

# Enhancement of Temporal and Spatial Synchronization of Entorhinal Gamma Activity by Phase Reset

Clayton T. Dickson and Marco de Curtis\*

*Department of Experimental Neurophysiology, Istituto Nazionale Neurologico Carlo Besta, Milan, Italy*

**ABSTRACT:** The synchronization of cortical gamma oscillatory activity (25–80 Hz) is thought to coordinate neuronal assemblies in the processing and storage of information. The mechanism by which independently oscillating and distantly located cortical zones become synchronized is presumed to involve activity in corticocortical connections, although evidence supporting this conjecture has only been indirect. In the present study, we show that activation of synaptic inputs within and to the medial entorhinal cortex (mEC) of the *in vitro* isolated guinea pig brain preparation resets the phase of ongoing gamma activity induced by muscarinic receptor agonism with carbachol (frequency:  $24 \pm 2$  Hz at 32°C). Phase reset was associated with a transient enhancement of the synchronization of gamma activity recorded at distant (>1 mm) mEC sites, across which low coherence (>0.75) was observed before stimulation. This increase in synchronization, as measured by cross-correlation analysis, was restricted to a maximal period of 200 ms after either local mEC or CA1 afferent stimulation. The results provide direct evidence that synaptic activation can enhance the rhythmic synchronization of spatially remote, independently oscillating neuronal assemblies in the mEC through a mechanism of synaptically evoked phase reset. Dynamic functional grouping of oscillatory discharges across long distances in the mEC may underlie coding processes involved in the integration and storage of incoming information and thus may be important for the role of this region in memory processes. *Hippocampus* 2002;12:447–456. © 2002 Wiley-Liss, Inc.

**KEY WORDS:** 40-Hz oscillation/rhythm; cholinergic; isolated whole-brain; guinea pig; binding

## INTRODUCTION

Fast oscillatory activity at 25–80 Hz (gamma) is a spontaneous cortical population rhythm of the “activated” electroencephalogram (Ribary et al., 1991; Steriade et al., 1991; Llinás and Ribary, 1993; Bragin et al., 1995; Maloney et al., 1996; Menon et al., 1996; Steriade et al., 1996; Chrobak and Buzsáki, 1998; Leung, 1998; Gross and Gotman, 1999) that may reflect a basic neuronal processing state of the brain (Basar et al., 1999). Gamma activity can also be evoked or induced in corresponding sensory cortical regions by olfactory, visual, auditory, and somatosensory stimuli. Its syn-

chronization is thought to underlie the formation of temporally correlated discharges in cortical neuronal ensembles that may en masse form the neural representation of the stimulus itself (for reviews, see Freeman, 1975; Singer and Gray, 1995; Laurent, 1996; Gray, 1999; Singer, 1999; Tallon-Baudry and Bertrand, 1999). As with spontaneous gamma activity, sensory evoked or induced gamma oscillations show state dependence, appearing with larger amplitude and showing more robust rhythmicity and synchronization in the awake state or during activation of the ascending cholinergic activating system (Ribary et al., 1991; Galambos, 1992; Metherate et al., 1992; Llinás and Ribary, 1993; Munk et al., 1996; Herculano-Houzel et al., 1999). In addition to its proposed role in the perception of sensory stimuli, dynamic synchronization of gamma activity across different and distant brain regions has also been associated with brain processes involved in attention, sensorimotor integration, cognition, consciousness, learning, and memory (Murthy and Fetz, 1992; Llinás and Ribary, 1993; Tiitinen et al., 1993; Joliot et al., 1994; Buzsáki and Chrobak, 1995; Lisman and Idiart, 1995; Singer and Gray, 1995; Gray, 1999; Chrobak et al., 2000; Fries et al., 2001).

The mechanisms by which synchronization of gamma oscillatory activity across large cortical distances takes place have only indirectly implied a function for synaptic interactions between independent generators (Engel et al., 1991a; Eckhorn et al., 1992; Jefferys et al., 1996; Traub et al., 1996b; Gray, 1999; Fuchs et al., 2001). We have previously reported that muscarinic stimulation induces gamma activity in the medial entorhinal cortex (mEC) of the isolated whole brain preparation (van der Linden et al., 1999; Dickson et al., 2000a), which closely approximates gamma activity observed in the EC *in vivo* (Chrobak and Buzsáki, 1998). Interestingly, in this preparation, gamma activity shows limited synchronization across the tangential dimension, suggesting that it may function to spatially organize the activity of mEC neurons in modules across the surface of the cortex. In the present study, we demonstrate that synaptic volleys evoked either by stimulation of the superficial associative mEC fiber system or by stimulation of hippocampal area CA1 transiently enhanced the synchronization of gamma activity recorded in spatially separated mEC modules through a mechanism of phase reset. In the mEC, such

Grant sponsor: European Community (VSAMUEL); Grant number: IST 99-1-1-A; National Institutes of Health; Grant number: P41-RR09754.

\*Correspondence to: Marco de Curtis, Dipartimento di Neurofisiologia Sperimentale, Istituto Nazionale Neurologico Carlo Besta, via Celoria, 11 20133 Milan, Italy.

E-mail: decurtis@istituto-besta.it

Accepted for publication 24 August 2001

DOI 10.1002/hipo.10013

transient oscillatory binding may be important for the processing, organization, and storage of information in the medial temporal lobe memory system.

## MATERIALS AND METHODS

The guinea pig isolated whole brain preparation has been previously documented in detail (Llinás et al., 1981; de Curtis et al., 1991; Muhlethaler et al., 1993; de Curtis et al., 1998) and is only briefly described in the present study. After barbiturate anesthesia (thiopentothal sodium 20 mg/kg), young adult guinea pigs (150–300 g) were perfused through the heart with a cold (4–10°C) carbogenated (95% O<sub>2</sub>–5% CO<sub>2</sub>) solution composed of (in mM): 126 NaCl, 3 KCl, 1.2 KH<sub>2</sub>PO<sub>4</sub>, 1.3 MgSO<sub>4</sub>, 2.4 CaCl<sub>2</sub>, 26 NaHCO<sub>3</sub>, 15 glucose, 2.1 HEPES, 0.4 thiourea, 0.5 ascorbic acid, and 3% dextran (MW 70,000). After rapid and careful removal, the brain was submerged in a recording chamber filled with the same solution maintained at a temperature of 16°C. After cannulation of the basilar artery, the preparation was perfused through the existing brain vasculature at a rate of 5.5 ml/min. Leaky vessels were ligated and the brain was gradually (0.2°C/min) warmed to a final temperature of 32°C. This protocol was reviewed and approved by the Committee on Animal Care and Use and by the Ethics Committee of the Istituto Nazionale Neurologico.

In all preparations, before any experimental procedures, the response of the piriform or lateral entorhinal cortex to electrical stimulation of the lateral olfactory tract was assessed in order to verify the viability of the brain. As described previously (van der Linden et al., 1999; Dickson et al., 2000a), fast oscillatory field activity was elicited in the mEC by the application of carbachol (CCh), either by arterial perfusion (25–100 μM in perfusion solution) or through local diffusion from recording pipettes (100 mM in 0.9% NaCl, positioned at a depth of 500 μm). As previously reported, the average frequency of the oscillatory activity described in this model (~24 Hz) increased to 40–45 Hz (gamma band) when the temperature of the preparation was increased to 36°C (van der Linden et al., 1999; Dickson et al., 2000a). Experiments were performed at 32°C to minimize spontaneous activity and to prolong the functional preservation of the preparation.

When metal electrodes were used for intracortical recording and stimulation, electrolytic lesions induced by 10 mA current application for 5–10 s were made in order to mark the location of the electrode tips. Subsequently, the brains were fixed overnight with a 4% paraformaldehyde solution in a 0.1 M sodium phosphate buffer and then sectioned at 100 μm by vibratome. Sections were mounted, stained with thionin, and inspected for the location of lesions. All chemicals were obtained from BDH (Poole, UK), and all drugs were obtained from Sigma (St. Louis, MO). Dextran was obtained from SIFRA (Isola della Scala, Italy) or Pharmacia (Milan, Italy).

### Recordings

Extracellular field recordings were made with (1) glass micropipettes with a 10-μm diameter tip filled with a NaCl solution

(0.9%); (2) 16-site linear silicon probes (50-μm contact separation, kindly provided by Jamille Hetke of the Center for Neural Communication Technology, University of Michigan, Ann Arbor, MI); or (3) tungsten 4 × 1 matrix microelectrode arrays (410-μm tip separation; FHC, Bowdoinham, ME). All single-electrode recordings, unless otherwise noted, were made at a depth of 500 μm from the pial surface; recordings at multiple sites were carried out simultaneously. Extracellular signals were amplified at a gain of ×1,000, using a DC amplifier (Biomedical Engineering, Thornwood, NY) and bandpass-filtered at 0.2–500 Hz. All signals were stored on DAT tape (DTR 2602 Biologic, Claix, France) and were digitized both online and offline, using customized software (CLAMPVIEW) developed by Gerardo Biella, in collaboration with SIDeA (alliance member of National Instruments, Milan, Italy). Signals were digitized at a frequency of 1,000 Hz.

Stimulation of the lateral olfactory tract was conducted with bipolar silver wires, insulated along their length except at the tips. Intracortical stimulation was performed with single or double stimuli separated by 20–30 ms, using thin insulated tungsten bipolar electrodes (10 μm at the tip; FHC).

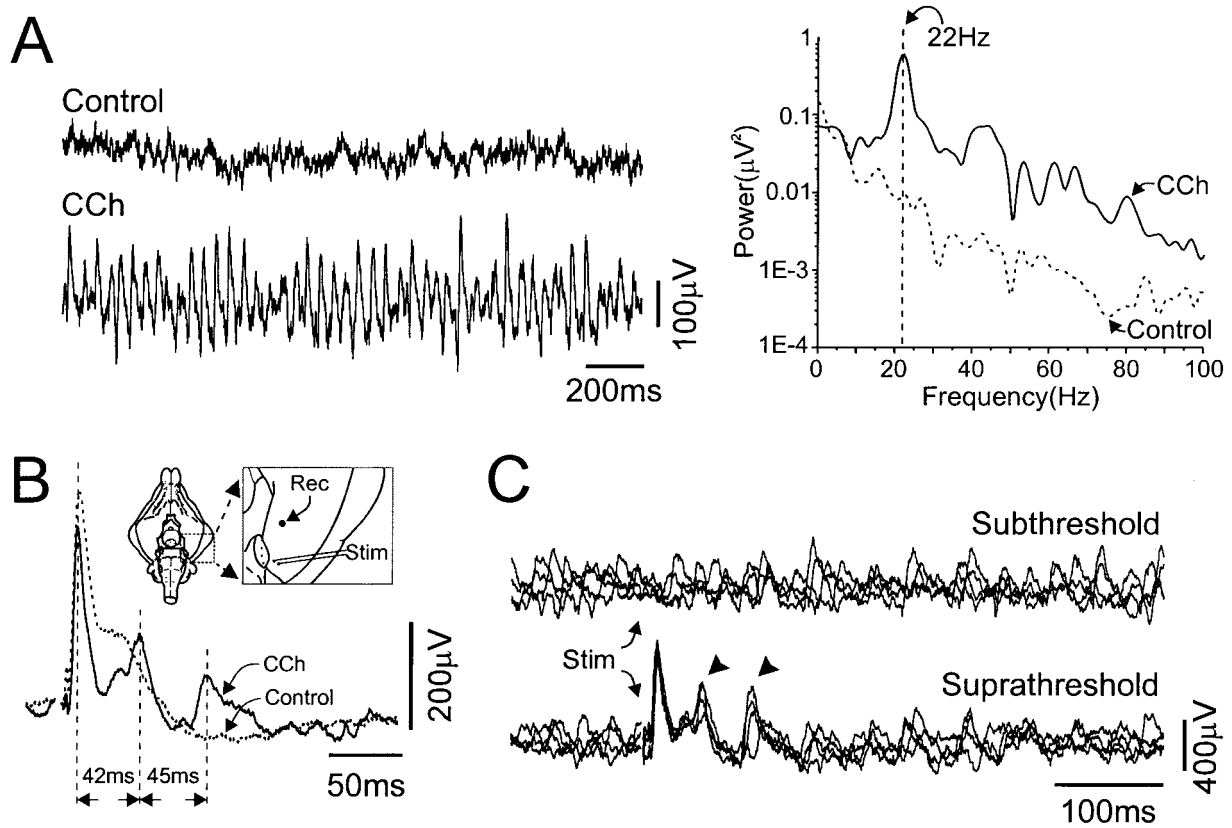
### Analysis

Offline analysis, including digital filtering, spectral and cross-correlation analysis were conducted using MATLAB (Mathworks, Natick, MA). Field signals were digitally filtered with a third- to fourth-order Butterworth function in a 2.5-Hz bandwidth around line frequency (50 Hz) to minimize spectral artifacts. By comparing the same signals with and without filtering, we determined empirically that our digital filter was adequate in eliminating line frequency artifacts without producing major alterations in the frequency band of interest (25–30 Hz). Auto-, cross-, and coherence spectra were conducted within and between all signals. Cross-correlation analysis was conducted within time windows varying from 70 to 120 ms between selected signals.

A laminar profile of spontaneous gamma activity was constructed by averaging characteristic gamma waveforms in all channels recorded from the linear silicon multiprobe. A signal template consisting of at least two cycles of the gamma oscillation was chosen, and subsequent samples (≥16, but more often 32) that fit this template well, as determined by visual inspection, were included in an average computed for every channel. This averaged profile was then plotted with respect to the position (depth) of the recording site on the linear multiprobe.

## RESULTS

As previously described in the isolated whole brain preparation (van der Linden et al., 1999; Dickson et al., 2000a), robust gamma oscillatory field activity with a frequency range of 20–30 Hz (mean ± SD: 24 ± 2 Hz) was induced in the medial entorhinal cortex (mEC) by arterial perfusion of carbachol (CCh: 50 μM) (Fig. 1A). Gamma activity evoked by muscarinic receptor stimu-

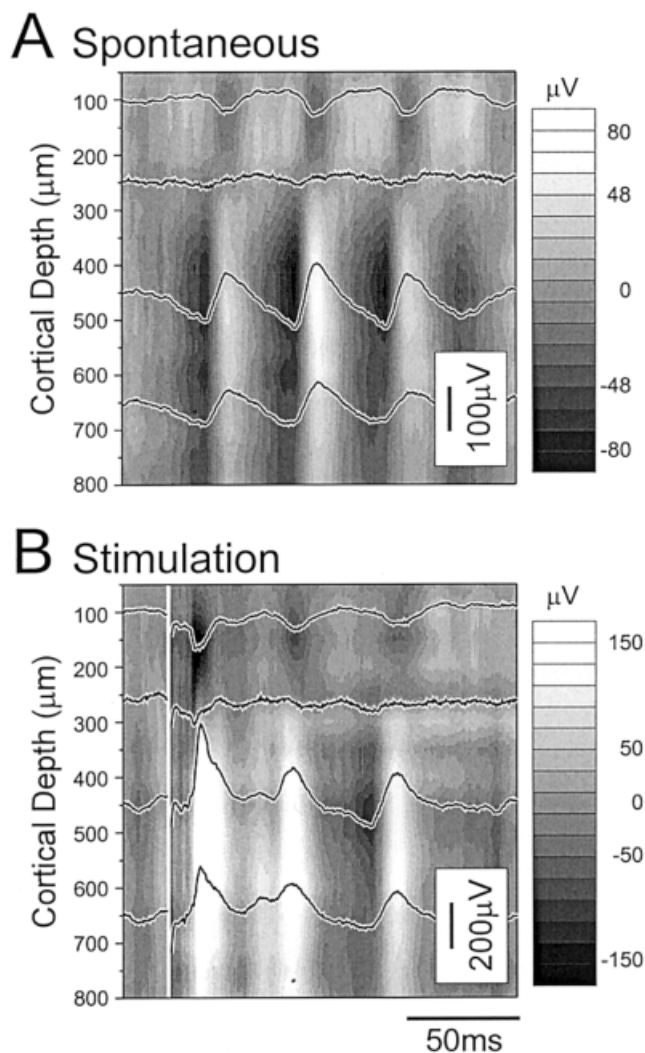


**FIGURE 1.** Stimulation of superficial associative fibers induces stimulus-locked phase reset of ongoing carbachol (CCh)-elicited gamma activity in the mEC. **A:** Spontaneous traces of medial entorhinal cortex (mEC) field activity recorded at a depth of 500  $\mu\text{m}$  (left panel) and corresponding power spectra (right panel) before and after arterial perfusion of 50  $\mu\text{M}$  CCh. Prominent fast oscillatory activity at a peak frequency of 22 Hz was observed after CCh application. **B:** In the same experiment, averaged potentials evoked by electrical stimulation in the superficial layers of the mEC before and after CCh (dotted and continuous lines, respectively) are shown. During spontaneous gamma, distinct rhythmic afterpotentials with a peak-to-peak period identical to that of spontaneous gamma ( $\sim 45$  ms) could be observed after the initial evoked potential. The inset shows a schematic diagram of the ventral view of the brain with an expansion of the entorhinal region and the relative positions of recording and stimulation electrodes. **C:** Superimposed individual traces during stimulation trials with subthreshold (upper panel) and suprathreshold (lower panel) stimulation intensities. Temporal overlap was poor between traces throughout the entire trial in subthreshold stimulation conditions as well as before suprathreshold stimulation. In contrast, consistent temporal overlap of rhythmic traces existed between trials for a transient period after the initial evoked potential in suprathreshold stimulation conditions, suggesting that stimulation induced a phase reset of ongoing gamma activity.

lation have also been successfully used to generate fast oscillations in vitro brain slices (Buhl et al., 1998; Fisahn et al., 1998; Traub et al., 2000). Interestingly, CCh application modified not only the pattern of spontaneous field activity in the mEC but also the averaged evoked field potential response to electrical stimulation of superficial associative fibers within the mEC (Fig. 1B). Under control conditions, (dotted line, Fig. 1B) stimulation of the superficial mEC evoked a waveform consisting of a prominent positive-going potential peaking at an average latency of  $13.5 \pm 3.0$  ms ( $n = 13$ ). Although application of CCh was often observed to alter the amplitude and duration of this positive potential (solid line: Fig. 1B; see also Konopacki et al., 1987), even more notable were the presence of positive rhythmic after-potentials which followed, at consistent latencies, the initial evoked potential. These rhythmic afterpotentials were only present after (never before) to the elicitation of gamma activity. On average, and as shown in Figure 1B, the period between the evoked potential and the first afterpotential (measured peak-to-peak) was highly similar to the average gamma period

( $41.0 \pm 4.3$  vs.  $41.2 \pm 3.9$  ms;  $n = 15$  experiments). In fact, using a paired samples comparison, there was no significant difference between these values (mean difference:  $-0.24 \pm 5$  ms,  $t(14) = -0.19$ ,  $P > 0.1$ ).

When individual stimulation trials were superimposed (Fig. 1C), the appearance of rhythmic afterpotentials in the average response corresponded to a locking of the phase of ongoing gamma activity directly after the evoked potential. Rhythmic overlap of traces across individual trials typically appeared for two cycles after the evoked potential. This phenomenon was independent of the phase of the gamma activity occurring before stimulation and was not apparent in trials in which the amplitude of electrical stimulation was lowered to a subthreshold level, at which no initial evoked potential was elicited (cf. Fig. 1C, upper and lower traces) ( $n = 11$ ). These results suggested that suprathreshold stimulation promoted a phase-locked response in the circuitry responsible for the generation of ongoing gamma activity. The duration and robustness of this phase reset of ongoing gamma activity was sufficient for



**FIGURE 2.** Laminar profiles demonstrate similar depth reversals for spontaneous and reset gamma. Recordings were performed simultaneously using silicon multisite probes along a path normal to the pial surface at 16 sites with an inter-contact spacing of 50  $\mu\text{m}$ . **A:** Gray-scale contour plot of the laminar profile of averaged spontaneous gamma activity (for details on averaging see Methods section). Four representative traces at cortical depths indicated by their alignment with the y-axis are superimposed for clarity. Consistent with our previous work, this oscillatory activity reversed phase at a cortical depth of 250  $\mu\text{m}$ , corresponding to layer II of the medial entorhinal cortex (mEC). **B:** Laminar profile of the evoked potential to mEC stimulation during spontaneous gamma activity in the same experiment. Four traces at similar depths as in (A) are superimposed. The prominent rhythmic afterpotentials after the initial evoked potential showed a similar frequency to spontaneous gamma and also reversed at the same cortical depth (250  $\mu\text{m}$ ).

the alignment of gamma waveforms after stimulation to be apparent in the average evoked potential (Fig. 1B).

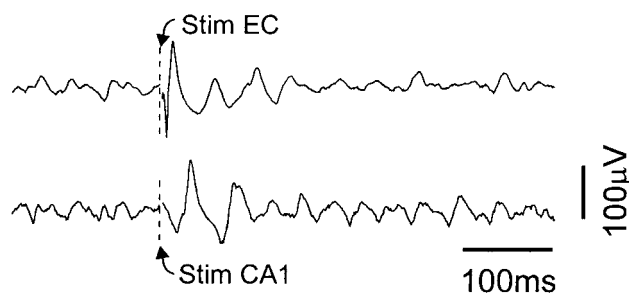
Our previous work has demonstrated that the phase of CCh-evoked gamma activity in the mEC reverses at an average depth of  $\sim 250$   $\mu\text{m}$  from the pial surface, corresponding to layer II (van der Linden et al., 1999; Dickson et al., 2000a). In the present study, we recorded the laminar profiles of both spontaneous gamma activity

and stimulus-evoked reset of gamma activity in the same experiments with the 16-site linear probe, which allowed us to make simultaneous recordings at different depths normal to the cortical laminations ( $n = 3$ ). The averaged spontaneous gamma traces (Fig. 2A) showed a similar depth reversal as the averaged afterpotentials evoked by superficial mEC stimulation (Fig. 2B). This provided further confirmation that the evoked afterpotentials indeed represented reset of ongoing gamma.

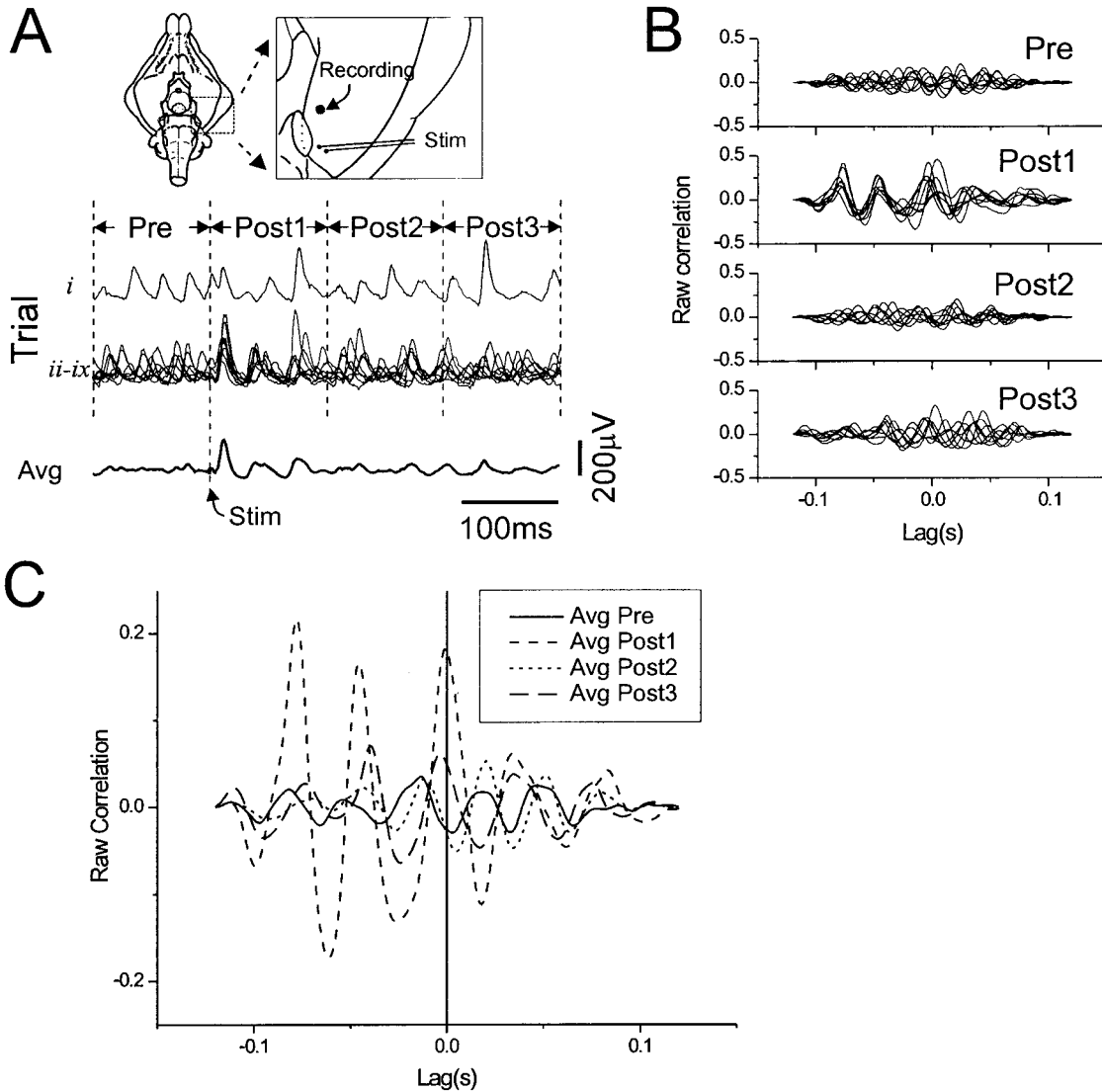
Stimulation-induced gamma reset was not only limited to activation of the superficial associative fibers system of the mEC. Stimulation of hippocampal field CA1, which induced a prominent evoked potential in the mEC, could also induce phase reset of ongoing gamma (lower trace, Fig. 3;  $n = 4$ ) in a similar fashion to local mEC stimulation (upper trace, Fig. 3). Consistent with polysynaptic activation, the latency of the positive potential in the evoked response to CA1 stimulation was  $41.0 \pm 3.0$  ms, markedly longer than for the local mEC evoked responses.

In order to better characterize the temporal aspects of gamma phase reset we divided stimulation trials into discrete time windows of approximately 100 ms before and after the onset of stimulation and compared the phase relationship of gamma activity of a given signal within these time windows across different stimulation trials (Fig. 4). By simple visual inspection of the individual traces plotted as overlapping trials (i to ix in Fig. 4A), as well as in the average trace (lower trace in Fig. 4A) a consistent match between different stimulation trials for the first  $\sim 100$  ms directly after stimulation was observed. By performing cross-correlation analysis within each time window between the signal obtained during the first stimulation trial and all others we were able to evaluate this phase relationship more systematically.

Before stimulation, individual cross-correlation functions (CCFs) were low in amplitude and showed a lack of overlapping correspondence (Fig. 4B, upper panel). In addition, there tended to be a lack of a central (zero lag) peak in individual CCFs. These results emphasize the random phase conformity of the gamma signal across trials before stimulation. In the first window directly after stimulation (Post1), however, there was both an increase in the amplitude of CCFs as well as a systematic and consistent overlap of individual CCFs (Fig. 4B, panel Post1), each of which dem-



**FIGURE 3.** Gamma reset evoked by stimulation of intrinsic and CA1 afferent pathways. Average evoked potentials recorded from the mEC during carbachol (CCh)-induced gamma activity after local mEC (upper panel) and CA1 stimulation (lower panel). Although both types of stimulation induce reset, the latency of the initial evoked potential to CA1 stimulation is notably delayed with respect to the response to local (medial entorhinal cortex [mEC]) stimulation.

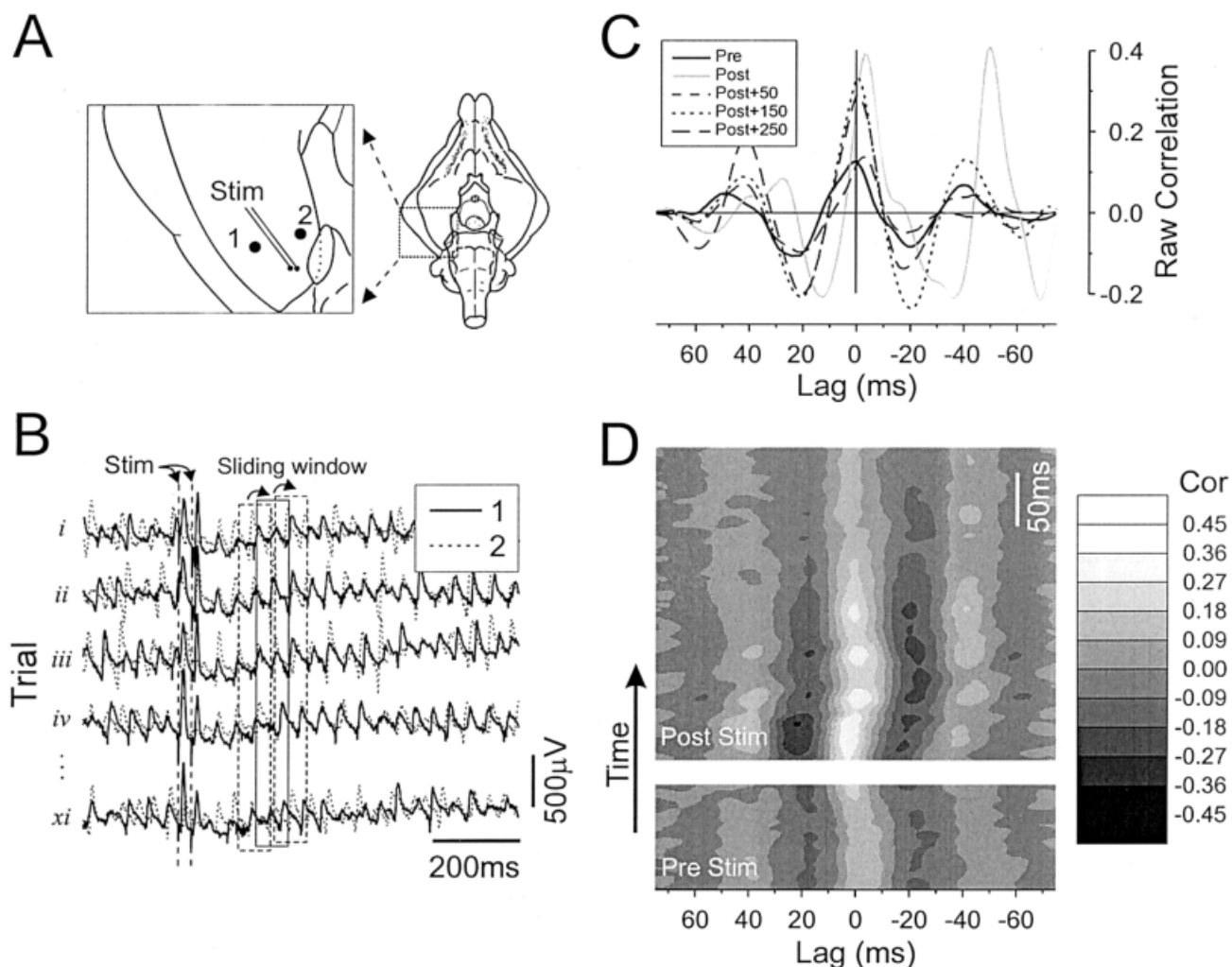


**FIGURE 4.** Transient stimulation-induced gamma phase reset. **A:** Top: Schematic representation of recording and stimulation electrode locations. Bottom: Extracellular field traces corresponding to individual stimulation trials (i to ix) and their average (thicker trace at the bottom). Traces were divided into equal time portions corresponding to approximately 100-ms periods pre- and post-stimulation. A consistent temporal overlap between individual traces during the first 100 ms after stimulation (Post1) was observed, in contrast to the random overlap occurring before and after this period. **B:** Cross-correlation functions (CCFs) conducted within each window between the first trial and all subsequent trials are plotted according to the windows denoted in A. Consistently high, rhythmic and overlapping CCFs were observed only during the period corresponding to the first 100 ms after stimulation (Post1: second graph from top), suggesting that rhythmic gamma reset is highly consistent between trials during this period. **C:** Average plot of CCFs for each window. While other windows show only random fluctuations, the CCF during the Post1 period has a larger amplitude and a prominent central (zero lag) peak and flanking peaks at the period of gamma suggesting that gamma reset during this period is temporally consistent between stimulation trials.

onstrated prominent central (zero-lag) peaks flanked by peaks at time lags (range 29–47 ms) corresponding to the period of ongoing gamma activity (in this case, 34 ms). Therefore, during the immediate post-stimulation period (Post1), the trial to trial phase relationship of gamma rhythmicity was consistent. Furthermore, these results imply that the peak-to-peak latencies of the evoked potential and the subsequent gamma waves are temporally locked. In contrast, these results were not observed in the subsequent time windows (Post2 and Post3), which showed a decrease in amplitude and a random alignment of individual CCFs, resembling those seen in the time window before stimulation. The averaged CCFs

for the data in each time window are shown in Figure 4C. A substantial enhancement in amplitude existed only for the average CCF for the time window directly after the stimulation (Avg Post1) when compared to all other time windows. The amplitude increase was observed both in the central (zero lag) peak and the flanking (gamma period) peaks. A similar pattern was seen in all other experiments analyzed in this way (n = 11), suggesting that the period during which consistent phase reset of gamma occurs is transient, on the order of approximately 100 ms.

Since the initial evoked potential itself provides a synchronizing influence across trials that could bias the CCF, we repeated this



**FIGURE 5.** Gamma phase reset enhances the synchronization of gamma activity recorded at remote sites. **A:** Schematic diagram of recording and stimulation locations. Constitutive gamma coherence before stimulation between sites 1 and 2 was 0.36. **B:** Extracellular field traces corresponding to individual stimulation trials (roman numerals on the left). Overlapping traces correspond to signals simultaneously recorded for each trial from sites 1 (continuous line) and 2 (dotted line). In this case, paired pulse stimulation of the medial entorhinal cortex (mEC) was conducted at an inter-stimulus delay of 30 ms. A window of 75-ms duration was slid across the traces for each trial in 1-ms increments and cross-correlation functions (CCFs) were calculated between traces in each window for every trial. CCFs were then averaged across all trials for every window. Windows which included the stimulus artifact (and in this case the first evoked potential) were omitted from the final analysis. **C:** Trial averaged CCFs for selected time windows pre- and post-stimulation derived from signals shown in B. The numbers represent the time (in ms) from the onset of the stimulation pulse at which the analysis was conducted. The average amplitude of the CCF (notably the central and flanking peaks) was increased after the stimulation protocol and this increase lasted for a period of 150–250 ms. **D:** Surface plot of the trial averaged CCF across all analyzed windows, pre- and post-stimulation, for the same experiment. The gray scale on the right indicates the cross-correlation values. The increase in the amplitude of the CCF function, and therefore the enhancement of gamma synchronization between the two recording sites, lasted for a maximum period of ~200 ms after stimulation.

analysis in all experiments excluding the initial 20–25 ms in the Post1 window to eliminate the evoked potential. The amplitude of the resulting CCF function still demonstrated an increase with respect to the period before stimulation (in addition to all others) for both the central and flanking (gamma period) peaks, and was only slightly reduced with respect to the CCF which included the initial evoked potential (not shown). Therefore, enhancement of the CCF appears to be specific for the phase locking of the gamma rhythm itself and is not merely due to the alignment of the evoked potentials.

The consistency of this reset phenomenon across trials suggested that this type of mechanism might enhance the synchronization of gamma signals recorded simultaneously across the surface of the mEC. As we have previously demonstrated (Dickson et al., 2000a), the spontaneous synchronization (as measured by coherence) of gamma activity decreases with distance between recording sites, across the tangential surface of the mEC. Therefore, we tested the influence of reset-inducing stimulation on synchronization of gamma signals that showed a low constitutive coherence ( $<0.75$ ) before stimulation. These sites tended to be distant from each

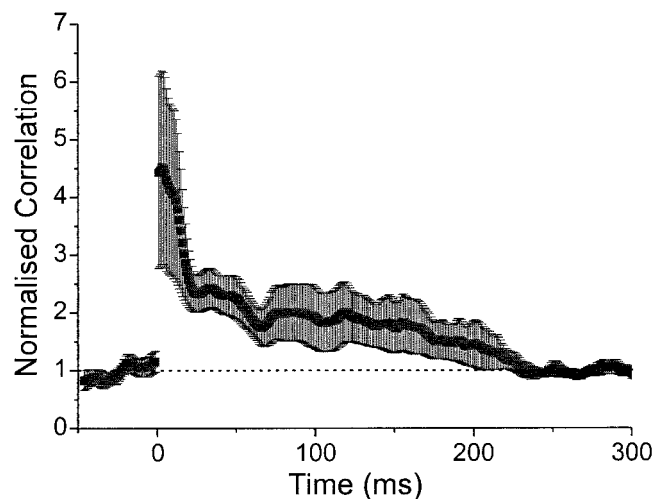
other, >1–2-mm horizontal separation. A similar form of windowed cross-correlation analysis as used above proved especially useful for this analysis. Stimulation trials were first divided into two portions, corresponding to the periods before and subsequent to stimulation. A time window of 75-ms duration was swept in millisecond increments across these samples and cross-correlation analysis was conducted within the windowed interval between the two simultaneously recorded signals of the same trial. These results were plotted according to the time at which the window began and were compared for windows taken both before and after stimulation.

Figure 5 illustrates typical results obtained by applying this type of analysis to gamma signals elicited by localized diffusion of CCh (100 mM; see Materials and Methods) from recording pipettes positioned at sites 1 and 2 (see diagram, Fig. 5A). As shown in the raw traces obtained from single trials (roman numerals in Fig. 5B), before the stimulation there were prominent examples of mismatch between rhythmic gamma signals at the two sites, consistent with low coherence (in this case, a value of 0.36) (Dickson et al., 2000a). After stimulation, however, individual signals tended to show enhanced gamma phase correspondence. This effect was most evident using the cross-correlation analysis, shown in Figure 5C. The individual plots reflect the averaged CCFs across all nine trials in representative time windows pre- and post-stimulation. There was a dramatic transient increase in the amplitude of CCFs after stimulation, which is also apparent in the surface plot of the same CCFs across time during the periods pre- and post-stimulation (Fig. 5D). Directly after stimulation, the CCF amplitude was dramatically enhanced; this increase lasted for a period of ~200 ms.

The increase in amplitude of the CCF after stimulation was observed in all experiments ( $n = 6$ ; 4 mEC and 2 CA1 stimulation experiments). In order to compare the time period during which the amplitude increase occurred, the amplitude of CCFs was normalized to the baseline (prestim) amplitude of the central (zero lag) peak for each experiment and then averaged across all experiments for every windowed time point. These values are plotted in Figure 6. The average increase in synchronization, as measured by the increased amplitude of the central peak, was maintained for a maximal post-stimulation period of 200 ms. Although the data reported above were obtained exclusively from signal pairs showing low coherence, this type of analysis was also conducted between signals from sites showing higher initial coherence values for spontaneous gamma activity ( $0.90 < r > 0.75$ ;  $n = 3$ ; not shown). As for the results illustrated above, stimulation was also found to transiently enhance the correlation between these signals. However, consistent with a ceiling effect, these increases tended to be on a smaller scale.

## DISCUSSION

We have recently demonstrated that muscarinic activation elicits spontaneous gamma oscillatory activity in the mEC of the iso-



**FIGURE 6.** Duration of transient reset-induced gamma synchronization. Pre- and post-stimulation plot of averaged (normalized) cross-correlation values for six cases including both mEC ( $n = 4$ ) and CA1 ( $n = 2$ ) stimulation experiments. Cross-correlation functions (CCFs) were computed as described in Figure 5 and the amplitude of the central (zero-lag) peak was normalized with respect to the baseline (pre-stimulation) condition and averaged over all experiments for all time points pre- and post-stimulation. These averages are displayed ( $\pm$ SE). The increase in synchronization, as measured by the increase in correlation values  $>1$ , lasted for a maximum period of 200 ms post-stimulation.

lated whole brain preparation (van der Linden et al., 1999), an *in vitro* model that provides the advantage of preserving the three-dimensional architecture of cortical structures. We observed that CCh-induced gamma activity was synchronized within, but not between, spatially localized mEC “modules” having a diameter of ~500  $\mu$ m (Dickson et al., 2000a). The present study extends our previous results by demonstrating that gamma synchronization between different mEC spatially distant modules could be transiently increased by stimulation of associative connections within and to the mEC. This increase was mediated through phase reset of the ongoing gamma activity and, as such, provides a direct demonstration of the modulation of rhythmic synchronization via synaptic input. As spontaneous gamma is a ubiquitous cortical phenomenon, we suggest that synaptically mediated oscillatory reset may provide a generalized mechanism for oscillatory synchronization in the brain.

Oscillatory reset is a physical property that allows timing adjustments in periodic processes and which therefore can allow for the synchronization of independent but coupled oscillators (Bendat and Piersol, 1986). Reset has been described for neuronal activity at the level of membrane potential oscillations (Pedroarena and Llinás, 1997; Desmaisons et al., 1999; Dickson et al., 2000c), rhythmic discharge (Buño et al., 1978; Cobb et al., 1995) and oscillating networks (Buño et al., 1978; Givens, 1996). What is novel and notable in the present study is that stimulation of afferent pathways was observed to enhance the synchronization of quasi-independently oscillating neural networks across the surface of the mEC. Synaptic entrainment via phase reset of ongoing activity may, in general, be achieved through two mechanisms. The first is

through functional interconnections between independently oscillatory neural assemblies. In this way, interactions of distant and relatively independent oscillating neural assemblies may be able to impose a mutual and dynamic state of synchronization by virtue of bidirectional phasic communication that provides a timing (phase-locking) signal. Consistent with this proposal, previous studies have shown that synchronous oscillatory responses in distantly located neural ensembles in visual cortex are correlated with specific reciprocal anatomical connections (Eckhorn et al., 1992; Gray, 1999). Furthermore, interhemispheric oscillatory neural discharge shows little to no synchrony after lesions to the corpus callosum (Engel et al., 1991b). A complementary and alternative putative synchronizing mechanism could be mediated through activity in a parallel distributed afferent system originating from a common source. In this case, synchronization would be achieved by an extrinsic and unique pace-setting signal that activates independently oscillating neural assemblies. A prominent example of this is the parallel medial septal innervation of the hippocampus and EC (Alonso and Köhler, 1984), which is necessary for the expression of coherent theta rhythm in these limbic cortical structures (Bland, 1986; Bland and Colom, 1993; Dickson et al., 1994). Furthermore, hippocampal theta activity has been shown to be reset by stimulation of the medial septal nucleus (Buño et al., 1978).

A mechanistic explanation of the induction of gamma oscillatory phase reset remains hypothetical. However, it is clear that stimulation of fiber pathways within the superficial layers of the mEC evokes a field potential dependent on excitatory glutamate receptor activation that is robustly blocked by local infusion of the AMPA receptor antagonist CNQX (Dickson et al., 2000a). Furthermore, intracellular recordings in superficial EC neurons have revealed that the activation of intrinsic associative connections of the EC elicits both feedforward excitation and inhibition (Finch et al., 1988; Jones and Heinemann, 1988; Jones, 1993, 1994; Jones and Buhl, 1993; Dickson et al., 2000b). Given that the generation of gamma activity in the mEC, like that described for archi-, paleo-, and neocortex (Freeman, 1975; Ketchum and Haberly, 1993; Whittington et al., 1995; Traub et al., 1996a; Wang and Buzsáki, 1996; Buhl et al., 1998; Fisahn et al., 1998; Leung, 1998; Zhang et al., 2000) involves an interaction between excitatory and inhibitory neuronal elements (Buzsáki and Chrobak, 1995; Chrobak and Buzsáki, 1998; Dickson et al., 2000a,b), it is likely that a synaptic volley such as that evoked by electrical stimulation acts to alter the timing of the network elements generating the oscillation. In support of this idea, laminar profile analysis (see Fig. 2) showed that the events subtending reset were temporally and spatially similar to those subtending the gamma oscillation itself. However, a definitive mechanistic explanation awaits a detailed and combined extra and intracellular study of both gamma and its reset.

Several *in vivo* studies have demonstrated that sensory stimuli can evoke transient (~100–200 ms) gamma responses, time-locked to stimulus presentation and elicited nonspecifically by elemental (simple) stimuli (Galambos et al., 1981; Ribary et al., 1991; Basar and Bullock, 1992; Galambos, 1992; Llinás and Ribary, 1993; Barth and MacDonald, 1996; Tallon-Baudry et al.,

1996; Schurmann et al., 1997; Karakas and Basar, 1998; Fries et al., 2001). These differ from induced gamma responses, which are not locked to the onset of stimulation, are longer-lasting (seconds) and are dependent on the complex configuration of the stimulus (cf. Basar and Bullock, 1992). It has been suggested that the evoked cortical gamma response at a single site could reflect resetting of ongoing spontaneous gamma rhythm (Ribary et al., 1991; Llinás and Ribary, 1993; Steriade, 1997). Given the similarity between evoked gamma and our results concerning gamma reset in the mEC this conclusion is likely to be correct. In a similar vein, electrical stimulation of hippocampal fiber systems *in vivo* can also evoke stimulus-locked transient gamma-frequency afterpotentials, which are dependent on behavioral state (Leung, 1992, 1998). Although not explicitly stated, it is also likely that these potentials reflect resetting of ongoing hippocampal gamma.

Since the EC is at the heart of a cortical-limbic loop that plays a role in both the integration and storage of diverse information (Van Hoesen, 1982; Amaral, 1987; Amaral and Witter, 1989; Witter et al., 1989; Squire and Zola-Morgan, 1991; Zola-Morgan and Squire, 1993; Squire and Alvarez, 1995; Nadel and Moscovitch, 1997), the generation of cholinergically mediated, state-dependent, or stimulus-related rhythmic synchronized activity in this region may have a crucial role for the process of memory consolidation and retrieval performed in the medial temporal lobe (Hasselmo and Bower, 1993; Lisman and Idiart, 1995; Chrobak and Buzsáki, 1998; Chrobak et al., 2000). Nadel and Moscovitch (1997) proposed that the components of a memory trace for a particular event are represented in a diffuse neuronal ensemble that includes elements in the hippocampus, the parahippocampal cortex and the neocortex. According to this model, the hippocampal formation (including the EC) serves as the unit that binds all different information in a coherent memory trace. On the basis of our results, it is tempting to speculatively ascribe to gamma activity within the EC the ability to extract an information set in response to an incoming input. The modulation of coherence and synchronization of fast oscillatory activity in the EC could be the mechanism by which selected pools of information continuously exchanged between the hippocampus and the neocortex acquire a spatial and temporal relevance that allows reinforcement of a synaptic route (link of neuronal ensembles) and promote consolidation of a particular memory trace. Enhancement of synchronization across neuronal assemblies via synaptic interactions could, indeed, provide a Hebbian reinforcing mechanism in the imprinting of neural associations (Hebb, 1949) between pre- and post-synaptic elements activated together upon repetition of the same input, through a process of synaptic enhancement (likely through long-term potentiation). Enticingly, stimulus parameters that are most robust in inducing long-term potentiation in limbic cortices mimic the state-dependent expression of rhythmic oscillatory discharge of limbic neurons (Larson and Lynch, 1986; Larson et al., 1986).

## Acknowledgments

C.T.D. was the recipient of a short-term HFSP fellowship, SF 9/99. Multi-channel silicon probes were kindly provided by the

University of Michigan Center for Neural Communication Technology, sponsored by NIH NCRR grant P41-RR09754.

## REFERENCES

- Alonso A, Köhler C. 1984. A study of the reciprocal connections between the septum and the entorhinal area using anterograde and retrograde axonal transport methods in the rat brain. *J Comp Neurol* 225:327–343.
- Amaral DG. 1987. Memory: anatomical organization of candidate brain regions. In: Plum F, editor. *Handbook of physiology*. Section 1: The nervous system. Oxford University Press, New York. p 211–294.
- Amaral DG, Witter MP. 1989. The three-dimensional organization of the hippocampal formation: a review of anatomical data. *Neuroscience* 31:571–591.
- Barth DS, MacDonald KD. 1996. Thalamic modulation of high-frequency oscillating potentials in auditory cortex. *Nature* 383:78–81.
- Basar E, Bullock TH, editors. 1992. *Induced rhythms in the brain*. Boston: Birkhauser.
- Basar E, Basar-Eroglu C, Karakas S, Schurmann M. 1999. Oscillatory brain theory: a new trend in neuroscience. *IEEE Eng Med Biol Mag* 18:56–66.
- Bendat JS, Piersol AG. 1986. *Random data: analysis and measurement procedures*. 2nd ed. New York: John Wiley & Sons.
- Bland BH. 1986. The physiology and pharmacology of hippocampal formation theta rhythms. *Prog Neurobiol* 26:1–54.
- Bland BH, Colom LV. 1993. Extrinsic and intrinsic properties underlying oscillation and synchrony in limbic cortex. *Prog Neurobiol* 41:157–208.
- Bragin A, Jandó G, Nádasdy Z, Hetke J, Wise K, Buzsáki G. 1995. Gamma (40–100 Hz) oscillation in the hippocampus of the behaving rat. *J Neurosci* 15:47–60.
- Buhl EH, Tamás G, Fisahn A. 1998. Cholinergic activation and tonic excitation induce persistent gamma oscillations in mouse somatosensory cortex in vitro. *J Physiol* 513(Pt 1):117–126.
- Buño W, Garcia-Sanchez JL, Garcia-Ausús E. 1978. Reset of hippocampal rhythmic activities by afferent stimulation. *Brain Res Bull* 3:21–28.
- Buzsáki G, Chrobak JJ. 1995. Temporal structure in spatially organized neuronal ensembles: a role for interneuronal networks. *Curr Opin Neurobiol* 5:504–510.
- Chrobak JJ, Buzsáki G. 1998. Gamma oscillations in the entorhinal cortex of the freely behaving rat. *J Neurosci* 18:388–398.
- Chrobak JJ, Lörincz A, Buzsáki G. 2000. Physiological patterns in the hippocampo-entorhinal cortex system. *Hippocampus* 10:457–465.
- Cobb SR, Buhl EH, Halasy K, Paulsen O, Somogyi P. 1995. Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. *Nature* 378:75–78.
- de Curtis M, Biella G, Buccellati C, Folco G. 1998. Simultaneous investigation of the neuronal and vascular compartments in the guinea pig brain isolated in vitro. *Brain Res Protocol* 3:221–228.
- de Curtis M, Pare D, Llinás RR. 1991. The electrophysiology of the olfactory-hippocampal circuit in the isolated and perfused adult mammalian brain in vitro. *Hippocampus* 1:341–354.
- Desmaisons D, Vincent J-D, Lledo P-M. 1999. Control of action potential timing by intrinsic subthreshold oscillations in olfactory bulb output neurons. *J Neurosci* 19:10727–10737.
- Dickson CT, Trepel C, Bland BH. 1994. Extrinsic modulation of theta field activity in the entorhinal cortex of the anesthetized rat. *Hippocampus* 4:37–52.
- Dickson CT, Biella G, de Curtis M. 2000a. Evidence for spatial modules mediated by temporal synchronisation of carbachol induced gamma rhythm in medial entorhinal cortex. *J Neurosci* 20.
- Dickson CT, Biella G, de Curtis M. 2000b. Interaction of excitatory and inhibition in the generation of gamma ( $\gamma$ ) activity in medial entorhinal cortex (mERC). *Soc Neurosci Abs* 26:703.
- Dickson CT, Magistretti J, Shalinsky M, Hamam B, Alonso A. 2000c. Oscillatory activity in entorhinal neurons and circuits: mechanisms and function. *Ann NY Acad Sci* 911:127–150.
- Eckhorn R, Schanche T, Brosch M, Salem W, Bauer R. 1992. Stimulus-specific synchronizations in cat visual cortex: multiple microelectrode and correlation studies from several cortical areas. In: Basar E, Bullock TH, editors. *Induced rhythms in the brain*. Boston: Birkhauser. p 47–80.
- Engel AK, König P, Kreiter AK, Singer W. 1991a. Interhemispheric synchronization of oscillatory neuronal responses in cat visual cortex. *Science* 252:1177–1179.
- Engel AK, Kreiter AK, König P, Singer W. 1991b. Synchronization of oscillatory neuronal responses between striate and extrastriate visual cortical areas of the cat. *Proc Natl Acad Sci USA* 88:6048–6052.
- Finch DM, Tan AM, Isokawa-Akesson M. 1988. Feedforward inhibition of the rat entorhinal cortex and subicular complex. *J Neurosci* 8:2213–2226.
- Fisahn A, Pike FG, Buhl EH, Paulsen O. 1998. Cholinergic induction of network oscillations at 40 Hz in the hippocampus in vitro. *Nature* 394:186–189.
- Freeman WJ. 1975. *Mass action in the nervous system*. New York: Academic Press.
- Fries P, Reynolds JH, Rorie AE, Desimone R. 2001. Modulation of oscillatory neuronal synchronization by selective visual attention. *Science* 291:1560–1563.
- Fuchs EC, Doheny H, Faulkner H, Caputi A, Traub RD, Bibbig A, Kopell N, Whittington MA, Monyer H. 2001. Genetically altered AMPA-type glutamate receptor kinetics in interneurons disrupt long-range synchrony of gamma oscillation. *Proc Natl Acad Sci USA* 98:3571–3576.
- Galambos R. 1992. A comparison of certain gamma band (40 Hz) brain rhythms in cat and man. In: Basar E, Bullock TH, editors. *Induced rhythms in the brain*. Boston: Birkhauser. p 201–216.
- Galambos R, Makeig S, Talmachoff P. 1981. A 40 Hz auditory potential recorded from the human scalp. *Proc Natl Acad Sci USA* 78:2643–2647.
- Givens B. 1996. Stimulus-evoked resetting of the dentate theta rhythm: relation to working memory. *NeuroReport* 8:159–163.
- Gray CM. 1999. The temporal correlation hypothesis of visual feature integration: still alive and well. *Neuron* 24:31–47.
- Gross DW, Gotman J. 1999. Correlation of high-frequency oscillations with the sleep-wake cycle and cognitive activity in humans. *Neuroscience* 94:1005–1018.
- Hasselmo ME, Bower JM. 1993. Acetylcholine and memory. *Trends Neurosci* 16:218–222.
- Hebb DO. 1949. *The organization of behavior*. New York: John Wiley & Sons.
- Herculano-Houzel S, Munk MHJ, Neuenschwander S, Singer W. 1999. Precisely synchronized oscillatory firing patterns require electroencephalographic activation. *J Neurosci* 19:3992–4010.
- Jefferies J, Traub R, Whittington M. 1996. Neuronal networks for induced “40Hz” rhythms. *Trends Neurosci* 19:202–208.
- Joliot M, Ribary U, Llinás R. 1994. Human oscillatory brain activity near 40 Hz coexists with cognitive temporal binding. *Proc Natl Acad Sci USA* 91:11748–11751.
- Jones RSG. 1993. Entorhinal-hippocampal connections: a speculative view of their function. *Trends Neurosci* 16:58–64.
- Jones RSG. 1994. Synaptic and intrinsic properties of neurons of origin of the perforant path in layer II of the rat entorhinal cortex in vitro. *Hippocampus* 4:335–353.
- Jones RSG, Buhl EH. 1993. Basket-like interneurons in layer II of the entorhinal cortex exhibit a powerful NMDA-mediated synaptic excitation. *Neurosci Lett* 149:35–39.

- Jones RSG, Heinemann V. 1988. Synaptic and intrinsic responses of medial entorhinal cortical cells in normal and magnesium-free medium "in vitro." *J Neurophysiol* 59:1476–1496.
- Karakas S, Basar E. 1998. Early gamma response is sensory in origin: a conclusion based on cross-comparison of results from multiple experimental paradigms. *Int J Psychophysiol* 31:13–31.
- Ketchum KL, Haberly LB. 1993. Synaptic events that generate fast oscillations in piriform cortex. *J Neurosci* 13:3980–3985.
- Konopacki J, Maciver MB, Bland BH, Roth SH. 1987. Theta in hippocampal slices: relation to synaptic responses of dentate neurons. *Brain Res Bull* 18:25–27.
- Larson J, Lynch G. 1986. Induction of synaptic potentiation in hippocampus by patterned stimulation involves two events. *Science* 232:985–988.
- Larson J, Wong D, Lynch G. 1986. Patterned stimulation at the theta frequency is optimal for the induction of hippocampal long-term potentiation. *Brain Res* 368:347–350.
- Laurent G. 1996. Dynamical representation of odors by oscillating and evolving neural assemblies. *Trends Neurosci* 19:489–496.
- Leung LS. 1992. Fast (beta) rhythms in the hippocampus: a review. *Hippocampus* 2:93–98.
- Leung LS. 1998. Generation of theta and gamma rhythms in the hippocampus. *Neurosci Biobehav Rev* 22:275–290.
- Lisman JE, Idiart MAP. 1995. Storage of  $7 \pm 2$  short term memories in oscillatory subcycles. *Science* 267:1512–1515.
- Llinás R, Ribary U. 1993. Coherent 40-Hz oscillation characterizes dream state in humans. *Proc Natl Acad Sci USA* 90:2078–2081.
- Llinás R, Yarom Y, Sugimori M. 1981. Isolated mammalian brain in vitro: new technique for analysis of electrical activity of neuronal circuit function. *Fed Proc* 40:2242–2245.
- Maloney KJ, Cape EG, Gotman J, Jones BE. 1996. High-frequency  $\gamma$  electroencephalogram activity in association with sleep-wake states and spontaneous behaviors in the rat. *Neuroscience* 76:541–555.
- Menon V, Freeman WJ, Cuttillo BA, Desmond JE, Ward MF, Bressler SL, Laxer KD, Barbaro N, Gevins AS. 1996. Spatio-temporal correlations in human gamma band electrocorticograms. *Electroencephalogr Clin Neurophysiol* 98:89–102.
- Metherate R, Cox CL, Ashe JH. 1992. Cellular bases of neocortical activation: modulation of neural oscillations by the nucleus basalis and endogenous acetylcholine. *J Neurosci* 12:4701–4711.
- Muhlethaler M, de Curtis M, Walton K, Llinás R. 1993. The isolated and perfused brain of the guinea pig in vitro. *Eur J Neurosci* 5:915–926.
- Munk MHJ, Roelfsma PR, Konjig P, Engel AK, Singer W. 1996. Role of reticular activation in the modulation of intracortical synchronisation. *Science* 227:271–274.
- Murthy VN, Fetz EE. 1992. Coherent 25 to 35 Hz oscillations in the sensorimotor cortex of awake behaving monkeys. *Proc Natl Acad Sci USA* 89:5670–5674.
- Nadel L, Moscovitch M. 1997. Memory consolidation, retrograde amnesia and the hippocampal complex. *Curr Opin Neurobiol* 7:217.
- Pedroarena C, Llinás R. 1997. Dendritic calcium conductances generate high-frequency oscillation in thalamocortical neurons. *Proc Natl Acad Sci USA* 94:724–728.
- Ribary U, Ioannides AA, Singh KD, Hasson R, Bolton JPR, Lado F, Mogilner A, Llinás R. 1991. Magnetic field tomography of coherent thalamocortical 40-Hz oscillation in humans. *Proc Natl Acad Sci USA* 88:11037–11041.
- Schurmann M, Basar-Eroglu C, Basar E. 1997. Gamma responses in the EEG: elementary signals with multiple functional correlates. *NeuroReport* 8:1793–1796.
- Singer W. 1999. Neuronal synchrony: a versatile code for the definition of relations? *Neuron* 24:49–65.
- Singer W, Gray CM. 1995. Visual feature integration and the temporal correlation hypothesis. *Annu Rev Neurosci* 18:555–586.
- Squire LR, Alvarez P. 1995. Retrograde amnesia and memory consolidation: a neurobiological perspective. *Curr Opin Neurobiol* 5:169–177.
- Squire LR, Zola-Morgan S. 1991. The medial temporal lobe memory system. *Science* 253:1380–1386.
- Steriade M. 1997. Synchronized activities of coupled oscillators in the cerebral cortex and thalamus at different levels of vigilance. *Cerebral Cortex* 7:583–604.
- Steriade M, Curró Dossi R, Paré D, Oakson G. 1991. Fast oscillations (20–40 Hz) in thalamocortical systems and their potentiation by mesopontine cholinergic nuclei in the cat. *Proc Natl Acad Sci USA* 88:4396–4400.
- Steriade M, Amzica F, Contreras D. 1996. Synchronization of fast (30–40 Hz) spontaneous cortical rhythms during brain activation. *J Neurosci* 16:392–417.
- Tallon-Baudry C, Bertrand O. 1999. Oscillatory gamma activity in humans and its role in object representation. *Trends Cog Sci* 3:151–162.
- Tallon-Baudry C, Bertrand O, Delpuech C, Pernier J. 1996. Stimulus specificity of phase-locked and non-phase-locked 40 Hz visual responses in human. *J Neurosci* 16:4240–4249.
- Tiitinen H, Sinkkonen J, Reinikainen K, Alho K, Lavikainen J, Naatanen R. 1993. Selective attention enhances the auditory 40-Hz transient response in humans. *Nature* 364:59–60.
- Traub R, Whittington M, Colling S, Buzsáki G, Jefferys J. 1996a. Analysis of gamma rhythms in the rat hippocampus in vitro and in vivo. *J Physiol (Lond)* 493:471–484.
- Traub RD, Whittington MA, Stanford IM, Jefferys JGR. 1996b. A mechanism for generation of long-range synchronous fast oscillations in the cortex. *Nature* 383:621–624.
- Traub RD, Biggig A, Fisahn A, LeBeau FEN, Whittington MA, Buhl EH. 2000. A model of gamma-frequency network oscillations induced in the rat CA3 region by carbachol in vitro. *Eur J Neurosci* 12:4093–4106.
- van der Linden S, de Curtis M, Panzica F. 1999. Carbachol induces fast oscillations in the medial but not in the later entorhinal cortex of the isolated guinea pig brain. *J Neurophysiol* 82:2441–2450.
- Van Hoesen GW. 1982. The parahippocampal gyrus. New observations regarding its cortical connections in the monkey. *Trends Neurosci* 5:345–350.
- Wang X-J, Buzsáki G. 1996. Gamma oscillation by synaptic inhibition in a hippocampal interneuronal network model. *J Neurosci* 16:6402–6413.
- Whittington M, Traub RD, Jefferys JGR. 1995. Synchronized oscillations in interneuron networks driven by metabotropic glutamate receptor activation. *Nature* 373:612–615.
- Witter MP, Groenewegen HJ, Lopes da Silva FH, Lohman AHM. 1989. Functional organization of the extrinsic and intrinsic circuitry of the parahippocampal region. *Prog Neurobiol* 33:161–253.
- Zhang S, Jose JV, Tiesinga PHE. 2000. Model of carbachol-induced gamma-frequency oscillations in hippocampus. *Neurocomputing* 32/33:617–622.
- Zola-Morgan S, Squire LR. 1993. Neuroanatomy of memory. *Annu Rev Neurosci* 16:547–563.