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Dentate Gyrus Granule Cell Firing Patterns Can Induce Mossy Fiber Long-Term Potentiation In Vitro

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Abstract

Hippocampal granule cells transmit information about behaviourally-relevant stimuli to CA3 pyramidal cells via mossy fiber synapses. These synapses express a form of long-term potentiation (mLTP) that is non-Hebbian and does not require NMDA receptors. mLTP is thought to be induced and expressed presynaptically, hence, the major determinant of whether mLTP occurs is activity in the granule cells. However, it remains unclear whether mLTP can be induced by activity patterns that granule cells exhibit *in vivo*, and — if so — what context generates these patterns. To address these issues, we examined granule cell activity from *in vivo* recordings from rats during performance of a delayed nonmatch-to-sample (DNMS) task and found that granule cells exhibit a wide range of spike patterns. *In vitro* slice experiments in mice demonstrated that some, but not all, of these patterns of activity could induce mLTP. By further defining the activity thresholds for mLTP in hippocampal slices, we found that mLTP can only be induced by spike patterns that fire in high frequency bursts with a low average firing frequency. Using this information, we then screened for supra-threshold bursts of activity during the DNMS task. In a subset of cells, supra-threshold bursts occurred preferentially during the sampling phase of the task. If supra-threshold bursting took place later, during the delay phase, task performance was disrupted. We conclude that mLTP can be induced by granule cell spike patterns during a memory task, and that the timing of mLTP induction can predict task performance.

Keywords

Hippocampus; Synaptic plasticity; Delayed non-match to sample task; LTP; Electrophysiology

Introduction

The function of mossy fiber synapses in the hippocampus has remained a mystery despite considerable effort to elucidate their role. Many theories have been postulated based on their perceived role as “detonator” synapses, in which a brief burst of activity in a presynaptic granule cell reliably activates postsynaptic CA3 pyramidal cells (Bischofberger et al., 2006; Henze et al., 2000; Henze et al., 2002; McBain, 2008; Rolls, 2007). This detonator model of a mossy fiber synapse is based on the large size of mossy fiber boutons, which are in close proximity to CA3 cell somata (Acsady et al., 1998; Rolls, 2007), and the massive facilitation of glutamate release from mossy fiber synapses recruited by modest (>0.5 Hz) frequencies

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of granule cell activity (Klausnitzer and Manahan-Vaughan, 2008; Salin et al., 1996). Frequency facilitation of mossy fiber synapses is thought to be a critical component of detonation, as individual spikes in a presynaptic granule cell are insufficient to reliably evoke spiking in postsynaptic CA3 cells, but bursts of spikes in a granule cell reliably cause postsynaptic spikes (Henze et al., 2002). This not only enables reliable information transfer but also contributes to Hebbian LTP at CA3 associational/commissural synapses (Kobayashi and Poo, 2004). Thus, short-term synaptic plasticity is thought to play a central role at mossy fiber synapses.

In contrast, a role for mFLTP remains elusive (Huang et al., 1995; Wang et al., 2003), even though—like frequency facilitation—it is a non-Hebbian mechanism by which granule cell activity enhances glutamate release (Harris and Cotman, 1986; Zalutsky and Nicoll, 1990). mFLTP is thought to be induced and expressed presynaptically (Maccaferri et al., 1998; Mellor and Nicoll, 2001; Nicoll and Schmitz, 2005; Zalutsky and Nicoll, 1990), hence, the major determinant of whether LTP occurs is granule cell activity. However, it remains unclear whether mFLTP can be induced by activity patterns that granule cells exhibit *in vivo*, and — if so — what context generates these patterns. To address this issue, we first asked if granule cell activity patterns observed *in vivo* during the performance of a delayed nonmatch-to-sample (DNMS) task could induce mFLTP in the *in vitro* hippocampal slice preparation. We then used the slice preparation to define the minimal threshold of presynaptic activity required for the induction of mFLTP, and used this data to identify supra-threshold patterns of activity in recordings made from granule cells during the DNMS task, a hippocampus-dependent olfactory memory task that requires spatial discrimination (Otto and Eichenbaum, 1992). Finally, we examined whether these supra-threshold patterns of activity are correlated with behaviorally relevant cues or performance during the task.

Materials and methods

Granule cell recording

Hippocampal granule cell recordings from the dentate gyrus were previously performed by S. Wiebe and U. Staubli as described (Wiebe and Staubli, 1999; Wiebe and Staubli, 2001). Briefly, adult rats were implanted with multielectrode arrays to the hippocampus. Single unit recording and granule cell identification were confirmed using criteria described in detail in Supplemental Note 1 and Supplementary Figure 1. Granule cells were recorded from a total of 9 rats; the mean firing rates of granule cells did not differ significantly across different animals ($p > 0.05$; ANOVA on ranks). The DNMS task involved navigation through a sound-attenuating Y-shaped chamber surrounded by a grounded copper mesh. A nosepoke port was present at the end of each arm, a photodetector/LED beam was located at the entrance of each arm to record entry to or exit from them. Odorised air in either of two odors (Apple Oliffac or Careno scents from International Flavors and Fragrances, Inc.) was delivered to a given arm chamber using a flow-dilution olfactometer to control concentration and flow rate, and gated by a solenoid valve. Odors were removed by a ceiling fan and vacuum pump.

Behavioral trials were begun with the illumination of a cue light above the sample port, and the first nosepoke into this port led to a pre-odor initial phase that was a minimum of 2 seconds long. A second nosepoke into the sample arm port initiated a sample phase of at least 10 seconds of odor exposure in the sample arm, which was then terminated by a third nosepoke. This also marked the start of a variable 10–50 second delay phase. The test phase was initiated by a nosepoke taking place after the delay, and was signalled by switching the cue light off and delivering the test odors to the test arms. Test odors were delivered continually and could be detected prior to physical entry of the test arm, as the animal did not need to enter both test arms of the maze to solve the task. A nosepoke into the port at the end of a test arm signified the animal's choice in the maze and led to removal of the test

odors. The task was performed correctly if the animal picked the port in the test arm with the novel odor; a positive (0.5 mL of water) or negative (brief burst of light) reinforcer was applied, depending on whether the trial was performed correctly or not. The identity of the novel odor and the test arm to which it was applied were randomised between trials, and the interval between trials was standardised at 20 seconds. Timestamps were denoted during the course of the task for a number of different distinct cue-types, including location (entry to or exit from the arms of the maze), odor presentation (onset, duration, and offset) during the sample phase, and the nose poke in the chosen test arm, signifying completion of the task. Granule cells were identified as “responsive” to a given cue if there was a significant change in activity associated with the timestamp for that cue, as described (Wiebe and Staubli, 1999; Wiebe and Staubli, 2001).

Hippocampal slice recording

Transverse hippocampal slices (400–500 μm thickness) were prepared from p25–p30 male C57/Bl6 mice. Slices were cut in ice cold artificial cerebrospinal fluid (aCSF) containing: (mM) NaCl 87, Sucrose 75, Glucose 25, NaHCO_3 25, KCl 2.5, NaHPO_4 1.25, CaCl_2 0.5, MgCl_2 7 saturated with 95 % O_2 , 5 % CO_2 . Slices were then transferred to recording aCSF containing: (mM) NaCl 119, Glucose 10, NaHCO_3 26, KCl 2.5, NaHPO_4 1.2, CaCl_2 2.5, MgCl_2 1.3 saturated with 95 % O_2 , 5 % CO_2 and heated to 35°C for 30 minutes. Slices were thereafter stored at room temperature. For recording, slices were immersed in recording aCSF containing D-AP5 (50 μM) in a submerged recording chamber at 35°C perfused at 3–4 mL/minute on the stage of an Olympus BX51 microscope. Extracellular field potential recordings were made with borosilicate glass pipettes of resistance 2–6 M Ω filled with aCSF placed in *stratum lucidum*. Whole-cell voltage-clamp recordings at –70 mV were made from visually identified CA3 pyramidal cells using pipettes of resistance 2–6 M Ω ; filled with intracellular solution containing: (in mM) 117 CsMeSO₃, 10 HEPES, 0.2 EGTA, 4 Mg-ATP, 0.3 Na-GTP, 8 NaCl, 5 QX-314 and set to pH 7.4, 280–285 mOsm. For whole-cell recordings 50 μM picrotoxin was added to the aCSF.

A bipolar tungsten stimulating electrode (FHC) was placed in the granule cell layer to stimulate mossy fiber responses with stimulation intensity set at 5–20 V, duration 100 μs (Digitimer, UK). The purity of mossy fiber responses was tested before each experiment by paired pulse facilitation at an interval of 40 ms and frequency facilitation switching from 0.05 Hz to 1 Hz and at the end of each experiment by bath application of DCG-IV (2 μM). Experiments were discarded if DCG-IV produced less than 75 % depression of the fEPSP amplitude, paired pulse facilitation was less than 2 or frequency facilitation was less than 300%. Stimulus frequency was 0.05 Hz unless otherwise stated.

Spikes from *in vivo* granule cell recordings were converted to time series of pulses (sampling rate 10 kHz), which were used to drive a stimulation box to activate presynaptic axons. All data were sampled at 10 kHz, filtered at 5 kHz and recorded onto computer using Signal or Spike acquisition software (CED, UK). Analyses were performed with scripts written in this software environment. Values for changes in synaptic strength are taken at 21 – 30 minutes after the end of the induction period. Data are presented as mean $\pm$ SEM. Statistical tests varied according to the hypothesis being tested; differences between two unpaired groups were established by the Student's t-test (for normally distributed data) or the Kolmogorov-Smirnov test (for non-normal data), and paired tests were used where appropriate. Correlations were evaluated using a linear regression analysis or the Spearman Rank Order test as appropriate. Comparisons between a parameter during a given phase of the task and the parameter expected at random were calculated using ANOVA followed by post-hoc application of Dunnett's test (for normal data), or using ANOVA on Ranks followed by Dunn's test (for non-normal data). D-AP5, picrotoxin and DCG-IV were purchased from Tocris (Bristol, UK).

Modelling

All analysis of spike trains was performed using Neuroexplorer (Plexon, Inc.) and custom-written programs in SigmaPlot; cells were subdivided into different categories based on number and identity of cue-types to which they responded, as defined previously (Wiebe and Staubli, 1999; Wiebe and Staubli, 2001). Mean firing frequencies were calculated by dividing the total number of spikes by the total recording length, and ISI distributions were calculated after separating the data into individual trials of the task. Non-random bursting was estimated by calculating the difference between the joint ISI distribution of spike triplets for the original dataset, and for those obtained from the same dataset where the ISIs between spike pairs were shuffled.

The model for LTP/hr was based on the amount of LTP induced during brief, constant frequency trains at 10, 25, and 100 Hz. A 3-D mesh was constructed between spike number during the train, spike frequency during the train, and LTP. This mesh was then subjected to a low-pass filter, as suggested by recordings using the same LTP induction train during varying background rates of stimulation. Regions on both the mesh and the filter where no significant LTP was observed experimentally were set to an LTP value of 0. This model was then applied to the population of recordings from granule cells, in three phases: first, the mesh was applied to a 2-second window following each spike during each trial, to determine if that spike was part of a burst of activity that would be expected to induce LTP. Second, we identified which spike within that time-window represented the start of the burst that would generate the largest amount of LTP within that window; this was then used as the start of the burst for subsequent calculations. Finally, we applied the low-pass filter to all values according to the mean firing frequency of the cell, based on interpolation of the data in 3h. In rare cases, we found that a burst would continue for a longer number of spikes than could be interpolated by the mesh; we subdivided these long bursts into distinct smaller bursts for calculation purposes.

Results

In vivo recordings from individual granule cells have been conducted by various different groups (Buzsaki and Czeh, 1992; Jung and McNaughton, 1993; Leutgeb et al., 2007; Wiebe and Staubli, 1999). We analysed the activity patterns of 68 single-unit recordings of granule cells that were recorded from adult rats in a previously published study of hippocampal activity during an olfactory-based DNMS task (See Methods, Supplemental Note 1 and Supplementary Figure 1) (Wiebe and Staubli, 1999). Briefly, the rat was introduced into one of three arms in a Y-maze and exposed to a sample odor. The odor exposure was then followed by a variable delay. Two test odors were then introduced, one in each of the other arms of the maze. One of these test odors was the sample odor, the other was novel. The task was completed successfully if the rat pressed a panel at the end of the maze arm containing the novel odor, and was failed if the rat pressed a panel at the end of the maze arm containing the sample odor. Each trial of the task generally took between 30 and 90 seconds, and recordings covered 20 – 794 repeated trials of the task (See Methods). This task is dependent on the hippocampus rather than perirhinal cortex since only 2 distinct test odors were used (Brown and Aggleton, 2001).

Granule cells exhibit a gradient of firing patterns

Early studies of granule cells reported that these cells fire exclusively at very low frequencies (<0.2 Hz) (Jung and McNaughton, 1993), but more recent studies have reported that these cells can sustain firing over a wider range of activity, averaging around 1 Hz (Leutgeb et al., 2007). We found a wide spectrum of mean firing frequencies in the granule cells recorded during this task, comparable to the results of Leutgeb et al. (mean firing

frequency = 0.83 ± 0.1 Hz, $n = 68$; individual cells ranged from 0.04 Hz to 2.9 Hz). We observed that the pattern of firing gradually changed over this range, from a bimodal distribution of inter-spike intervals (ISIs) with a high proportion of short ISIs ($ISI \leq 30$ msec) in granule cells with lower mean firing frequencies, to a unimodal ISI distribution with a smaller proportion of the shortest ISIs in granule cells with higher mean firing frequencies (Figure 1a–b). Neither the mean firing frequency (Figure 1c) nor the ISI distribution as a function of mean firing frequency (Figure 1d) showed any sharp transition across the population of cells that would suggest that these different activity patterns were generated by fundamentally distinct subpopulations of neurons. An analysis of spike triplets during the task revealed that many cells had non-random bursting behavior that could not be accounted for by the ISI distribution of the cell (Supplementary Figure 2). This non-random bursting was more prevalent in cells that fired at low mean frequencies than in those that fired at high mean frequencies (Supplementary Figure 2e; $r = -0.33$, $p < 0.01$ by linear regression; $n = 68$), suggesting that cells with lower mean firing frequencies were more likely to contain high frequency bursts of spikes required for the induction of LTP.

Granule cell firing pattern correlates with the number, but not type, of responsive cues

We next examined if there was a correlation between the mean firing frequency (and therefore firing pattern) of a cell and the behavioral cues that activated the cell during the DNMS task. Previous studies have reported that granule cells can change activity in response to any of several types of cues during a task, including both spatial and non-spatial information (Buzsaki and Czeh, 1992; Jung and McNaughton, 1993; Leutgeb et al., 2007; Wiebe and Staubli, 1999; Wiebe and Staubli, 2001). Moreover, some granule cells respond specifically to only one type of cue, while others respond to multiple cue types, and others do not show an obvious response to any of the cues monitored during the task (Supplemental Note 2). There was a positive correlation between the number of monitored cues that a granule cell responded to and its mean firing frequency (Figure 2a; $r = 0.45$, $p < 0.001$ by Spearman Rank Order test; $n = 68$), so that non-responsive cells and cue-specific cells fired at lower mean frequencies than cells that responded to multiple cue types. There was also a significant inverse correlation between non-random bursting and the number of monitored cues to which a cell responded, as expected given that each of these parameters is correlated with mean firing frequency ($r = -0.23$, $p < 0.05$ by Spearman Rank Order test; $n = 68$).

We also examined if specific cue types were correlated with firing frequency by defining two mutually exclusive sets of cells; those that responded during the sampling phase of the DNMS task but not during transition from one arm of the maze to another (termed sample responsive cells), and those that responded to transit but not during the sample phase (termed transit responsive cells). The mean firing frequencies of sample responsive cells (0.97 ± 0.21 Hz; $n = 15$) and transit responsive cells (0.79 ± 0.14 Hz; $n = 16$) were not resolvably different (Figure 2b; $P > 0.4$ by Kolmogorov-Smirnov test). Thus, the mean firing frequencies are similar for granule cells responding to different cue types, while granule cells that respond to multiple different types of behavioral information have higher mean firing frequencies compared to those responding specifically to one cue type, or cells that did not respond to any of the cues monitored during the task. The higher firing frequency of granule cells that respond to multiple cues is also associated with more regular firing patterns and a reduced likelihood of non-random bursts. .

Granule cell firing patterns can induce mfLTP in hippocampal slices

LTP induction at the mossy fiber synapse has classically been shown to require a burst of presynaptic spikes (Harris and Cotman, 1986; Maccaferri et al., 1998; Mellor and Nicoll, 2001; Zalutsky and Nicoll, 1990) (Supplemental Note 3). Granule cells that fire in bursts also tend to fire at low basal frequencies and respond to few cues, leading us to the

hypothesis that patterns of activity from these cells would be more likely to induce mfLTP. To test this hypothesis, we replayed patterns of granule cell activity into mossy fiber axons in hippocampal slices from mice via an extracellular stimulating electrode to see if they would elicit mfLTP. We recorded mossy fiber synaptic responses with extracellular field potential recordings (see methods). Whilst *in vitro* recording is not an exact replication of the *in vivo* situation, this method has been used extensively to study mfLTP and is the best model currently available to us. In addition, because mfLTP is believed to be induced and expressed presynaptically, the only determinant of whether mfLTP is induced is activity in the presynaptic granule cell (Supplemental Note 3).

After a 20 minute stable baseline, a period of spike activity was replayed by the presynaptic stimulation electrode (Figure 3). We chose periods of activity from 4 granule cells with low mean firing frequencies that showed burst firing episodes (Figure 3, cells a, b, c & d). The activity from cells a, b & c induced significant LTP (cell a, $134 \pm 10\%$, $n = 7$; cell b, $118 \pm 6\%$, $n = 6$; cell c, $128 \pm 10\%$, $n = 8$, $p < 0.05$ by t-test) whereas the activity from cell d did not ($107 \pm 9\%$, $n = 6$). We also used activity from granule cells with higher mean firing frequencies and more regular firing patterns (Figure 3, cells e & f). Neither of these activity patterns induced LTP (cell e, $105 \pm 9\%$, $n = 7$; cell f, $106 \pm 7\%$, $n = 6$). Notably, the period of activity and number of spikes in the pattern from cell e was similar to that from cell c, but the two different patterns differed in their ability to induce LTP. Even more strikingly, the period of activity in the pattern from cell d was actually longer and contained more spikes than those from cells a, b & c, but this pattern was not sufficient to induce LTP.

To determine whether LTP induced by granule cell activity patterns was expressed presynaptically and therefore shares mechanisms with classical mfLTP we analysed the paired pulse ratio and coefficient of variance in synaptic response amplitude before and after the induction of LTP by the spike activity used in Figure 3a. During field potential recordings, the paired pulse ratio was reduced from 3.01 ± 0.21 to 2.62 ± 0.24 ($n = 7$, $p < 0.05$, paired t-test). The same spike activity also induced significant LTP in whole-cell experiments ($135 \pm 9\%$, $n = 9$, $p < 0.05$, paired t-test) and a commensurate reduction in the paired pulse ratio from 3.79 ± 0.42 to 2.74 ± 0.26 (Figure 4a,b). We also found that LTP caused a substantial increase in the quantal content contributing to the EPSC, as assessed by changes in the coefficient of variance of the EPSC (Figure 4c, $n = 9$, $p < 0.05$, paired t-test).

Thus, classical presynaptically expressed mfLTP was induced by some, but not all, patterns of activity seen in granule cells *in vivo* during task performance. LTP was seen only in response to those patterns from cells with low mean firing frequencies and burst firing episodes. However, even within this subset of cells, not all patterns of activity were sufficient to induce LTP. Moreover, the patterns of activity from the 6 granule cells chosen for analysis here may not be representative of the entire population. To address these issues, we defined the threshold for LTP induction using a more systematic approach.

mfLTP threshold is dependent on stimulus number and frequency

To define the threshold for mfLTP induction, we first applied brief tetanic trains with a constant frequency to mimic high-frequency bursting *in vivo*, and systematically varied the frequency and number of stimuli during the tetanus. Using 10 Hz tetani, we found that LTP was never induced even when 48 stimuli were given during the induction protocol. When 25 Hz or 40 Hz stimulation was used, 12 stimuli were sufficient to induce LTP whereas 9 stimuli were insufficient. When the frequency of the tetanus was increased to 100 Hz, 9 stimuli induced a significant LTP whereas 6 stimuli did not (Figure 5a and Supplementary Figure 3). We then interpolated from this data to create a mesh represented by a 3D plot relating the amount of LTP induced during a single burst, given a wide range of stimuli number over a wide range of frequencies (Figure 5b).

Induction of mfLTP is dependent on baseline stimulation frequency

A second factor that we anticipated would strongly affect LTP induction in response to patterns of activity seen *in vivo* is the mean firing frequency of the granule cells. Many of these cells had firing rates known to induce a significant amount of frequency facilitation (Klausnitzer and Manahan-Vaughan, 2008; Salin et al., 1996) and mossy fiber LTD (Kobayashi et al., 1996). Mossy fiber LTD is thought to be a reversal of mossy fiber LTP (Tzounopoulos et al., 1998), and LTP is known to partially occlude frequency facilitation at the mossy fiber synapse (Salin et al., 1996), with the functional effect of reducing the dynamic range of the synapse (Gundlfinger et al., 2007). We therefore thought it possible that LTP induction would be impaired by the confounding influence of these other forms of plasticity during sustained activity at rates in the range above 0.1 Hz. However, this has never been directly tested.

To address this issue, we investigated the effect of sustained activity on LTP by increasing the baseline stimulation frequency from 0.05 Hz to 1 Hz. This induced a greater than 4 fold increase in the fEPSP amplitude (Figure 6a; $431 \pm 56\%$, $n = 5$) and a commensurate reduction in the paired pulse ratio (0.05 Hz, PPR = 3.02 ± 0.30 ; 1 Hz, PPR = 1.60 ± 0.10) indicating the presynaptic origin of the fEPSP increase (Salin et al., 1996). After establishing a stable baseline, tetanic stimulation of 100 stimuli at 25 Hz failed to induce significant LTP (Figure 6b; $114 \pm 11\%$, $n = 8$, $p > 0.05$). This same tetanic stimulation induced robust LTP if the baseline frequency of stimulation was reduced to 0.05 Hz (Figure 6b, $158 \pm 15\%$, $n = 9$, $p < 0.05$). This was then repeated over a range of baseline stimulation rates. We were unable to elicit a significant level of LTP at baseline stimulation rates ≥ 0.33 Hz (Figure 6c; 0.167 Hz, $120 \pm 6\%$, $n = 7$, $p < 0.05$; 0.333 Hz, $112 \pm 6\%$, $n = 8$, $p > 0.05$). The blockade of LTP at a stimulation frequency of 1 Hz is likely due to the reversal of LTP as shown previously (Tzounopoulos et al., 1998). We tested this directly by stimulating at a frequency of 1 Hz for 15 minutes immediately after tetanic stimulation before reverting to 0.05 Hz for the rest of the experiment. This completely reversed LTP and resulted in a small depression in the synaptic response elicited by 0.05 Hz stimulation (Figure 6d, $74 \pm 6\%$, $n = 6$, $p < 0.05$).

To incorporate these data into our analysis, we created a low-pass filter based on the data in Figure 6c (see methods) that was then applied to the mesh in Figure 5b to obtain a final model relating the pattern of granule cell activity with the induction of mfLTP. When applied to the complex patterns of activity used to elicit LTP in figure 3, the amount of LTP predicted by the model was well-correlated with the observed amount of LTP for all 6 patterns (Figure 6e; $r^2 = 0.69$; $p < 0.05$ by linear regression).

Bursts sufficient to induce mfLTP occur during the DNMS memory task

We used this model of LTP induction to screen through the entire dataset of granule cell recordings and identify which cells had the combined low mean firing frequency and high-frequency bursting required for LTP induction. 29 out of 68 granule cells had mean frequencies of less than 0.333 Hz, and 26 out of these 29 cells had at least one burst of activity expected to elicit LTP over the course of recording. Representative raster plots of activity from 3 cells with mean frequencies in the appropriate range are shown in Figure 7a; spike bursts that crossed the threshold for LTP induction are shown in red. The bursts that are predicted to elicit LTP were often brief, and on average lasted for < 0.5 seconds (Figure 7b).

In order to compare different cells that were recorded for differing lengths of time, we normalized the amount of LTP predicted by the model per hour of task-related activity recorded in each cell. We also restricted our subsequent analysis to the 29 cells that had

mean firing frequencies below 0.33 Hz, as these are the only ones that are expected to show any LTP induction. Cells within this range varied substantially in their predicted expression of LTP, and the magnitude of LTP/hr predicted in a given cell was not significantly correlated with the cell's mean firing frequency ($p > 0.15$ by Spearman Rank Order test). Cells with little or no predicted LTP ($< 5\%$ LTP/hr) could be observed over the entire range of mean firing frequencies, as could cells with more substantial levels of predicted LTP (Supplemental Figure 4). In contrast, the magnitude of LTP predicted in a cell was strongly correlated with the proportion of short ISIs (< 100 msec) in the ISI distribution (Figure 7c; $r = 0.78$, $p < 0.0001$ by Spearman Rank Order test, $n = 29$). There was no significant correlation between the number of cues to which a cell responded and the magnitude of LTP predicted in that cell ($p > 0.05$ by Spearman Rank Order test; see Figure 7c), although such a correlation would also have been unusually difficult to resolve given that there were so few cells in this low frequency range that responded to multiple cues (2 out of 29; see Figure 7c).

We next examined whether the patterns of activity seen in cells that responded to different cues would be differentially effective in eliciting LTP. We focused our attention on two clearly distinct and mutually exclusive sets of cells: those that responded during application of the sample odor, but not during movement between arms of the maze (referred to as sample-responsive cells), and those that responded during movement between the arms of the maze but not during the application of the sample odor (referred to as transit-responsive cells). Sample-responsive cells had patterns of activity that would be expected to elicit robust LTP, while transit-responsive cells did not (Figure 7d, left; sample cells, $18 \pm 4\%$ LTP/hr, $n = 5$; transit cells, $1 \pm 1\%$ LTP/hr; $n = 4$; $p < 0.05$ comparing sample- to transit-responsive cells, t-test). This result could not be explained by differences in the mean firing frequency between the two sets of cells (Figure 7d, right; sample cells, 0.17 ± 0.05 Hz, $n = 5$; transit cells, 0.16 ± 0.04 Hz, $n = 4$; $p > 0.8$ by t-test).

We next separated the DNMS task into four phases: an initial phase, after the animal is placed in the maze but prior to being given the information required to solve the task; a sample phase, when the sample odor is presented and must be memorized; a delay phase, when the odor is no longer present but must be remembered; and a test phase, in which the test odors are presented and the animal can navigate the maze to complete the task (See Methods). We then measured the fraction of supra-threshold bursts that occur in each phase compared to the fraction of time that was spent in each phase. If the bursts occur at random throughout the task, then the fraction of bursts in a given phase would be equal to the fraction of time spent in that phase, and the difference between the two fractions would be zero. In sample responsive cells, we found that supra-threshold bursts occurred during the sample phase significantly more often than expected at random (Figure 7e, filled bars, $n = 5$, $p < 0.05$ by ANOVA with post-hoc correction). In contrast, cells that did not respond to any of the monitored cues during the task had supra-threshold bursting that occurred at random throughout the task, as expected (Figure 7e, open bars, $n = 13$; $p > 0.1$ by ANOVA).

Finally, we examined the correlation between supra-threshold bursting and animal performance in the memory task during trials with supra-threshold bursting in any task phase (Figure 8a). We did not observe a significant improvement in task performance during trials with supra-threshold bursting during the sample phase in sample-responsive cells; however, the overall success rate for the task was already high ($\sim 80\%$), so it would be difficult to resolve a significant increase in the success rate above this value on the relatively few trials in which one of the few sample-responsive cells had a supra-threshold burst during the sample phase of the task. Given this constraint, a significant increase in the error rate would be much more readily resolvable. Analysis of the error rates revealed a significant impairment in task performance on trials where supra-threshold bursts occurred in any granule cell during the delay phase of the task (Figure 8b; $p < 0.05$ by ANOVA with

post-hoc correction, $n = 26$). Thus, the timing of supra-threshold bursting in individual granule cells can predict behavioral task performance, with supra-threshold bursting that occurs during the delay phase associated with a significant disruption in task performance.

Discussion

Our study indicates that the induction of mFLTP depends on a specific type of activity in granule cells that combines a low mean frequency of firing with high-frequency bursts. Most of the granule cells that responded to cues that could be clearly associated with the DNMS task in this dataset did not show this pattern of activity, but those that did were almost all cells that responded during the sample phase of the task, and the bursts that would elicit LTP also occurred selectively during this time period. Thus, patterns of granule cell activity that are expected to elicit mFLTP during this task take place during acquisition of the information that must be remembered in order to complete the task successfully, at the time when this information is present.

Remarkably, supra-threshold bursts that occur during the delay phase predict a subsequent failure of the animal to perform that trial of the task correctly. This raises the possibility that not only the occurrence of mFLTP but also the timing of mFLTP induction is important for task performance and may explain why the complete ablation of mFLTP has produced conflicting results in various forms of learning and memory (Huang et al., 1995; Wang et al., 2003; Zhang et al., 2008). However, we cannot exclude the alternative that a distinct process coinciding with the induction of mFLTP is the true determinant of task performance, while the LTP itself is a correlative marker rather than a causal agent. In either case, it is striking that supra-threshold bursting in individual granule cells can so strongly predict an animal's trial-to-trial performance of a complex behavioral task.

Our finding that many granule cells fire at a mean rate that is too high to sustain mFLTP is surprising, and it is of interest to note that these cells also fire at a rate where frequency facilitation will be tonically engaged. This suggests that the dynamic range of mossy fiber synapses from many granule cells is likely to be smaller than expected. As the dynamic range of the synapse is the feature that drives detonation, this suggests that different granule cells, and potentially different mossy fiber synapses (Galimberti et al., 2006), may perform distinct roles in hippocampal processing. Alternatively, the range of activity in granule cells may represent a maturational continuum, as these cells undergo continual neurogenesis. It is not yet clear whether cells would respond to greater or fewer cues with age (Schmidt-Hieber et al., 2004; Tashiro et al., 2007; Toni et al., 2008), making it difficult to relate the maturational state of granule cells with their overall activity and propensity for supra-threshold bursts.

The observation that supra-threshold bursts occur in granule cells that respond during the sampling phase is particularly striking in light of recent data supporting distinct roles for granule cells of the dentate gyrus and CA3 pyramidal cells in "pattern separation" and "pattern completion", respectively. According to this model, mossy fibers transmit sparse representations of highly specific aspects of an episodic stimulus to the CA3 pyramidal cells (Leutgeb et al., 2007; McHugh et al., 2007). In this context, it is interesting to note that cells responding to fewer distinct cues, and therefore exhibiting better pattern separation, are more likely to be able to induce mFLTP. The mossy fibers then activate the recurrent network of CA3 pyramidal cells to form a full representation of the episodic event associated with the stimulus (Fyhn et al., 2002; Marr, 1971; Nakazawa et al., 2002; Nakazawa et al., 2003). In this framework, mFLTP would be expected to amplify the strength of the episodic stimulus (e.g. odor in this task) required to solve the task in a

specifically non-Hebbian fashion. This stimulus would then be a more effective trigger for task recall in its entirety by the CA3 network (Rolls and Kesner, 2006).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Hippocampal granule cells exhibit a wide range of spike patterns and frequencies

a The timing of spikes that occur during consecutive DNMS trials in 4 example granule cells with increasing mean firing frequency (a₁, a₂, a₃ and a₄). Timing of spikes is indicated by vertical lines.

b The overall inter-spike interval (ISI) histograms for the same cells in (a) demonstrates that bursts of spikes tend to occur in cells with low mean firing frequencies. Note that there is a continuum of firing patterns from low to high frequency in which the cells shift from bimodal to unimodal ISI distributions as the firing frequency increases.

c The cumulative relative distribution of mean firing frequencies is shown for all granule cells recorded.

d ISI distributions as a function of mean firing frequency are shown for cells grouped together according to mean firing frequency in half log-unit bins. The relative frequency of each ISI shown in pseudocolor. Although cells at the highest and lowest mean firing frequencies have clearly distinct ISI distributions, there is a smooth continuum from one to the other. We note that this distribution distinguishes the population of granule cells in this analysis from the population of CA3 pyramidal cells from the same dataset, which have a substantially smaller representation of short-duration ISIs even at low frequencies of firing (Frerking et al., 2005).

Figure 2. Granule cell firing patterns depend on the number, but not type, of responsive cues

a Mean firing frequency increases as granule cells respond to a larger number of distinct cue-types during the DNMS task (non-responsive cells in black, cells responsive to one cue-type in red, and cells responsive to multiple cue-types in blue).

b Mean firing frequency distribution is similar for cells responding only to the sample odor or transit between arms of the maze (sample responsive cells in black, transit responsive cells in red).

Figure 3. Some, but not all, spike patterns recorded from granule cells *in vivo* can induce mFLTP *in vitro*

In vivo recordings from 6 different granule cells (**a–f**) show a range of mean firing frequencies and ISI distributions (top row).

Sections of activity were excised to produce similar ISI distributions (second row; the entire duration of granule cell recording was used for cell **f**).

Granule cells that have low mean firing frequencies exhibit pronounced burst firing activity whereas cells with high mean firing frequencies have more regular spike firing patterns as illustrated by the instantaneous spike frequency during the excised section of activity (third row).

Spike activity from burst firing granule cells with low mean firing frequencies (**a–c**) induced mFLTP in slice experiments whereas regular firing cells with high mean firing frequencies (**d–f**) did not induce LTP (bottom row). The activity shown in the third row was replayed at the time indicated by the arrow for each cell. fEPSP amplitude was recorded for 30 minutes after replay to measure LTP after which the mGluR group II agonist DCG-IV (2 μ M) was applied to every slice as a test for the purity of the mossy fibre response. Example traces show the average mossy fibre fEPSP response during baseline (black) and 20–30 minutes after the replay of granule cell activity (red). Scale bars are 0.3 mV (**a**, **e** and **f**), 0.5 mV (**b**), 0.2 mV (**c**), 0.15 mV (**d**), 10 ms.

Figure 4. mfLTP induced by granule cell firing patterns is expressed presynaptically

a mfLTP is induced during whole-cell recording from CA3 pyramidal cells by stimulation with the granule cell firing pattern used in Figure 3a ($n = 9$). Example traces show response to paired pulse stimulation before (black) and after (red) mfLTP induction. Scale bars are 50 pA and 20 ms.

Paired pulse ratio (**b**) and coefficient of variance (**c**) are reduced significantly following mfLTP induction.

Figure 5. Induction of mflLTP is dependent on the spike number and frequency during tetanic stimulation

a Systematic variation of the frequency and number of stimuli during the LTP induction protocol revealed the minimum threshold for LTP induction at each frequency. 10 Hz stimulation does not induce mflLTP for 12, 18, 24 and 48 stimuli ($n = 5, 5, 6$ & 5 respectively). 25 Hz stimulation induced LTP for 12 or more stimuli ($n = 8, 8, 6, 6$ & 4 for 6, 9, 12, 18 and 24 stimuli respectively). 40 Hz stimulation induced LTP for 12 or more stimuli ($n = 11, 12, 8, 5$ & 5 for 6, 9, 12, 18 and 24 stimuli respectively). 100 Hz stimulation induced LTP for 9 or more stimuli ($n = 7, 5, 5$ & 4 for 6, 9, 12 and 18 stimuli respectively). Baseline stimulation frequency was 0.05 Hz. * denotes statistical significance from baseline ($p < 0.05$).

b LTP mesh showing how the amount of LTP (shown in pseudocolor scale) induced depends on the frequency and stimulus number during the high frequency train. Warmer colours indicate increasing amount of LTP.

Figure 6. Induction of mFLTP is dependent on the baseline stimulation frequency

a Mossy fiber synaptic release probability increases with stimulus frequency. Switching stimulation frequency from 0.05 Hz to 1 Hz results in an approximate 4 fold increase in fEPSP amplitude. Scale bars for example traces are 0.3 mV, 10 ms.

b mFLTP cannot be induced if the baseline stimulation frequency is 1 Hz but robust LTP is induced when the baseline stimulation frequency is reduced to 0.05 Hz. Scale bars for example traces (black pre-tetanus, red post-tetanus) are 0.4 mV (0.05 Hz), 0.2 mV (1 Hz), 10 ms.

c Baseline stimulation frequency must be <0.333 Hz for mFLTP to be induced. LTP is induced at baseline stimulation frequencies of 0.05 Hz and 0.167 Hz ($n = 9, 7$ respectively) but not at frequencies of 0.333 Hz or 1 Hz ($n = 8, 8$ respectively).

d mFLTP is reversed by switching the stimulation frequency from 0.05 Hz to 1 Hz for 15 minutes immediately after tetanic stimulation. Scale bars for example traces (black pre-tetanus, red post-tetanus and 1 Hz) are 0.3 mV, 10 ms.

e Predictions for the amount of LTP induced by natural stimulus patterns calculated from the LTP mesh in Figure 5b agree with observed amounts of LTP found in Figure 3 for each of the 6 patterns tested.

Figure 7. Bursts of activity that would be expected to induce mossy fiber LTP occur in granule cells that respond during the sample phase of the DNMS task

a Raster plots are shown of activity in representative granule cells: one that did not respond to any monitored cues during consecutive trials of the DNMS task (a_1), one that responded selectively during the sample phase of the task (a_2), and one that responded selectively during movement between arms of the maze (a_3). Symbols indicate the time of each spike, with red symbols indicating the initial spike in a burst that crossed the threshold for LTP induction. The average fraction of time spent in each phase of the task (initial (i), sample (s), delay (d) and test (t)) is denoted using dashed vertical lines. Trials were normalized according to both trial and phase duration, to reflect the fact that these time periods varied for each trial.

b Thirty representative supra-threshold bursts from a representative cell are shown, aligned at their initial spike (upper traces). The majority of these bursts were caused by relatively high-frequency firing for a very short (~250 ms) period of time. The average of all bursts from the cell is also shown (lower trace), binned into 20 ms intervals. Note that the probability of spiking peaks at multiple spikes in the first 20 ms, then monotonically decays to baseline firing rates.

c The amount of predicted LTP induced per hour of recording increases in cells that have a larger proportion short ISIs in the overall ISI distribution. Cells are grouped into those that respond to no cues, those that respond to one type of cue and those that respond to more than one type of cue.

d Granule cells that respond during the sample phase but not during transit between arms of the maze are predicted to have significantly more LTP/hr than cells that respond to transit but not during the sample phase. Both populations of cells have similar mean firing frequencies. * $p < 0.05$.

e Sample-responsive cells have supra-threshold bursts preferentially during the sampling phase of DNMS trials (black bars). Cells that do not respond to any of the recorded cues fire supra-threshold bursts at random throughout the trials (white bars). * $p < 0.05$.

Figure 8. The timing of bursts that induce mfLTP predicts task performance

a The timing of supra-threshold bursts is shown in a representative cell, phase of the task (initial (i), sample (s), delay (d) and test (t)) is denoted using dashed vertical lines. Different colours indicate whether the trial ended with successful task completion (green) or not (red). Note that most trials with supra-threshold bursts during the delay phase were not completed successfully. This contrasts sharply with the observation that most trials ended with successful task performance (~80%).

b The fraction of supra-threshold bursts during trials that ended with incorrect task performance was calculated for different phases of the task; supra-threshold bursting during the delay phase was selectively associated with a substantial increase in errors during the task ($n = 26$ cells). Dashed line indicates the overall expected task performance in all 26 cells, for comparison. * $p < 0.05$.