Carbachol Induces Fast Oscillations in the Medial but not in the Lateral Entorhinal Cortex of the Isolated Guinea Pig Brain

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van der Linden, Solange, Ferruccio Panzica, and Marco de Curtis. Carbachol induces fast oscillations in the medial but not in the lateral entorhinal cortex of the isolated guinea pig brain. J. Neurophysiol. 82: 2441–2450, 1999. Fast oscillations at 25–80 Hz (gamma activity) have been proposed to play a role in attention-related mechanisms and synaptic plasticity in cortical structures. Recently, it has been demonstrated that the preservation of the entorhinal cortex is necessary to maintain gamma oscillations in the hippocampus. Because gamma activity can be reproduced in vitro by cholinergic activation, this study examined the characteristics of gamma oscillations induced by arterial perfusion or local intracortical injections of carbachol in the entorhinal cortex of the in vitro isolated guinea pig brain preparation. Shortly after carbachol administration, fast oscillatory activity at 25.2–28.2 Hz was observed in the medial but not in the lateral entorhinal cortex. Such activity was transiently associated with oscillations in the theta range that showed a variable pattern of distribution in the entorhinal cortex. No oscillatory activity was observed when carbachol was injected in the lateral entorhinal cortex. Gamma activity in the medial entorhinal cortex showed a phase reversal at 200–400 μm, had maximal amplitude at 400–500 μm depth, and was abolished by arterial perfusion of atropine (5 μM). Local carbachol application in the medial entorhinal cortex induced gamma oscillations in the hippocampus, whereas no oscillations were observed in the amygdala and in the piriform, periamygdaloid, and perirhinal cortices ipsilateral and contralateral to the carbachol injection. Hippocampal oscillations had higher frequency than the gamma activity recorded in the entorhinal cortex, suggesting the presence of independent generators in the two structures. The selective ability of the medial but not the lateral entorhinal cortex to generate gamma activity in response to cholinergic activation suggests a differential mode of signal processing in entorhinal cortex subregions.

INTRODUCTION

Fast oscillatory activity in the gamma range (25–80 Hz) has been described in several cortical regions (for review see Farmer 1998; Jefferys et al. 1996; Singer and Gray 1995) and has been interpreted as an attention and arousal mechanism that promotes associative binding between large ensembles of neurons in the neocortex (Gray et al. 1989; Llinas and Ribary 1993; Murthy and Fetz 1996; Steriade et al. 1996). The presence of fast oscillations in the gamma range has been demonstrated in vivo in the hippocampus (Bragin et al. 1995; Penttonen et al. 1998; Traub et al. 1996) and in the entorhinal cortex (ERC) of the rat (Chrobak and Buzsaki 1998; Eckman and Freeman 1990), the cat (Boeijinga and Lopes da Silva 1988), and the guinea pig (Charpak et al. 1995) and can be reproduced in vitro either by orthodromic high-frequency stimulation (Funahashi and Stewart 1998; Stanford et al. 1998; Whittington et al. 1995, 1997) or by pharmacological cholinergic activation (Fisahn et al. 1998). It is commonly accepted that the cholinergic system plays a crucial role in determining fast activity and in sustaining propagation of such activity within the cortex. Indeed, fast cortical activity at ~40 Hz is enhanced during states of cortical arousal (Llinas and Ribary 1993; Maloney et al. 1996), increases after stimulation of the brainstem cholinergic ascending system (Steriade et al. 1991, 1996; Munk et al. 1996) and after pharmacological activation of the basal forebrain cholinergic nuclei (Cape and Jones 1998), and can be induced by muscarinic agonists (Fishan et al. 1998), as mentioned earlier.

Gamma activity in limbic cortices has been proposed to provide a functional setting that facilitates a condition during which synaptic plasticity occurs (Traub et al. 1998). A differential function and possibly a hierarchic organization between cortical structures in the control of limbic fast oscillation is suggested by the observation that surgical removal of the ERC has a modulatory influence on gamma activity in the hippocampus (Bragin et al. 1995; Charpak et al. 1995). The demonstration that fast activity in the ERC leads to or entrains gamma hippocampal oscillations led us to study gamma activity in this cortical region and to evaluate its relation with other limbic structures in an in vitro isolated guinea pig brain preparation (de Curtis et al. 1991; Muhlethaler et al. 1993). The regional distribution of fast oscillations in the ERC was evaluated after pharmacological stimulation of the preparation with the muscarinic receptor agonist, carbachol. Surprisingly, during our study the fast oscillations induced by carbachol were observed exclusively in the medial-septal region of the ERC and not in its lateral-temporal portion that borders the rhinal sulcus. Preliminary results have been presented in abstract form (van der Linden and de Curtis 1998).

METHODS

Guinea pig brains were isolated from young adult animals (150–250 g) and were maintained in vitro according to a technique previously described in detail (de Curtis et al. 1991, 1994, 1998; Llinas et al. 1991; Muhlethaler et al. 1993). The animals were anesthetized with 20 mg/kg thiopental sodium (Pentothal) and were perfused intracardially with ice-cold (10°C), carbogenated (95% O₂,5% CO₂) saline solution consisting of 126 mM NaCl, 3 mM KCl, 1.2 mM KH₂PO₄, 1.3 mM MgSO₄, 2.4 mM CaCl₂, 26 mM NaHCO₃, 15 mM glucose, 2.1 mM HEPES, 0.4 mM thiourea, and 3% dextran, molecular weight 70,000 (SIFRA, Isola della Scala, Italy). The brain was removed from the skull after an extensive craniotomy performed under hypothermic conditions and was positioned ventral side up in a perfusion chamber cooled at 15°C. A polyethylene cannula was inserted and secured to

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one vertebral artery, and perfusion with the solution described was started at a rate of 5.5 ml/min. The contralateral vertebral artery, the carotid artery, and the hypophyseal system were tied with silk threads to establish the perfusion of the whole brain through the basilar system through the circle of Willis. The temperatures of both the chamber and the perfusate were gradually increased to 30°C at a rate of 0.1°C/min. The experimental protocol was reviewed and approved by the Committee on Animal Care and Use and by the Ethics Committee of the Istituto Nazionale Neurologico.

Extracellular recordings were performed either with glass micropipettes filled with a 0.9% NaCl solution (≈10 MΩ input resistance) or with stainless steel microelectrodes (5–10 MΩ; FHC, Brunswick, ME). The signals were amplified and high-pass filtered at 0.2 Hz through an extracellular amplifier (Biomedical Engineering, Thornwood, NY) and were stored by means of a digital tape recorder (DTR 2602, Biologic, Claix, France) for off-line analysis with a Pentium computer. The software for acquisition and analysis was developed in our laboratory by Gerardo Biella in collaboration with Marco Fiorengetti (SIDeA, Milan, an alliance member of National Instruments, Italy). In general, recordings were performed at 4–5 different sites simultaneously. The location of the recording electrodes in different ERC regions were decided and reproduced in different experiments with reference to brain surface markers, using as a guide the guinea pig brain atlas by Luparello (1978). A bipolar silver wire electrode was used to stimulate the lateral olfactory tract (LOT) with 0.1-ms current pulses of variable amplitudes delivered at low frequencies (<0.2 Hz). LOT stimulation evoked large-amplitude field potentials in the lateral ERC (LERC), whereas in the medial ERC (MERC) small responses that showed a decreasing amplitude gradient from rostral to caudal were recorded. The potentials evoked by LOT stimulation were used to evaluate the viability of the preparation during the experiment. When stainless steel electrodes were used for recordings, the location of the electrode tip was marked by electrolytic lesions performed by passing a 100-μA current for 2–5 s at the tip of the electrode after the electrophysiological experiment. The brains were fixed in 4% formaldehyde in 0.1 M phosphate buffer (pH 7.4) for 1 wk and 100-μm coronal sections were cut, mounted on slides, and stained with thionine to verify the location of the lesions.

Carbachol (Sigma) was applied either by local pressure injection or by arterial perfusion. Local applications at a cortical depth of 500 μm in either the LERC or the MERC were achieved by a single 10-s microinjection of 5–50 mM carbachol in 0.9% NaCl through a glass pipette (10 μm external tip diameter) connected to a graduated syringe. These parameters corresponded to the injection of ~50 μl of solution. Extracellular recordings were performed from the pipette used for the injection of carbachol. When applied arterially, carbachol was dissolved in the perfusate at a concentration of 100 μM. The muscarinic antagonist atropine (Sigma) was dissolved in the Ringer solution (5 μM) and administered by arterial perfusion.

To better characterize the presence of gamma activity in the ERC, power spectral density was estimated by means of a bivariate autoregressive (AR) parametric model, based on a regression method (Dumermuth and Molinari 1991; Marple 1986). The main advantages of the AR method are that it does not need the averaging procedure required by the more commonly applied fast Fourier transform and that the frequency resolution of the spectrum does not depend on the duration of the analyzed epoch (Panzica et al. 1999). In a bivariate AR parametric model, the two signals are represented through the following linear relationship

\[ X(t) + A_1 X(t-1) + A_2 X(t-2) + \ldots + A_p X(t-p) = E(t) \]

where \( X(t) = (x_{1,t}, x_{2,t}) \) is the bidimensional vector representing the sample of the two signals at the discrete time \( t; A_1, A_2, \ldots, A_p \) are the \( 2 \times 2 \) matrices of the model coefficients, \( E(t) = (e_{1,t}, e_{2,t}) \) is the vector of the two-dimensional white-noise process with zero mean and covariance matrix \( R \), and \( P \) is the order of the AR model. Given

**Fig. 1.** Differential effect of local intracerebral injection of carbachol in the medial entorhinal cortex (MERC) and the lateral entorhinal cortex (LERC). A: single 10-s injection of carbachol (50 mM) in the intermediate MERC (iMERC, *) induced fast rhythmic oscillations in the gamma range (~10 min) that gradually increased in amplitude and propagated to the rostral and caudal MERC (trMERC, cMERC). In the rostral and caudal lateral ERC (rLERC, cLERC), no gamma activity was observed. The locations of the recording electrodes on the ERC surface are shown in the ventral view of the guinea pig brain (A, left). B: injection of carbachol into the rLERC (*) after washout of the iMERC-injection effect evoked no fast activity. In this figure, Fig. 2, and Fig. 3, ERC recordings at five sites were performed simultaneously.

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the set of parameters describing the AR modeling, the power spectral density can be estimated by the following formula

\[ S(f) = H(f) \overline{R} H(f)^T \]

where

\[ H(f) = \left( I - \sum_{k=1}^{p} A_k e^{-i2\pi(f+k\Delta f)} \right) \]

\[ I \] is the identity matrix, \( \overline{H} \) is the complex conjugate of \( H \), the apical \( T \) denotes the transposition of the matrix, and \( \Delta T \) is the sampling interval. The AR model order was determined by using the multichannel version of the Akaike AIC criterion (Marple 1987). The coherence function is defined as

\[ C_{xy}(f) = \frac{(S_{xy}(f))^2}{(S_{xx}(f))(S_{yy}(f))} \]

FIG. 2. Effect of local injection of carbachol in the MERC. A: single 10-s injection of carbachol (20 mM) in the iMERC (+) induced transient rhythmic sequences at 8–12 Hz in the rMERC and rLERC simultaneously to gamma activity in the iMERC (5 min). The 8–12-Hz activity decreased within 10 min after carbachol injection, and gamma oscillations become larger and propagated to the entire MERC, but were not observed in the LERC. Positions of the recording electrodes are shown in the ventral view of the brain (A, left). Arrowheads indicate the responses evoked by afferent stimulation of the lateral olfactory tract. B: power spectra obtained from recordings performed in the same experiment as in A before (dotted lines) and 5 min (dashed lines) and 25 min (continuous lines) after carbachol injection into the iMERC. Peak of 8–12-Hz activity was present in all recording sites at 5 min (+) and disappeared in all sites except the rLERC at 25 min. Inset, top left (rMERC): gamma activity when the power density scale was uniformed to that used for cMERC and iMERC.
where $S_{xx}(f)$ and $S_{yy}(f)$ were the power spectral densities of the two channels, $(x$ and $y$ respectively), and $S_{xy}(f)$ is the cross-spectral density.

RESULTS

Extracellular recordings before and after carbachol application were performed from different ERC regions in 29 guinea pig brains maintained in vitro. Considering the similarities between the cortical organization of the guinea pig and the rat, we used the cytoarchitectonic criteria described in the rat to divide the guinea pig ERC in two areas that we designated MERC and LERC (Insausti et al. 1997; Menno Witter, personal communication; see DISCUSSION for details). In the large majority of the experiments electrodes were positioned (under direct visual control with a stereoscopic microscope) in the rostral and the caudal part of the LERC (rLERC and cLERC) and in the rostral, the intermediate, and the caudal part of the MERC (rMERC, iMERC, and cMERC; see inset in Fig. 1A) at a cortical depth of 400–500 μm. The predicted location of the electrodes was consistently confirmed after histological verification in 14 experiments (see Figs. 4 and 5 for ERC locations).

In a set of experiments the effect of carbachol (5–50 mM) locally injected for 10 s at 400–500-μm depth in the LERC and the MERC was first analyzed (Fig. 1). When injections were performed, no detectable change in the responses evoked by LOT stimulation was observed at any of the recording sites. Carbachol injections in the iMERC generated rhythmic field activity at 26.5–32.0 Hz ($n = 18$). The gamma activity appeared first in the iMERC 5–10 min after drug injection and subsequently was observed in the rMERC and cMERC, but never in the LERC (Fig. 1A). The involvement of the rMERC in the generation of gamma oscillation was inconsistent and depended on the location of the electrode (description follows). The activity had a continuous character, increased progressively to reach maximal amplitude within 30 min, and persisted ≥2 h after a single 10-s injection of carbachol. The frequency of the fast oscillations increased to 41.7–45 Hz when the temperature of the preparation was raised from 32 to 37°C ($n =$
5). When carbachol was applied locally to the LERC (either in the rLERC or in the cLERC; \( n = 6 \)), no rhythmic activity was observed (Fig. 1B). Figure 2 illustrates in detail the events that followed iMERC application of 20 mM carbachol. In 8 of 18 experiments field oscillations at 8–12 Hz transiently appeared in the rMERC at the same time that fast oscillation was initiated in the iMERC (Fig. 2A, 5 min); 8–12-Hz oscillations lasted 5–15 min. Frequency analysis demonstrated that such carbachol-induced rhythmic activity (see DISCUSSION) was transiently observed throughout the ERC, as illustrated by the frequency peaks of the spectra recorded 5 min after carbachol injection (dashed lines in Fig. 2B; note the different scales in the four diagrams). Concurrent with 8–12-Hz activity, faster oscillations in the gamma range appeared in the MERC, but not in the LERC. In the illustrated experiment small-amplitude gamma activity was observed in the rMERC (inset in the top left panel in Fig. 2B). The histological verification performed afterward showed that the recording electrode was not precisely positioned in the rMERC, but at the border with the rLERC (described later).

In a subsequent set of experiments, carbachol (50–100 \( \mu \text{M} \)) was administered by arterial perfusion (\( n = 5 \)). The drug was diluted in the perfusate and applied for 10 min. As for the local MERC application, 8–12-Hz activity that showed a continuous pattern at the onset was observed (Fig. 3A, 16 min); such activity subsequently grouped in prolonged 2–10-s bursts (Fig. 3A, 25 min) that recurred in 20–30-s intervals. Eventually, 8–12-Hz activity decreased in amplitude and disappeared within 10–30 min. When 8–12-Hz activity sequences were observed, small-amplitude gamma activity also appeared in the MERC. Its amplitude increased progressively with time and reached maximal values in the intermediate-caudal MERC ~30 min after perfusion began, regardless of the duration of the carbachol application, which usually did not last longer than 10 min (Fig. 3A, 1 h). The mean peak frequency of the fast activity recorded in different ERC regions is reported in Fig. 3B (\( n = 5 \)).

Regarding the local carbachol injections, gamma activity was expressed in the MERC exclusively. When the perfusion with carbachol was maintained for a period >10 min (\( n = 4 \)), epileptiform discharges characterized by ictal bursts of large-amplitude population spikes that propagated throughout the limbic cortices, the hippocampus, and the amygdala were observed (not shown). Fast oscillatory activity in the MERC was observed also when epileptiform activity was induced. To sort out whether the gamma oscillations recorded simultaneously in cMERC and iMERC were related to each other, we performed coherence analysis between pairs of recordings. This procedure revealed a very low degree of coherence between the cortical regions. The correlation coefficient calculated on the oscillatory activity recorded simultaneously at different MERC locations was 0.226 for the local carbachol iMERC injections (\( n = 6 \)) and 0.155 for the arterial carbachol perfusions (\( n = 4 \)).

Figures 4 and 5 illustrate the histological verification of the ERC recording sites. Figure 4 shows the locations of three electrolytic lesions performed during an experiment in which carbachol was ejected in the iMERC. Gamma activity was observed in the iMERC (\( \bigcirc \)) and in the cMERC (\( \triangle \)), but not in the cLERC (\( \bullet \); Fig. 4B). In Fig. 5 the distribution of the electrolytic lesions performed in 14 experiments is illustrated. The location of the lesions (represented by the symbols) were superimposed on sampled coronal ERC sections representative of the rostrocaudal levels at which the lesions were observed. The open symbols represent the sites where gamma activity was recorded. No gamma oscillatory activity was observed in the sites illustrated by the filled symbols. The caudal, intermediate, and rostral portions of the LERC and MERC are described by different symbols (see legend). The results confirm that fast oscillatory activity was generated exclusively in the caudal and medial part of the ERC. The lesions corresponding to the recording electrodes directed at the most rostral part of the MERC were found in a cortical region that showed the cytoarchitectonic features of the rLERC in three of five experiments, due to the broadening of the most rostral part of the

**FIG. 4.** Histological controls of MERC and LERC recording electrodes. A: electrolytic lesions in the cMERC (\( \bigcirc \), left) and in the iMERC and cLERC (\( \bigcirc \), \( \bullet \) right) were induced after recording the activity illustrated in B. Thionine-stained 100-\( \mu \text{m} \) thick coronal section. B: carbachol was ejected in the iMERC, and gamma oscillations were recorded in the cMERC and in the iMERC, but not in the cLERC. In A, calibration bar: 1 mm.
LERC, which could be visualized by the medial extension of the patchy appearance of layer II in Fig. 5, D, E, and F. In the experiments in which the displacement of the electrode in the LERC instead of the targeted rMERC was histologically demonstrated, no gamma activity was observed (filled symbols).

To identify the depth location of the generators of the fast oscillatory activity in the MERC, depth profiles were performed in the iMERC by inserting an electrode perpendicular to the cortical surface and by advancing it in 100-mm steps, while a reference recording electrode was placed close by at 500 mm. During the penetrations a phase reversal of the gamma activity was observed at 200–400 mm depth (Fig. 6A and B), which corresponds to layer II (n = 5). The amplitude of the gamma activity was maximal at 400–500 mm.

When gamma activity was established in the MERC after local iMERC injection, simultaneous extracellular recordings were performed from different rhinencephalic structures in five experiments. No carbachol-induced gamma activity was found in the anterior and posterior piriform cortices, in the amygdala, in the perirhinal (PRC) and postrhinal (PoRC) cortices ipsilateral to the carbachol injection, or in the contralateral ERC (Fig. 7). Fast oscillatory activity in the CA1 region of the hippocampus was observed in three of four experiments. Such activity started earlier in the MERC and showed higher frequency in the hippocampus (23–30 Hz) than in the iMERC (22–25 Hz), suggesting the existence of independent local gamma activity generators in the two cortical structures (Fig. 7). When carbachol was applied by arterial perfusion (n = 3), fast activity appeared simultaneously in the amygdala, the hippocampus, and the MERC, but not in the LERC, PC, PRC, and PoRC (data not shown).

Previous in vitro and in vivo studies have demonstrated that the effects of carbachol application are abolished by the antagonist of the cholinergic muscarinic receptor atropine. Accordingly, the fast oscillations induced by carbachol in the MERC completely disappeared after arterial perfusion of atropine (5 μM; n = 7), whereas the LOT-evoked field response was not affected or was slightly increased in amplitude in both LERC, which could be visualized by the medial extension of the patchy appearance of layer II in Fig. 5, D, E, and F. In the experiments in which the displacement of the electrode in the LERC instead of the targeted rMERC was histologically demonstrated, no gamma activity was observed (filled symbols).

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the MERC and LERC (Fig. 8C). The atropine effect reverted after washout in four tests.

**DISCUSSION**

Recently Insausti and co-workers (1997) proposed to use cytoarchitectonic and connectivity criteria to distinguish ERC subfields. According to this study, the portions of the ERC composed of six distinct layers located caudally and medially correspond to the previously defined medial entorhinal area (Hjorth-Simonsen and Jeune 1972; Steward and Scoville 1976), whereas the strip of cortex close to the rhinal sulcus that broaden rostrally and shows a dense layer II formed by neurons grouped in clusters or islands separated by acellular bands corresponds to the lateral entorhinal area. The ERC subdivision in two principal regions on the basis of cytoarchitectonic features is also present in the guinea pig and was used in our study to identify the regions that are capable of generating carbachol-induced gamma oscillations. The medial part of the ERC that corresponds to the medial and caudal ERC of Insausti et al. (1997) produced high-frequency oscillations, whereas the lateral and intermediate parts of the ERC close to the rhinal sulcus did not generate gamma rhythmic activity after either local or arterial application of carbachol. It should be noted that the LERC and the MERC also differ substantially in their patterns of afferent and efferent connections. Retrograde tracing studies of the cortical extrahippocampal projections to the ERC demonstrate that in the rat and the monkey the lateral strip of ERC adjacent to the rhinal sulcus projects diffusely to those neocortical areas (Kosel et al. 1982; Swanson and Köhler 1986) that are also connected to the PRC (Burwell et al. 1995; Kosel et al. 1982), whereas sparse retrograde labeling was observed in the MERC when neocortical injections were performed. Moreover, the LERC shares with the PRC the pattern of projection into the subiculum (van Haeften and Witter 1997). Further indications of the functional independence between MERC and LERC are provided by the existence of separate anatomic projections to either one of the two major subdivisions of the ERC. For instance, the presubicular projections to the ERC are confined to the medial and dorsal portions of the guinea pig ERC (Shipley 1975) and the projection from the olfactory bulb and the piriform cortex terminate preferentially in the LERC (Krettek and Price 1978). These findings suggest that the MERC and the LERC can be considered as anatomically and functionally distinct structures.

The ERC receives a cholinergic input from the basal forebrain predominantly on layer II and layer V (Alonso and Köhler 1984; Eckenstein et al. 1988; Gaykema et al. 1990). Cholinergic inputs to the cortex are commonly interpreted as an arousing signal. In fact, it has been demonstrated that cortical gamma frequency activity is enhanced either by electrical stimulation of the mesopontine cholinergic nuclei (Steriade et al. 1991, 1996) or by pharmacological activation of the basal forebrain (Cape and Jones 1998). Moreover, functional brain states such as wakefulness and paradoxical sleep correlate to an increase of gamma frequencies in the electroencephalogram (Maloney et al. 1996). The ultimate evidence that establish the involvement of the cholinergic system in the control of fast oscillations is the recent demonstration that gamma activity can be induced by application of the muscarinic receptor agonist carbachol in slices of hippocampus and neocortex maintained in vitro (Buhl et al. 1998; Fisahn et al. 1998). Our findings confirm that fast oscillations in the gamma range are activated by muscarinic activation in the MERC,
Our understanding of the MERC function is based on a wealth of anatomic data and physiological studies performed in vivo and in vitro. According to these studies it can be concluded that even if neurons in the MERC are able to generate fast oscillations in the gamma range (Chrobak and Buzsáki 1998), such activity cannot be accounted for exclusively by intrinsic membrane properties. Indeed, neurons that exhibit intrinsic fast oscillatory activity such as those described in thalamus and neocortex (Gray and McCormick 1996; Llinás et al. 1991; Steriade et al. 1996) were never observed in the MERC neurons of layer II (Alonso and Klink 1993; Alonso and Llinás 1989), layer III (Dickson et al. 1997; van der Linden and Lopes da Silva 1998), and layer V (Jones 1993; Jones and Heinemann 1988). Moreover, the lack of studies that specifically describe the network and intrinsic properties of LERC neurons does not allow any conclusion to be drawn about the role of differential expression of electroresponsive properties in explaining the different ability of MERC and LERC to generate gamma oscillations in response to cholinergic activation.

The possibility should be mentioned that the absence of gamma in the LERC could be due to the experimental conditions we used—that is, the use of cholinergic agonists to induce fast oscillation. It is feasible that sustained gamma activity in the hippocampus could synaptically entrain fast oscillations in the LERC. A careful evaluation of the coherence relationship between gamma rhythms in limbic structures performed in vivo will help to clear up this issue.

The demonstration of a low coherence between fast oscillations recorded simultaneously at different sites in the MERC after either local or arterial perfusion of carbachol suggests that gamma activity is not a population event that synchronizes the entire cortical region, but rather a local event generated at multiple sites within the MERC. As for CA1 and the subiculum (Bragin et al. 1995; Penttonen et al. 1998; Stanford et al. 1998; Traub et al. 1996; Whittington et al. 1995), the gamma oscillations in the ERC were proposed to be elicited in principal neurons by rhythmic inhibitory postsynaptic potentials (IPSPs) imposed by a tonic excitation on mutually inhibitory interneurons (Chrobak and Buzsáki 1998). Interactions between local interneurons (together with a reciprocal excitation among pyramidal cells) also play a major role in sustaining the oscillations induced by carbachol in the CA3 region of the hippocampus (Fisahn et al. 1998). If local interneurons are involved in the generation of fast oscillatory activity in the MERC, our data on the absence of coherence between gamma activity recorded in different MERC locations and between MERC and hippocampus support the idea that segregated pools of interneurons may entrain patches of cortex independently. This assumption is in agreement with the conclusion reached in a recent study on carbachol-induced epileptiform discharges in the MERC, which demonstrated that giant IPSPs activated by muscarinic stimulation probably result from the postsynaptic effect of synchronous firing of interneurons on layer II principal neurons (Dickson and Alonso 1997). These IPSPs were asynchronous at sites separated by more than 200 μm, suggesting that the events were generated by discrete and independent pools of neurons in the ERC. A careful pharmacological characterization of the events observed in our experiments will reveal whether a similar network organization could sustain gamma generation and propagation in the MERC.

Studies performed in slices have demonstrated that layer II neurons in the MERC have the intrinsic capability to produce subthreshold membrane oscillations in the theta range (Alonso and Klink 1993; Alonso and Llinás 1989). Rhythmic oscillations at 5–12 Hz can be induced in vitro by perfusing cortical slices with carbachol (Bland and Colom 1993; Konopacki et al. 1987). In our experiments 8–12 Hz activity occurred simultaneously with gamma activity but was prevalent in most rostral portions of LERC and MERC, in contrast with the gamma oscillations that were found exclusively in the MERC and were observed only transiently (5–15 min), whereas gamma activity lasted several hours. Such theta-like activity showed peculiar features reminiscent of carbachol-induced hypersynchronous discharges described in the hippocampus (Traub et al. 1992; Williams and Kauer 1997), the ERC (Dickson and Alonso 1998; Klink and Alonso 1997), and the neocortex (Lukatch and McIver 1997) in vitro. The functional significance of this activity is still unresolved and its possible correlation with the theta activity recorded in vivo is controversial.

Early anatomic studies demonstrated that layer II neurons of the MERC and LERC in the rat project to different portions of the molecular layer in the dentate gyrus (i.e., to the middle (proximal) and the outer (distal) dendrites of granule cells, respectively) (Hjorth-Simonsen and Jeune 1972; Steward and Scoville 1976; Witter 1993). Such a distinct projection pattern was subsequently confirmed electrophysiologically (Canning and Leung; 1997; Dickson and Alonso 1998; Leung et al. 1995; McNaughton 1980; Wilson and Steward 1978). The laminar segregation of MERC and LERC inputs to the dentate gyrus may be related to the functional meaning of the differential expression of gamma oscillations in the two ERC regions. We speculate that if both MERC and LERC receive simultaneously afferent inputs from different brain regions, the reciprocal weight of the ERC outputs transmitted to the hippocampus through the dentate gyrus could be different whether fast oscillations in the MERC are present or not. The cholinergic modulation of layer II MERC neurons during gamma oscillations can enhance the probability that these neurons will reach firing threshold, as demonstrated in the hippocampus (Fishan et al. 1998). It has been demonstrated that layer II neurons in the MERC are able to endure high-frequency firing at 40 Hz during synaptic activation (Gioveli et al. 1997). Repetitive high-frequency firing of the MERC may lead to a sustained synaptic depolarization of the proximal dendrites of the granule cell, which could heterosynaptically facilitate a separate synaptic input simultaneously impinging on the distal dendrites of the dentate neuron through the concomitant activation of the LERC input.

The present findings represent the first demonstration of a functional distinction between the MERC and the LERC that may underlie different mechanisms of information transfer from the neocortex to the hippocampus. Cholinergic modulation may influence the processes of memory formation and/or retrieval by way of inducing gamma oscillations in the MERC that could work as a switch to enhance the strength of the synapses formed by the medial perforant path on the dentate granular cells.
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