Hippocampus Retains the Periodicity of Gamma Stimulation In Vivo

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¹A. I. Virtanen Institute and ²Department of Computer Science and Applied Mathematics, University of Kuopio, FIN-70211 Kuopio, Finland; and ³Department of Psychology, University of Connecticut, Storrs, Connecticut 06268

Received 23 July 2002; accepted in final form 24 July 2002

Mikkonen, J. E., T. Grönfors, J. J. Chrobak, and M. Penttonen. Hippocampus retains the periodicity of gamma stimulation in vivo. J Neurophysiol 88: 2349–2354, 2002; 10.1152/jn.00591.2002. Several behavioral state dependent oscillatory rhythms have been identified in the brain. Of these neuronal rhythms, gamma (20–70 Hz) oscillations are prominent in the activated brain and are associated with various behavioral functions ranging from sensory binding to memory. Hippocampal gamma oscillations represent a widely studied band of frequencies co-occurring with information acquisition. However, induction of specific gamma frequencies within the hippocampal neuronal network has not been satisfactorily established. Using both in vivo intracellular and extracellular recordings from anesthetized rats, we show that hippocampal CA1 pyramidal cells can discharge at frequencies determined by the preceding gamma stimulation, provided that the gamma is introduced in theta cycles, as occurs in vivo. The dynamic short-term alterations in the oscillatory discharge described in this paper may serve as a coding mechanism in cortical neuronal networks.

INTRODUCTION

Hippocampus exhibits two distinct operational states defined by specific oscillatory rhythms. The “unaroused” hippocampus exhibits bursts of high-frequency (70–200 Hz) ripples interspersed by lower beta (12–20 Hz) and delta (1–3 Hz) frequency waves, whereas the “aroused” hippocampus exhibits gamma (20–70 Hz) frequencies nesting within the theta (3–12 Hz) rhythm (Bragin et al. 1995; Chrobak and Buzsaki 1998b; Traub et al. 1996). Theta-modulated gamma occurs during periods of focused attention, exploratory movement, and rapid-eye-movement sleep, thus coinciding with flow of sensory-dependent patterns of neural activity into the hippocampus from the neocortex. Thus it is likely that theta-modulated gamma relates to a period of “information acquisition” within hippocampal circuits (Chrobak and Buzsaki 1998a).

Hippocampal gamma oscillations reflect the underlying intrinsic interneuronal network rhythm of interneuron-interneuron connections, which are stabilized by excitatory connections (Whittington et al. 2000). The interneuronal network gamma oscillation appears to create and coordinate time windows for synchronized firing within networks of pyramidal cells (Buzsaki and Chrobak 1995; Penttonen et al. 1998; Whittington et al. 2000). Although gamma frequency time frames (14–50 ms) have been linked to hippocampal loop times (Pare and Llinás 1995) and delay lines (Bi and Poo 1998), the presence of prominent gamma oscillations in hippocampal slices (Doheny et al. 2000; Whittington et al. 1997) and in local hippocampal ensembles in vivo suggest that at least part of the gamma oscillation is generated in situ. Pyramidal cell membrane depolarization levels (Penttonen et al. 1998), time constants (Volgushev et al. 1998), and ephaptic field effects (Bracci et al. 1999; Whittington et al. 2001) have been proposed as local generators of gamma oscillations at various frequencies. In addition, the frequency of the interneuronal network gamma oscillations has been shown to depend on GABA_A decay time constants (Olypher 1998; Traub et al. 1998; Wang and Buzsaki 1996; Whittington et al. 1995). In the present work, we recorded, both extracellularly and intracellularly, rat CA1 pyramidal neurons in vivo and examined their response to contralateral fimbria fornix stimulation at natural gamma nested theta frequency patterns. Our results show that CA1 pyramidal cells can retain the gamma pattern induced by gamma/theta-patterned fornix fornix stimulation.

METHODS

In vivo methodology

Experiments were conducted on 25 Kuopio Wistar rats (250–350 g) anesthetized with 1.1–1.4 g/kg urethan. The methods used in the experiments have been approved by the Provincial Government of Eastern Finland (Approval No. 99-61). The animal was placed in a stereotaxic instrument (Kopf series 962), the scalp was removed, and small bone windows were drilled above the target structures (Fig. 1). A pair of stainless steel wires (100 μm diam) with 0.2- to 0.4-mm tip separation was placed in the fimbria fornix [anteroposterior (AP), –1.3 mm from bregma; lateral (L), +1.0 mm from midline; ventral (V), –4.0 mm from cortical brain surface] to stimulate commissural afferents to the contralateral CA3 region. The intensity of the 0.2-ms electrical pulse stimulation (Master6 pulse generator and Iso-flex stimulus isolator, AMPI, Jerusalem, Israel) was twice the threshold (T) capable of inducing compound action potentials (CAPs) in more than two consecutive trials. The stimulation intensity was between 140 and 300 μA. In six experiments, higher stimulation intensities (3T and 4T) were tested to determine the possible intensity-related alterations in induced CAP frequencies. Micropipettes for intracellular recordings (R = 80–120 MΩ) were pulled from 1-mm filimented quartz capillary glass (P2000 Sutter Instruments, Novato, CA) and filled with 3 M potassium acetate, whereas single tungsten wire (60 μm in diameter) was used for extracellular field potential recordings. Vertical positioning to the CA1 pyramidal layer for the extracellular (25 animals; AP, –3.6; L, –4.0 at 30° angle) and intracellular (5 animals; AP, –3.6; L, –2.2) recording electrodes were estimated from the polarity of the field response, the shape, and firing patterns.
We constructed an in vivo pattern-resembling stimulation protocol using naturally co-occurring gamma and theta frequencies (Fig. 1). There is considerable variance in the frequency range definitions of different frequency bands in the literature. In our experiment, we selected the frequency band values that, in our opinion, most accurately describe the representative frequency bands decelerated by urethane anesthesia (Penttonen et al. 1998; Ylimen et al. 1995a). Each stimulation epoch had a total of 64 pulses that were spaced so that four gamma pulses (30–60 Hz) were embedded in a theta cycle that was repeated four times at 3- to 7.5-Hz frequencies. This 16-pulse gamma/theta pattern was then repeated a maximum of four times at 0.3–0.5 Hz (see Figs. 1 and 3). In an individual stimulation epoch, gamma and theta frequencies were kept constant. The stimulation frequencies were varied across stimulation epochs so that each animal received at least three different gamma frequencies, and each of them twice, from lower to higher to lower frequency (e.g., 30-40-50-40 Hz) or vice versa (50-40-30-40-50 Hz). Stimulation theta frequency was constant during such gamma frequency manipulation. The stimulation epochs were repeated at 3- to 10-min intervals for 1 h, after which the animal was left unstimulated for 30–60 min. Each animal experienced two to three 1-h stimulation sessions. The final recordings from 10 animals involved prolonged 10- to 20-s stimulations with an increased number of stimulation pulses. These stimulations had an increased number of cycles (6–8) in one or more of the stimulation time frames, or used a reduced protocol with only 3- to 5-s gamma stimulations or 5- to 10-s theta modulated gamma stimulations.

The intracellular signal was 10-fold amplified using an Axoclamp 2B amplifier (Axon Instruments, Foster City, CA), then further fourfold amplified with a Cyberamp380 (Axon Instruments). The EEG signal was 1000-fold amplified using a custom-made amplifier. Thereafter, both signals were low-pass filtered at 6 kHz (Cyberamp380, Axon Instruments) and finally sampled with 16-bit precision at 10 kHz (Digidata 1320A; Axon Instruments). The data were stored on a computer hard disk using Axoscope 8.0 data-acquisition software (Axon Instruments).

Analyses

The extracellular signal was digitally low-pass filtered [finite impulse response (FIR) filter] at 1 kHz and subjected to an additional high-pass FIR filtering at 100 Hz to distinguish CAPs. The selection of events was performed using filtered data superimposed on unfiltered data to ensure that filtering artifacts would not contaminate the analysis. Large-amplitude (over ±200 μV) oscillatory activity was considered CAP-like and was accepted into the analyses. The original 10-kHz data were additionally high-pass filtered at 200 Hz to identify stimulation artifacts. Because we used FIR filtering to prevent phase distortions, we could accurately combine the two differently filtered data to separate stimulation time-locked evoked CAPs from induced data that were not temporally bound to stimulation. The first CAP occurred in a time window of 7–12 ms after a stimulation pulse and was considered evoked, whereas later CAPs of up to 2 s were counted as induced. Previous studies have indicated an approximate 3-s time frame between stimulation induced burst- and epilepsy-like discharges (Gluckman et al. 2001). In addition, tetanically induced gamma oscillations do not extend beyond 1.5 s. Consequently, we discarded events occurring later than 2 s after the stimulation as epileptiform. In the intracellular recordings, only neurons with overshooting APs and resting membrane potentials below −55 mV were included in the analysis. The intracellular APs were classified accordingly.

The instantaneous frequency was calculated from the interval between the negative (extracellular) or positive (intracellular) peaks of two consecutive induced CAPs or APs, respectively. The interspike interval data were then divided into gamma stimulation frequency groups. Data analysis consisted of computations of autocorrelations for each group and inter-group analysis using bivariate correlation analysis and one-way ANOVA with a Tukey test for pairwise comparisons within the gamma band CAPs.

RESULTS

In this experiment, extracellular and intracellular membrane potential changes and APs of the hippocampal CA1 cells were
recorded in response to in vivo patterned fimbria fornix stimulation epochs of embedded gamma, theta, and slow frequencies (Figs. 1–3). We shall refer to this stimulation paradigm as gamma/theta-patterned. Bragin et al. (1995) have shown that each theta cycle can contain approximately 10 gamma cycles. To create such a temporal structure, we embedded four gamma stimulation pulses into a theta cycle. In other words, we created an in vivo resembling situation where four stimulation gamma cycles and six “empty” gamma cycles constituted each theta cycle. The vertical arrows below recording traces in Figs. 2 and 3 demonstrate the groups of four stimulation gamma cycles and subsequent empty cycles. The stimulation part of the design corresponds to the depolarized phase of natural hippocampal theta oscillation when the firing of the neurons most likely occurs. Stimulatory theta frequency was similarly embedded into a slow 0.5-Hz frequency as depicted below the schematic illustration of hippocampus in Fig. 1. The slow frequency was implemented to reduce pulse numbers. In addition, the slow rhythm further emphasized the in vivo nature of the stimulations because the hippocampal gamma oscillations have been shown to coincide with 0.5-Hz frequencies (Penttonen et al. 1999). In an epoch, each stimulation frequency band, gamma, theta, and slow, was repeated four times. Thus a total of 64 stimulation pulses were applied at a given gamma frequency but interspersed with nonstimulatory phases corresponding to theta and slow frequencies. In total, 1,237 stimulation epochs from 371 10-min sessions were recorded. Neither intensity nor frequency of the stimulation was varied during the course of the 64-pulse epoch. Although fimbria fornix has connections to both CA1 and CA3 (Fig. 1), we did not observe direct stimulation effects in the CA1 area indicated as antidiromic CAPs or APs 3–5 ms after the stimulation. However, antidiromic CAPs (3–5 ms after the stimulation, and 4–7 ms before CA1 CAPs) were evident in the electrodes lowered into the CA3 area of the hippocampus (data not shown). The recordings were conducted in the gamma frequency order of low-high-low or high-low-high and randomly to reduce systematic error. The patterned stimulation-evoked CAPs were recorded from 25 animals. Poststimulatory, induced responses were evident in 85% (317) of the 10-min recording sessions. In general, the patterned gamma/theta stimulation resulted in one or two induced CAPs retaining the periodicity of the stimulation gamma frequency. These CAPs appeared after the final stimulation pulse in a gamma series, delayed by the periodicity of the stimulation frequency. Additionally, in every animal, at least one recording exhibited a short burst of CAPs or prominent oscillations at or close to 0 (±2.5 ms) the gamma stimulation interval (Figs. 3E and 2A; combining examples from 6 animals stimulated at 40- or 60-Hz gamma/theta pattern). Experiments with higher than twice the threshold stimulations yielded similar results albeit with more frequently occurring double CAPs at 200 Hz and prolonged attenuation of the unit activity.

Retention of the stimulation gamma periodicity was additionally present in 15 of the 20 intracellular recordings (Figs. 3 and 2B; 4 individual pyramidal cells stimulated with 40- and 60-Hz gamma/theta patterns). The five unresponsive cells may have been injured during the insertion of the electrode because, despite having overshooting APs, all of these cells were lost during the first 15 min of recording. Interestingly, the amplitude of the intracellular APs in the cells responding to the stimulation declined rapidly in the course of the gamma stimulations (Fig. 3, A–C). The reduction in the AP amplitude coincided with the permanent depolarization (indicated by thick arrows in the Fig. 3, B and C) of the cell membrane above the firing threshold of approximately −56 mV. The AP amplitudes recovered after the cell returned to normal membrane potential or after it was hyperpolarized by current injection (data not shown). In general, the AP frequencies and burst durations were similar to the CAP responses described in the preceding text.

Altering only the stimulatory theta frequency did not significantly affect induced CAP or AP frequencies. Rather, the induced gamma frequency was determined by the gamma component of the stimulation (Fig. 3, D and E), and the induced theta frequency was 4.4 ± 1.6 (SE) Hz irrespective of the stimulatory theta or gamma frequencies. However, in experiments excluding the theta frequency, no retention of the gamma stimulation frequency was evident. Therefore the underlying stimulatory theta frequency was necessary, at least for the detection of retention of the stimulatory gamma frequency, although it did not affect the frequency of that retention.

Prolonged (10–20 s) stimulation epochs with an increased pulse number were tested in 10 animals. In eight of these animals, the duration of CAP firing increased to tens of sec-
onds, but the periodicity of the stimulation was no longer recognized. Even though there was a short-term retention of the stimulation periodicity evident in the CAPs during the initial phase of the prolonged stimulation, the prolonged responses declined to the beta frequency band (12–20 Hz) with interruptions at 1.4–2 Hz (Fig. 3F). The findings on beta transition are in accordance with previous experiments (Pare and Llinas 1995; Pare et al. 1992). It has been suggested that this decline from gamma to beta frequencies may result from prolonged recovery from inhibition (Bracci et al. 1999; Traub et al. 1999) or habituation (Whittington et al. 2000). By limiting the number of pulse sequences to four and additionally limiting the time frame of induced CAPs accepted into the analysis to 2 s, we eliminated the longer beta frequency afterdischarges from the analysis. In addition, tetanically induced gamma oscillations in CA1 in vitro have been shown to persist for up to 1.5 s and are in the same range as the in vivo gamma oscillations induced by visual stimulation or those occurring spontaneously in monkey sensorimotor cortex (Traub et al. 1999). Therefore our CAP results display neuronal behavior that can be considered to fall within the limits of normal hippocampal physiology, although the phenomenon is mainly revealed at the population level.

To study the generality of the preceding results, the post-stimulatory induced CAPs of all the recorded animals were combined into gamma stimulation frequency groups for further analysis. In each group, the autocorrelation function of the data peaked at the stimulation frequency (Fig. 4A) and, as is typical of CA1 pyramidal neurons, at 200 Hz. We wanted to further specify the gamma response and selected only the gamma frequency CAPs to study whether there were additional differences between the gamma stimulation frequency groups. The results from 1-way ANOVA and correlation analysis (Fig. 4E) were similar to the results obtained from the autocorrelation functions. There was a tight correlation between stimulatory and induced rhythms at gamma stimulation frequencies below 60 Hz. The 60-Hz gamma stimulation frequency group, on the other hand, peaked in the autocorrelation function at 60 Hz but showed a remarkably lower gamma frequency mean of 42.8 Hz. This can be explained by the inability of the anesthetized animal to repeat high frequencies (Penttonen et al. 1998) and a subsequent increase in sub-harmonic frequencies since 60 Hz/2 = 45 Hz. Indeed, the decline into sub-harmonic frequencies was more evident in the higher stimulatory gamma frequencies (data not shown). Furthermore, the artificial division


Previous experiments have described precise gamma synchronization in the hippocampal formation (Bracci et al. 1999; Chrobak and Buzsaki 1998a; Fisahn et al. 1998; Penttonen et al. 1998). Here, we have shown that not only are there synchronous gamma frequency ensembles in the hippocampus, but there is a capability to retain a gamma frequency pattern, defined by prior gamma/theta stimulation. This capability was demonstrated as the retention of the gamma periodicity of the in vivo patterned gamma/theta stimulation. The frequency of the underlying induced theta oscillation did not significantly interact with the gamma frequency retention. Furthermore, the induced CAPs were frequency locked into the gamma component of the stimulation, irrespective of the frequency of the underlying theta component of the stimulation. Therefore the gamma/theta patterned fimbria fornix stimulation did not affect the hippocampal theta frequency output in the structures primarily responsible for hippocampal theta activity, the entorhinal cortex (Ylinen et al. 1995b), medial septum (Dragoi et al. 1999), and raphe nucleus (Varga et al. 2002). In addition, CA3 has been identified as an intrahippocampal theta rhythm generator (Buzsaki 2002). In our recordings, CA3 may have influenced theta current generation, but it was unable to modify the frequency of the rhythm. On the other hand, our experiment was designed to reveal gamma frequency related changes in the hippocampus, and within each stimulation epoch we had 16 opportunities for induced retention of gamma frequencies compared with only 4 occasions for induced theta (Fig. 1). Interestingly, however, the theta component of the stimulation was necessary to induce gamma frequency firing.

Millisecond-range variability in the induced oscillation around the stimulatory gamma timing indicates that the mechanism of action is not precise within the sub-millisecond range. Such retention of temporal relations with an in-built degree of variability could be formed by depolarization level dependent resonance of the participating pyramidal neurons (Penttonen et al. 1998). The preservation of the temporal information may not be restricted to the pyramidal cell resonance in the CA1 but may incorporate other parts of the hippocampal formation that supply CA1 with excitatory or inhibitory inputs. The network drive imposed to the CA3 interconnections by the gamma/theta-patterned stimulation could be strong enough to locally override the synaptic suppression. Therefore the induced stimulation frequency specific rhythm could be retained in the associative CA3 network and transmitted into CA1 after the stimulation. The proposed transfer of the rhythm from CA3 to CA1 has been described in vitro (Fisahn et al. 1998) and in vivo at a higher frequency band (Csicsvari et al. 2000).

The interneuronal network can operate at variable gamma frequencies depending on GABA A decay time and network properties at a given state (Whittington et al. 1995). Our gamma/theta-patterned stimulation could entrain the interneuronal network to oscillate at stimulatory gamma frequencies. If this oscillation persists beyond the end of the stimulation, the entrained set of interneurons could retain the frequency by rhythmic inhibition (McBain and Fisahn 2001; Wang and Buzsaki 1996). Because the intrinsic firing of the hippocampal interneurons occurs at gamma frequencies, this depolarization-dependent interneuronal gamma frequency modulation would represent a cost-efficient way of achieving frequency retention in the hippocampus. The initial sub-harmonic CAPs evident in Fig. 2, right, could result from increased interneuronal activity hyperpolarizing the pyramidal cells sufficiently to omit the first cycle of 60-Hz induced rhythm. In addition, the reduced level of glutamate-mediated excitation under urethan anesthesia (Heltovics et al. 1995) further emphasizes the role of the interneuronal network. The issue of interneuron actions in vivo should be further examined in future experiments.

The variation in the autocorrelation function suggests that not all the pyramidal cells in the CA1 area are driven into the rhythm, but the stimulation frequency is retained in groups of cells with possibly favorable intrinsic resonance or appropriate interneuronal connections (Wang and Buzsaki 1996). However, we could produce stimulation frequency-specific population responses at varying gamma frequencies during a single recording. This indicates that the same pyramidal cells were receptive to different gamma frequencies. Furthermore, the intracellular recordings demonstrated that individual cells were responding accurately to several stimulatory gamma frequencies. Therefore we are not selecting neurons responsive to specific frequencies but rather tuning the peak frequency of the network. The hypothesis is further supported by the fact that CAPs retaining the periodicity of the stimulation frequency were sporadic. This is in accordance with previous experiments where pyramidal cells have been shown to fire in approxi-
We have demonstrated a mechanism for short-term storage of the temporal structure of hippocampal oscillations. In our experiment, the CA1 hippocampal network retained the gamma periodicity of the in vivo-patterned gamma/theta frequency stimulation. We believe this preservation of temporal relations evolves from the interplay between the underlying hippocampal interneuronal network and the associative reverberant connections between CA3 pyramidal cells. The intrinsic properties or resonance of the interneuronal network were able to repeat the periodicity of the gamma stimulation, whereas the pyramidal cells recovering from inhibition again excited the interneurons, thus reinforcing the induced rhythm. Because the fimbria fornix stimulation had reduced the synaptic noise, this phenomenon was visible in the CA1 network. Given that the beginning of the stimulation temporarily shuts the nerve cells (Fig. 3, A and B), it is possible that this initial CAP firing is a result of the simultaneous recovery of the majority of the pyramidal cells. Therefore the early uncorrelated spiking (spikes not entrained to the stimulation frequency, Fig. 3F, 1) could be due to the excitatory recurrent connections overriding the inhibition (Bragin et al. 1995). In contrast, later subharmonic action potentials most likely result from increased inhibitory interneuronal activation forcing the pyramidal cell membrane potential below the firing threshold during one or two consecutive gamma cycles (Wang and Buzsáki 1996). We conclude that the in vivo patterned gamma/theta stimulation induces short-term self-sustaining alterations in the temporal properties of the hippocampal CA3-CA1 network gamma oscillations. This dynamic retention of gamma timing could link the spatially synchronized cell assemblies into a single temporal domain in the hippocampus. Thus the short-term temporal specificity would combine different units of the hippocampal neuronal network into a functional ensemble for effective information coding.

We thank K. Kaila and H. Tanila for comments on earlier versions of this paper.

This work was supported by the Finnish Ministry of Education (J. E. Mikkonen).

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