Long-Term Potentiation/Depotentiation Are Accompanied by Complex Changes in Spontaneous Unit Activity in the Hippocampus

AKIHISA KIMURA1 AND CONSTANTINE PAVLIDES2
1Department of Physiology, Wakayama Medical College, Wakayama 641-0012, Japan; and 2The Rockefeller University, New York, New York 10021

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Kimura, Akihisa and Constantine Pavlides. Long-term potentiation/depotentiation are accompanied by complex changes in spontaneous unit activity in the hippocampus. J Neurophysiol 84: 1894–1906, 2000. Typically, long-term potentiation (LTP) has been assessed as long-lasting changes in field potentials or intracellularly recorded postsynaptic potentials evoked by activation of a set of afferents. In the present experiment, we determined changes in spontaneous unit activity in the dentate gyrus (DG) following high-frequency (HFS) or low-frequency stimulation (LFS) of the medial perforant pathway. Experiments were performed in anesthetized rats. Field potentials and unit recordings were obtained alternatively from the same recording electrode. Of 39 single units isolated (from 25 independent sessions), the spontaneous discharges of 13 units (33%) increased, while 7 units (18%) decreased their discharges following HFS that induced significant LTP of the field potentials. Such opposing modulations of unit discharges following HFS were observed on simultaneously recorded units. LFS applied following HFS also induced bi-directional effects on unit discharges. Of 20 single units isolated from a subset of recordings (12 experiments) to which LFS was applied, 6 units increased and 4 units decreased their discharges. LFS produced a long-lasting (>20 min) depotentiation, to the baseline level, on field potentials in four recording cases. The autocorrelation functions indicated that the isolated unit discharges were comparable to those of the putative DG granule cells and interneurons, shown in previous studies. The results suggest that changes in synaptic efficacy following HFS or LFS produce rather dynamic changes in cell activity in the DG.

INTRODUCTION

Changes in synaptic weights within a neuronal network and the subsequent alteration of cell activity have been hypothesized as a neuronal substrate for learning and memory. In the hippocampus, discharge patterns of an individual neuron or an ensemble of neurons encode sensory and/or contextual information related to learning and memory (Deadwyler et al. 1996; Eichenbaum et al. 1987; Sakurai 1994, 1996; Vidyasagar et al. 1991; Wilson and McNaughton 1993; Young et al. 1994). A number of previous studies have shown modulation of hippocampal cell discharges in the course of learning and memory formation (Berger and Thompson 1978; Deadwyler et al. 1979; Segal and Olds 1972; West et al. 1981). The most putative cellular mechanism underlying learning and memory is long-term potentiation (LTP) and depression (LTD), which represent activity-dependent changes in synaptic efficacy (Collingridge and Bliss 1995; Linden and Connor 1995). In the hippocampus, various forms of synaptic plasticity have been revealed in both the well studied “tri-synaptic” circuit and local synaptic connections involving both principal neurons and interneurons (Bear and Abraham 1996; Bliss and Collingridge 1993; Grunze et al. 1996; Maccaferri and McBain 1996; Xie and Lewis 1995). Plasticity in the hippocampus is expressed on both excitatory and inhibitory synaptic connections, and subsequent cell interactions on polysynaptic connectivity are also likely to be affected by the primary change of synaptic efficacy (Buzsáki 1988; Mott et al. 1993; Xie and Lewis 1995; Yeckel and Berger 1998). Such ubiquitous and diverse plasticity leads to a view that the modulation of hippocampal cell activity is very dynamic in the presumed information processing related to learning and memory. However, despite the great number of studies and wealth of information concerning the mechanisms underlying synaptic plasticity, it is less clear how LTP induction may affect individual cell firing in a highly complex network, such as exists in the hippocampus.

The most common measure of neuronal plasticity has been assessed as long-lasting changes in field potentials or intracellularly recorded postsynaptic potentials evoked by activation of a set of afferents. Thus far, very little attention has been focused on possible dynamic modulations of cell discharges. It has been shown that LTP of a given set of excitatory afferents in the hippocampus is accompanied with an increase of cell discharge probability to a subsequent afferent activation by electrical stimulation (Andersen et al. 1980; Buzsáki and Edelberg 1982).

The modulation of cell discharges driven by naturally incoming synaptic inputs, as in actual information processing, has yet to be examined. Activity-dependent modification of synaptic connectivity has been implied, for example, in the reactivation of specific cell interactions during sleep after waking experience (Pavlides and Winson 1988; Skaggs and McNaughton 1996; Wilson and McNaughton 1994). Of particular interest is whether the conditioning of a given set of inputs, which induces changes in synaptic efficacy, would also bring about modifications in connectivity and cell interactions that would produce specific patterns of cell activities as may be seen in actual information processing.
It has previously been reported that tonic activation of the entorhinal cortex induces a long-lasting increase of spontaneous discharges of hippocampal cells (Deadwyler et al. 1976). It has been postulated that the overall spontaneous discharge probability of a given cell is determined by spatio-temporal interactions of synaptic inputs converging on the cell. Therefore possible modifications of cell interactions brought about by the induction of LTP could have significant effects on spontaneous cell activity. Given that the induction of LTP should produce plasticity of both mono- and poly-synaptic connections, it would be predicted that a diverse modulation of cell activity must take place. We recorded both field potentials and spontaneous cell discharges in the dentate gyrus (DG) and examined the effect of high-frequency stimulation (HFS) of the perforant path. HFS induced LTP of field potentials evoked by the perforant path stimulation and, in the majority of cases, a long-lasting increase of spontaneous cell discharges, concomitantly. A subgroup of cells, however, decreased their spontaneous firings despite the induction of LTP of field potentials. In a subset of experiments, low-frequency stimulation (LFS) was applied following LTP induction. Similar to the findings with HFS, LFS induced either decrement or an enhancement of spontaneous cell discharges.

**METHODS**

Thirty-two adult albino rats (6 Sprague Dawley and 26 Wistar) of both sexes weighing 125–340 g were anesthetized with Chloropent (15 mg/100 g body wt ip) and placed on a stereotaxic apparatus. Stainless steel screws were placed on the skull and were used for stimulation and recording reference and ground. A stimulating electrode (tungsten-in-glass, 0.2–0.5 MΩ impedance) was placed in the medial perforant pathway (coordinates: AP: β +7.9 mm; ML: 4.0–5.0 mm). A recording electrode (tungsten-in-glass or glass capillary filled with 4% biocytin in saline, impedance 1–3 MΩ) was aimed at the granular cell/hilar area in the dentate gyrus (coordinates: AP: β +3.9 mm; ML: 2.3 mm). Electrode placement was aided by monitoring the depth profile of evoked field potentials, and the final positions of the stimulating and recording electrodes were adjusted to produce maximum field potentials. The intensity of test stimulation (25–500 μA) was adjusted to evoke field potentials ~30% of the maximum on the basis of an input/output function. A test stimulus was delivered five times, once every 10 s, to obtain an averaged field potential. Field potentials were band-pass filtered (3 Hz to 3 kHz), digitized (10 kHz; MIO-16X, National Instruments) and averaged (5) on-line using LabVIEW (National Instruments) custom-built software. Both the excitatory postsynaptic potential slope (measured in the initial positive rise) and population spike amplitude of each averaged field potential were measured. Recordings of field potentials evoked by the perforant path stimulation were performed every 2–7 min, while spontaneous unit discharges were continuously recorded through the same recording electrode and stored on video tape, via a digital recorder (VR-100A, Instrutech) for future off-line analysis. The unit signals were filtered (300 Hz to 3 kHz), digitized (20 kHz), and stored on disk. Using LabVIEW programs, single units (each consisting of negative-positive spikes) were isolated from the digitized data on the basis of the following four parameters: amplitude of negative peak, time from onset to negative peak, amplitude difference between negative peak and positive peak, and time from negative peak to positive peak (Kimura et al. 1996). To characterize an isolated single unit, we assessed the autocorrelation function of unit discharges during baseline recordings and the spike width, which was defined as the average time from negative to positive peak.

After recording a stable baseline of field potentials and spontaneous unit activity for ~10 min, high-frequency stimulation (HFS, 400 Hz, 10–50 pulses, 5 or 10 trains, 10 s apart) was delivered to the perforant path at the same intensity as test stimulation. The effect of HFS on spontaneous cell discharges was assessed by counting the number of discharges in blocks of 10 s of a continuous recording (duration, 90–180 s). The recording was then interrupted for the recording of field potentials. In a subset of experiments, after recording for ≥25 min following HFS, LFS (1 Hz for 5 or 10 min) was applied, and the effect of LFS was assessed in the same way. Statistical significance in the alterations of unit discharges and field potentials was examined by a Mann-Whitney rank-sum test. Statistical significance was set at P < 0.001 and P < 0.05, for unit activity and field potentials, respectively.

Some of the recording sites were verified histologically (8 animals) by injecting biocytin iontophotographically (anodal 5–10 μA, 7 s on-off, 20–30 min) after the recordings (King et al. 1989). Following completion of the injection, the electrode was removed, and the wound was closed. The animals were administered antibiotics and allowed to recover. After a survival period of 1–2 days, the animals were anesthetized deeply with an overdose of pentobarbital sodium (100 mg/kg ip) and perfused transcardially with saline followed by phosphate buffered 4% paraformaldehyde. After postfixing overnight and storing at 4°C in 30% sucrose-phosphate buffer solution for 3 days, the brains were cut at 60-μm coronal sections. The sections were processed with biotin-avidin complex (ABC kit, Vector Labs) to visualize the biocytin-labeled cells by horseradish peroxidase histochemical reaction (Horikawa and Armstrong 1988). The sections were counterstained with neutral red and examined under a light microscope. The injection sites were located in the upper blade of stratum granulosum (Fig. 6) or the border between the hilus and the upper blade of stratum granulosum. Some were observed in the crest of stratum granulosum.

**RESULTS**

Data analysis was carried out on 25 recording sessions. Only units adhering strictly to the isolation criteria were included in the final analysis. Signals of units adhering to the four parameters (defined in the preceding text) were monitored off-line throughout the blocks of continuous recordings (90–180 s). Units showing fluctuations in the four parameters were discarded as their recordings could be affected by electrode drift even though the field potentials were judged to be stable. The total number of single units obtained from 25 recording sessions was 39. Of these, simultaneous isolations of two to three units were made for analysis in 12 recording sessions. HFS was applied in all the recording sessions. LFS was also applied following HFS in 12 cases that involved 20 units. Furthermore a second HFS was applied in seven recording cases that involved 14 units (Table 1).

Using our HFS parameters, significant potentiation of field potentials was evidenced more consistently in population spike amplitudes (mean, 191%, maximum, 572% of baseline levels) than in excitatory postsynaptic potential (EPSP) slopes (mean, 107%, maximum, 125%), as measured at 20 min after conditioning to the end of recordings or the next conditioning (maximum 70 min). HFS induced significant potentiation (P < 0.05) of both population spike amplitudes and EPSP slopes in 20 cases (Table 1). In three cases (recording sessions 5, 7, and 12), a significant potentiation of population spike amplitudes was observed, but not of the EPSP slopes. In the other two cases, HFS did not induce significant changes (recording sessions 10 and 20). In contrast to HFS, LFS produced a long-lasting (>20 min) depotentiation, to the baseline level (i.e., prior to HFS) in four cases (Fig. 7). A long-lasting decrease in field potentials (although not a complete depotentiation to
baseline levels), was induced in six cases (Fig. 5). In the other two cases, no significant long-lasting changes were observed (Table 1).

Spike characteristics

Autocorrelation functions of spontaneous unit discharges were assessed in baseline recordings. They indicated that the isolated units were heterogeneous in discharge pattern as well as in spike width. A subgroup of units had relatively wide spike widths (>300 μs) and a clear propensity to fire in bursts with short interspike intervals, as indicated by a tall peak in the center of the autocorrelation function (Figs. 1, 3, 5, and 7, unit B). In contrast, units with relatively short spike widths (<300 μs) did not have a propensity to fire in bursts (Fig. 7, unit A; except 1 case, unit 2, in Table 1). The spike widths of units with a propensity to fire in bursts (n = 16) were significantly wider (P < 0.05, Mann-Whitney rank-sum test) than those of the other units (n = 23). There were two cases in which two different types of units, one with a long spike width and a propensity to fire in bursts and the other with a short spike width and no propensity to fire in bursts, were obtained simultaneously (Fig. 7). Further, we recorded from a unit that fired rhythmically as indicated by a clear oscillation (4–5 Hz) in the autocorrelation function (Fig. 2). Discharge rates in baseline recordings ranged from 0.03 to 5.99 Hz (Table 1). There was no significant difference in discharge rate between the units with a propensity to fire in bursts and the nonbursting units.

Effects of HFS on spontaneous unit discharges

Of particular interest was the finding that the effects of HFS were bi-directional, i.e., either an increase or a decrease of discharges, following conditioning stimulation. In the two cases presented in Figs. 1 and 2 (recording sessions 6 and 1, Table 1), an increase of discharges of a single unit was induced immediately following HFS, which lasted until the end of the recording (~25 and 55 min, respectively). The two

### TABLE 1. Discharge characteristics and effects of HFS/LFS

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Average discharges were in baseline recordings. LFS, low-frequency stimulation; +, increase; −, decrease; t+, transient increase; t−, transient decrease; =, no change; high-frequency stimulation (HFS) was applied twice; (spike), potentiation only in population spike amplitudes. * Unit discharges were evoked by trains of stimuli in HFS.
units isolated in these recordings had characteristic firing patterns. The example shown in Fig. 1 was a “typical” type of unit that had a relatively long spike width (381 μs, negative to positive peak) and fired mainly in bursts. A burst of discharges consisted of two to six spikes over a 5- to 30-ms duration (Fig. 1). In contrast, the unit in Fig. 2 had a short spike width (287 μs) and fired rhythmically as indicated by a clear oscillation (4–5 Hz) in the autocorrelation function (Fig. 2). In the other case (recording session 4, Table 1), an intense HFS (400 Hz, 50 pulses, 10 trains) increased the discharges of unit C, whereas it depressed the discharges of unit B. The second more intense HFS (400 Hz, 50 pulses, 10 trains) increased the discharges of unit A, which had not been affected by the first HFS, whereas it depressed the discharges of units B and C. All three units had wide spike widths (>350 μs). Unit A had a propensity to fire in bursts, but the other two did not have characteristic firing patterns. A cluster of granule cells labeled by biocytin indicated that the recording site was in the middle of stratum granulosum (Fig. 6).

Thus the effects of HFS on spontaneous unit discharges were diverse and bi-directional as summarized in Table 1. An in-

![Field Potentials](image1)

**FIG. 1.** Long-term potentiation (LTP) of field excitatory postsynaptic potentials (EPSPs) and unit discharges of a complex spike cell. A: high-frequency stimulation (HFS) induced significant potentiation of the population spike (178%) and the EPSP slope (103%), as measured after 20 min following the HFS. The traces of the field potentials were taken at the times indicated by the letters. B: HFS induced an immediate increase of discharges, for the unit shown, that also lasted the entire recording period. The value in the ordinate indicates the total number of discharges in 10 s. Unit discharges were counted in 90-s continuous recordings that were interrupted every 3–4 min, while field potentials were evoked by test stimuli (intensity, 50 μA). C: the autocorrelation function indicates the isolated unit fired almost exclusively in bursts with short interspike intervals. The entire baseline recording period was used for the assessment of the autocorrelation function. Top right inset: a burst of discharges is shown. The average spike width of the unit was 381 μs.

![Unit Discharges](image2)

![Autocorrelation](image3)

![Field Potentials](image4)

**FIG. 2.** LTP of field EPSPs and unit discharges, of a putative theta cell. LTP of field potentials (A) was accompanied with potentiation of single-unit discharges (B). A: the traces of the field potentials were taken at the times indicated by the letters. B: the discharge rate was significantly high (B) compared with that of the unit depicted in Fig. 1. Unit discharges were counted in 150-s continuous recordings that were interrupted every 4–7 min, while field potentials were evoked by test stimuli (intensity, 120 μA). C: the isolated single unit fired rhythmically as indicated by an oscillation (4–5 Hz) in the autocorrelation function. The dots under the raw trace (top right inset) indicate discharges of the isolated single unit. The average spike width was 287 μs.
significant changes in the other 14 units. A further decrease of discharges was induced by LFS in three units. A long-lasting increase of discharges was observed following LFS in four units; two of them showed a decrease from the enhanced activity induced by the preceding HFS and an increase of discharges in three units that had been augmented by the preceding HFS (Table 1). Figure 5 (recording session 14, Table 1) depicts a case (recording session 14, Table 1) in which opposing effects of LFS were observed on a pair of units (units A and B), which were of different types, in terms of spike width and firing patterns (Fig. 7C). HFS (400 Hz, 30 pulses, 5 trains) induced potentiation of field potentials and an increase of discharges of unit A, while the discharges of unit B were not affected. The enhanced discharges of unit A were further augmented by LFS (1 Hz, 10 min), which depotentiated field potentials to the baseline level. On the other hand, the discharges of unit B were depressed by LFS. The second more intense HFS (400 Hz, 50 pulses, 5 trains), applied after LFS, potentiated field potentials again, but it depressed the enhanced discharges of unit A. No significant effects were observed on the suppressed discharges of unit B. A noteworthy observation was that the potentiation of field potentials following the second HFS was preceded by a transient stagnation of field potentials as in the above-mentioned case (Fig. 3).

Thus the effects of LFS on spontaneous unit discharges were also diverse and bi-directional as summarized in Table 1. Twenty units were obtained from the subset of recordings to which LFS was applied. LFS induced a further increase of discharges in three units, which had been augmented by the preceding HFS and an increase of discharges in three units that had been depressed by the preceding HFS. A decrease of discharges was observed following LFS in four units; two of them showed a decrease from the enhanced activity induced by the preceding HFS. The discharges of one unit had been depressed transiently by the preceding HFS and the other units had not been affected by the preceding HFS. No significant effect by LFS was observed on the other 10 units. Opposing effects on a pair of units isolated simultaneously were also observed in two recording sessions.

Unit discharges during conditioning

Unit discharges were recorded continuously during HFS. In the case shown in Fig. 7, the discharges of unit A were activated at ~200 ms following each of the five trains of stimuli in the second HFS (Fig. 8), although the first HFS did not produce a significant effect on the unit discharges. The discharges of unit B (white arrows in Fig. 8B) were not affected by HFS. Such an activation of discharges during HFS was observed in 11 units (Table 1). The activation took place with a time lag [100–300 ms, except unit 31 (10 ms) and unit 21 (500 ms)] and an increase of discharge probability lasted for 200 to 1,500 ms (mostly <500 ms). A transient suppression of discharges followed the activation in this case but subsequent
repetitive paroxysmal discharges or epileptic afterdischarges (Bragin et al. 1997; Somjen et al. 1985) were not observed. In six units, the HFS that activated unit discharges, however, did not produce any significant effect on spontaneous activity afterward. These results suggest that there is no significant relation between the activation of unit discharges during HFS and the alterations of spontaneous activity. Of significance was the observation that only two of these units activated during HFS were the type of units showing a propensity to fire in bursts. It, therefore appeared that the type of units without a propensity to fire in bursts were more susceptible to activation during HFS.

**DISCUSSION**

The results show that cell activity in the DG could be modulated in either direction, i.e., either potentiation or depression, by HFS of the perforant path. LFS, which was applied after HFS in the present study, was also potent in modulating cell activity in opposite directions. The most striking finding is that the direction of the long-lasting change in discharge probability of a given cell in the DG was not always in accordance with the direction of a change in field potentials; a potentiation of field potentials by HFS could be accompanied with a depression of discharges and a depotentiation of field potentials by LFS could occur coincidentally with a potentiation of discharges. Changes of population spike amplitudes represent overall changes of discharge probability of a group of units in response to synaptic activation of a set of afferents. In contrast, changes in spontaneous unit discharges are thought to represent changes of spatial and temporal interactions of synaptic inputs driving discharges of a given cell in the group, which were shown to not always be similar as those of field potentials. The results indicate that the conditioning of a given set of afferents is in fact potent in reorganizing spatio-temporal characteristics of the neuronal circuit that...
determines cell activity in the DG. It is, further, suggested that activity-dependent changes in synaptic efficacy modify the DG neuronal circuit to produce specific patterns of cell interactions and activities in actual information processing.

Effects of HFS on spontaneous unit discharges

The observation of a decrease in spontaneous discharges, however, was inconsistent with the changes in homosynaptic LTP of excitatory inputs. Other changes in mono- and polysynaptic connections, especially those involving the modification of inhibitory synaptic inputs, could be assumed to take place. Various interneurons in the DG [which are mainly inhibitory GABAergic neurons (Obenaus et al. 1993; Seress and Ribak 1983; Sloviter and Nilaver 1987)] are recipients of perforant path synaptic inputs (Deller et al. 1996) and mediate feed-forward inhibitory connections (Buzsáki and Eidelberg 1982; Scharfman 1991). The E-S potentiation as a component of homosynaptic LTP by HFS is thought to involve a concomitant modulation of the feed-forward inhibitory inputs (Tomisolo and Ramirez 1993; Tomasulo and Steward 1996; Tomisulo et al. 1991; Wilson 1981; Wilson et al. 1981) along with possible changes of firing properties of postsynaptic cells (Taube and Schwartzkroin 1988a,b; Wathey et al. 1992). While in the DG a decrease of feed-forward inhibition has been shown (Kanda et al. 1989; Tomasulo and Ramirez 1993), HFS of the perforant path induces homo- and heterosynaptic potentiation of interneuron activity (Buzsáki and Eidelberg 1982; Tomasulo and Steward 1996) and, consequently, an increase in feed-forward inhibition on granule cells (Kairiss et al. 1987; Tomasulo and Ramirez 1993; Xie and Lewis 1995). A positive net change of synaptic weight in response to an artificial activation of the perforant path by electrical stimulation would...
augment the discharge probability of granule cells and interneurons (Andersen et al. 1980; Buzsáki and Eidelberg 1982).

For the spontaneous activity of a given cell, however, it is also possible that depending on the route and timing of incoming synaptic inputs in the neuronal circuit, potentiated inhibition would lead to a reduction of the overall discharge probability that could not be assessed in the discharges elicited by an activation of a specific group of synaptic inputs by electrical stimulation. Of interest is the observation that in the cases in which a depression of discharges was seen, a transient stagnation of field potentials, especially in the population spike amplitude, occurred following relatively intense HFS (Figs. 3 and 7). According to Tomasulo and Ramirez (1993), high-intensity conditioning of the perforant path is liable to potentiate feed-forward inhibition. Therefore a speculative hypothesis is that the transient stagnation of population spike amplitude, which was originally reported by Bliss and Lømo (1973), is suggestive of a significant recruitment of feed-forward inhibition by strong conditioning and the consequently augmented inhibition might depress tonically the activity of a given cell. On the other hand, tetanic stimulation of the perforant path depresses feed-back inhibition (Maru 1989) that is mediated by various interneurons receiving mossy fiber inputs (Ácsády et al. 1998) and controls the excitability of DG cells (Halasy and Somogyi 1993; Han et al. 1993; Sik et al. 1997) along with feed-back excitation (Scharfman 1995, 1996). The modulation of inhibitory synaptic weights in feed-forward and -back circuits is thus postulated to be pivotal for the bi-directional alterations of discharge probability. In 11 units, trains of stimuli during HFS provoked a transient activation of discharges (Fig. 8), suggesting some recruitment of excitatory driving force in these circuits. The detailed mechanism of this activation is unclear at this moment because there was no consistent relation between the activation and the change of spontaneous activity. It is indicated, however, that HFS, including that which was subthreshold to drive neurons, (like the first HFS in the case shown in Fig. 7), could have significant effects on the neuronal circuits.

Finally, a reduction of spontaneous discharges could follow HFS due to an induction of heterosynaptic LTD (Abraham and Goddard 1983; Abraham et al. 1985; Christie et al. 1995; Krug et al. 1985; White et al. 1988, 1990; Zhang and Levy 1993) or heterosynaptic depotentiation (Doyère et al. 1997; Levy and Steward 1979), if the main driving force of spontaneous discharges of a given cell is provided by an unconditioned excitatory synaptic input. Then, homo- and heterosynaptic plasticity produced by associative interactions of conditioned and unconditioned afferents (Tomasulo et al. 1993; White et al. 1990) might bring about diverse modulations of overall discharge probability, which could be further complicated due to the modulation of the lateral inhibition arising from association pathways (Sloviter and Brisman 1995). Using the present techniques, it would not be possible to determine the source of the changes in neuronal excitability. It is noteworthy to state, however, that the heterogeneity in the modifications of synaptic connectivity was observed even in adjacent neurons isolated
with the same electrode (Figs. 4 and 7). Furthermore the overall discharge probability of each cell was consistently stabilized at a new static level. This implies that the neuronal circuit in the DG consists of the spatio-temporal structure that is modifiable but also consistently stabilized, in terms of each cell activity, which might be a fundamental property of the neuronal circuit to preserve information in specific patterns of cell activities.

**Effects of LFS on spontaneous unit discharges**

It has been controversial whether LFS of the perforant path induces homosynaptic LTD or depotentiation in the DG (Bear and Abraham 1996), despite being a relatively reliable paradigm in the CA1 field (Barrionuevo et al. 1980; Dudek and Bear 1993; Errington et al. 1995; Fujii et al. 1991; Mulkey and Malenka 1992; Staubli and Lynch 1990). There have been several in vitro studies documenting homosynaptic LTD in-
duction by LFS in the DG (O’Mara et al. 1995; Wang et al. 1997); however, in vivo, it is thought that LFS is not capable of inducing LTD or depotentiation (Abraham et al. 1996; Errington et al. 1995) except under certain conditions when afterdischarges follow LFS (Abraham et al. 1996; Bramham and Srebro 1987) or LFS is applied within a short time (<2 min) after the HFS (Martin 1998). Nonetheless homosynaptic LTD or depotentiation itself can be produced in the DG in vivo by HFS applied on the negative phase of theta rhythm (Pavlides et al. 1988) or in conjunction with the activation of adrenal steroid receptors (Pavlides et al. 1995). It is therefore pertinent to consider tentatively that the DG contains at least some neuronal properties that diminish or reset synaptic efficacy of excitatory inputs (Rick and Milgram 1996). In the present study, LFS (1 Hz) induced a depotentiation of field potentials to the baseline level lasting ≥20 min, in 4 of 12 cases where afterdischarges were not observed following LFS. Since this observation was limited in time and number of cases, we cannot conclude that the depotentiation observed in the present study was truly a long-term effect of LFS. However, it should be noted that spontaneous cell discharges were also affected by LFS as long as some effect was exerted on the field potentials. This observation substantiates the potential effect of LFS on synaptic efficacy in the DG.

As has been suggested for the mechanisms underlying E-S potentiation, the concomitant modulation of synaptic efficacy in inhibitory connections is considered to play a pivotal role in molding the effect of LFS as well (Wagner and Alger 1996). Homosynaptic LTD and depotentiation by LFS in the CA1 are accompanied by E-S potentiation (Bernard and Wheat 1995), and it was reported in a study examining habituation of responses to repeated stimuli that LFS (1 Hz) of the perforant path also resulted in E-S potentiation (Abraham and Bliss 1985). The neuronal mechanisms of depotentiation are also considered to involve the GABAergic system that is modulated by the prior conditioning (Wagner and Alger 1995). It is, therefore possible that in the spontaneous cell activity the concomitant modulation of excitatory and inhibitory synaptic weights by LFS results in the bi-directional alterations of overall discharge probability of a given cell, depending on the route and timing of incoming inputs.

**Identity of isolated units**

A question that arises concerns the identity of neurons in which spontaneous activity was modulated in the present study. Previous extracellular recording studies categorized units in the DG into those of putative granule cells and inhibitory interneurons. A number of studies reported that the putative granule cells have electrophysiological characteristics similar to those of theta cells that fire rhythmically at relatively high rates and do not exhibit bursts of discharges (Buzsáki et al. 1983; Foster et al. 1987; Rose et al. 1983). Other studies, however, suggest that the putative granule cells exhibit both rhythmic as well as burst firing (Bland et al. 1980; Suzuki and Smith 1985) or categorized the putative granule cells and interneurons as nontheta and theta cells, respectively (Fox and Ranck 1975). Recently Jung and McNaughton (1993) defined the putative granule cells as those that fire in bursts at extremely low rates, while the putative inhibitory interneurons as those that fire rhythmically at high rates. They further suggested that the putative granule cells and inhibitory interneurons have relatively wide and short spike widths, respectively, although the difference was not statistically significant. Their definitions were derived from the criteria of Mizumori et al. (1989) based on electrophysiological characteristics such as the response latency to the perforant path stimulation and the effect of paired pulse inhibition. However, according to Mizumori et al. (1989), the majority of cells recorded in stratum granulosum do not have characteristic firing patterns under pentobarbital sodium anesthesia.

In the present study, we obtained a subgroup of units that fired mostly in bursts with short interspike intervals and had a relatively wide spike width (Table 1). In line with the classification by Jung and McNaughton (1993), those units with an especially low firing rate are likely to be granule cells although the overall discharge rates we observed for this subgroup were relatively higher and were not distinct from the discharge rates of the other units. An example of such a unit, with a propensity to fire in bursts and with a relatively long spike width, was recorded in the middle of the granule cell layer (Fig. 6). A single unit exhibiting a clear theta oscillation in firing (Fig. 2) had a short spike width (280 μs) and high firing rates (~6 Hz during baseline and 10 Hz following HFS), which are characteristics similar to those of the putative interneurons. Other units, in the present study, with relatively short spike widths (~300 μs) did not exhibit burst discharges (Table 1), suggesting that they may constitute another major subgroup. Other units, however, lacked characteristic features and could not be categorized. Taken together, the present result suggest that our neuronal population consisted of both granule cells and interneurons.

**Functional considerations**

Because the synaptic and biophysical bases of spontaneous cell activity are not well understood, spontaneous cell activity is usually regarded as noise, unrelated to information processing. Yet it has been shown in the sensory system that spontaneous cell activity signifies the functional connectivity and its reorganization for information processing (deCharms and Merzenich 1996; Dinse et al. 1993; Johnson and Alloway 1996). Another view is that spontaneous cell activity itself is functionally significant ongoing network dynamics that has a major influence on sensory processing in its specific interactions with the activity evoked by sensory inputs (Arieli et al. 1995, 1996). In this sense, the modifiability of the neuronal circuit indicated by the alterations of spontaneous cell activities could be fundamental also for cell activities that encode actual information. However, because of the unpredictable diversity in the results, the activity-dependent modifiability of cell discharges shown in the present study does not appear feasible as a neuronal basis for the functionally rational reorganization of cell activities in actual information processing. As mentioned in the preceding text, the diverse results could be ascribed to the ambiguity inherent to the experimental paradigm using an artificial activation of synaptic inputs that must have consequently involved many factors affecting cell activities in a highly complex neuronal circuit. In actual information processing, it is postulated that subtle activation of specific synaptic inputs in a spatial frame (Moser 1996) and probably in a specific time sequence of activation (Christie and Abraham 1992; Otani and


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