

Orthogonal arrangement of rhythm generating microcircuits in the hippocampus

Tengis Gloveli*[†], Tamar Dugladze*, Horacio G. Rotstein[‡], Roger D. Traub[§], Hannah Monyer[¶], Uwe Heinemann*, Miles A. Whittington^{||} and Nancy J. Kopell[‡]

*Institute of Neurophysiology, Charité – Universitätsmedizin Berlin, Tucholskystr. 2, 10117 Berlin, Germany

[‡]Department of Mathematics & Center for Biodynamics, Boston University, 111 Cummington Street, Boston, MA, 02215, USA

[§]Department of Physiology & Pharmacology, SUNY Health Science Center, Brooklyn, New York 11203, USA

[¶]Univ. Hospital Neurol., Dept. Clin. Neurobiol. Im Neuenheimerfeld 364 Heidelberg D-69120, Germany

^{||}School of Neurology, Neurobiology & Psychiatry, University of Newcastle, Newcastle upon Tyne, NE2 4HH, UK

[†]To whom correspondence should be addressed at:

Institute of Neurophysiology
Charité – Universitätsmedizin Berlin
Tucholskystr. 2, 10117 Berlin
Germany
Phone: ++49 30 450 528 214
Fax: ++49 30 450 528 962
e-mail: tengis.gloveli@charite.de

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Abbreviations: O-LM, oriens lacunosum-moleculare; KA, kainate; FP, field potential

As a structure involved in learning and memory, the hippocampus functions as a network. The functional differentiation along the longitudinal axis of the hippocampus is poorly demarcated in comparison to the transverse axis. Using patch clamp recordings in conjunction with posthoc anatomy we have examined the pattern of connectivity and the functional differentiation along the long axis of the hippocampus. Here we provide anatomical and physiological evidence that the prominent rhythmic network activities of the hippocampus, the behavior-specific gamma and theta oscillations, are seen predominantly along the transverse and longitudinal axes respectively. This orthogonal relationship is the result of the axonal field trajectories and the consequential interaction of the principal cells and major interneuron subtypes involved in generating each rhythm. Thus, the axonal arborization patterns of hippocampal inhibitory cells may represent a structural framework for the spatiotemporal distribution of activity observed within the hippocampus.

The hippocampus is required for the encoding of new information and for retrieving information shortly after acquisition (1). Spatial information is coded at theta frequencies (2) by clusters of neurons segmentally distributed along the longitudinal axis of the hippocampus (3). Gamma oscillations are also generated by the hippocampus nested within these theta rhythms and are thought to be involved in transient neuronal assembly formation (4), information transmission and storage (5).

As previous observation has shown, the hippocampus is made up of multiple lamellae organized in parallel across the long axis, in which each lamella contains a functionally independent transverse circuit (6). The well defined connections between subregions along the transverse axis facilitates the investigation of crucial neurobiological processes such as network oscillatory activity in the in vitro preparation. Although the functionally segregated subregions of the long axis of the hippocampal formation may cooperate with each other during memory formation (for review see 7), morphological evidence about their interconnectivity is sparse. While CA3 neurons connect to other CA3 neurons (8, 9), and excitation of interneurons occurs along the longitudinal axis (10), the involvement of hippocampal interneurons in the functional circuit along the longitudinal axis of hippocampus is still unclear.

Interneurons serve a wide variety of functions in the brain amongst them generating and maintaining local or large scale coherent activity (for review see 11). They also have a particularly pivotal role in driving inhibition-based rhythms, such as gamma and theta frequency network oscillations (12-16). Theta rhythms are seen as a major operational mode of the hippocampus (for review see 17), and clusters of neurons that encode information required to perform spatial and nonspatial short-term memory tasks during theta rhythms are

distributed in distinct segments along the length of the hippocampus (3). The circuit analysis of functional neuronal networks along the long axis may therefore provide a unique insight into the role played by the hippocampus in cortical information processing and memory formation.

Using whole-cell patch-clamp recordings in conjunction with posthoc anatomy we investigated the three-dimensional axonal arborisation patterns of different hippocampal CA3 interneurons. In contrast to all other recorded CA3 interneurons single distal dendrite targeting oriens lacunosum-moleculare (O-LM) cells arborized most extensively in the longitudinal axis as revealed by 3D reconstruction of these cells. Therefore, the axon plexus of this cell type innervates distal dendrites of pyramidal CA3 cells that are distributed in distinct segments along the length of the hippocampus. The experimental (electrophysiological) results and computational models suggest that these morphological features may underlie the observed orthogonal arrangement of rhythm generating microcircuits in the hippocampus.

Material and Methods

Slice preparation. Transverse, coronal and longitudinal slices (450 μm thickness) obtained from ventral, middle and whole hippocampus respectively (see inset Ai in Figs.1-3) were prepared from C57 mice (P 18-25) in accordance with the UK Animals Scientific Procedures Act. Perisomatic interneurons were studied using transgenic mice that expressed enhanced fluorescent protein under the control of the parvalbumin promoter. Slices were incubated at room temperature for at least 1 h in a holding chamber and then transferred to the recording chamber. The solution used during incubation and recording contained (in mM): NaCl, 126;

KCl, 3; NaH₂PO₄, 1.25; CaCl₂, 2; MgSO₄, 2; NaHCO₃, 24; glucose, 10, saturated with 95% O₂ and 5% CO₂.

Extracellular field recordings. Experiments were performed in an interface (at 34⁰C, dual channel field potential (FP) recordings) or in a submerged-type (at 29⁰C, patch clamp recordings) chamber. Kainate (KA) was applied either by bath (400 nM, in the interface chamber) or by pressure pulse (1 mM, 60 psi, duration 10-60 ms, in an submerged chamber) to get a network oscillations. We employed a pharmacological gamma-induction paradigm to study network oscillations in submerged slices (16). Ejected volume was generally correlated with oscillatory peak power and duration, whereas the peak frequency remained constant for any given slice. Single or dual (up to 8 recording sites separated by ~ 100 μm) channel FP recordings and pressure application of KA were done in the stratum radiatum of the CA3 using patch pipettes. FPs from dual channel were low pass filtered at 1 kHz with a custom-made Bessel filter, digitized at 4 kHz using an ITC-16 A/D board (Instrutech, USA) and analysed using WinTida software (Heka, Lambrecht/Pfalz, Germany). Oscillatory peak frequency was determined by averaging several consecutive Fourier transforms, contained within a 2-30 s epoch. The spectral analysis of the FP recordings was conducted off-line using Matlab (Mathworks, Natick, MA). To exclude noise, the signal was digitally filtered using an infinite impulse response notch filter at 50 Hz. The power spectral density, cross-spectral density, phases and coherence spectra were computed for all signals and signal pairs. The power spectra were calculated by Fourier Transformation. Coherence (between 0 and 1) estimates the degree to which phases at the frequency of interest are dispersed. To compare the relation between frequencies in one recording site, bispectrum and bicoherence was computed (18). Bispectral analysis quantifies the degree of phase coupling between the frequencies of a signal. Bicoherence provides information about the phase-coupling at different frequencies independent of the amplitude obtainable from the power spectrum (see

supplementary material). For further analysis parameters for both theta (4-12 Hz) and gamma (25-45 Hz) bands (maximal and mean values) were extracted from the spectral- and bispectral/bicoherence analysis. The Student's *t*-test was used for statistical comparisons, differences were considered statistically significant if $p < 0.05$. Average values are expressed as the mean \pm SEM.

Whole-cell recordings. Concomitant with the extracellular FP whole-cell patch-clamp recordings in current clamp mode were obtained from stratum oriens/pyramidal interneurons and pyramidal cells of area CA3 visualized by infrared differential interference contrast videomicroscopy (Zeiss Axioscope). Whole-cell recording pipettes (3-5 M Ω) were filled with a solution containing (in mM): K-gluconate, 135; KCl, 5; ATP-Mg, 2; GTP-Na, 0.3; HEPES, 10; plus biocytin, 0.5% (pH 7.3 and 280 mOsm). An Axopatch 1D or Multiclamp 700B amplifier (Axon Instruments) was used for current-clamp recordings. The holding potential was -60 mV unless indicated otherwise. The seal resistance before establishing whole-cell mode was ≥ 2 G Ω . The series resistance (range 12-18 M Ω) was monitored repeatedly during the experiment by measuring the amplitude of the capacitive current in response to a -10 mV pulse.

Signals were filtered at 5 kHz (whole-cell recording) or 0.002-1 kHz (extracellular FP recording in submerged conditions) with an external 8-pole Bessel filter (Applegarth Electronics, Oxford, UK) and digitized at 10 kHz using an ITC-16 A/D board.

Anatomical identification of interneurons. Slices with a biocytin-filled interneuron were processed as have been described (16). Subsequently, the cells were reconstructed with the aid of a NeuroLucida 3D reconstruction system. The absolute extension of axonal collaterals within and between adjacent axonal clusters was measured using a NeuroExplorer program (NeuroLucida).

Modeling and simulations. The minimal network model studied in this paper consists of two O-LM (O) cells, two fast spiking interneurons (I) and a population of pyramidal cells represented by one somatic (E) and one dendritic (D) compartment which are electrically coupled. The O- and I- cells are modeled as a single compartment. All cells are modeled using the Hodgkin-Huxley (HH) formalism. In what follows we will refer to the somatic and dendritic compartments as E- and D- cells respectively. The I-, E- and D- cells are modeled using the standard HH (19-21) currents (transient sodium, delayed rectifier potassium and leak), using the same equations as in (22). The I-, E-, and D- cells differ only in the applied bias current; i.e. in their natural frequency. The O-cells have, in addition to the standard HH currents, an A-current and a hyperpolarization-activated (h-) current. We use a model adapted from a multicompartmental model proposed in (23). Since the spiking E-cell represents the output of all the pyramids, we use the subthreshold behavior of the D-cell to compare to measured variations in voltage of non-spiking E-cells (see Results).

In the network the cells are connected as schematically shown in Fig. 5D. The decay time of the I IPSPs are chosen to be 10 ms (24). The IPSPs originating from the O-cells onto pyramidal cells was taken to be 20 ms (25). The decay time of the EPSPs onto O- and I- cells was taken to be 4 ms. In experiments gap junctions have been shown to be important for generating theta and gamma rhythms. However, their proposed role in propagating excitatory and inhibitory signals in large networks (26) is not directly applicable to the small-scale models used in the present study. So we are not including them in our simulations. The simulations have been performed using the XPP package (cvscode method, tolerance= 10^{-5} , dt = 0.02 (27)

The purpose of the simulations is to show that the switches in rhythms associated with changes in orientation of the slice can be replicated in a simple network model in which the change of orientation is reflected in the conductances of the synapses. We model the

transverse “slice” as having a larger E-X conductance (where X represents O or I) than the longitudinal “slice” to reflect the predominantly transverse trajectory of E-cell axon collaterals. The O-Z conductance (where Z is I or E) is larger in the longitudinal “slice” than in the transverse “slice” to reflect the predominantly longitudinal distribution of O-LM axon collaterals (see Fig. 4B,C). The coronal “slices” have values that are in between. All other parameters are unchanged among simulations (see Appendix in Supplementary Material). In the absence of synaptic connections each O-cells fires at about 10 Hz, each I-cell fires at about 30 Hz, the E-cell (population) fires at 65 Hz and the dendritic compartment (D) is silent.

We note that we are using one E-cell to represent the population, whereas, *in vitro*, the E-cells fire very sparsely. Therefore, we cannot compare the somatic traces in the experiment with the behavior of the somatic compartment (E) in the model. However, the “subthreshold” behavior seen in the experimental traces shown in Fig. 5A-C is captured in the non-spiking dendritic compartment (D-cell) of the model. In the experiments, the voltage variations arise from the inhibition from interneurons, which fire when population of pyramids spikes. Since the interneurons are excited by the population of pyramidal cells, we expect the D-cell voltage to co-vary with the somatic variation shown in the experimental panels, but with reversed signs. This is indeed what we see in Fig. 5.

Results

Bath application of KA (400 nM) in hippocampal transverse slices induced gamma frequency field oscillations in area CA3 (mean frequency 36.7 ± 2.0 Hz, $n=7$, Fig. 1A-C, see also (28)). To characterize the spatial distribution and synchronization of gamma activity across the horizontal extent of area CA3 dual extracellular recordings were performed in the present

study. Recordings were conducted with two FP electrodes, one serving as a reference whilst the second electrode was moved from a closer site ($\sim 100 \mu\text{m}$) to 6 consecutively more distant sites in $\sim 100 \mu\text{m}$ steps (Fig. 1A). When a close distance (100-200 μm) separated the electrodes, the coherence of the gamma signals across both was high (~ 0.9). When the separation between the electrodes increased, the coherence of gamma activity decreased substantially (Fig. 1D,E). Since gamma frequency power was variable along the recording sites (Fig. 1A,B) and this may have had an effect on the coherence measurements, a regression analysis was conducted between the cross-spectral amplitude and coherence for each of the signal pairs. No significant linear relationship between these two parameters was found ($p > 0.05$; $n=4$, data not shown) suggesting that changes in coherence are independent of the gamma amplitude. In contrast to coherence, the phase shift of gamma waves in the transverse slices was very low (within $\pm 10^\circ$) and remained unchanged in all distances measured (Fig. 1F).

To characterize the dynamics of network activity along the longitudinal axis, similar spectral analysis was undertaken in longitudinal hippocampal slices (Fig. 2A-F). In contrast to transverse slices, the dominant field network activity in the longitudinal slices was in the theta frequency range (Fig. 2Aii, B). This activity remained highly coherent along all distances measured (Fig. 2E). Similarly, low phase shift of theta waves, observed in the longitudinal axis remain unchanged across all recording sites (Fig. 2F).

Selective manifestation of theta and gamma frequency activity, in the longitudinal and transverse axes respectively, suggested an orthogonal relationship between the local circuits responsible for the generation of the two rhythms. We therefore tested the possibility that intermediate orientations of hippocampal slice may contain sufficient local circuitry to support both gamma and theta rhythms concurrently. Using coronal slice preparations in which pseudotransverse sections of the hippocampus can be seen extended in the longitudinal

plane by 30 – 60° we investigated KA-induced network activity (Fig. 3A). Both theta and gamma population rhythms were manifest in this preparation with power spectra and cross spectra demonstrating two clear peaks, one in each frequency band (Fig. 3B,C). The coherence values for gamma band reduced strongly as the interelectrode distance was increased, while the theta coherence values changed significantly less (Fig. 3D,E). In contrast, phase coupling showed little variance ($<25^{\circ}$) in both frequency bands (Fig. 3F). To investigate the correlation (phase coupling) between FP frequency components in the coronal slice, we performed bispectral and bicoherence analyses. When bispectral analysis was used, we found clear peaks in the theta and gamma frequency band suggesting the high coupling powers in the same (theta/gamma) frequency (Fig. 3G). A further peak in the bispectral analysis indicates strong coupling tendency also between theta and gamma frequencies (Fig. 3G). Similarly, bicoherence analysis showed a high level of phase coupling between these two frequencies across all recording distances (Fig. 3H). This indicates a strong functional interaction between the generators of theta and gamma frequency oscillations in slices with intermediate orientation.

To elucidate the cellular and synaptic mechanisms underlying field network activity in the transverse and longitudinal axes of the hippocampus, we further analyzed the pattern of connectivity and the functional differentiation along the long axis of the hippocampus. In particular we focused on the morphological and physiological properties of O-LM interneurons, shown previously to generate theta rhythms as part of a local circuit (14). Using whole-cell patch-clamp recordings in conjunction with posthoc anatomy we investigated the three-dimensional axonal arborisation patterns of different hippocampal CA3 interneurons recorded in transverse slices. Parvalbumin containing perisomatic targeting interneurons, along with trilaminar and bistratified cells (16), show a clear tendency to arborize widely within the transverse plane (the maximal extensions of the projections of perisomatic

targeting cells in transverse and longitudinal planes were: $727.5 \pm 54.4 \mu\text{m}$ and $310.0 \pm 18.3 \mu\text{m}$, $n=5$, data not shown). However, distal dendrite targeting O-LM cells arborized most extensively in the longitudinal plane (the maximal axonal spread was $223.7 \pm 12.2 \mu\text{m}$ and $430.3 \pm 4.4 \mu\text{m}$ in transverse and longitudinal directions, respectively, $n=6$) forming 2 (4/6 cells) or 3 (2/6 cells) clusters in this direction (Fig.4B-C). The axon branches of O-LM interneurons ($n=6$) in the stratum lacunosum-moleculare tended to cluster in narrow bands both in the plane containing the cell soma (cluster width $125.9 \pm 11.2 \mu\text{m}$, $n=6$) and also further along the longitudinal direction (width $118.7 \pm 10.9 \mu\text{m}$, $n=6$). The longitudinal intercluster interval (between adjacent edges) was $92.2 \pm 17.1 \mu\text{m}$ ($n=6$). Therefore, the axon plexus of this cell type innervates distal dendrites of pyramidal CA3 cells that are distributed in distinct segments along the length of the hippocampus. In contrast to axonal arborizations in stratum lacunosum-moleculare, the axon branches of O-LM cells in stratum oriens form a single cluster at the level of their somata (Fig. 4C).

We further examined the intrinsic and synaptic properties of O-LM interneurons in transversal hippocampal slices. During depolarizing current injection O-LM cells show subthreshold membrane potential oscillations in the theta frequency range between 4.9 Hz and 11.9 Hz (mean frequency – $8.1 \pm 1.8 \text{ Hz}$, $n=7$, data not shown). In addition, during KA-induced field gamma oscillations these interneurons discharged with firing rates in the theta frequency range ($8.2 \pm 2.1 \text{ Hz}$, $n=9$, Fig. 4D, see also 16). The gamma frequency field oscillation was no longer present in the longitudinal slices. Instead, a low frequency oscillation in the theta frequency range (mean frequency $8.1 \pm 2.0 \text{ Hz}$, $n=14$) was the dominant activity in this preparation. Similar to the transverse slices, O-LM interneurons continued to fire at theta frequency during KA application in the longitudinal slices (Fig. 4E,ii,iii). However, significant decreases in the frequency and amplitude of KA-induced excitatory postsynaptic potentials (EPSPs) were observed in comparison with the transverse slice (Fig. 4D-E,ii,iii). O-

LM cells are likely to provide a theta-frequency patterned output to distal dendritic regions of pyramidal cells along the long axis of hippocampus.

Simulations. The results of our simulations are given in Fig. 5. Fig. 5A (Model) corresponds to the transverse slice, using parameter set I of the Appendix. Here the E- and I- cells generate a synchronized pattern at gamma frequencies, while the O-cells fire at theta. The $O \rightarrow I, D$ connections prevent the E-I pattern from being completely periodic; the bottom panel shows that the gamma field is slightly modulated at theta frequencies. In the experiments application of KA resulted in IPSPs at gamma frequency in pyramidal cells (Fig. 5A, Experiment). Fig. 5B (Model) corresponds to the longitudinal slice, using parameter set II of the Appendix. Here II cells fire at theta frequencies, though not in a synchronous manner, and the FP shows a clear theta rhythm. Experimental recordings from pyramidal cells in longitudinal slices demonstrate rhythmic trains of IPSPs at theta frequency (Fig. 5B, Experiment). Fig. 5C (Model) corresponds to the coronal slice, using parameter set III of the Appendix. Here the E- and I- cells generate a synchronous gamma pattern with periodic interruptions at theta frequencies. These interruptions are stronger than in Fig. 5A, due to the greater strength of the $O \rightarrow I, D$ connections. The FP now displays a clear gamma rhythm modulated at theta frequencies. Whole cell patch-clamp recordings from pyramidal cells show the gamma frequency IPSPs modulated at theta frequency (Fig. 5C, Experiment).

Comparing Figs 5A, 5B and 5C, it is apparent that an increase in I-cell frequency from theta to gamma does not necessarily suppress the O-cells firing in our “minimal network” model (Fig. 5D). Due to the presence of an h-current, the inhibitory interaction between fast spiking interneurons and O-LM cells has a dual inhibitory and excitatory effect that helps maintain the rhythm (22). For higher I-cell frequencies and/or larger values of the maximal synaptic conductances the O-cells are effectively suppressed. The value of the frequency at which this suppression occurs is model dependent (22).

Discussion

Our experiments and model indicate an orthogonal arrangement of gamma and theta rhythm-generating microcircuits in hippocampal area CA3. Theta frequency oscillations are the dominant network activity along the long axis of the hippocampus interconnected via CA3 pyramidal cells and O-LM interneurons. In contrast, gamma-band oscillations are observed predominantly within the transverse axis of the hippocampus.

Local gamma generating microcircuits have been seen *in vitro* in transverse sections of hippocampus involving both recurrent inhibition (12) and heterogeneous networks of interneurons and principal cells (13, 16). *In vivo* area CA3, particularly subregion CA3a-b, has been suggested to be the principal source of intrahippocampal gamma rhythms (29). These gamma oscillations project to area CA1 along the transverse axis of the hippocampal slice (13, 14), albeit with a relatively steep drop off in coherence even within area CA3 (30, see Fig. 1), suggesting that they may conform functionally to the lamellar organization originally proposed for the hippocampal formation (6).

In contrast, neurons in area CA3c of the hippocampus project in the longitudinal direction (9) and in this study we demonstrate that axonal projections from O-LM interneurons, capable of sustaining a field theta rhythm with tonic excitation (14), also project in this direction. In longitudinal slices the preservation of these projections facilitated the generation of theta rhythms with robust coherence over large distances (see Fig. 2). This pattern of theta coherence has also been reported in the freely moving rat *in vivo* (30, 31). Generally, slower oscillations *in vivo* are spatially more extended (32). The almost complete absence of power in the gamma band in the horizontal section strongly suggests an orthogonal arrangement of the local hippocampal circuits responsible for the generation of the two

rhythms. It should be noted that CA1 O-LM interneurons have been shown to extend up to 800 μm in both transverse and longitudinal planes (33) suggesting that this orthogonal arrangement, as described here, is specific for area CA3.

In the intact hippocampal formation gamma activity is nested within the theta rhythm and is larger during theta-associated behaviours than during immobility (30, 34). Thus we took advantage of the selective orientation possible with hippocampal slices to investigate the interplay between these two rhythms with orientations intermediate between the transverse and horizontal axis. Using coronal sections it was possible to elicit both persistent gamma and theta rhythms. Bispectral and bicoherence analyses of these simultaneously expressed oscillations showed strong coupling in the theta-gamma wave bands. The field recordings still lacked the overt, large amplitude theta waves seen in vivo (see above) but this was not surprising given the absence of medial septum-diagonal band of Broca input (35). However, the phase locking of spatially distributed regions of hippocampus can occur in the absence of this input.

On the basis of morphological, electrophysiological and computational analyses we suggest that at least two factors are responsible for the orthogonal arrangement of rhythms in the hippocampus. Firstly, most pyramidal cells of the CA3 region participate in the strong “gamma-generating” recurrent system in the transverse as opposed to longitudinal axis. The significantly lower amplitude and frequency of interneuron-EPSPs in longitudinal slice than those in the transverse slice during KA induced network activity (see Fig. 4) support this notion. Secondly, “theta-generating” O-LM interneurons show at least 2 fold larger axonal spread in the longitudinal rather than transverse plane. They may therefore provide much stronger (at least by a factor of 2) theta frequency patterned output to the distal parts of the pyramidal cells as well as to the interneurons in the longitudinal plane.

Co-existent gamma and theta rhythms may, therefore, represent a two-dimensional strategy for processing hippocampal inputs. The apparent orthogonal arrangement of theta and gamma generating microcircuits within the hippocampal network may allow activity within functional lamellae, along the classical trisynaptic circuit, to be temporally coordinated at gamma frequencies whereas activity distributed between distinct lamellae (e.g., see 3) is temporally coordinated at theta frequencies.

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Figure legends:

Figure 1. Properties of KA-induced gamma oscillations in transverse hippocampal slices.

Ai, Schematic illustration of location of field electrodes (1-6) along the transverse axis of the hippocampal CA3 region. Ai, inset, 3D sketch of the hippocampus with the cutting planes in transverse direction. Aii, Example of gamma frequency oscillations recorded from the two FP electrodes, one serving as a reference whilst the second electrode was moved from a closer site ($\sim 100 \mu\text{m}$) to 6 consecutively more distant sites in $\sim 100 \mu\text{m}$ steps (as illustrated in Aii). B-D, Power (B) and cross-spectral (C) density plots, and coherence spectra (D) for the gamma activity from all recording sites (data are obtained from 1 min recordings). Peak gamma power for this slice was 33 Hz. E, Mean gamma coherence as a function of interelectrode distance ($n=4$). F, Phase of KA-induced gamma waves in the transverse slices. In contrast to coherence, there was no clear phase shift of gamma waves in these slices.

Figure 2. KA-induced theta-like network oscillations in longitudinal slices.

(Ai) Schematic illustration showing the placement of recording electrodes (1-6) within the area CA3 of the longitudinal hippocampal slice. Ai, inset, 3D sketch of the hippocampus with the cutting planes in longitudinal direction. (Aii) Representative extracellular recordings following bath-applied KA shows a theta-like activity. The oscillatory activity does not substantially change across the long axis of the hippocampus. B-D, Power (B) and cross-spectral (C) density plots, and coherence spectra (D) for the theta-like activity from all recording sites. The power spectrum shows a distinct peak at 8 Hz. E-F, Mean theta coherence and phase in the longitudinal axis as a function of interelectrode distance ($n=7$). The theta-like oscillatory activity showed no clear differences in both coherence (E) and phase (F) for different distance between the recording electrodes (1-2 to 1-9).

Figure 3. Simultaneous expression of gamma and theta frequency oscillations in the coronal slices.

A, Schematic illustration of the coronal slices with the recording sites (i) and the representative FP recordings of the oscillatory activity (ii) following bath applied KA. Ai, inset, 3D sketch of the hippocampus with the cutting planes in coronal direction. The power spectral (B) and cross-spectral (C) density plots demonstrates two clear peaks at theta (8 Hz) and gamma (38 Hz) frequency range. D, Representative example of coherence spectra obtained from different recording sites along the coronal slices. Note a strong dependence of values of coherence on the recording distance for gamma waves but not for theta waves. Mean coherence (E) and phase (F) of theta and gamma waves in the coronal slices. In contrast to coherence, phase of theta and gamma oscillations were little changed as function of the recording distance. G, 2D graph of the results of bispectral analysis. The (theta/gamma) peak indicates that theta and gamma power wax and wane in a correlated way. (H) Averaged theta/gamma bicoherence pattern during the network oscillations in the coronal slices (n=5). High values of bicoherence indicates the strong functional interaction between the generators of theta and gamma oscillations.

Figure 4. Morphological and electrophysiological characterization of O-LM interneurons in hippocampal slices.

A, 3 D representation of the hippocampus illustrating the orientation of the transverse and longitudinal axis. B, NeuroLucida reconstruction of biocytin-filled O-LM cell in area CA3 from transverse slice. The soma and dendrites are drawn in red, whereas the axon is in yellow. C, Superimposed neuroLucida reconstruction of 6 rotated O-LM interneurons from transverse slices showing the axonal arborization in the longitudinal direction (axons of cell in B are

yellow, axons of all other O-LM cells are superimposed in green). D, Typical example of extracellular FP (i) and concomitant current clamp recordings (ii) in an O-LM cell following induction of oscillatory activity with KA in transverse slice. iii, corresponding power spectra (60 s epoch) from field (black) and current clamp (red) recordings. E, Corresponding recordings from longitudinal slice following KA.

Figure. 5. Models of gamma, theta and theta/gamma network activity.

A-C (Model), The network contains 2 O-LM cells – “O”; 2 fast spiking perisomatic targeting basket cells – “I” and 1 pyramidal cell “E” representing a population. The pyramidal cell has 2 compartments: soma (E_s) and dendrite (E_d). To get from theta (B) to theta/gamma (C) to gamma (A) the OI and OD connections are decreased whilst the EI and EO connections are increased. In V_E (A-C) action potentials and afterhyperpolarizations are truncated for clarity.

A-C (Experiment), Whole cell patch-clamp recordings from pyramidal cell soma (V_E) illustrate inhibitory postsynaptic potentials (IPSPs) (from a membrane potential of -20 mV) during kainite induced network oscillations. D, Minimal network for investigating of gamma, theta and theta/gamma rhythms.