Gamma oscillations dynamically couple hippocampal CA3 and CA1 regions during memory task performance

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The hippocampal formation is believed to be critical for the encoding, consolidation, and retrieval of episodic memories. Yet, how these processes are supported by the anatomically diverse hippocampal networks is still unknown. To examine this issue, we tested rats in a hippocampus-dependent delayed spatial alternation task on a modified T maze while simultaneously recording local field potentials from dendritic and somatic layers of the dentate gyrus, CA3, and CA1 regions by using high-density, 96-site silicon probes. Both the power and coherence of gamma oscillations exhibited layer-specific changes during task performance. Peak increases in the gamma power and coherence were found in the CA3–CA1 interface on the maze segment approaching the T junction, independent of motor aspects of task performance. These findings signify that gamma oscillations can dynamically coordinate hippocampal networks according to behavioral demands. Based on these findings, we hypothesize that gamma oscillations may serve as a physiological mechanism by which CA3 output can coordinate CA1 activity to support retrieval of hippocampus-dependent memories.

Results

LFPs were recorded simultaneously in the dentate, CA3, and CA1 regions of the dorsal hippocampus by using a two-dimensional silicon probe array with 96 monitoring sites. In agreement with previous observations, the in situ recording sites in the various regions and layers could be determined with high spatial resolution (±30 μm) by using a combination of spontaneous LFP patterns, multiple unit activity, evoked potentials in response to perforant path and commissural stimulation and posthoc histological identification of the anatomical position of each recording shank [Fig. 1; see supporting information (SI) Fig. 6A for electrode positions in all animals] (23, 29).

To engage hippocampal networks, rats (n = 4) were trained on a hippocampus-dependent, delayed spatial alternation task (Fig. 2A) (24). This task requires rats to encode their spatial response on each trial and, after a 10-second delay, retrieve this information to appropriately choose the opposite arm from the one entered on the previous trial. Although retrieval of previous trial information could occur in this task at any time before crossing the T junction, rats typically ran smoothly through the center arm and the T junction in one swift trajectory, suggesting that retrieval processes likely occur before reaching the T junction. All rats performed the task at high levels of proficiency (>85% correct).

Layer-Specific Gamma Power Increase on the Center Arm of the T Maze. We investigated the involvement of different hippocampal networks during performance of the spatial alternation task by...
probe sites have a vertical spacing of 100 μm and a horizontal spacing of 300 μm. The anatomical position of recording electrodes in the different layers in the dentate gyrus and CA1 and -3 regions of the hippocampus was determined by using 1,1’-dioctadecyl-3,3,3,3’-tetramethylindocarbocyanine (DiI) labeling of electrode tracks (A) matched with known electrophysiological and anatomical characteristics of the hippocampus for each recording session. (B and C) Event-triggered averages of the LFP (traces) and CSD (color) centered on the estimated position from which the activity was recorded. (B) Ripple-triggered average responses (centered on ripple event, n = 179). Note the large sink (blue) associated with a sharp wave in CA1 str. radiatum. (C) Average evoked activity in response to perforant path stimulation (left aligned to stimulation, n = 3). Note the large sinks in the molecular layer of the dentate gyrus.

Fig. 1. On-line calibration of recording sites. A 96-site silicon probe with recording pads spaced regularly over a 1.5 mm × 1.5 mm area is shown. The probe sites have a vertical spacing of 100 μm and a horizontal spacing of 300 μm. The anatomical position of recording electrodes in the different layers in the dentate gyrus and CA1 and -3 regions of the hippocampus was determined by using 1,1’-dioctadecyl-3,3,3,3’-tetramethylindocarbocyanine (DiI) labeling of electrode tracks (A) matched with known electrophysiological and anatomical characteristics of the hippocampus for each recording session. (B and C) Event-triggered averages of the LFP (traces) and CSD (color) centered on the estimated position from which the activity was recorded. (B) Ripple-triggered average responses (centered on ripple event, n = 179). Note the large sink (blue) associated with a sharp wave in CA1 str. radiatum. (C) Average evoked activity in response to perforant path stimulation (left aligned to stimulation, n = 3). Note the large sinks in the molecular layer of the dentate gyrus.

measuring the power (amplitude) of gamma oscillations simultaneously in various layers, across different portions of the task. In agreement with previous findings, maximum LFP gamma power was recorded in the hilar region of the dentate gyrus (25, 30). However, the relative power changes in the separate maze regions varied differentially across layers. As illustrated in Fig. 2 B and C, the amplitude of the gamma oscillation (40–120 Hz; SI Fig. 7), recorded from the middle of CA1 stratum (str.) radiatum, showed a selective increase on the center arm that was not reliably observed in other hippocampal layers. Relative changes in gamma power were also observed in areas outside the CA1 str. radiatum in other segments of the maze, suggesting that the various hippocampal regions and afferents are involved in various task components (see SI Movie 1), but the present behavioral paradigm did not reliably isolate these events.

Because the running pattern of the rat was not identical in the different segments of the maze, and because previous research has shown speed and acceleration-dependent changes of hippocampal unit and field activity (31, 32), we first addressed the issue of whether overt motor behavior could account for the fluctuations in gamma power. Gamma power measurements were fit separately for each recording site with a general linear model (GLM), including as explanatory variables, running speed, acceleration, and maze region (see Methods). Similar to an analysis of covariance, GLM analysis dissects the trial-by-trial variance in gamma-power measurements that can be accounted for by the variables of interest (33, 34). GLM analysis showed that gamma power only weakly correlated with running speed and acceleration, as indicated by the consistently low r² values (“explained variance,” <10%) across all recording sites (Fig. 3A). Maze region, on the other hand, could explain >30% of the observed variance in gamma power at a number of sites. Especially striking was the degree to which the effect of maze region respected the laminar anatomy of the hippocampus, with the highest r² values confined to the CA1 str. radiatum. β values of the GLM analysis, showing the direction and magnitude of the change in gamma power for each maze region (Fig. 3B), revealed increased gamma power in CA1 str. radiatum on the center arm of the maze. Because the dominant projection to CA1 str. radiatum derives from the CA3 region (8), the increase of gamma power in this layer suggests an enhancement of CA3 output to CA1 on the center arm of the T maze.

To statistically compare task-related changes in the anatomical distribution of gamma power across animals, recordings sites were assigned to specific layers (as shown in SI Fig. 6B), and the distribution of β values from each layer across all four rats was tested for reliable behavior-related changes (two-tailed t tests). Fig. 3C shows group statistics revealing differential increases in gamma power across hippocampal layers on the center arm versus other segments of the maze. Although significant increases in gamma power were observed in several hippocampal layers on the center arm of the maze, the gamma power increase in CA1 str. radiatum was larger than in all other layers except for the CA3 pyramidal layer (ANOVA, Tukey post hoc test, P < 0.05). Further statistical analysis, including the effect of running speed, acceleration and all maze regions is shown in SI Fig. 8. We also tested whether gamma patterns were different on “error” versus “correct” trials (data not shown) but found no reliable difference, possibly because of the low number of error trials, high behavioral variability on error trials, and/or the foiled engagement of cognitive processes, e.g., retrieval mechanisms that return the wrong information.

Even though the center-arm increase in LFP gamma power shown in Fig. 3C was concentrated in specific layers, the LFP may be contaminated by volume conduction of activity in other layers. To reduce the contribution of possible volume-conducted fields, we first performed a one-dimensional current source density (CSD) analysis on the LFP data and repeated the GLM calculations on the derived data (Fig. 3D). This analysis revealed that the increased gamma oscillations in the CA3 pyramidal layer and CA1 str. radiatum were generated by local currents rather than volume conducted from the dentate gyrus or direct entorhinal input to the CA1 region (i.e., str. lacunosum-moleculare). CSD gamma power showed significant increases in the dentate molecular layer, possibly reflecting a change in entorhinal input, local circuitry, or CA3 back-projections to the inner molecular layer of the dentate gyrus (35). Gamma power also increased in
the CA1 pyramidal layer, possibly reflecting the entrainment of CA1 gamma oscillations by CA3 input (26, 36) or str. radiatum return currents unmasked from the volume conduction bias caused by anatomical curvature.

Enhanced CA3–CA1 Gamma Coherence on the Center Arm. Because power analysis suggested the engagement of specific intrahippocampal networks on the center arm of the T maze, we further examined the functional connectivity by analyzing the coherence of gamma oscillations between hippocampal regions during alternation task performance. The mean gamma coherence was highest between sites within the same layer (see “Constant” term, SI Fig. 8C), revealing that within-layer processing is highly coordinated even over large physical distances (>1.5 mm). Fig. 4A shows the changes in CSD gamma coherence between a reference site in the CA3 pyramidal layer and two example sites in CA1 str. radiatum and the dentate molecular layer averaged with respect to spatial position on the T maze (see also SI Fig. 9). The anatomical distribution of the center arm-related changes in gamma coherence between the CA3 pyramidal layer and all other hippocampal recording sites are shown in Fig. 4B (see also SI Fig. 8 and SI Movie 2). Gamma coherence between site pairs was separately fit with GLM statistics for all rats. The matrices in Fig. 4C and D show the group statistics for gamma coherence between all hippocampal layer pairs. Extending the gamma power results, the coherence analysis showed increased coordination of intra hippocampal circuits, reflected by the maximal gamma coherence increase between the CA3 and CA1 regions on the center arm of the maze.

Task-Specific Enhancement of CA3–CA1 Gamma Synchrony. Although the GLM statistics are specifically designed to dissect individual explanatory variables (33), we performed additional control experiments to isolate the hypothesized mnemonic contribution to the observed changes in gamma oscillations. In addition to the alternation task, each rat was trained and tested on one of three control tasks (SI Fig. 10) possessing no memory requirement and no dependence on the hippocampus to control for motor and nonmnemonic cognitive aspects of task performance. Similar to the alternation task, in each control task the rat was held in a small delay area and then released to run for water reward. The initial segment of these control tasks showed similar speed and acceleration profiles to the center arm of the alternation task (SI Fig. 10). Thus, comparison of these initial maze segments of control tasks to the center arm of the alternation task controls for basic route “planning” operations that may accompany the start of a journey and further controls for the effects of speed and acceleration. As evidence against these alternatives, we found significantly higher gamma power in the CA1 str. radiatum LFP in the alternation task relative to tasks with no mnemonic demand (Fig. 5A; also see SI Fig. 11). Separate analysis of CSD traces also revealed increased gamma power in the CA1 pyramidal layer and confirmed that the str. radiatum gamma oscillation was locally generated, indicating that the LFP gamma power effects in CA1 str. lacunosum-moleculare were likely due to volume-conducted contamination from str. radiatum (Fig. 5B). Analysis of gamma coherence revealed enhanced CA3–CA1 coordination in the alternation task compared with controls. Smaller increases in coherence between CA1 and other layers were also observed, but, notably, effects were weak with the CA1 str. lacunosum-moleculare and between the CA3 pyramidal layer and dentate layers, indicating that the other effects may have been mediated multisynaptically via the dominant changes in CA3–CA1 synchrony. These results provide evidence that gamma coordination of CA3–CA1 networks is enhanced by the specific cognitive demands of the delayed alternation task, independent of motor performance.

Discussion

The major finding of these experiments is that gamma oscillations are differentially modulated by aspects of behavior in the various hippocampal networks. Both the power and coherence of gamma oscillations at the CA3–CA1 interface were selectively enhanced by the cognitive demands of the spontaneous alternation task.

Gamma oscillations have been suggested to assist various operations, including the binding of sensory attributes (20), synaptic plasticity (25), working memory (37, 38), sensory-motor coordination (21), attention (39), formation of cell assemblies (40), memory coding (22, 41, 42), and even consciousness (43, 44). These diverse putative functions indicate that gamma oscillations per se cannot be explicitly assigned to specific behavioral events. Instead, changes in the gamma oscillations recorded from a particular brain structure may be taken as an indicator that the supporting circuitry is engaged in a particular mode of computation.

Fig. 2. Changes in gamma power during performance of a delayed spatial alternation task. Rats were trained to perform a hippocampus-dependent, delayed spatial alternation task on a modified T maze (shown in A). (B) Single-trial example of the task-related fluctuations in LFP gamma oscillations from recording sites positioned in CA1 str. radiatum (CA1 rad) and the dentate molecular layer (DG mol). The filtered (40–120 Hz) gamma trace (gray) is overlaid on the normalized spectrogram (color). Note the burst of gamma power in CA1 str. radiatum but not the dentate molecular layer on the center arm of the T maze. (C) Normalized LFP gamma power averaged over one recording session (20 trials) from the same layers with respect to spatial position on the maze.
CA3 and CA1 Networks Are Dynamically Coordinated by Gamma Oscillations. Gamma oscillations have diverse expression throughout the hippocampal networks. They are generated by a consortium of mechanisms and depend primarily on the activity of fast spiking basket neurons and GABA_A receptor-mediated inhibition and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated excitation (19, 45–50). Gamma oscillations in the CA1 str. lacunosum-moleculare and the dentate gyrus are mainly under the control of entorhinal input (25, 51). However, evidence from in vitro and in vivo studies suggests that the CA3 region can generate gamma oscillations from the interactions between CA3 pyramidal cells and basket interneurons (25–27). The gamma phase-locked firing of CA3 projection neurons can in turn power and coherence of CA3 and CA1 gamma oscillations. Some analyses also implicated specific layers in the dentate gyrus, although future experiments with dual entorhinal-hippocampal recordings will be needed to precisely determine the extent to which these effects were related to direct entorhinal involvement, local activity, or CA3-dentate projections. Because our recordings principally sampled the CA3b and -c subregions, we cannot speculate about the role of the CA3a subregion from these data, given the differential wiring patterns of these subregions (35). Nevertheless, our results provide further support for a role of the CA3 region in generating self-organized gamma activity, independent of the gamma oscillations in other hippocampal regions. Additionally, our results provide evidence that the temporal coordination of CA3 and CA1 networks is dynamically regulated via gamma synchronization, according to behavioral demands.

Mnemonic Function of CA3–CA1 Gamma Coupling. Numerous lesion studies in humans and other animals link hippocampal networks to memory (2, 3). Previous computational models (9–11, 52), lesion studies (13, 14), and genetic knockout studies (15) have implicated CA3–CA1 communication in retrieval of hippocampus-dependent memories. We found a consistent pattern of changes in the power and coherence of gamma oscillations, showing increased synchrony in the CA3–CA1 interface on the center arm of the T maze. Using statistics and analysis of control tasks to rule out the contribution of overt behavior and other nonmnemonic variables, we found that the increased CA3–CA1 gamma coordination was specifically enhanced on the center arm of the T maze by cognitive aspects of alternation task performance. Performance of the delayed spatial alternation task requires, on each trial, that rats retrieve information about the previous journey to choose the opposite arm upon reaching the T junction. Although the exact spatial position(s) at which retrieval processes occur likely varies from trial to trial (as does the CA3–CA1 gamma synchrony increase seen in SI Movies 1 and 2), the fact that rats typically run through the center arm and the T junction in one swift trajectory suggests that retrieval and
decision-making usually occur on the maze before the T junction. This observation is corroborated by previous studies finding that turn-selective neuronal firing emerges in the CA1 network during traversal of the center arm in similar tasks (refs. 4–7; also see refs. 24 and 53). Based on our results and convergent evidence pointing to the mnemonic role of the hippocampus and its subregions, we hypothesize that the increased CA3–CA1 gamma coordination on the center arm of the T maze may reflect retrieval of previous trial information. Overall, our findings suggest that gamma oscillations may provide a mechanism by which hippocampal networks can dynamically coordinate to perform specific mnemonic operations.

Our results also posit specific predictions for future experiments investigating the changes in CA3 and CA1 unit firing that accompany retrieval processes. The observed increase in CA1 str. radiatum gamma power on the center arm of the T maze is suggestive of accompanying changes in bursting and/or gamma synchrony of CA3 units. Increased bursting leads to a nonlinear increase in neurotransmitter release (54), thereby increasing postsynaptic currents and the amplitude of LFP oscillations. Similarly, increased synchrony of unit firing increases the amplitude of LFP oscillations through greater temporal summation of postsynaptic currents (55, 56). Local modulation of specific CA1 layers could also facilitate CA3 inputs, but this must occur at fast (ostensibly ionotropic) receptors to mediate the observed gamma fluctuations. The observed increase in gamma coherence between CA3 and CA1 suggests that the synchrony between CA3 and CA1 unit firing increases at short (monosynaptic) and gamma time scales during the retrieval process. Because hippocampal principal neurons fire in restricted spatial locations, and because the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated effects between pyramidal cells are weak (57), assessment of the CA3–CA1 connections by monitoring unit activity would require recording very large numbers of neurons simultaneously. In contrast, by using LFP recording, the gamma power and coherence provide information about the collective behavior of neurons and the mode of operation of a given network.

In summary, our findings show that gamma oscillations dynamically coordinate the activity of hippocampal networks during performance of a hippocampus-dependent memory task. The most robust change in network activity was increased gamma coordination between CA3 and CA1 regions during the portion of the task associated with the retrieval of prior experience. Although the specific content of the retrieved memory may not be accessible without large-scale single-cell recordings, our results show that monitoring gamma oscillations with sufficient spatial resolution and proper behavioral control is an effective tool for identifying specific circuit dynamics involved in cognitive operations.

Methods

Animals and Behavior. Four Long–Evans rats (male, 300–400 g) were water-deprived and trained to run in a hippocampus-dependent continuous delayed nonmatch to place (“alternation”) task (24) and a control task. All rats performed the alternation task well (>85% correct). The control tasks included a C-shaped (n = 2 rats) and a Z-shaped (n = 1 rat) linear track, requiring the rats to run back and forth for water reward, and a cue task (n = 1 rat), requiring the rat to run in a path similar to the alternation task; but, on each trial, the rat’s trajectory was randomly cued left or right by a large block that was visible after the delay period. To constrain the behavioral and cognitive variability, trials in which the rat engaged in rearing, excessive sniffing, grooming, or immobility were eliminated from further analysis.

Spectral and GLM Analysis. Gamma-band (40–120 Hz) power analysis was performed by using the filter–rectify–smooth method, and spectral power and coherence analysis was performed by using Morlet wavelet analysis (courtesy of Aslak Grinsted, Arctic Centre, University of Lapland, Rovaniemi, Finland) (see SI Detailed Methods). Fitting data from correct trials in the alternation task (errors were rare and often associated with exploratory behavior), the distribution of spectral estimates (either power or coherence) for each electrode site was fit with a linear model (ANOVAR, Type 3 SS; MatLab; The Mathworks, Natick, MA): G = βconstant + βrunning speed + βrunning acceleration + βmaze region (4 arms) + ε.

To perform group statistics across animals, resulting β values from one electrode site per shank per animal were sampled from each layer (SI Fig. 6B). The resulting sampled distribution of β values from each anatomical layer was subsequently tested for a statistical difference from zero (t tests, P < 0.001, Bonferroni corrected). The normality assumption of the t test and assumptions of the general linear model, including normality of residuals, absence of interaction effects, and uncorrelated residuals, were assessed. Similar analyses were performed to compare physiological parameters on the center arm of the alternation task to those in the corresponding region of a control task (SI Fig. 10).

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