of the dentate and CA3: a revised model of the phase precession. *Neural Netw*. 18:1191-1201.
- Manahan-Vaughan D, Braunewell KH. 1999. Novelty acquisition is associated with induction of hippocampal long-term depression. *Proc Natl Acad Sci U S A*. 96:8739-8744.
- McNaughton B, Morris R. 1987. Hippocampal synaptic enhancement and information storage within a distributed memory system. *Trends Neurosci*. 10:408-415.
- Morris RG, Moser EI, Riedel G, Martin SJ, Sandin J, Day M, O'Carroll C. 2003. Elements of a neurobiological theory of the hippocampus: the role of activity-dependent synaptic plasticity in memory. *Philos Trans R Soc Lond B Biol Sci*. 358:773-786.
- Nakao K, Ikegaya Y, Yamada MK, Nishiyama N, Matsuki N. 2002. Hippocampal long-term depression as an index of spatial working memory. *Eur J Neurosci*. 16:970-974.
- Nakazawa K, Sun LD, Quirk MC, Rondi-Reig L, Wilson MA, Tonegawa S. 2003. Hippocampal CA3 NMDA receptors are crucial for memory acquisition of one-time experience. *Neuron*. 38:305-315.
- O'Reilly RC, McClelland JL. 1994. Hippocampal conjunctive encoding, storage, and recall: avoiding a trade-off. *Hippocampus*. 4:661-682.
- Paxinos G, Watson C. 1998. *The rat brain in stereotaxic coordinates*. 4th ed. New York: Academic Press.
- Skaggs WE, McNaughton BL. 1996. Replay of neuronal firing sequences in rat hippocampus during sleep following spatial experience. *Science*. 271:1870-1873.
- Straube T, Korz V, Frey JU. 2003. Bidirectional modulation of long-term potentiation by novelty-exploration in rat dentate gyrus. *Neurosci Lett*. 344:5-8.
- Treves A, Rolls ET. 1992. Computational constraints suggest the need for two distinct input systems to the hippocampal CA3 network. *Hippocampus*. 2:189-199.
- Vazdarjanova A, Guzowski JF. 2004. Differences in hippocampal neuronal population responses to modifications of an environmental context: evidence for distinct, yet complementary, functions of CA3 and CA1 ensembles. *J Neurosci*. 24:6489-6496.
- Zalutsky RA, Nicoll RA. 1990. Comparison of two forms of long-term potentiation in single hippocampal neurons. *Science*. 248:1619-1624.