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Mossy fibre synaptic NMDA receptors trigger non-Hebbian long-term potentiation at entorhino-CA3 synapses in the rat

Masako Tsukamoto, Takuya Yasui, Maki K. Yamada, Nobuyoshi Nishiyama, Norio Matsuki and Yuji Ikegaya

Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan

Hippocampal CA3 pyramidal cells receive two independent afferents from the enthorinal cortex, i.e. a direct input via the temporoammonic pathway (TA, perforant path) and an indirect input via the mossy fibres (MF) of dentate granule cells. In spite of past suggestions that the TA is assigned an important role in exciting the pyramidal cells, little is known about their physiological properties. By surgically making an incision through the sulcus hippocampi and a small part of the dentate molecular layer, we succeeded in isolating TA-mediated monosynaptic responses in CA3 stratum lacunosum-moleculare. The TA–CA3 synaptic transmission was completely blocked by a combination of D,L-2-amino-5-phosphonopentanoic acid (AP5) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), NMDA and non-NMDA receptor antagonists, respectively, and displayed paired-pulse facilitation and NMDA receptor-dependent long-term potentiation, which are all typical of glutamatergic synapses. We next addressed the heterosynaptic interaction between TA–CA3 and MF–CA3 synapses. The TA–CA3 transmission was partially attenuated by single-pulse MF pre-stimulation at inter-pulse intervals of up to 70 ms. However, surprisingly, burst stimulation of the MF alone induced long-lasting facilitation of TA–CA3 synaptic efficacy. This non-Hebbian form of synaptic plasticity was efficiently prevented by local application of AP5 into the MF synapse-rich area. Therefore, MF-activated NMDA receptors are responsible for the heterosynaptic modification of TA–CA3 transmission, and thereby, the history of MF activity may be etched into TA–CA3 synaptic strength. Our findings predict a novel form of spatiotemporal information processing in the hippocampus, i.e. a use-dependent intersynaptic memory transfer.

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Corresponding author Y. Ikegaya: Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. Email: ikegaya@tk.airnet.ne.jp

CA3 pyramidal cells form an autoassociative network with tens of thousands of counterparts in both the ipsilateral and contralateral hippocampi via their associational/commissural (AC) axons (Amaral & Witter, 1995). This network serves as a pivotal input device of the hippocampus; two distinct inputs from the enthorinal cortex converge on the apical dendrite of a CA3 pyramidal cell (Amaral & Witter, 1995). One consists of axons arising from layer II/III enthorinal cortical neurons, termed the temporoammonic (TA) pathway. At the distal portion of the apical dendrite in stratum lacunosum-moleculare, each CA3 pyramidal cell synapses directly with about four thousand TA afferents. The enthorinal cells also send their axon collaterals to the dentate gyrus, in which granule cells relay neocortical information to CA3 via their mossy fibre (MF) axons. Thus, the MF is an indirect input pathway from the enthorinal cortex to the hippocampus. A target pyramidal cell makes synaptic contacts with only a few dozen MF axons, most of which are aligned in the proximal segment of the apical dendrite in the stratum lucidum (Henze *et al.* 2000).

Unlike any other excitatory synapses, MF–CA3 synapses exhibit several unique properties, e.g. giant presynaptic terminals, multiple transmitter release sites and prominent paired-pulse facilitation (Henze *et al.* 2000). They display long-term potentiation (LTP) of synaptic efficacy in response to high frequency stimulation, but exceptionally, the induction is independent of NMDA receptor activation (Nicoll & Malenka, 1995). Thus, the functional significance of MF postsynaptic NMDA receptors remains unclear.

The direct TA input transmits significant cortical information to the hippocampus. The denervation of MFs spares selective firing of CA3 pyramidal cells for particular locations in animal's environment (McNaughton *et al.* 1989), suggesting that 'place' representation is rendered principally by TA signal. Indeed, the TA input is capable of driving CA3 pyramidal cells to fire, probably more efficiently than MFs (Urban *et al.* 2001). Nonetheless, less information is available on TA–CA3 synaptic properties, the main reason being the difficulty in isolating TA–CA3

monosynaptic responses. Most of the previous studies on TA–CA3 synapses have utilized anaesthetized animals (Yeckel & Berger, 1990; Wu & Leung 1998; Do *et al.* 2002; Martinez *et al.* 2002), yet these *in vivo* experiments could not exclude a possible contamination with polysynaptic components. Unfortunately, this is the case in entorhino-hippocampal slice preparations (Berzhanskaya *et al.* 1998; Urban & Barrionuevo, 1998; McMahon & Barrionuevo, 2002), in which simple TA stimulation would unexpectedly cause disynaptic activation of CA3 pyramidal cells through recruitment of dentate granule cells via the perforant path. In the present study, therefore, we establish a method of recording TA monosynaptic responses *in vitro* and find that the TA net activity is dynamically regulated by heterosynaptic MF signals. Here we report that NMDA receptors at MF synapses help to inscribe the suprathreshold MF activity into a plastic change in TA–CA3 synaptic efficacy. Thus, the present study has important implication for network operation in the CA3 region.

METHODS

Materials

D,L-2-Amino-5-phosphonopentanoic acid (AP5), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and NMDA were purchased from Sigma (St Louis, MO, USA). [2*S*,2′*R*,3′*R*]-2-(2′3′-dicarboxy-cyclopropyl)glycine (DCG IV) was obtained from Tocris (Cookson, Ballwin, MO, USA). Tetrodotoxin was from Wako Chemicals (Osaka, Japan).

Hippocampal slice preparation

According to the Japanese Pharmacological Society guide for the care and use of laboratory animals (Ikegaya & Matsuki, 2002), postnatal 17- to 27-day-old Wistar/ST rats (SLC, Shizuoka, Japan) were deeply anaesthetized with ether and immediately decapitated as described previously (Ueno *et al.* 2002). Briefly, the brain was quickly removed and immersed in ice-cold artificial cerebrospinal fluid (ACSF), consisting of (mM): 124 NaCl, 25 $NaHCO_3$, 3 KCl, 1.24 KH_2PO_4, 1.4 $MgSO_4$, 2.2 $CaCl_2$, 10 glucose and 7 μM picrotoxin, continuously bubbled with 95 % O_2 and 5 % CO_2. Transverse hippocampal slices (400 μm thick) were prepared using a ZERO-1 vibratome (Dosaka, Osaka, Japan).

Electrophysiological recording

Slices were preincubated in a 95 % O_2–5 % CO_2-saturated ACSF for at least 1 h at 32 °C, placed in an interface recording chamber and perfused with the same ACSF (32 °C). Test stimuli were delivered to TA every 30 s through bipolar tungsten electrodes positioned near the hippocampal fissure, and field excitatory postsynaptic potentials (fEPSPs) were recorded from CA3 stratum lacunosum-moleculare by a glass microelectrode filled with 0.15 M NaCl (resistance ~1 Ω). The stimulus intensity was set to produce fEPSPs with a half-maximal slope, and the baseline was recorded for at least 30 min to ensure the stability of the response. In some experiments, two other stimulating electrodes were positioned in the stratum granulosum and CA3 stratum radiatum for conditioned stimulation of MF and AC, respectively (stimulus intensity 300 μA). Either AP5 or NMDA was applied at a rate of ~300 μl min^{-1} through a local perfusion pipette (280 μm inner diameter) positioned within ~100 μm of the slice surface of the stratum lucidum. In the experiments shown in Fig. 7, slices were attached onto a MED-P515A probe (Alpha MED Sciences, Chuo-ku, Tokyo, Japan) with a wired mesh weighing 140 mg and perfused with ACSF. Two of the planar microelectrodes out of the 64 available were used to stimulate TA and MF (bipolar constant current pulses, 15–50 μA, 100 μs). The fEPSPs evoked at the remaining 62 sites were simultaneously recorded at a 20 kHz sampling with a MED64 multichannel recording system (Alpha MED Sciences; Oka *et al.* 1999; Shimono *et al.* 2000). Synaptic responses were evaluated by measuring changes in the maximal slopes of fEPSPs, but the fEPSP amplitudes were adopted for positive-going (source) responses (Fig. 6) or 'small' negative-going (sink) responses (less than 0.2 mV of peak amplitude) (Figs 2, 3*A* and 4) because it was hard to estimate their slopes precisely. Unless otherwise specified, slices were continuously disinhibited by a low concentration of picrotoxin (7 μM) in order to induce homosynaptic LTP at TA synapses (McMahon & Barrionuevo, 2002). This dose is insufficient for complete blockade of GABAergic inhibition, but neither the probability of LTP induction nor the degree of LTP magnitude was changed in the range 7 to 120 μM picrotoxin; the effect was preliminarily checked at concentrations ranging from 0.5 to 120 μM (data not shown). Because we hoped that our experiments were conducted under physiological conditions, the present study adopted ACSF containing 7 μM picrotoxin.

RESULTS

Isolation of TA monosynaptic responses

In all experiments, a cut was made along the sulcus hippocampi and across the edge of the dentate molecular layer using a small, curved scalpel under stereo-microscopic controls (Fig. 1*A*) in order to prevent contamination with monosynaptic and polysynaptic components via the classical perforant path, i.e. entorhino-dentate connections. In these slices, no apparent responses were any longer elicited in the dentate gyrus by stimulation of the enthorinal cortex (data not shown), indicating complete lesions of the perforant path. Under these conditions, single-pulse stimulation of the TA pathway evoked a characteristic negative field potential (Fig. 1*B*). The input–output relationship was constructed in the range 10–400 μA of stimulus intensity. The maximal slope (Fig. 1*C*), the peak amplitude (Fig. 1*D*) and the width at half height (Fig. 1*E*) of the negative potential increased with increasing stimulus intensity, but the latency from the TA stimulus to the sink peak was always around 5–6 ms and almost unchanged by the intensity (Fig. 1*F*), suggesting that the sink potentials reflect monosynaptic responses.

The synaptic responses were slightly attenuated by bath perfusion with 50 μM AP5, a NMDA receptor antagonist, and completely abolished by additional application of 20 μM CNQX, a non-NMDA receptor antagonist (Fig. 2*A*). Therefore, they proved to be ionotropic glutamate receptor-mediated fEPSPs; the main component was produced by AMPA receptors, but NMDA receptors were also partly involved in the baseline transmission. This is

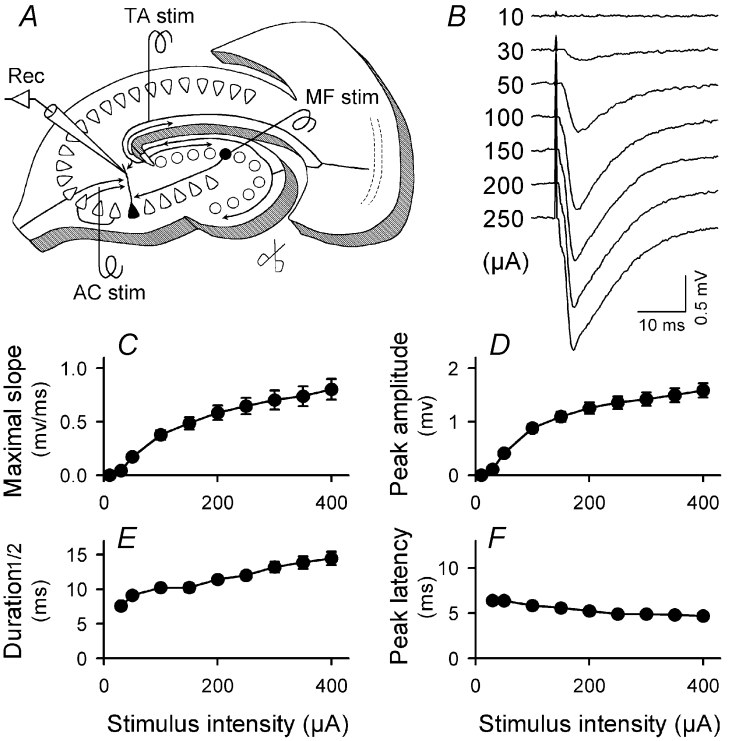

Figure 1. Recording of TA-CA3 fEPSPs

A, recording set up. A recording electrode was positioned in CA3 stratum lacunosum-moleculare of a slice incised along the sulcus hippocampi and across the edge of the dentate molecular layer (scissors). Three stimulating electrodes were placed in the proximate of the hippocampal fissure, the stratum granulosum and CA3 stratum radiatum to stimulate TA, MF and AC, respectively. *B*, representative traces of fEPSPs evoked by applying gradually increased intensity of the TA stimulus, ranging from 10 to 250 μA. *C–F*, input–output relationships of the slope of fEPSPs (*C*), the amplitude of fEPSPs (*D*), the width of fEPSPs at half-height (*E*) and the latency from TA stimulation to reaching the fEPSP peak (*F*). Almost no shift in the peak latency (only a 21.9 ± 18.7 % decrease) was detected by increasing stimulus intensity, confirming that the fEPSPs reflect TA–CA3 monosynaptic responses. All data represent means ± S.E.M. of 24 slices; where no error bars are apparent they have been obscured by the symbol.

consistent with a report of TA–CA1 synapse responses, which also contain relatively large NMDA receptor-mediated components (Otmakhova *et al.* 2002).

Immunohistochemical studies indicated that group II metabotropic glutamate receptors are highly expressed in the terminal field of TA, i.e. the stratum lacunosum-moleculare of the Ammon's horn (Petralia *et al.* 1996; Shigemoto *et al.* 1997). In CA1, bath application of DCG IV (1 μM), an agonist of this type of metabotropic receptors, decreases TA-evoked synaptic responses by about 80 % (Kew *et al.* 2001). We confirmed that in CA3 as well, the same concentration of DCG IV induced a 77.7 % decrease in the fEPSPs (Table 1, Fig. 2*B*). It is possible that an AC-mediated component is disynaptically merged on TA-evoked fEPSPs when the TA pathway is strongly stimulated. Thus, we checked the effect of DCG IV on fEPSPs when the TA was maximally activated by stimulation at 400 μA. However, the suppressive effect of DCG IV did not depend on the intensity of TA stimulation (Fig. 2*B*). This indicates that our stimulus conditions produced few AC contaminations. DCG IV is shown to have no effect on AC–CA3 responses (Table 1; Ueno *et al.* 2002) but does inhibit MF–CA3 responses (Table 1; Kamiya *et al.* 1996). However, TA-evoked fEPSPs displayed only < 130 % of paired-pulse facilitation ratios

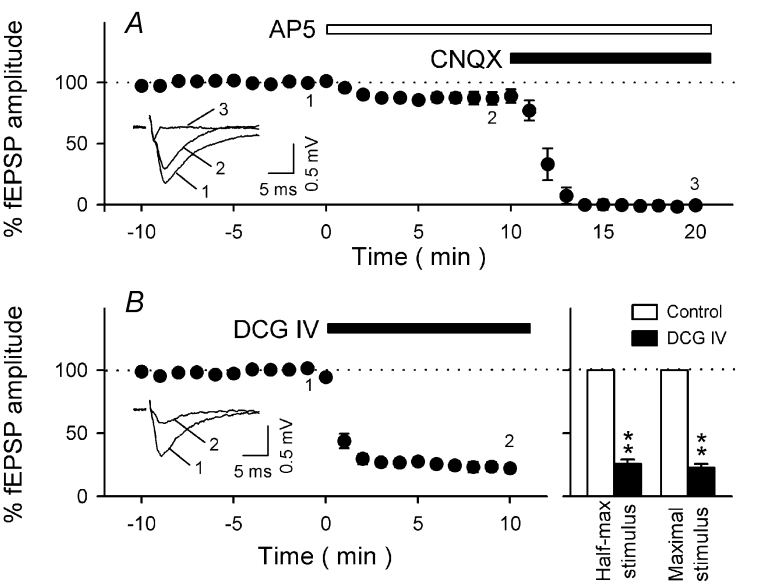

Figure 2. Pharmacological characterization of TA–CA3 fEPSPs

A, time courses of TA–CA3 responses following bath application of 50 μM AP5 alone and then both 50 μM AP5 and 20 μM CNQX. Application of AP5 decreased TA–CA3 fEPSPs by 11.1 ± 5.5 %, and the remaining component was completely abolished by additional perfusion with CNQX. These results indicate that basal neurotransmission at TA–CA3 synapses are mediated not only by AMPA receptors but also partly by NMDA receptors. Representative recordings at times −2, 8 and 20 min (marked 1, 2 and 3 in the figure) are shown in the inset. *B*, Effect of DCG IV on TA–CA3 fEPSPs. Bath perfusion with 1 μM DCG IV attenuated TA responses. Representative recordings at times −2 and 10 min (marked 1 and 2 in the figure) are shown in the inset. The data are summarized in the right panel. The TA was activated at a stimulus intensity that produced either half-maximal or maximal fEPSPs. The suppressive effect of DCG IV did not depend on stimulus intensity. Data represent means ± S.E.M. of 5 slices.

(Table 1, Fig. 3*A*), while MF–CA3 transmission was characterized by a high value for this ratio (Table 1; Claiborne *et al.* 1993). Thus, the fEPSPs were not contaminated with a MF component. Because GABAergic transmission was not completely blocked in our experimental conditions, the remnant inhibitory influence might affect the ratio of paired-pulse facilitation. However, even when slices were completely disinhibited by 100 μM picrotoxin, the paired-pulse facilitation ratio of TA-evoked fEPSPs was 129.5 ± 6.1 % at a 50 ms interval (mean ± S.E.M. of 4 slices), which was almost the same as that at a low concentration of picrotoxin (Table 1). Taken together, we concluded that TA–CA3 monosynaptic fEPSPs were purely isolated by the optimized incision of entorhino-hippocampal slices.

When the TA was tetanized at 100 Hz for 1 s fEPSPs were immediately enhanced, and homosynaptic LTP was induced (Fig. 3*B*). In the presence of 50 μM AP5, the tetanus caused no change in fEPSPs without slight post-tetanic potentiation in the immediate aftermath (Fig. 3*B*). Thus, the LTP is NMDA-receptor dependent.

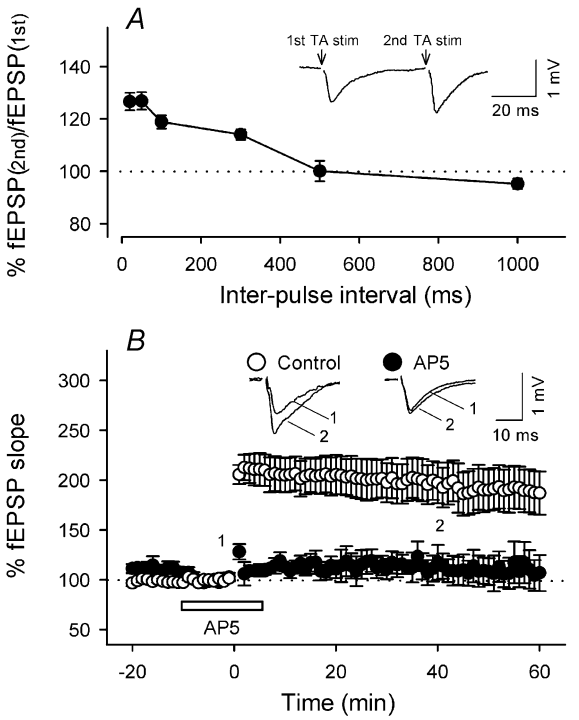

Figure 3. Homosynaptic plasticity of TA–CA3 transmission

A, paired-pulse facilitation of TA–CA3 fEPSPs. The ordinate indicates the ratio of the second fEPSP amplitude to the first one at each inter-pulse interval (n = 3–7). A typical field response evoked by paired-pulse stimuli with a 50 ms interval is shown in the inset. *B*, time course of changes in fEPSP slopes following TA tetanic stimulation (100 Hz for 1 s). The tetanus was delivered to TA in the absence (open circles, n = 7) or presence (closed circles, n = 5) of 50 μM AP5. AP5 was continuously perfused from −10 min to 5 min (open bar). Representative recordings at times 0 and 40 min are shown in the inset. Data represent means ± S.E.M. of n cases.

Non-Hebbian interaction between MF and TA

Theoretical analyses have predicted that a functional interplay between MF and TA inputs is crucial for memory storage and retrieval in the hippocampus (Treves & Rolls, 1992; Lisman, 1999). Experimental evidence has revealed that these two signals are actively summed in the apical dendrite of a CA3 pyramidal cell (Urban & Barrionuevo, 1998). We therefore addressed the possible interaction of TA synaptic efficacy with MF and AC inputs.

As single-pulse simulation was delivered to stratum granulosum, a small 'source' signal emerged in the field potentials recorded from CA3 stratum lacunosum-moleculare (Fig. 4), which displayed a high degree of paired-pulse facilitation and was attenuated by DCG IV (Table 1). Thus, MF synapses were successfully excited under our conditions. When the TA stimulus was immediately preceded by the MF stimulus, the TA-evoked fEPSP was significantly depressed (Fig. 4). The depression occurred when the MF stimulus preceded the TA stimulus by up to 70 ms, suggesting temporal summation of the TA- and MF-elicited EPSPs (Fig. 4). In contrast, prestimulation of AC exerted no apparent effect on TA-evoked fEPSPs. The validity of the AC stimulus was readily confirmed by a larger source response elicited in the field potential, compared with the MF-evoked source signal (Fig. 4). This displayed a low ratio of paired-pulse facilitation and was resistant to DCG IV (Table 1).

After confirming the stability of the baseline responses for at least 30 min, burst stimulation (100 Hz for 1 s) was delivered to the MF in the absence of TA stimulation.

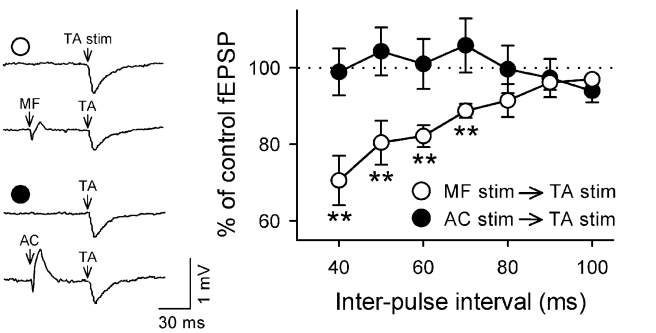

Figure 4. Heterosynaptic modulation of TA–CA3 synaptic transmission by MF inputs

MF (open circles, n = 8) or AC (closed circles, n = 5) was stimulated (each 1 pulse at 300 μA) prior to TA stimulation. The ordinate indicates the ratio of TA-evoked fEPSPs following MF/AC stimulation to control fEPSPs without the prior conditioned stimulus. When the MF stimulus was immediately preceded by TA activation, TA–CA3 transmission was significantly depressed. The depression occurred as a function of the time by which the MF stimulus precedes the TA stimulus. The AC stimulus was virtually ineffective. Representative field potentials with (each lower trace) or without (each upper trace) the conditioned stimulus at an inter-pulse interval of 40 ms are shown in the inset. ** $P < 0.01$ *vs.* no conditioned stimulus. Data represent means ± S.E.M. of n cases.

Thereafter, surprisingly, TA-evoked fEPSPs increased gradually and reached a steady state after ~5 min, and this enhancement was maintained for > 60 min (Fig. 5*A*). Thus, MF activation heterosynaptically induced long-lasting facilitation of TA–CA3 fEPSPs. This heterosynaptic LTP was completely abolished by transverse incision of the stratum oriens, pyramidale and lucidum of CA3c, which was made in order to transect the MF pathway (Fig. 5*C*). Thus, activation of MF synapses causes TA potentiation but it is also possible that MF tetanus elicited disynaptic AC activation via firing of CA3 pyramidal cells, indirectly affecting TA transmission. However, the non-associative form of LTP was not produced by a 100 Hz tetanus of AC (Figs 5*B* and 6*B*), even when the stimulus intensity was increased up to 400 μA ($n = 3$, data not shown). We therefore concluded that heterosynaptic TA modulation is produced specifically by the MF afferents.

Using our experimental system, we also examined the interaction between other CA3 inputs. The tetanization of the TA pathway did not affect AC–CA3 fEPSPs (Fig. 6*A*). Likewise, AC tetanus did not change MF–CA3 fEPSPs (Fig. 6*C*) nor did MF tetanus affect AC–CA3 fEPSPs (Fig. 6*D*). Thus, there was no heterosynaptic interaction between TA → AC, AC → MF or MF → AC. In contrast with Fig. 6*D*, Bradler & Barrionuevo (1990) have indicated that burst stimulation of the MFs induced long-lasting facilitation of AC–CA3 transmission. This phenomenon probably resulted from disynaptic recruitment of AC–CA3 synapses, and we therefore consider that in a broad sense, their finding represents one form of 'homosynaptic' LTP induction. This difference in our and

Table 1. Characterization of TA-, AC- and MF-evoked fEPSPs

Stimulated pathway	% inhibition by DCG IV	% paired-pulse facilitation
TA	77.7 ± 3.3 (5)	126.9 ± 3.3 (7)
AC	−4.7 ± 1.7 (4)	124.2 ± 2.9 (4)
MF	70.9 ± 4.8 (4)	247.9 ± 48.1 (4)

Summary of the effect of 1–2 μM DCG IV and paired-pulse facilitation ratio of TA–CA3, AC–CA3 and MF–CA3 synaptic transmission. All fEPSPs were extracellularly recorded from the stratum lacunosum-moleculare after single-pulse stimulation of the hippocampal fissure (TA) stratum radiatum (AC) and stratum granulosum (MF). The ratios of paired-pulse facilitation were measured at 50 ms interpulse interval. The number of slices tested is indicated in parentheses.

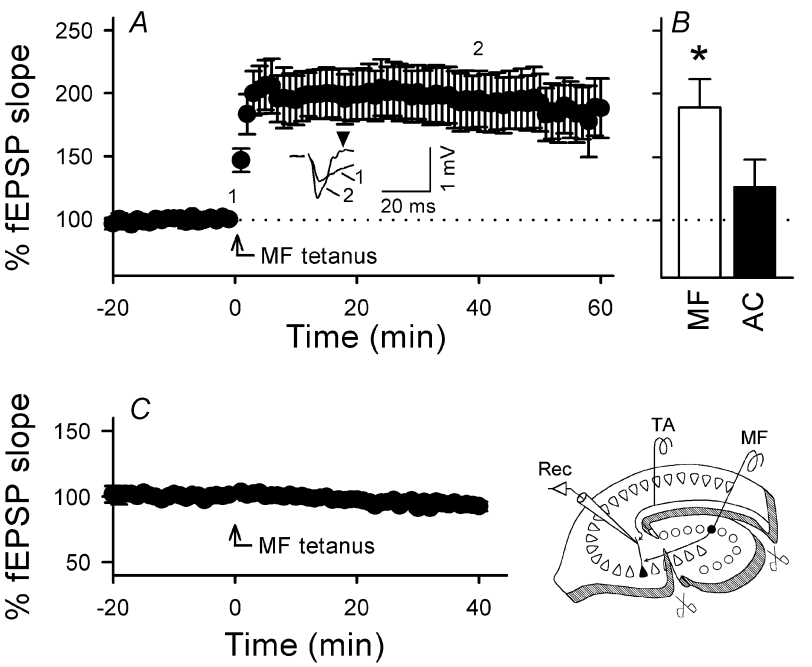

Figure 5. Heterosynaptic induction of TA–CA3 LTP by MF inputs

A, time courses of TA–CA3 responses following MF tetanization (100 Hz for 1 s). The tetanus was applied without TA stimulation. TA-evoked fEPSPs gradually increased up to about 200 % and were maintained for at least 60 min. Representative traces at times 0 and 40 min are shown in the inset. After MF tetanus, a TA stimulus that was previously ineffective to evoke a spike elicited spike-relevant responses (arrowhead) in most cases tested (> 90 %), which suggests that MF activation gates TA inputs. *B*, the average fEPSP slopes from 40 to 60 min after tetanic stimulation of MF (white column, $n = 6$) or AC (black column, $n = 6$). A significant increase in TA-evoked fEPSPs was obtained only for MF tetanization. *C*, in stratum lucidum-transected slices (as shown in the schematic drawing), MF tetanization (100 Hz for 1 s did not cause the induction of heterosynaptic LTP ($n = 4$). Data represent means ± S.E.M. of *n* cases.

others' observations means that under our experimental conditions, the MFs were more purely stimulated, and thus the undesirable contamination of the disynaptic AC component was minimal. Taken together, the non-Hebbian interplay seems likely to be specific between MF → TA.

Because recording with a single glass electrode alone could not precisely estimate the spatial distribution of the areas that were influenced by MF tetanization, we sought to determine the spatial spread of the non-Hebbian LTP by using an 8 × 8 electrode-array multichannel probe (Fig. 7*A*). Instead of the proximity of the hippocampal fissure, the middle part of stratum lacunosum-moleculare was stimulated to activate TA synapses efficiently because these microelectrodes were thin and planar. We confirmed again that MF tetanization induced heterosynaptic LTP in fEPSPs recorded from CA3 stratum lacunosum-moleculare (Fig. 7*B*). No potentiation was observed in fEPSPs in CA1

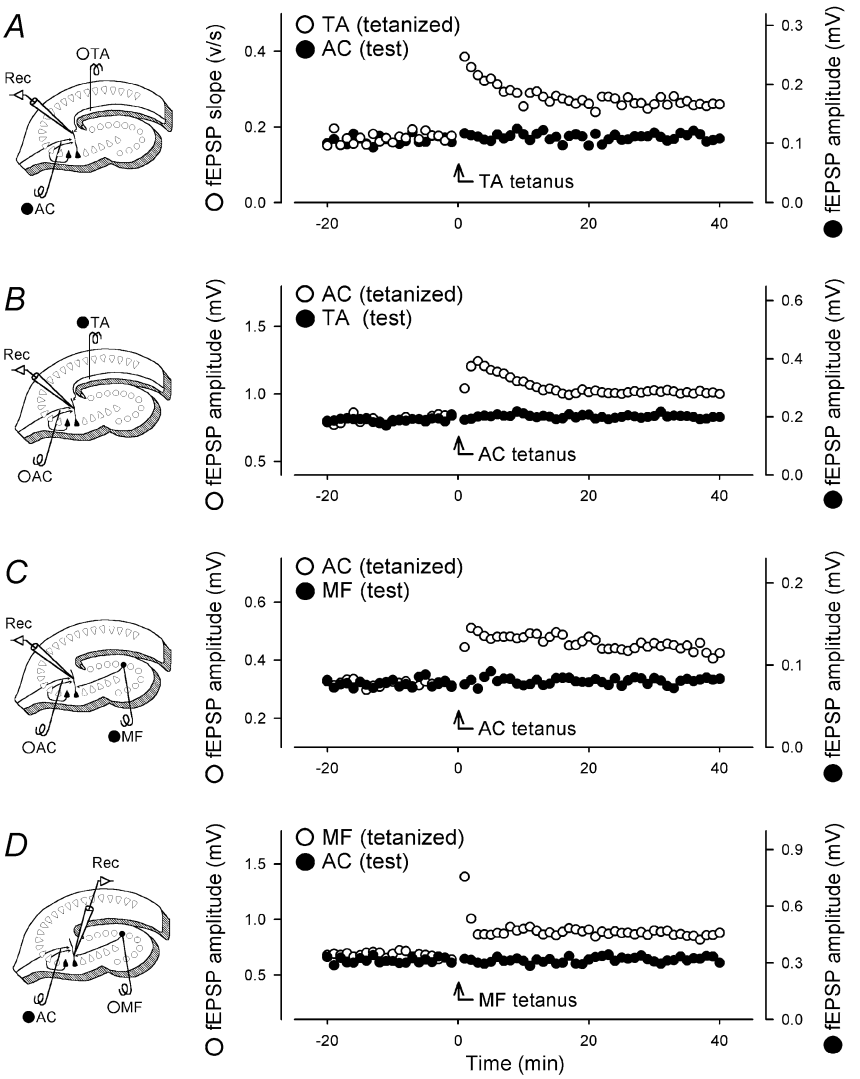

Figure 6. No heterosynaptic interaction between other combinations of three CA3 afferents

Two different afferents were alternatively stimulated at 15 s intervals, and tetanic stimulation (100 Hz for 1 s) was delivered to one pathway (open circles) at time 0. *A*, field responses were recorded from the stratum lacunosum-moleculare. Thus, stimulation of the TA and AC results in a negative-going (sink) field potential and a positive-going (source) field potential, respectively. The afferents to produce sink potentials were set as a tetanized pathway, that is, the tetanized pathway (open circles) was TA, and the control pathway (closed circles) was AC. *B*, field responses were recorded from the stratum radiatum. The tetanized pathway was AC, and the test pathway was TA. *C*, field responses were recorded from the stratum radiatum. The tetanized pathway was AC, and the test pathway was MF. *D*, field responses were recorded from the stratum lucidum. The tetanized pathway was MF, and the test pathway was AC. Although MF-evoked responses did not display marked post-tetanic potentiation, we confirmed that 2 μM DCG IV inhibited these responses. Experiments were repeated with 3–5 slices, producing similar results. Data show one representative case. We did not conduct normalization or averaging because the baseline values, particularly of the test pathway, varied considerably among experiments.

stratum lacunosum-moleculare, i.e. TA–CA1 synaptic responses (Fig. 7*B*), suggesting that MF-induced, heterosynaptic LTP was restricted to TA–CA3 synapses.

To determine whether NMDA receptors at MF synapses are involved in the non-Hebbian LTP, AP5 was locally applied into CA3 stratum lucidum, pyramidale and oriens, which contain the terminal field of the MF. The area that drugs were perfused over is illustrated in Fig. 8. In this experimental system, we recorded MF-evoked source responses with amplitude 0.11 ± 0.04 mV, whereas the responses were 0.13 ± 0.03 mV in amplitude in Fig. 5 (means ± S.E.M. of 5 and 6 slices, respectively, $P > 0.1$). Therefore, almost the same amount of MFs were stimulated in the experiments illustrated in Fig. 8 and Fig. 5. The local application of 2 μM DCG IV attenuated MF-evoked responses by 72.2 ± 11.8 % without apparently affecting TA-evoked fEPSPs (0.0 ± 1.7 %, means ± S.E.M. of 4 slices). In addition, the local application of 50 μM AP5 did not alter the baseline responses nor inhibit TA tetanus-induced homosynaptic LTP (Fig. 8*A*). These data indicate that the local perfusion could spatially separate MF and TA synaptic regions. Under these well-controlled conditions, local AP5 prevented the induction of MF-induced TA LTP (Fig. 8*A*). This form of LTP is, therefore, dependent on activation of heterosynaptic NMDA receptors. To examine whether or not NMDA receptor activation alone elicits the non-Hebbian LTP, the agonist NMDA, dissolved in Mg^{2+}-free ACSF containing 1 μM tetrodotoxin, was locally applied without MF activation. The NMDA perfusion did not affect TA-evoked fEPSPs at as high a concentration as 1 mM (Fig. 8*B*).

DISCUSSION

Accumulating evidence indicates that direct cortical input plays an important role in hippocampal function. Rats with lesions of intrahippocampal CA3–CA1 connections

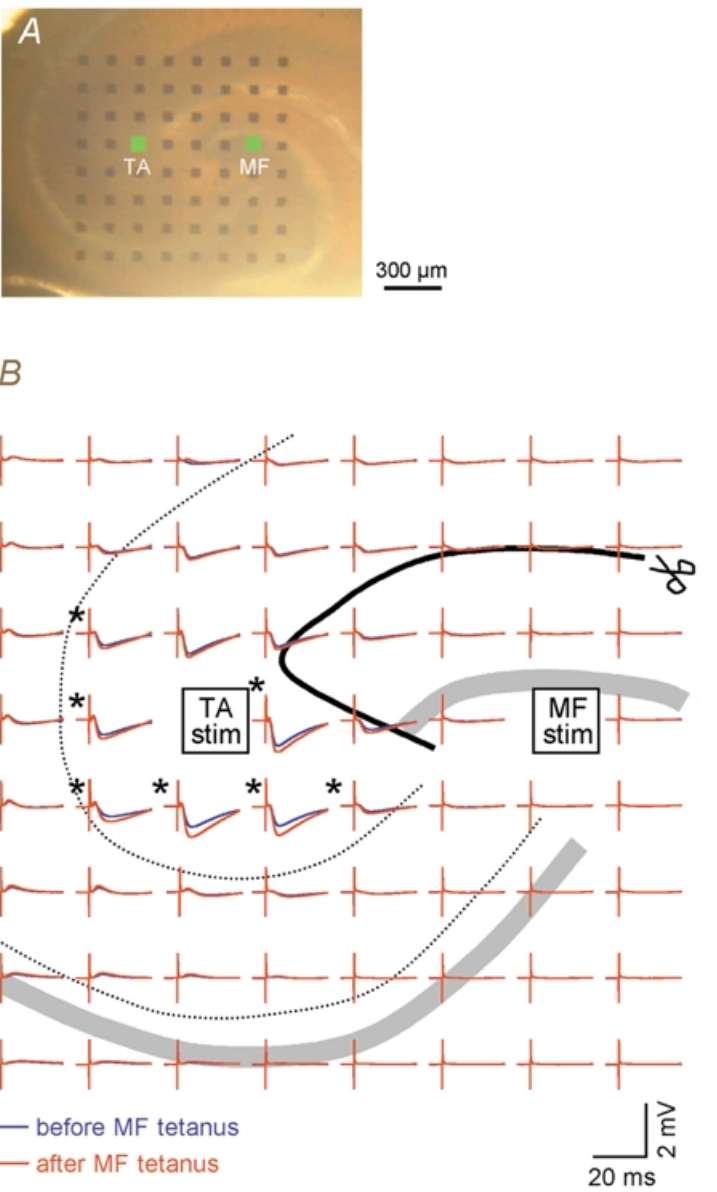

Figure 7. MF activation induces heterosynaptic LTP of TA–CA3, but not TA–CA1, transmission

A, micrograph of a hippocampal slice placed onto a 8 × 8 electrode-array probe, with interelectrode spacing of 150 μm, centred in the apical dendritic field of CA3 pyramidal cells. The electrodes cover the stratum lacunosum-moleculare of CA1 as well as CA3. *B*, samples of TA-evoked fEPSPs immediately before (blue) and 60 min after (red) MF tetanization (100 Hz for 1 s). Each trace represents an average waveform of 10 successive responses recorded by the location-matched electrode in *A*. TA stimulation and MF tetanization were applied through the electrodes indicated by the green squares in *A*. The acceptable level of a defined LTP is a more than 18.7 % increase in fEPSP slopes (asterisks), which corresponds to 2.58 × S.D. of baseline responses ($P < 0.01$). MF tetanization induced heterosynaptic TA–CA3 LTP but did not affect TA–CA1 fEPSPs. Experiments were repeated with a different seven slices, producing the same results. The scissors and thick black line indicate the incision made in this slice.

display normal location-specific activity of CA1 pyramidal cells and spatial recognition task performance (Brun *et al.* 2002), while TA lesions result in severe disruption of spatial memory encoding and retrieval (Lee & Kesner, 2001). Thus, the direct TA system is essential for place recognition as well as place-specific neuronal activity. Previous studies on the TA pathway have focused mainly on synapses onto CA1 neurons (Empson & Heinemann; 1995; Levy *et al.* 1998; Dvorak-Carbone & Schuman; 1999), yet less is known about the properties of TA–CA3 transmission. Electrophysiological studies have recently revealed that TA–CA1 synapses do not only exhibit NMDA-dependent LTP but their activity can also modulate the input efficacy from CA3 and thereby gate information outputs from the hippocampus (Remondes & Schuman, 2002). In CA3, on the other hand, three major afferents, i.e. MF, AC and TA, terminate on the same apical dendrite, forming a more complicated laminar network (Amaral & Witter, 1995). Elucidating the computational properties of the CA3 local circuit is, therefore, critical for understanding of hippocampal information processing. Indeed, theoretical analyses have proposed that a dynamic interplay of these CA3 afferents contributes to the efficacy of memory storage and retrieval (Treves & Rolls, 1992; Lisman, 1999). We have shown for the first time that prior MF activation causes transient depression of TA–CA3 synaptic efficacy, while conversely, burst stimulation of MF elicits long-lasting facilitation of TA–CA3 fEPSPs. No heterosynaptic interaction was detected for AC stimulation. Thus, TA–CA3 transmission is selectively susceptible to MF synaptic activity.

In the first part of the present study, we have established a method for isolating TA-mediated monosynaptic responses, in which slices were surgically incised through the sulcus hippocampi and the edge of dentate molecular layer in order to cleave the classical perforant path. Without this incision, stimulation of the stratum granulosum would not only excite MFs but also antidromically activate the perforant path, which would in turn cause direct activation of TA synapses. Likewise, stimulation of the hippocampal fissure would result in orthodromic and antidromic activation of granule cells. These undesirable contaminations with monosynaptic and polysynaptic components might lead to different observations. McMahon & Barrionuevo (2002) showed that MF tetanization, the conditions of which might directly stimulate a substantial number of TA synapses as well, caused a transient heterosynaptic depression of TA–CA3 fEPSPs, but we found no evidence for such

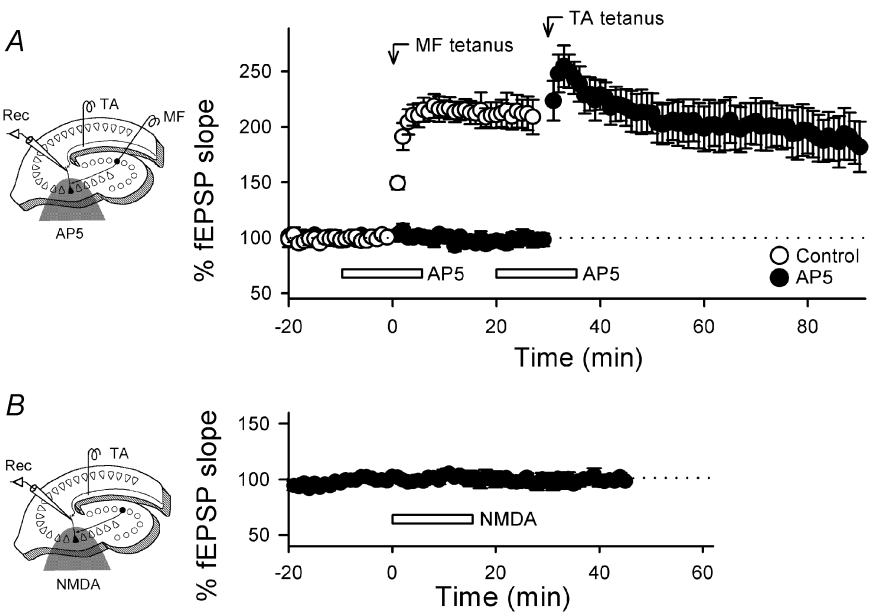

Figure 8. Requirement of activation of NMDA receptors at MF synapses for non-Hebbian TA–CA3 LTP

A, effect of local application of AP5 on MF-induced TA–CA3 LTP. The half-tone meshing in the schematic drawing indicates the drug-perfused area, which includes the stratum lucidum, a MF-terminal zone. Two tetani (each 100 Hz for 1 s) were delivered to MF and then TA at time 0 and 30 min, respectively, in the absence (open circles) or presence (closed circles) of 50 μM AP5 ($n = 6$). AP5 was twice applied through a silicon microtube each for 15 min during the time indicated by the open bars. AP5 inhibited the induction of MF-evoked, heterosynaptic LTP but not of TA-evoked, homosynaptic LTP. No changes in basal responses were produced by AP5, confirming that the local application successfully separated the TA- and MF-synaptic zones. *B*, effect of local application of NMDA on TA-evoked fEPSPs. Mg^{2+}-free solution containing 1 mM NMDA and 1 μM tetrodotoxin was locally applied for 15 min, causing no apparent change in TA synaptic efficacy ($n = 4$). Data represent means ± S.E.M. of *n* cases.

depression. With our method that ensures separate stimulation of TA and MF, we reproducibly found that the tetanization led to heterosynaptic facilitation of TA–CA3 fEPSPs, i.e. non-Hebbian LTP.

Cellular basis for non-Hebbian interaction between MF and TA

How does MF activation induce LTP at distant synapses? MF and TA axons innervate the same apical dendrite of a CA3 pyramidal cell. Because TA–CA1 synapses were unaffected by MF tetanus, the MF appears to interact with TA synapses via its postsynaptic dendrite. Recent evidence indicates that synapses are capable of influencing others via dendritic action potentials, which can bidirectionally propagate across the length of the dendrites (Stuart *et al.* 1997). The dendritic spikes participate in the induction of associative and cooperative synaptic plasticity at TA–CA1 synapses (Magee & Johnston 1997; Golding *et al.* 2002). Strong MF activation probably generates postsynaptic spiking and thereby may modulate TA synaptic efficacy. Recent evidence shows that postsynaptic depolarization alone induces long-term changes in MF–CA3 synaptic strength (Berretta *et al.* 1999, 2000). This non-Hebbian interaction may be mediated by intrasynaptic ephaptic feedback (Berretta *et al.* 2000; Kasyanov *et al.* 2000). It is possible that this mechanism operates at TA–CA3 synapses, although it does not appear to work at AC–CA3 synapses (Berretta *et al.* 2000).

Another possibility can also be raised. The dendrites contain the intracellular Ca^{2+} stores, the surface of which expresses ryanodine and inositol trisphosphate receptors (Berridge, 1998). Both the receptors evoke Ca^{2+} release from the stores in response to a rise of intracellular Ca^{2+} itself, yielding Ca^{2+}-mediated, regenerative spikes across the endoplasmic reticular membrane (Berridge, 1998). The intracellular Ca^{2+} wave propagates as slowly as 40 μm s^{-1} but can reach the distal part of the dendrite (Berridge, 1998), probably regulating heterosynaptic plasticity in the hippocampus (Nishiyama *et al.* 2000). Kapur *et al.* (2001) have demonstrated that burst stimulation of MFs evokes Ca^{2+} release from the internal stores in the thorny excrescences of CA3 pyramidal cells, i.e. MF synaptic spines, and this Ca^{2+} signal spreads into the adjacent dendrite. It is plausible, therefore, that intracellular Ca^{2+} spikes, produced by MF activation, are transduced to TA synapses, resulting in TA LTP. Kullmann *et al.* (1992) reported that at CA1 synapses, a rise in postsynaptic Ca^{2+} alone is sufficient to cause a transient (< 30 min) increase in synaptic efficacy and that presynaptic co-activation converts this transient potentiation into a sustained form. Importantly, the heterosynaptic induction of our TA LTP did not require TA stimulation, but under our conditions, spontaneous TA activity remained intact. Evaluating the contribution of basal TA activity to TA–CA3 synaptic changes is now underway in our laboratory. Further investigation would be necessary to clarify the inter-synaptic dynamics between MF and TA inputs

Although NMDA receptors are present at MF postsynaptic sites (Weisskopf & Nicoll, 1995; Vogt *et al.* 2000; Reid *et al.* 2001), they are not indispensable for the induction of MF–CA3 LTP, which may rather be explained by presynaptic mechanisms alone (Nicoll & Malenka, 1995). During development, NMDA receptors at MF synapses begin to function before AMPA receptors emerge (Ikegaya *et al.* 2002). Their activity may, therefore, be required for subsequent acquisition of AMPA receptors. Even in the adult hippocampus, however, the MF synaptic NMDA receptors are kept active (Weisskopf & Nicoll, 1995; Vogt *et al.* 2000; Reid *et al.* 2001). Therefore, their functional significance has remained unclear. The present study demonstrated that MF-induced TA LTP was inhibited by pharmacological blockade of NMDA receptors in the MF synapse-rich area. MF activation might also disynaptically recruit AC axons to some extent. Indeed, Bradler & Barrionuevo (1990) reported that MF tetanus leads to heterosynaptic LTP induction at AC–CA3 synapses (but see Fig. 6*D*). However, AC tetanus *per se* was proven virtually ineffective in inducing a plastic change in TA fEPSPs. We conclude, therefore, that NMDA receptors at MF synapses are responsible for the heterosynaptic LTP. Exogenous application of NMDA to MF synapses did not induce TA LTP. Thus, activation of MF synaptic NMDA receptors is necessary but not sufficient to trigger TA LTP. These receptors may serve as a threshold detector of MF activity for the non-Hebbian LTP.

Functional significance of non-Hebbian TA LTP in CA3 circuits

Non-Hebbian forms of synaptic plasticity, e.g. the spread of LTP beyond synapses, have recently been implicated as an integral part of global changes in neural networks (Bi & Poo, 2001). Our findings of a use-dependent LTP propagation through postsynaptic dendrites, therefore, provide novel insights into information processing by the CA3 circuit. Cortical information is doubly conveyed into CA3 via MF and TA, but the MF signal is previously processed in the dentate network. Our present study suggests that when a CA3 pyramidal cell receives a strengthened MF signal via the prior dentate processing, the TA input into the same neuron is also reinforced, thereby acquiring a more potent ability to excite the neuron. Thus, the dentate local circuits may gate direct cortical input into the hippocampus. In this case, all TA synapses may be equally affected since their presynaptic activity is apparently not required. This places strong limitation on the kind of information storage that is possible by such a mechanism. However, the lifespan of MFs is perhaps extremely short (Gould *et al.* 2001) and thus, the concomitant loss of information encoded in their

synapses must be transferred to more stable storages. MF NMDA receptor-triggered, non-Hebbian TA LTP may represent such a heterosynaptic memory transfer system. The strength of TA–CA3 synapses may epitomize the history of MF activity.

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