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Gamma oscillations induced by kainate receptor activation in the entorhinal cortex *in vitro*

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Abstract

Gamma frequency (30 – 80 Hz) oscillations are recordable from human and rodent entorhinal cortex. A number of mechanisms used by neuronal networks to generate such oscillations in the hippocampus have been characterised. However, it is as yet unclear as to whether these mechanisms apply to other anatomically disparate brain regions. Here we show that the medial entorhinal cortex (mEC) in isolation in vitro generates gamma frequency oscillations in response to kainate receptor agonists. Oscillations had the same horizontal and laminar spatiotemporal distribution as seen in vivo and in the isolated whole brain preparation. Oscillations occurred in the absence of input from the hippocampal formation and did not spread to lateral entorhinal regions. Pharmacological similarities existed between oscillations in the hippocampus and mEC in that the latter were also sensitive to $GABA_A$ receptor blockade, barbiturates, AMPA receptor blockade and reduction in gap junctional conductance. Stellate and pyramidal neuron recordings revealed a large GABAergic input consisting of gamma frequency IPSP trains. Fast spiking interneurons in the superficial mEC generated action potentials at gamma frequencies phase locked to the local field. Stellate cells also demonstrated a subthreshold membrane potential oscillation at theta frequencies which was temporally correlated with a theta-frequency modulation in field gamma power. Disruption in this stellate theta frequency oscillation by the Ih blocker ZD7288 also disrupted theta modulation of field gamma frequency oscillations. We propose that similar cellular and network mechanisms to those seen in the hippocampus generate and modulate persistent gamma oscillations in the entorhinal cortex.

Introduction

Cortical gamma band (30-80 Hz) oscillations are thought to grant large ensembles of neurones the ability to engage in associative binding, in particular during processing of incoming sensory signals (Gray et al., 1989; Singer & Gray, 1995; for review see Verala et al., 2001). Thus, gamma activity has been recorded in sensorimotor (Murphy & Fetz, 1996), auditory (Barth & MacDonald, 1996) and visual cortices (Roelfsema et al., 1997), Gamma frequency oscillations have been reported in the entorhinal cortex (EC) of humans during wakefulness (Hirai et al., 1999; Uchida et al., 2001), as well as in rodents *in vivo* (Charpak et al., 1995; Chrobak & Buzsáki, 1998) and in an isolated whole brain preparation (van der Linden et al., 1999; Dickson et al., 2000).

The EC occupies a unique position in the temporal lobe, in that it acts as an interface between neocortical regions and the cornu ammonuis as well as other associated limbic structures such as the subiculum and dentate gyrus (Van Hoesen, 1982; Witter et al., 1989; Witter et al., 2002). Superficial neurones of the EC supply the main neocortical input to the hippocampus via the perforant pathway (Steward & Scoville, 1976; Witter & Groenewegen, 1984; Amaral & Witter, 1995), whilst the deep layer neurones receive output from the hippocampus via CA1 and the subiculum (Naber et al., 2001; Witter et al., 2001). In this respect, the EC occupies a pivotal position in the process of gating information flow along the neo-archicortical axis. Hence, the EC is assumed to act as a supermodal associational cortical region, where inputs from a number of sensory areas can assemble (Van Hoesen, 1982; Amaral & Witter, 1989; Witter et al., 1989) and engage in a functional interplay of sensory information with the hippocampus (Fernandez et al., 1999; Fell et al., 2001). The importance of the EC is further emphasised by the fact

that it is an early and selective site of pathological changes which occur in disorders of memory function such as Alzhemier's disease (Hyman et al., 1984; Van Hoesen et al., 1991; Brakk, 1991) and schizophrenia (Beckmann & Senitz, 2002).

Gamma oscillations have been described in a number of *in vitro* slice preparations. In a number of these metabotropic receptor activation has induced this activity (Whittington et al., 1995; Fisahn et al., 1998) or application of nanomolar concentrations of kainate (Hájos et al., 2000; Hormuzdi et al., 2001). However attempts to elicit gamma activity in an *in vitro* preparation of the EC with application of the metabotropic cholinergic agonist, carbachol occasionally resulted in the generation of epileptiform activity (Dickson & Alonso, 1997; Gloveli et al., 1999). Here we present data, which demonstrates that the activation of kainate receptors can generate persistent gamma activity *in vitro* in the EC. This activity was associated with sparse activation of principal cell soma but large-scale phasic interneuronal output. Population gamma frequency activity was associated with trains of inhibitory postsynaptic potentials in a similar manner to that seen in models of hippocampal gamma oscillation. Preliminary data from this study has been published in abstract form (Cunningham et al., 2002a; Cunningham et al., 2002b).

Methods

Preparation of slices

Transverse EC-hippocampal slice (450 μm) were prepared from adult Sprague-Dawley rats, anaesthetized with inhaled isoflurance, immediately followed by an I.M. injection of ketamine (≥ 100 mg kg^{-1}) and xylazine (≥ 10 mg kg^{-1}). Animals were

intracardically perfused with ~50 ml of modified artificial cerebrospinal fluid (ACSF), which was composed of (in mM): Sucrose (252), KCl (3), NaH_2PO_4 (1.25), $NaHCO_3$ (24), $MgSO_4$ (2), $CaCl_2$ (2) and glucose (10). All salts were obtained from BDH (Poole, UK). The brain was removed and submerged in cold (4-5°C) ACSF during dissection, and horizontal slices were cut using a Campden vibroslice. Slices were then transferred to either holding chamber or directly to recording chamber. Here, they were maintained at 34 ^{o}C at the interface between a continuous stream (1.2ml/min) of ACSF (composition in mM: NaCl (126), KCl (3), NaH_2PO_4 (1.25), $NaHCO_3$ (24), $MgSO_4$ (2), $CaCl_2$ (2) and glucose (10)), and warm, moist carbogen gas (95% O_2 : 5% CO_2). Slices were permitted to equilibrate for 45 minutes before any recordings commenced.

Drugs

All drugs were bath applied at known concentrations: kainic acid (2*S*,3*S*,4*R*)-carboxy-4-(1methylethenyl)-3-pyrrolidineacetic acid) 200-400 nM; NBQX (2,3- dioxo-6-nitro-1,2,3,4-tetrehydrobenzo[*f*]quinoxaline-7-sulfonamide) 20 μM; carbachol 20-50 μM; domoic acid ([2*S*-[2a,3b,4b(1Z,3E,5R,]]-2-Carboxy-4-(-5-carboxy-1-methyl-1,3-hexadienyl)-3-pyrrolidineacetic acid) 100 nM; bicuculline methochloride 2 μM, ZD7288)4-ethylphenylamino-1,2-dimethyl-6-methylaminopyridinium chloride), 10 μM, were all obtained from Tocris Cookson (UK), pentobarbital 20 μM and carbenoxenlone 100 μM were obtained from Sigma.

Recording, data acquisition and analysis

Extracellular recordings electrodes were pulled from borosilicate glass (Harvard Apparatus, UK) filled with ACSF and had resistances in the range of 2~5 MΩ. For the purpose of intracellular recordings, electrodes were pulled from borosilicate glass filled

with KCH_3SO_4 and had resistances in the range of 70~130 MΩ. Three types of neuron were recorded from in the entorhinal cortex, each identified by the following specific electrophysiological parameters: Stellate cells: pronounced sag observed with hyperpolarizing current step, with action potentials present on rebound depolarization. Long duration depolarizing current steps (500 – 800 ms) revealed action potentials of stellates cells had a fast afterhyperpolarization (fAHP) which was followed by a depolarizing after-potential (DAP) and action potential had half-widths >1 ms. There was also accommodation of action potentials. Upon tonic depolarisation, stellate cells exhibited a sub-threshold membrane oscillation. Pyramidal cells: displayed little or no sag in response to hyperpolarizing current, a rebound depolarization was not observed. No sub-threshold membrane oscillation was apparent. Depolarizing current revealed action potentials of pyramidal cells displayed a fAHP but no DAP and had half-widths <1.5 ms. Action potential accommodation was also observed (van der Linden & Lopes de Silva, 1998). Maximum firing frequencies on current injection >400 Hz, no Action potential accommodation, action potential half-widths <0.6 ms. Peak frequency and power values were obtained from power spectra generated with Fourier analysis in the Axograph software package (Axon Instruments). Power for a given frequency band was determined as the area under the peak in the power spectra between 20 and 80 Hz for gamma frequency oscillations. All values are given as mean ± S.E. Power spectra were constructed off line from digitised data (digitization frequency 10 kHz) using a 60 second epoch of recorded activity. MatLab (Mathworks, USA) was used to generate spectrograms. The kinetics of IPSPs were measured using MiniAnalysis (Jaejin, USA), >200 IPSPs were obtained per slice and pooled for further analysis.

Results

Kainate receptor agonists induced gamma activity in the mEC

When slices of EC were bath perfused with nanomolar concentrations of kainic acid (200-400 nM) rhythmic (50-200 μV) field activity was observed in all slices studied (n=36, fig. 1*A*). Activity in the superficial and deep layers had a mean frequency of 46.4 ± 1.9 and 45.4 ± 2.0 Hz, respectively, whilst the mean area power was 622.0 ± 116.6 and 215.9 ± 43.6 $\mu V^2.s^{-1}$, respectively. In all cases the power of the oscillation was significantly greater in superficial layers than with that in the deep layers ($P < 0.001$)(Fig. 1*A*), whilst there was no significant difference in mean frequencies ($P < 0.1$). The activity between superficial and deep layers of the EC was in anti-phase (see inset Fig. 1*A*). Once initiated, the network activity was stable for many hours (~ 4/5 h). In addition, the more potent kainate receptor agonist, domoic acid (domoate) (100 nM) was also able to elicit network activity in both layers of the EC (n=5) (Fig. 1*B*). The activity in superficial and deep layers had a mean frequency of 48.3 ± 3.9 and 48.3 ± 3.5 Hz (n = 5), and a mean area power of 828.4 ± 383.8 and 415.0 ± 171.3 $\mu V^2.s^{-1}$, respectively. A cross-correlogram of the activity produced by domoate revealed, in agreement with kainate experiments, that a sharp 180° phase reversal was seen in layer III (see also Fig. 3).

Spatial profile of gamma activity in the EC

Previously, Fisahn (1999) has demonstrated that the bath application of kainate can generate gamma activity in the hippocampus *in vitro*. When 400 nM kainate was bath applied to the combined slice preparation gamma activity was observed in stratum pyramidale in both CA3 and CA1 in this present study, (Fig. 2*A*). The gamma activity

had a significantly lower mean frequency (CA3 = 32.6 ± 1.8 Hz, CA1 = 33.7 ± 1.1 Hz, n=9, $P < 0.05$ compared with mEC data above). The EC and the hippocampus have extensive and robust reciprocal connections (Witter et al., 1986; Witter et al., 1989). Therefore, in order to determine whether the EC was capable of generating gamma activity independent of the hippocampus we carried out a number of microlesions. Control activity in the EC was recorded before subsequent microlesions. The control activity (superficial/deep) had a mean area power of 537.0 ± 122.5 and 310.7 ± 68.6 $\mu V^2.s^{-1}$. Lesion of area CA3 from the rest of the slice (lesion 1, fig.2) produced a reduction in mean area power in the mEC (superficial = 408.6 ± 118.0, deep = 232.6 ± 77.6 $\mu V^2.s^{-1}$) but this was not significant ($P > 0.05$, n=6). Further lesion of area CA1 from the mEC recording sites (lesion 2, fig. 2) had no significant effect on mean area power (superficial = 371.2 ± 107.1, deep = 238.8 ± 83.8 $\mu V^2.s^{-1}$, $P > 0.05$, n=6).

In vivo and whole-brain studies also demonstrated that entorhinal gamma activity was spatially localized across the horizontal plane of the mEC. To test this in vitro a reference electrode was orientated at the most medial portion of the mEC and a second electrode was then moved in 50 μm steps towards the lateral EC. In the superficial layers, the amplitude and area power of gamma activity was consistent across 300 μm of the medial portion of the EC and sharply diminished on reaching the lateral EC (Fig. 3A). In the deep layers, gamma activity peaked in the medial portion and slowly diminished as the electrode was moved laterally (Fig. 3A). No change in peak frequency of kainate-induced oscillation was seen along the longitudinal axis of EC in either deep or superficial layers. Direct comparison of power in the gamma frequency range between

mEC and lateral EC showed a significant reduction in oscillations in the lateral part of EC ($P < 0.05$, fig. 3B, n=6).

In the isolated whole guinea pig brain preparation (van der Linden et al., 1999; Dickson et al., 2000) gamma oscillations were shown to have a marked phase reversal through the laminae. We tested this by placing one reference electrode in the sub-cortical white matter of the angular bundle and a second electrode close and perpendicular to the reference electrode in 100 μm steps towards the pia. Around the level of the perisomatic region (i.e., between layer II and III), a distinct phase reversal was observed (Fig 4. *A* & B). The maximal area power of gamma was also observed around the site of phase reversal, in layer III (1130.5 ± 174.6 $\mu V^2.s^{-1}$; n=22). In order to ascertain whether this phase shift was simply due to a polarity change of the extracellular field or a genuine phase difference between gamma generators in deep and superficial layers we carried out joint field and intracellular IPSP train measurements. Intracellular events in layer III pyramidal neurons and extracellular activity in layer II were found to be in phase (Fig. 5A), whereas intracellular events in layer III pyramidal neurons and field activity in layer III where in antiphase (Fig. 5B). Intracellular events in layer II stellate cells and field activity in layer III where also in antiphase (Fig. 5C). Thus principal neurons either side of the layer where the field phase shift was seen share the same temporal pattern of inhibitory drive despite changes in the phase of the field (see discussion).

GABAergic and glutamatergic pharmacology of gamma activity in the mEC

Gamma activity in the hippocampus is critically dependent on the rhythmic synchronous output of populations of interneurons (Whittington et al., 1995; Fisahn et al., 1998). To test whether this hypothesis also held for the entorhinal cortex we first reduced

fast GABAergic transmission. After inducing gamma activity with kainate, co-application of a low concentration of bicuculline (2 μM) resulted in the virtual abolition of the population gamma activity (Fig. 6A; n=7). Prolongation of the decay kinetics of $GABA_A$ receptor-mediated IPSPs has been shown to have a marked effect on gamma oscillations driven by tonic interneuronal excitation, but much less of an effect when phasic excitation is required (see Whittington et al., 2000). Pentobarbital (20 μM) (Fig. 6B; n=6) produced a small but significant reduction ($P < 0.001$) in the peak frequency of gamma activity (superficial; control: 45.8 ± 2.6, pentobarbital: 37.4 ± 2.8, washout: 45.4 ± 2.4 Hz, deep; control: 43.7 ± 2.6, pentobarbital: 34.9 ± 2.6, washout: 44.8 ± 2.8 Hz).

Fast phasic glutamatergic transmission has been demonstrated to influence persistent gamma activity in hippocampal and neocortical slices (Buhl et al., 1998; Fisahn et al., 1998). Thus, we investigated the possible involvement of this mechanism in mEC by blocking AMPA receptors. Addition of SYM 2206 (20 μM; n=3), a potent non-competitive AMPA receptor antagonist (Li et al., 1999; Behr et al., 2001) significantly reduced gamma frequency activity both the superficial and deep layers ($P < 0.05$, fig 7A). In superficial layers only this near abolition of activity in the gamma band was accompanied by a small but significant increase in activity at lower frequencies (4-12 Hz range) (peak frequency: control v. SYM 2206; 44.8 ± 2.5 v. 11.6 ± 1.6 Hz; $P < 0.001$). In a subsequent set of experiments the application of the mixed AMPA/kainate receptor antagonist NBQX (20 mM; n=3), abolished all rhythmic activity in both layers (Fig. 7*B*).

The precise timing of phasic excitatory inputs to interneurons during gamma oscillations in the hippocampus has been proposed to involve gap junctions between principal cell axons (Traub et al., 2000). In the mEC, application of the gap junction

blocker carbenoxolone (100 μM) significantly ($P < 0.05$; n=6) reduce gamma power in both the superficial (control *v*. drug; 399.9 ± 97.2 *v*. 115.1 ± 38.7 $\mu V^2.s^{-1}$) and deep (56.9 ± 10.1 *v*. 14.3 ± 5.1 $\mu V^2.s^{-1}$) layers (data not shown).

Characterisation of cellular and synaptic behaviour during γ-activity in the mEC

Using sharp microelectrodes we have examined the intrinsic and synaptic properties of both principal excitatory neurones and putative, fast spiking inhibitory interneurones. When stellate neurons in layer II and pyramidal neurons in layer III were recorded at depolarised holding potentials (≥ -40 mV, eg. see fig. 5) trains of large amplitude inhibitory postsynaptic potentials were seen (8.3 ± 0.2 mV; n = 5871 IPSPs from eight neurons). Spectral analysis demonstrated a peak in the gamma range (layer II *v*. layer III; 42.3 ± 2.3 *v*. 37.3 ± 1.1 Hz). With no holding current applied, stellate neurones in layer II and pyramidal neurons in layer III, fired spontaneous action potentials, with significantly different ($P < 0.05$) mean frequencies of 0.7 ± 0.1 and 5.0 ± 0.6 Hz (n=6, fig. 8A), respectively. When the membrane potential of neurones was adjusted to close to the reversal potential of IPSPs (≥ -70 mV; n=7), small amplitude EPSPs (1.4 ± 0.01 mV; n=5900) were observed. Analysis of these events demonstrated that they were generated over a faster frequency range than concurrent stellate/pyramidal cell output (stellate vs. pyramid; 7.6 ± 0.6 v. 11.8 ± 0.7 Hz, $P < 0.05$, fig. 8B,C). Recordings from fast spiking interneurons located in layer II (n=3), revealed that during field gamma oscillations these cells generated action potentials in the gamma range (45.1 ± 2.8 Hz). These interneurons received significantly larger EPSPs at faster frequencies than in the two principal neuron types studied. However, EPSPs were still present in

these cells at lower frequencies than their output (EPSP frequency was 29.3 ± 2.8 Hz cf. Action potential frequency above).

The origin of theta-frequency modulation of field gamma oscillations.

Unlike in vivo and whole brain mEC recordings, little overt theta frequency activity was seen in power spectral analysis of the gamma oscillations in this study. However, the amplitude of field gamma activity in the superficial mEC (layers II and III was strongly modulated at theta frequencies throughout kainate application (modulation frequency 3.2 ± 0.2 Hz; n=12, fig.9A,B). In contrast field gamma frequency activity in the deep layers demonstrated no such modulation (fig. 9C). This frequency modulation was significantly different from the pattern of synaptic excitation see in either principal cell type (3.2 *v*. 7.6; 3.2 *v*. 11.8; Hz $P < 0.001$). It has been previously shown that stellate cells in the EC have theta-frequency subthreshold membrane potential oscillations (Alonso and Llinas, 1989; Alonso and Llinas, 1993; Jones, 1994). In this model stellate cells demonstrated a subthreshold theta frequency membrane potential oscillation of 3.2 ± 0.1 Hz (n = 11) with a fixed phase relationship with the modulated concurrent field recordings (5.9 ± 1.1 ms, n=11). To investigate whether this behaviour in these cells was responsible for the observed modulation of gamma frequency oscillations we recorded from stellate cells concurrently with superficial EC field recordings in the presence and absence of ZD7288, 10 μM. The subthreshold membrane potential oscillation has been proposed to be, in part, mediated by the action of a hyperpolarisation activated current (Ih), which is blocked by ZD7288. Bath application of ZD7288 had no significant effect on overall field gamma power measured as area (control: 379.8 ± 134.9 $\mu V^2.s^{-1}$, ZD7288: 324.3 ± 121.0 $\mu V^2.s^{-1}$, P>0.1, n=8). However, no rhythmic pattern of modulation of field

gamma frequency was seen in the presence of this drug (fig. 10). Cross correlograms of subthreshold stellate cell activity and the field oscillations showed a significant reduction in central peak amplitude (control: -0.05 ± 0.00, ZD7288: -0.02 ± 0.01, $P < 0.05$, n=5) and a significant reduction in the peak frequency of stellate cell membrane potential oscillations from 3.2 Hz (above) to 2.0 ± 0.2 Hz ($P<0.05$) with a considerable broadening of the spectral peak within the theta frequency range.

Discussion

The entorhinal cortex plays a pivotal role in transferring information to and from the neocortex and the limbic regions of the brain (Fernandez et al., 1999; Fell et al., 2001). It has been proposed to act as a major associational area, taking information from many neocortical areas (Van Hoesen, 1982; Amaral & Witter, 1989; Witter et al., 1989). Associating activity from spatially distant brain regions in a single, separate region may be seen as an extension of the binding hypothesis for associating activity in areas coding for separate features of a sensory object. The role of rhythmogenesis (particularly at gamma frequencies) may therefore be seen as a critical tool used by the entorhinal cortex to temporally organise multiple inputs of diffuse spatial origin. Different forms of gamma frequency network activity have different properties relevant to the temporal organisation of local and spatially distributed firing patterns (e.g. see Whittington et al., 2000). Thus it is important to understand the mechanisms used by the entorhinal cortex to generate gamma frequency oscillations.

Gamma frequency rhythmogenesis in the hippocampal formation is becoming better understood and provides a reference point with which to compare entorhinal rhythms. The present data demonstrated that, at least superficially, the medial entorhinal

cortex can generate persistent field gamma rhythms in a similar manner to the hippocampus in the presence of kainate receptor activation. The lateral entorhinal cortex did not respond in such a manner in the slice as in whole brain studies (van der Linden et al., 1999; Dickson et al., 2000). There is a lack of evidence for gross anatomical and cytoarchitectonic differences between the lateral and medial entorhinal cortex (van Der Linden, 1999). However, the lateral entorhinal cortex does receive afferent input from different sources to the medial entorhinal cortex (Kosel et al. 1982; Swanson and Köhler 1986) further providing evidence for a functional difference between the two regions.

In vivo and whole brain studies also demonstrated a marked 180^{o} phase change between laminae II and III in the mEC. This observation was replicated in the slice preparation here but could not be ascribed to two independent, antiphase, gamma generators. Instead, principal neurons either side of the phase reversal point received temporally identical patterns of gamma frequency IPSPs. These data suggested that the phase reversal seen in field recordings may represent a source/sink interaction. The main polar neurons in the mEC are the pyramidal neurons found predominantly in LIII. Spatially discrete, perisomatic and proximal apical dendritic synaptic inputs from interneurons onto these cells, as seen for hippocampal pyramids (Papp et al., 2001), would be expected to generate the field phase relationship seen here and in the in vivo/whole brain situation.

In the hippocampus two main subtypes of GABA-dependent gamma frequency network activity can be seen experimentally. The first subtype, interneuron network gamma (ING), is seen transiently in response to brief periods of direct excitation of populations of interneurons (Whittington et al., 1995). It requires no phasic excitatory

input from principal cells and generates a gamma frequency network output on the basis of the kinetics of interneuron-interneuron IPSPs. As such it is exquisitely sensitive, in terms of frequency, to barbiturates and requires no gap junctional communication for local generation (Traub et al., 2001). The second type, pyramidal/interneuron network gamma (PING), is seen persistently and does require phasic synaptic excitation of interneurons via AMPA receptors (Fisahn et al., 1998; Traub et al., 2000; Hormuzdi et al., 2001). It is less sensitive to barbiturates, in terms of frequency, and requires gap junctional communication in conditions where individual principal cell firing rates are much lower than population frequency. The gamma oscillations seen in the mEC correspond more closely to PING than to ING: They require AMPA receptors and gap junctions and the frequency is altered only a little (but significantly) by barbiturates. However, the spike rates for LIII pyramidal cells was higher than that seen in CA3 and CA1 pyramids. Despite this the frequency of EPSPs invading fast spiking interneurons in the superficial entorhinal cortex was significantly lower than the spike frequency in these cells, The interneurons generated an output which matched the field gamma frequency suggesting that some action potentials were generated as a consequence of tonic excitatory drive and not through the phasic drive from population EPSPs - a situation reminiscent of ING models in the hippocampal formation.

Field theta oscillations are a prominent feature of in vivo recordings from the entorhinal cortex. However, these rhythms are thought to arise from inputs from the medial septum/diagonal band (Mitchell et al., 1982; Dickson et al., 1994; Jeffery et al., 1995; Leranth et al., 1999), a structure absent from the slices used in the present work. However, strong amplitude modulation of field gamma rhythms was seen at theta

frequencies suggesting that the EC can generate theta frequency oscillations intrinsically. The most likely source for this modulation comes from stellate cells in the superficial layers. No theta modulation was seen in the deep layers (fig. 9) and stellate cells have been proposed to generate theta frequency oscillations both alone, as a consequence of intrinsic conductances, and as a network of neurons coupled by recurrent excitation (Haas & White, 2002).

Intrinsic conductances such as I_h have been shown to be involved in generating theta frequency outputs from theta generating interneurons in the hippocampus (Gillies et al., 2002) and stellate cells express large amounts of this conductance (Alonso & Llinas, 1989; Alonso & Llinas, 1993; White et al., 1995; Dickson et al., 2000). Blockade of I_h with ZD7266 significantly disrupted both subthreshold stellate cell theta rhythms and the theta frequency modulation of field gamma amplitude. These data suggest that stellate cells may modulate gamma frequency activity in the superficial EC in addition to providing a population theta frequency output to the hippocampal formation via the perforant path (Holsheimer et al., 1983; Boeijinga & Lopes da Silva FH, 1988; Heynen & Bilkey, 1994; Bragin et al., 1995; Charpak et al., 1995; Chrobak & Buzsaki, 1998).

In summary, the medial entorhinal cortex in vitro generates persistent gamma oscillations in a manner similar to that seen in the cornu ammonis. Differential involvement of pyramidal neurons and stellate cells (in terms of mean firing frequency) suggests different patterns of input to the hippocampus via the temporo-ammonic and perforant pathways. The similarities in mechanisms used by the hippocampus and mEC to generate gamma frequency population oscillations may facilitate the temporal organisation of information passing along this axis.

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Figure Legends

Figure 1. Application of nanomolar concentrations of kainate receptor agonists induced persistent network oscillations in the mEC in vitro. *A*, Perfusion of kainate (400 nM) generated fast oscillations in the gamma range in the superficial and deep layers of the mEC. Field recordings in **Ai** demonstrate extracellular recordings from superficial and deep mEC during control and 60 minutes following the initial application of kainate. **Aii**. Pooled power spectra (n = 36) for 60s epochs of field recording before (dashed line) and after (solid line) kainate application in superficial (left hand graph) and deep (right hand graph) mEC. **Bi.** Addition of the more potent kainate receptor agonist, domoate (100 nM) also produced fast oscillations in the gamma range. **Bii**. Pooled power spectra of the signals before and after the application of domoate for superficial and deep

layers (n = 5). The inset *graphs* in both power spectra are cross-correlograms of the superficial and deep traces shown in **A(i)** and **B(i)**. Calibration: **Ai & Bi** 50 μV, 100 ms.

Figure 2. Gamma activity in the mEC is generated independently from the hippocampus. Ai. Recordings from hippocampal regions CA1 and CA3 (s.p. = stratum pyramidale) demonstrate ongoing gamma activity. **Aii**. Control field gamma activity (c) in the mEC, in the presence of 400 nM kainate in the intact slice. Following lesion of area CA3 (1) Field recordings marked **1** were recorded following the first lesion, which separated CA3 from CA1. Field recordings denoted **2** were made following the second lesion, which separated CA1 from the subiculum and mEC. *B*, Power spectrum of pooled experiments (n=6) for superficial (top) and deep (lower), illustrate that compared to control (*continuous line*), lesion **1** (*dashed line*) and lesion **2** (*dotted line*) did not significantly reduce ($P > 0.01$) gamma-activity recorded in both layers of the mEC. *C*, Schematic diagram of combined entorhinal-hippocampal slice demonstrates the location of both microlesions. Calibration: *A* 100 μV (top scale bar)/20 μV (lower scale bars), 100 ms.

Figure 3. Lateral profile of gamma-activity across superficial and deep layers. Electrode was moved in 50 μm steps from a reference position at the most medial portion of the mEC as illustrated in the diagram. **A.** Graphs show pooled data (n=6) for area power (upper two graphs), peak power and frequency (lower two graphs) across superficial and deep layers of the mEC. As the recording electrode is moved across the lamina to a more lateral position, the power of the activity significantly diminished. **B**.

Pooled power spectra (n=6) illustrating that there was little gamma-activity observed in the lateral EC (*dashed line*) during the application of kainate, when compared with the mEC (*continuous line*).

Figure 4. Laminar profile of gamma activity in the mEC. Profile was obtained by placing one electrode as a reference in the subcortical white matter, a second electrode was then moved in steps of 100 μm across the mEC laminae. **A.** Field recordings illustrating gamma-activity across all layers of the mEC in one experiment. Note phase change marked with asterisk, which occurred in the region between layer II and III. **B.** Graphs showing pooled data (n=12) for power, phase and frequency. Note that layer III exhibits the highest gamma activity and that the phase shift is confirmed, in addition the frequency of the activity was consistent across all layers. Calibration: **A** 50 μV, 50 ms.

Figure 5. Origin of the phase reversal of gamma activity observed in the mEC. **A**. *Upper trace*: intracellular recording of rhythmic IPSP activity in a layer III pyramidal cell during ongoing gamma oscillation, cell was depolarised to -40 mV by injection of current through the recording electrode. *Lower trace*: simultaneous extracellular field recording in layer II, i.e. across the phase reversal point illustrated in fig. 4. *Graph* shows pooled cross correlogram (n=6) of concurrently recorded field and intracellular IPSP data. The activity was in phase. **B.** IPSPs again recorded from layer III pyramidal neuron (membrane potential -40 mV), with concurrent field recordings were of gamma activity layer III, i.e. at the same side of the phase reversal point illustrated in fig. 4. Pooled cross-correlogram (n=6) demonstrates that the IPSPs and field are 180° out of phase. **C.** IPSPs

recorded from a layer II stellate neuron depolarised to -40 mV, the lower trace is a concurrent field recording from layer III of the mEC. Pooled cross-correlogram (n=6) shows the activity to be 180° out of phase. Calibration: **A,B,** ***& C*** *upper trace* 1 mV, 100 ms; *lower trace* 50 μV, 100 ms.

Figure 6. Gamma frequency oscillations in the mEC were critically dependent on $GABA_A$ receptor mediated activity. **A**. The $GABA_A$ receptor antagonist bicuculline (2 μM) virtually abolished gamma-activity in both superficial and deep layers of the mEC. Activity was restored on washout of the drug. Power spectrums located in the right panel illustrate pooled results for both layers (n=7). **B.** Traces show control activity from superficial and deep mEC. Application of pentobarbital (20 μM) caused the power of the activity, as well as a slowing of the frequency of the activity. This reduction in both superficial and deep layers was highly significant ($P < 0.001$) and the effect was reversible on washout. Power spectra located in the right panel demonstrate pooled power spectra (n=6). Calibration: **A & B** 50 μV and 100 ms.

Figure 7. Action of AMPA receptor antagonists on mEC gamma activity. A. Example traces showing the action of SYM 2206 (20 μM) on field gamma oscillations in superficial and deep mEC. Whilst gamma activity was abolished in deep layers, in the superficial addition of the drug abolished activity in the gamma band but revealed a small peak in the theta range. Power spectra illustrate the pooled data from these experiments (n=3). **B.** Control field recordings show gamma activity in superficial and deep layers of

the mECm before and after addition of NBQX. Pooled power spectra (n=3) are shown in the right panel. Calibration**: A & B**, 50 μV and 100 ms.

Figure 8. Pattern of action potential generation in three major cell types in the superficial mEC during gamma activity. **A.** Intracellular recordings from: *i* layer II stellate neuron, *ii* layer III pyramidal neuron and *iii* a layer II fast spiking interneuron during kainate-induced field gamma frequency oscillations. **B.** Recordings of EPSP trains in each cell type injected with hyperpolarising current to keep membrane potential at -70 mV. Examples of such recordings from a layer II stellate, layer III pyramid and layer II interneuron are illustrated in *i*, *ii* and *iii*. **C.** Combined action potential frequency histograms and EPSP power spectra illustrate the frequency of action potentials and EPSPs in each cell type. Cell firing frequencies are shown as the grey lines, whilst EPSPs power spectra are the black lines. Calibration: **A** 10 mV and 250 ms; **B** 2 mV and 250 ms.

Figure 9. Theta frequency modulation of field gamma frequency oscillations in superficial layers. A. Spectorgram of a three second period of field gamma activity in layer II of the mEC. *Lower trace* is raw data trace of the activity shown in spectrogram. **B.** Spectrogram of a three second period of field gamma activity in layer III of the mEC. Again, *lower trace* is raw data trace of the activity analysed. **C.** Spectrogram illustrating relative paucity of theta frequency modulation of field gamma oscillation power in the deep mEC. *Lower trace* is raw data. Calibration: **A,B & C** 50 μV, 500 ms. Note different scales on the spectrograms

Figure 10. Blockade of Ih disrupts theta frequency modulation of field gamma oscillations and subthreshold membrane potential oscillations in stellate cells. A. Control data. **i**. Concurrent recordings of field from LII (upper trace) and a LII stellate neuron (middle trace) demonstrating power modulation of field gamma response coinciding with subthreshold slow membrane potential oscillations in stellate neurons. Lower trace shows the same data from a stellate cell low pass filtered at 12 Hz. **ii**. Spectrogram of field gamma frequency activity illustrating quantal nature of the power in the gamma band. **iii**. Cross correlogram of field data vs. low pass filtered stellate membrane potential data. **B**. Effects of 10 μM ZD7288. **i**. Concurrent recordings of field from LII (upper trace) and a LII stellate neuron (middle trace) demonstrating the disruption of power modulation of field gamma response concurrently with disruption of the subhreshold slow membrane potential oscillations in stellate neurons. Lower trace shows the same data from a stellate cell low pass filtered at 12 Hz. **ii**. Spectrogram of field gamma frequency activity illustrating disrupted quantal nature of the power in the gamma band. **iii**. Cross correlogram of field data vs. low pass filtered stellate membrane potential data. Scale bars Ai, Bi; 20 μV (upper traces), 2 mV (lower traces), 250 ms.