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In vitro recorded theta-like activity in the limbic cortex: comparison with spontaneous theta and epileptiform discharges

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Abstract. The generation of EEG theta rhythm in the mammalian limbic cortex is a prime example of rhythmic activity that involves central mechanisms of oscillations and synchrony. This EEG pattern has been extensively studied since 1938, when Jüng and Kornmüller (1938) demonstrated the first theta recordings in the hippocampal formation of rabbits. In 1986 in collaboration with Drs. B.H. Bland, S.H. Roth and B.M. MacIver we demonstrated for the first time that bath perfusion of hippocampal slices with the cholinergic agonist, carbachol, resulted in theta-like oscillations. Since this initial demonstration of *in vitro* theta-like activity, we have carried out a number of experiments in an attempt to answer the basic question: what are the similarities between cholinergic-induced *in vitro* theta-like activity and theta rhythm which naturally occurs in the *in vivo* preparation. Thus far, our studies have provided strong evidence that theta-like activity recorded *in vitro* shares many of the physiological and pharmacological properties of theta rhythm observed *in vivo*. The question whether *in vitro* theta-like oscillations reflect features of epileptiform activity is also adressed in this review.

Review

Key words: theta rhythm, limbic cortex, *in vitro*, cholinergic receptors, GABAergic receptors

INTRODUCTION

Over the past few years there appeares to have been an increasing interest in the elaboration of basic mechanisms underlying neural oscillation and synchrony, particularly with respect to its involvement in the production of regular slow activity (RSA, theta rhythm). Theta rhythm is the largest (1-2 mV), most prominent, and best synchronized (3-12 Hz) electroencephalogram (EEG) generated by the mammalian brain. This EEG pattern is spontaneously generated and can be also evoked by different experimental procedures (Fig. 1). Commonly, theta activity has been associated with the hippocampal formation (HPC) since it is one of the most conspicuous activities recorded in this structure (Vanderwolf and Leung 1983, Bland 1986, Lopes da Silva et al. 1990, Bland and Colom 1993). However, a number of the *in vivo* reports have revealed that the HPC is not the only limbic cortical region involved in the production of theta activity. Theta oscillations have also been recorded from the entorhinal cortex (EC) and the cingulate cortex (CC) and recently from the posterior hypothalamic area (PH) in freely behaving or anesthetized animals (Mitchell and Ranck 1980, Alonso and Garcia--Austt 1987, Borst et al. 1987, Leung and Borst 1987, Dickson et al. 1994, Grabowski et al. 1996, Kocsis and Vertes 1997).

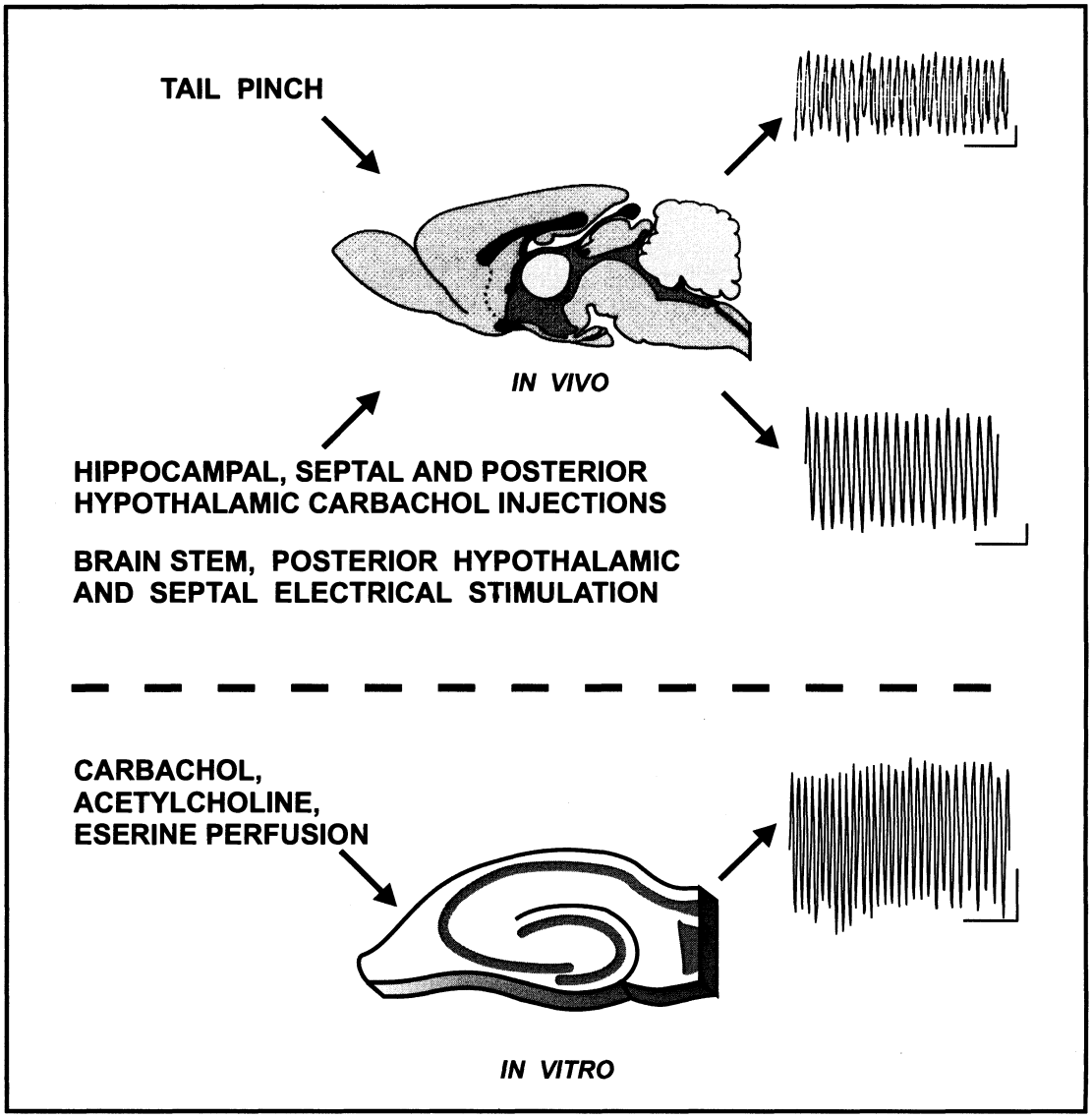

Fig. 1. Experimental methods of inducing theta field potentials in *in vivo* and *in vitro* preparation. Calibration: 1s and 200 μV.

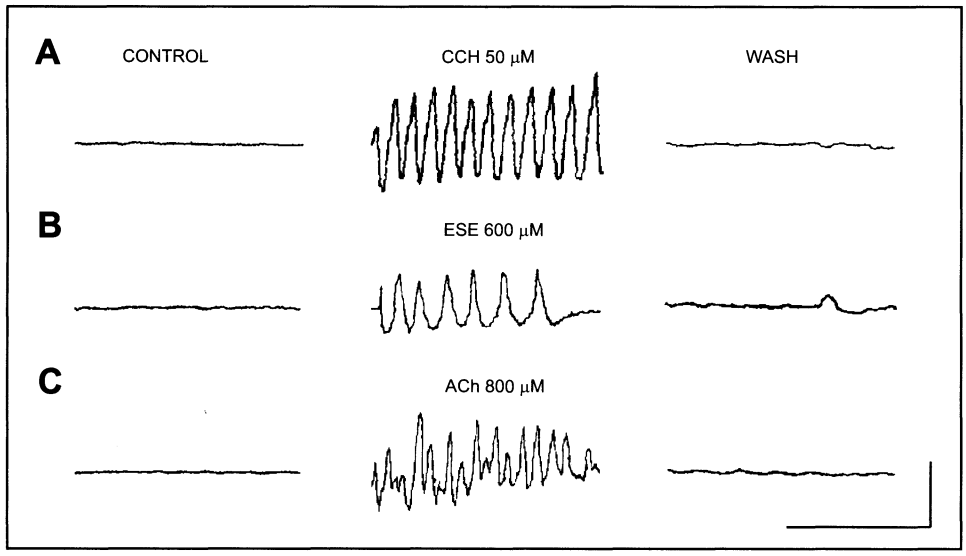

Fig. 2. Comparison of slow wave activity recorded from the molecular layer of the dentate gyrus after perfusion: carbachol (CCH, 50 μM), eserine (ESE, 600 μM) and acetylocholine (ACh, 800 μM). *In vitro* theta-like activity was usually reversed within a 60 min wash with artificial cerebrospinal fluid (WASH). Calibration: 1 s and 200 μV.

It is the intent of this review to demonstrate that the limbic cortex mechanisms underlying the production of oscillation and synchrony can also be succesfully investigated in complete isolation from the extrinsic input (i.e. in the *in vitro* maintained brain slice preparation obtained from the HPC and EC of rats and cats). Interestingly, more than 20 years ago Lynch and Schubert (1980) pointed out that one of the differences in the electrophysiology of the *in vitro* and in vivo limbic cortex "is that the synchronous slow waves characteristic of the hippocampus are not to be found *in vitro*". The results of the experiments presented in this review demonstrate that rhythmic slow waves (theta-like activity) are also present under certain conditions *in vitro* (Fig. 1). In addition, they provide evidence that in many aspects the *in vitro* recorded theta-like oscillations are similar to the physiological and pharmacological properties of the *in vivo* recorded theta rhythm.

The idea of recording the HPC theta rhythm *in vitro* dates back to the early 1970s, when Bland made the first *in vitro* observations of theta-like oscillations in Per Andersen's laboratory in Oslo, Norway. Thirteen years ago, in 1986, Konopacki with Bland and MacIver in Roth's laboratory (The University of Calgary) documented for the first time that the perfusion of hippocampal slices with the cholinergic agonist,

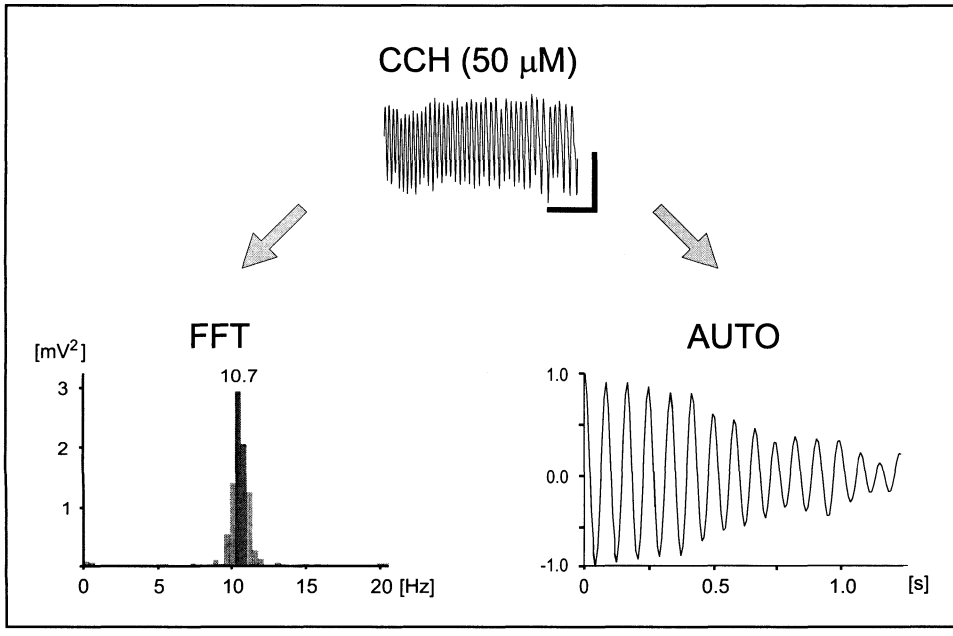

Fig. 3. Analogue example, power frequency (FFT) and autocorrelation (AUTO) analysis of theta-like activity recorded in the region of CA3 pyramidal cells in the presence of carbachol (CCH, 50 μM). Calibration: 1 s and 200 μV.

carbachol (CCH), resulted in the production of theta-like slow waves (Figs. 2A and 3, see also MacIver et al. 1986). They also observed *in vitro* theta-like activity in response to bath perfusion of acetylcholine and acetylcholine esterase blocker, eserine (Fig. 2B and C, see Konopacki et al. 1987b,c).

The cholinergic induced EEG activity was found to be reversible after 10-40 min. of washout with artificial cerebrospinal fluid (ACSF). It ranged in frequency from 3-12 Hz with amplitudes of 0.1-2.0 mV (Fig. 3), and typically appeared in trains, lasting 1-10 s.

After this initial demonstration of theta-like oscillations in rat hippocampal slices, the basic question arose regarding the similarities between the cholinergic induced *in vitro* theta-like activity and the theta rhythm occurring in the *in vivo* preparations. Further experiments were designed specifically to answer this question.

INTRAHIPPOCAMPAL GENERATORS AND PHARMACOLOGICAL PROFILE OF THE *IN VITRO* RECORDED THETA-LIKE ACTIVITY

In the first series of experiments we demonstrated that, as is the case for the cholinergically-mediated type 2 theta in the rat, CCH-induced *in vitro* theta-like oscillations can also be antagonized by the muscarinic blocker, atropine sulphate, but not by the nicotinic antagonist, D-tubocurarine (Fig. 4, see also Konopacki et al. 1987b). Similar results we also obtained in experiments performed with the use of the cat HPC slice preparation (Konopacki et al. 1992b).

The next experiments addressed the problem of generators of theta, localized in the HPC. The earlier *in vivo* studies suggested that neurons in the CA1 area of the HPC generated the currents underlying theta field potentials (Green and Machne 1955). Subsequent detailed topographic investigations performed *in vivo* reported two theta amplitude maxima, one in the stratum oriens of the CA1 area and another in the stratum moleculare of the dentate gyrus (DG) (Bland et al. 1975, Bland et al. 1979, Buzsáki et al. 1985, Vanderwolf et al. 1985). Our detailed mapping study and evaluation of amplitude profiles of the *in vitro* recorded theta-like activity (Konopacki et al. 1988a) supported previous *in vivo* studies. These results indicated that synaptic potentials both in the CA1 and DG areas were capable of independent theta generation, as proposed by the two generator hypothesis (Bland et al. 1975, Bland 1986). In addition, using the model of the transected slice (trans-slice) preparation (Fig. 5), in which the CA1 and DG regions were completely anatomically separated, we demonstrated that both the CA1 and DG regions were capable of inde-

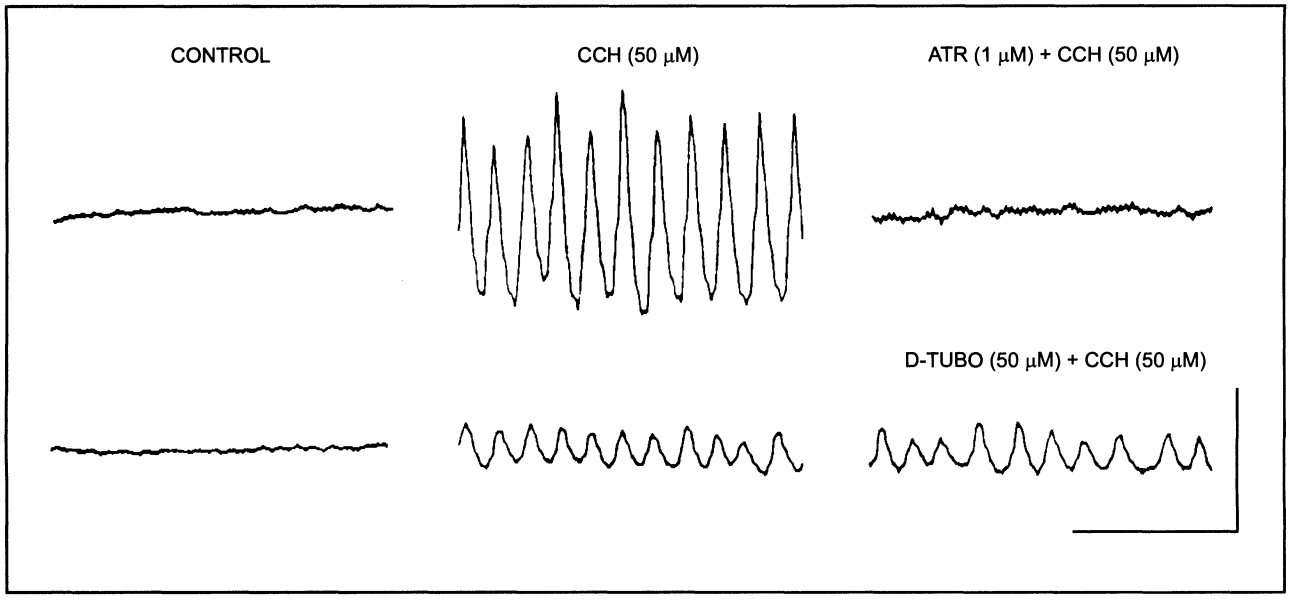

Fig. 4. Theta-like activity recorded from two separate experiments on different slices. Carbachol (CCH, 50 μM) induced theta-like activity was antagonized by atropine sulphate (ATR, 1 μM), but resistant to D-tubocurarine (D-TUBO, 50 μM). Calibration: 1 s and 200 μV.

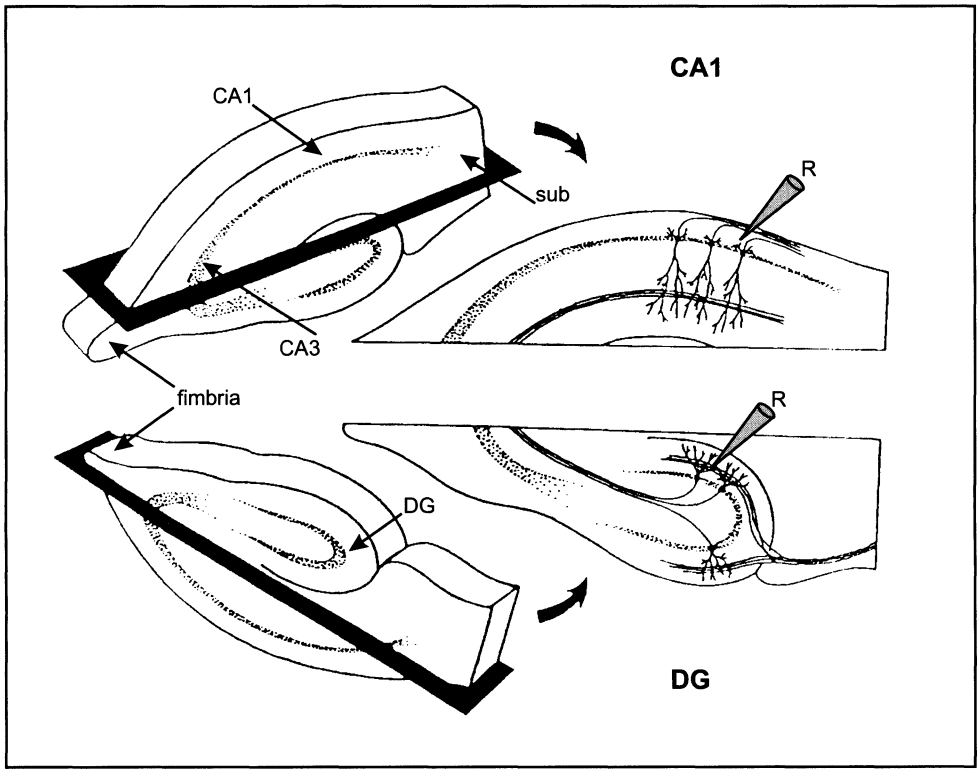

Fig. 5. The preparation of CA1 and DG-trans-slices of the rat hippocampal formation. Recording electrodes (R) were placed close to the cell body layers of CA1 or DG areas to record theta-like field potentials.

pendent generation of theta-like oscillations in the presence of continuous cholinergic stimulation (perfusion with CCH, Fig. 6A, B and C, see also Konopacki et al. 1987a). This finding was the first *in vitro* observation supporting the two generator hypothesis. Further physiological findings concerning the *in vitro* CA1 and DG theta-like activity were consistent with numerous earlier *in vivo* reports suggesting that the generator producing larger HPC type-2 theta was localized in the DG region (Stumpf et al. 1962, Winson 1976, Bland 1986). We also demonstrated *in vitro* that when the CA1 and DG generators were anatomically separated, they could independently generate theta-like oscillations of different amplitude, as shown in Fig. 6B.

The results of experiments conducted with the use of transected slices also revealed that integrity of the laminar, trisynaptic hippocampal circuit was not required for the generation of theta-like oscillations. Furthermore, pharmacological profiles of theta-like activity recorded from the isolated CA1 and DG area supported earlier *in vivo* findings that muscarinic receptors mediate this EEG response (Kramis et al. 1975, Bland 1986, Bland and Colom 1993). Both CA1 and DG theta-like oscillations recorded *in vitro* were antagonized by a muscarinic blocker, atropine sulphate, and were found to be completely resistant to the nicotinic antagonist, D-tubocurarine (Fig. 6C).

The transected slice technique was also found to be very useful in determining whether other regions of the HPC were capable of independent theta generation. Historically, Petsche and Stumpf (1962) were the first to record theta in the CA3 region of the hippocampus proper *in vivo*. This observation was supported later by Feder and Ranck (1973) and Buzsáki et al. (1985). Using our transected slices technique, we demonstrated later that CCH-induced theta-like activity could be recorded from the isolated population of CA3c pyramidal cells (Konopacki et al. 1988a).

Summing up, our studies utilizing transected slices provide strong evidence that there are in fact 3 anatomically separated intrahippocampal generators of cholinergic-induced theta-like oscillations, one localized in the basal part of the CA1 neurons (stratum oriens), the other in the stratum moleculare of the dorsal blade of the dentate gyrus, and a third in the CA3c region of the hilus. Experiments performed on the trans-slice preparation revealed that these generators could operate independently of one another.

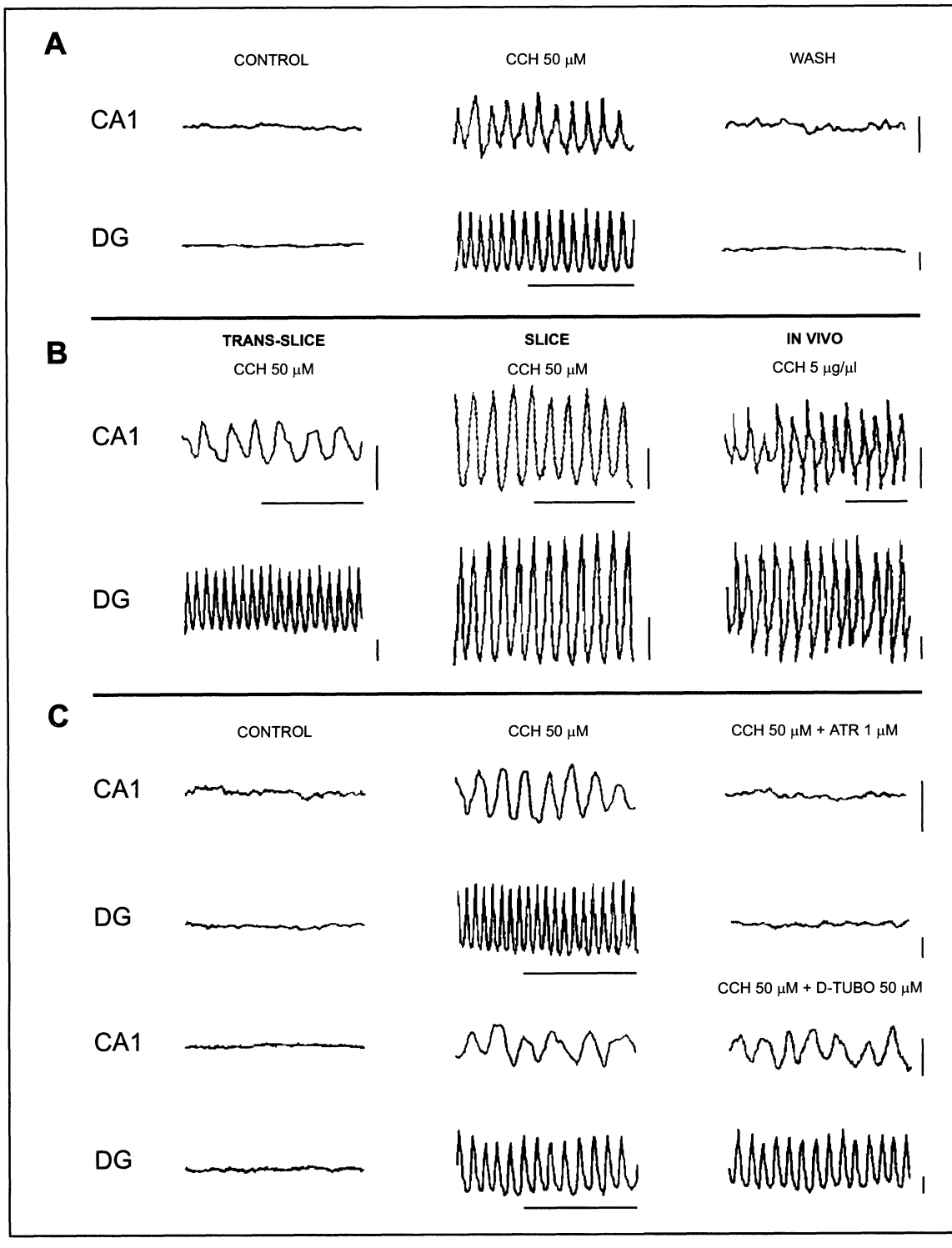

Fig. 6. Carbachol induced theta-like activity recorded in the hippocampal trans-slice preparations. A, carbachol (CCH, 50 μ M) induced theta-like activity both in the CA1 and DG trans-slices. The CCH effect was reversed within 15-60 min of wash with artificial cerebro-spinal fluid (WASH). B, comparison of CCH- induced theta-like activity recorded from the CA1 and DG areas in the hippocampal trans-slices, intact slices and slices from anesthetized rats (*in vivo*). C, CCH-induced theta-like activity from both the CA1 and DG area was antagonized by 1 μM of atropine (ATR), but unaffected by 50 μM of D-tubocurarine (D- TUBO). Calibration for A, B and C: 1s and 200 μV.

CELLULAR BASIS OF THETA-LIKE ACTIVITY RECORDED *IN VITRO*

The advantages of the *in vitro* brain slice preparation for intracellular recordings and pharmacological manipulations are well documented (Lynch and Schubert 1980). In the next stage of our studies we investigated cellular correlates of CCH-induced theta-like activity (Bland et al. 1988). Intracellular recordings were made in the CA1, CA3, and DG regions prior to, during, and after the application of CCH. More than 50% of cells tested were related to the extracellular theta-like activity. They exhibited clear membrane potential oscillations (MPOs, 5-28 mV) and multiple spike discharges occurring close to the peak positivity (Fig. 7). MPOs were always phase locked with extracellulary recorded theta-like field potentials and disappeared when extracellular theta-like oscillations were no longer observed (Konopacki et al. 1988). Similar *in vitro* observations were also noted by other authors (MacVicar and Tse 1989, Leung and Yim 1991, Garcia-Munoz et al. 1993, Bianchi and Wong 1994, Lukatch and MacIver 1997, McMahon et al. 1998, Strata 1998, Chapman and Lacaille 1999). Neural mechanisms responsible for *in vitro* observed MPOs remain an open question. An argument that MPOs arise from intrinsic membrane properties is based on the observation that these oscillations persist during the blockade of synaptic transmission by either low calcium, low sodium or the highly-selective sodium-channel blocker, tetrodotoxin (TTX) (Leung and Yim 1991). On the other hand, MacVicar and Tse (1989) demonstrated that the application of TTX or inorganic calcium-channel blockers abolished CCH-induced MPOs in the CA3 region of HPC slices. Future research must be focused at determining the contributions of intrinsic membrane properties and/or synaptic inputs in

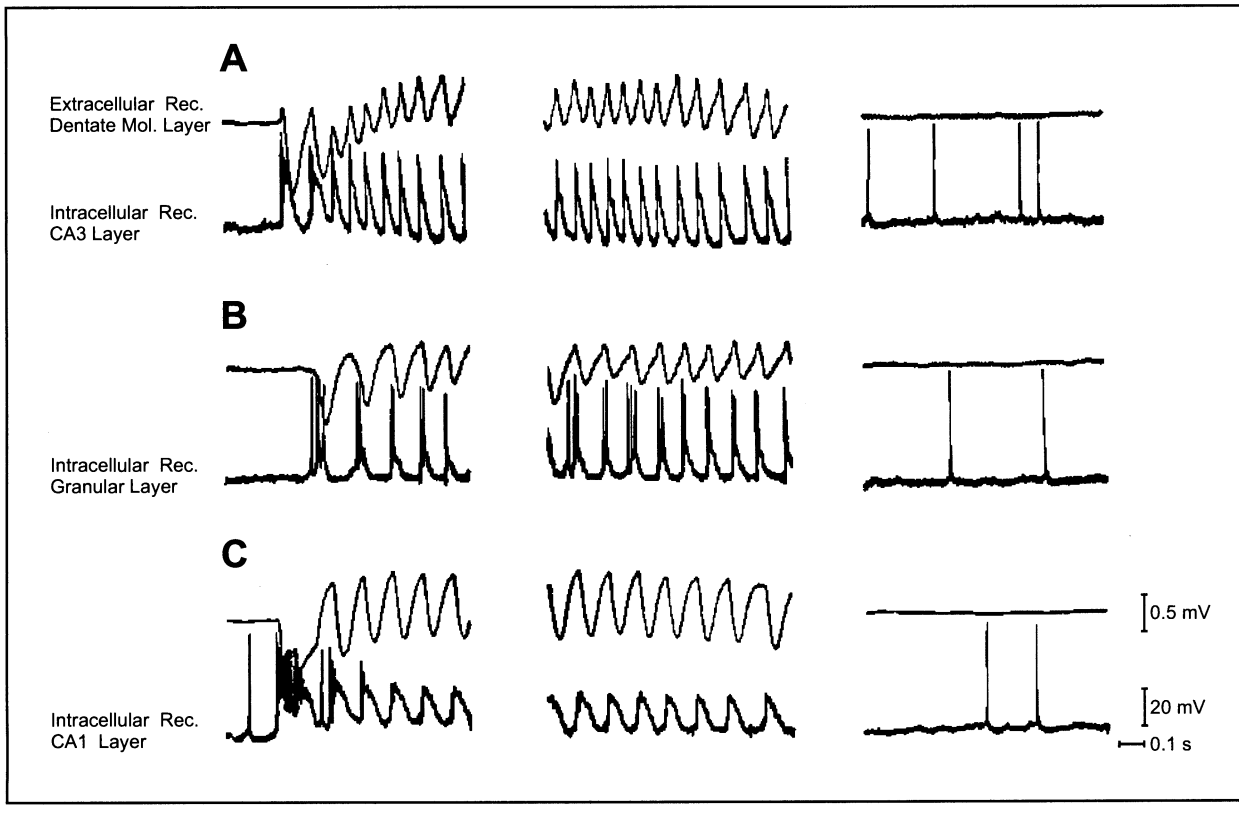

Fig. 7. Membrane potential oscillations (MPOs) and accompanying spike discharges in cells related with extracellular theta-like activity. A, the 3 panels were continuous recordings, from left to right. Note that the intracellular oscillations were large (25 mV) enough that the successive spike discharges in each burst were attenuated. B, an example of the dentate layer cell recording with smaller membrane potential oscillations, and less of a reduction in the number and height of successive spike discharges. C, an example of the CA1 layer cell recordings with large amplitude membrane potential oscillations (28 mV) and inactivation of spike discharges.

generation of MPOs. It should be emphasized that MPOs (intracellular theta rhythm) and rhythmic spike discharges are also observed *in vivo* in phasic "theta-on" and "theta-off" cells during extracellulary recorded theta (Fujita and Sato 1969, Artemenko 1973, Leung and Yim 1986, Núnez et al. 1987, Konopacki et al. 1992a, Bland and Colom 1993).

The above findings clearly demonstrated that CCH-induced *in vitro* theta-like activity has a strong cellular basis which closely resembles neuronal mechanisms responsible for the appearence of the *in vivo* theta rhyhm. In addition, the model of the *in vitro* recorded theta-like activity is particularly valuable for studying cellular processes underlying type 2 theta, offering all the advantages concomitant with the slice preparation.

POSTNATAL DEVELOPMENT OF THETA-LIKE ACTIVITY RECORDED *IN VITRO*

In the next stage of our *in vitro* study we analysed the postnatal development of CCH-induced theta-like activ-

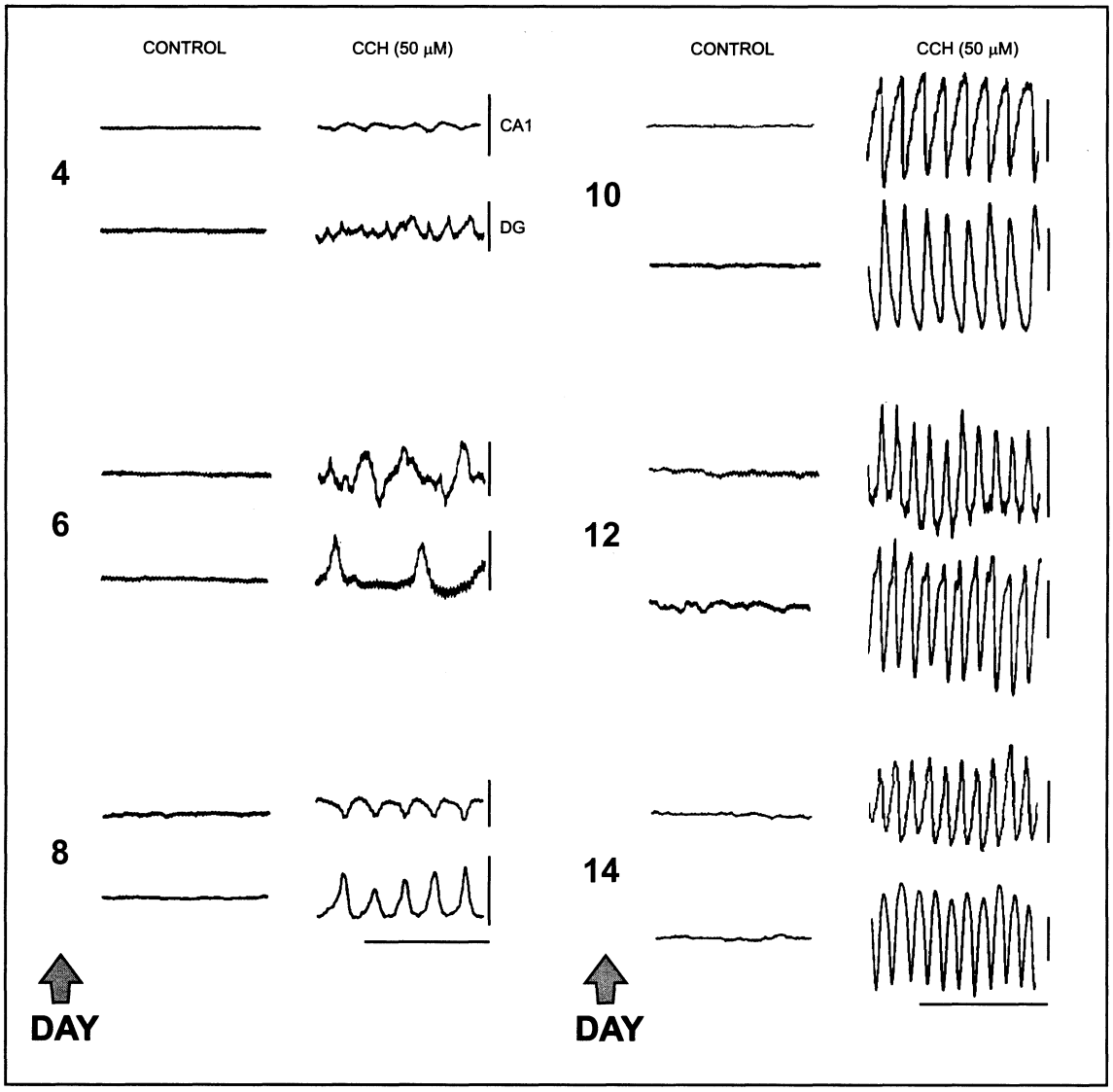

Fig. 8. Development of carbachol (CCH, 50 μM) induced *in vitro* theta-like activity in the CA1 and DG region of the hippocampal slices. At 4 and 6 days of age, perfusion of slices with CCH-induced irregular short-latency activity. Regular theta-like trains were induced in 8-days old slices. From 8 days onward an increase in frequency and amplitude was observed. Calibration: 1s and 200 μV.

ity and compared it with the pattern of development of spontaneous theta described earlier in neonatal rats. Leblanc and Bland (1979) demonstrated that type-2 theta appeared in rats around 10 days of age during voluntary movements and during rapid eye movement (REM) sleep and then increased in amplitude and frequency to the value typically seen in adult animals. Our *in vitro* experiments conducted on slice preparations obtained from neonatal (4, 6, 8, 10, 12, 14 days of age) and mature rats supported this observation (Fig. 8, see Konopacki et al. 1988b). Despite the difference in the time-course of neurogenesis between CA1 and DG regions (Bayer and Altman 1974), CCH-induced theta-like activity was observed in these two areas at about the same time (8-10 days after birth). At around 14 days of age, it reached the frequency and amplitude typical for rhythmical slow activity observed during CCH perfusion in slices delivered from adult rats (Konopacki et al. 1988b).

GABAERGIC / CHOLINERGIC INTERACTION IN THE PRODUCTION OF THE *IN VITRO* THETA-LIKE ACTIVITY

There is accumulating evidence for a GABAergic involvement in the neural mechanisms responsible for the

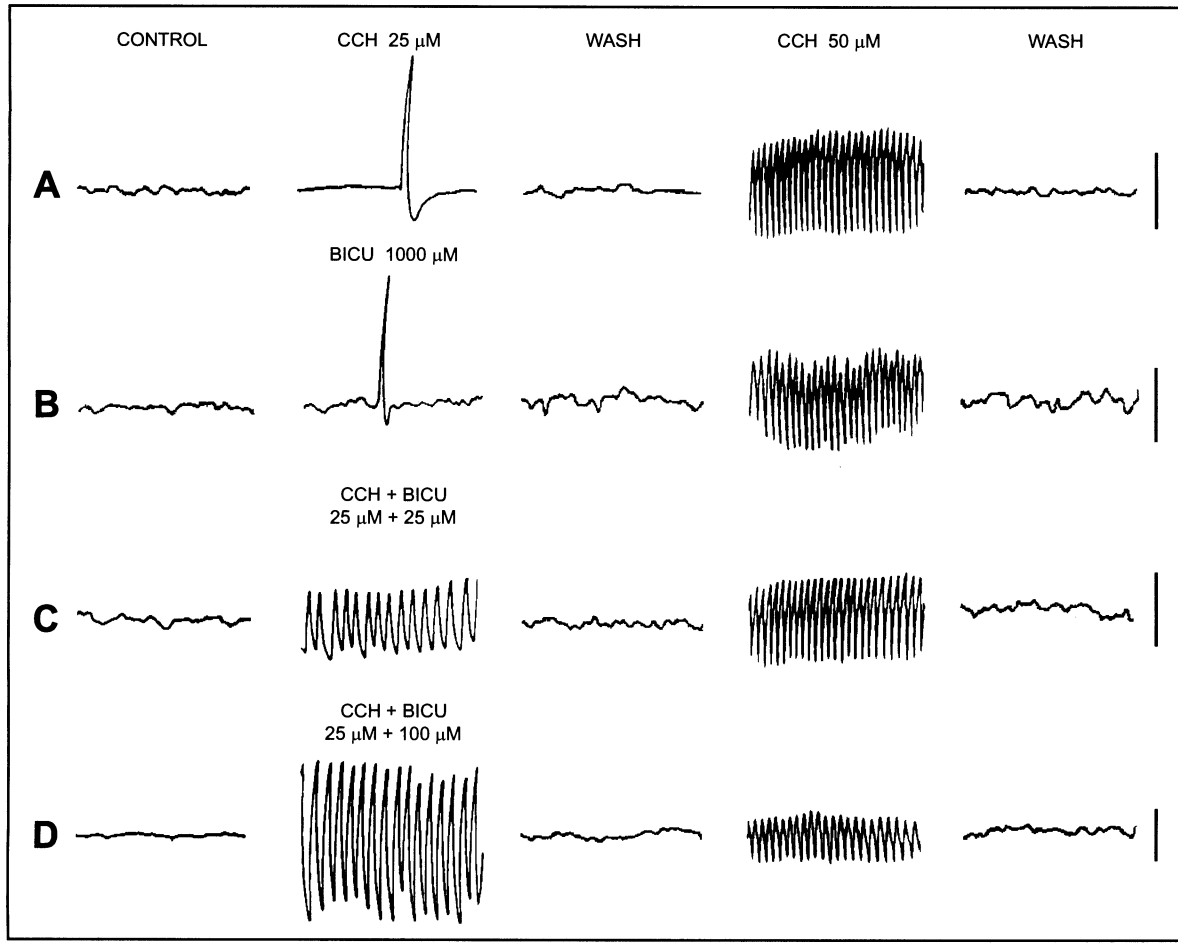

Fig. 9. Cholinergic/GABAergic interaction in the generation of theta-like activity in hippocampal slices. A and B these traces show a lack of rhythmical oscillations after the perfusion of a low concentration (25 μM) of carbachol (CCH) and high concentration (1,000 μM) of bicuculline (BICU). Note that the slices tested responded with theta-like slowe waves to 50-100 μM of carbachol (CCH). C, when 25 μm. of CCH was perfused in the presence of 25 μM BICU, theta-like oscillations could be observed. D, these traces show an increase in amplitude of CCH + BICU induced theta-like activity (vs. theta-like oscillations induced by 25 μM CCH + 25 μμ BICU) in the presence of 100 μM of BICU. The induced field potentials were usually reversal after 20-60 min. of wash with cerebro-spinal fluid (WASH). Calibration for A, B, C and D: 1 s and 500 μV.

generation of the hippocampal formation theta rhythm. It has been histochemically demonstrated that aproximately 30% of the fibres forming the septo-hippocampal projection are GABAergic (Lewis and Shute 1967, Alonso and Kohler 1984, Amaral and Kurz 1985). In addition, the HPC has been reported to contain a significant amount of glutamic acid decarboxylase (GAD) immunoreactive cells (i.e. the cells which possess GABA synthesizing enzyme, see Ribak et al. 1986). Recently, Cobb et al. (1995) have demonstrated that specific activation of GABAergic interneurons is capable of modulating the frequency of discharges of theta-related cells. It has also been recently demonstrated *in vivo* that intrahippocampal and intraseptal microinjections of muscimol, a GABA-A agonist, reversibly abolished theta field potentials and the hippocampal cell discharges (Smythe et al. 1992, Bland and Colom 1993). This muscimol effect was antagonized by bicuculline, a GABA-A antagonists (Smythe et al. 1992). It was also demonstrated that only combined intrahippocampal injections of carbachol and bicuculline or picrotoxin (GABA-A antagonists) were capable of producing trains of theta rhythm during the procaine suppression of the medial septum in urethanized rats (Colom et al. 1991, Heynen and Bilkey 1991). The authors suggested that the HPC type-2 theta resulted from a dynamic interaction between the cholinergic and GABAergic systems (Smythe et al. 1992). This was precisely what we observed *in vitro* (Fig. 9, see also Konopacki and Gołębiewski 1993). CCH at low concentrations (25 μM) never induced theta-like oscillations. The overall level of activation of the hippocampal neuronal network was probably insufficient for theta-like activity to appear (Fig. 9A). When the same concentration of CCH was perfused simultaneously with bicuculline (25 μM), well synchronized theta-like oscillations were observed (Fig. 9C). By blocking GABA-A receptors, bicuculline reduced hippocampal inhibition and this diminution of GABAergic inhibition together with the subthreshold excitation of the hippocampal cholinergic network produced the level of activity required for generation of theta-like oscillations. Further disinhibition of the hippocampal neuronal network by 100 μM bicuculline resulted in a pronounced increase in the amplitude of *in vitro* recorded theta-like activity (Fig. 9D).

In another set of experiments we provided additional evidence supporting a GABAergic/cholinergic interaction in mechanisms responsible for production of theta-like activity (Konopacki and Gołębiewski 1993). Muscimol, which diminishes overall hippocampal excitation by increasing the level of GABAergic inhibition,

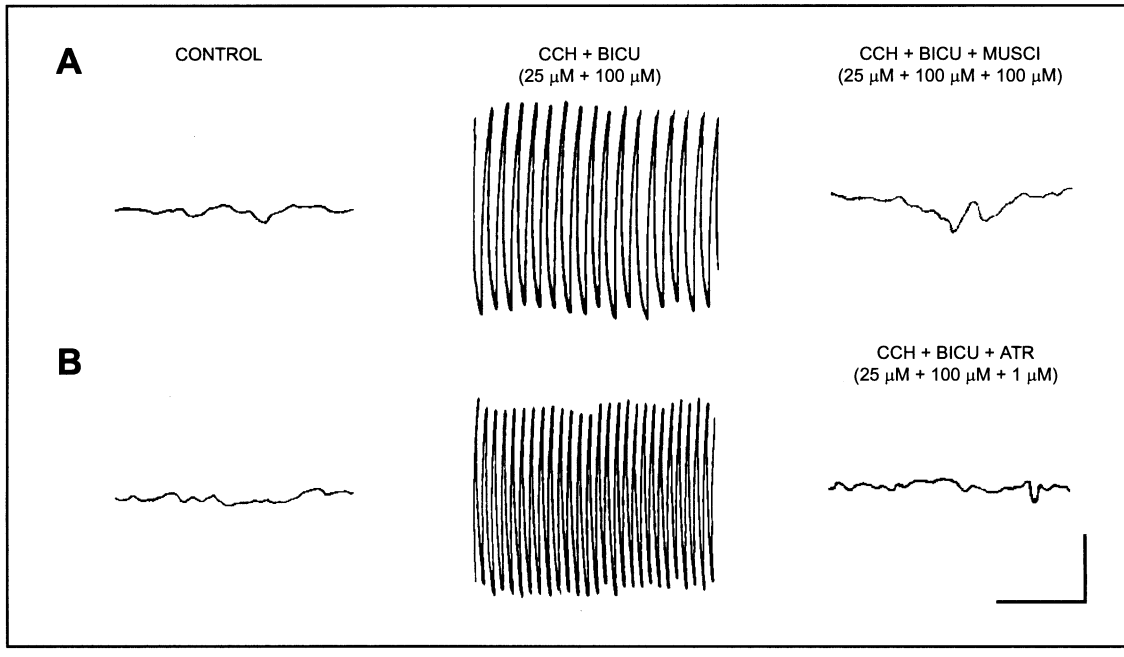

Fig. 10. The effect of muscimol (MUSCI) and atropine (ATR) on carbachol/bicuculline (CCH, 25 μM +BICU, 100 μM) induced theta-like activity. Both MUSCI (100 μM) and ATR (1 μM) antagonized the induced theta-like activity. Calibration: 1s and 500 μV.

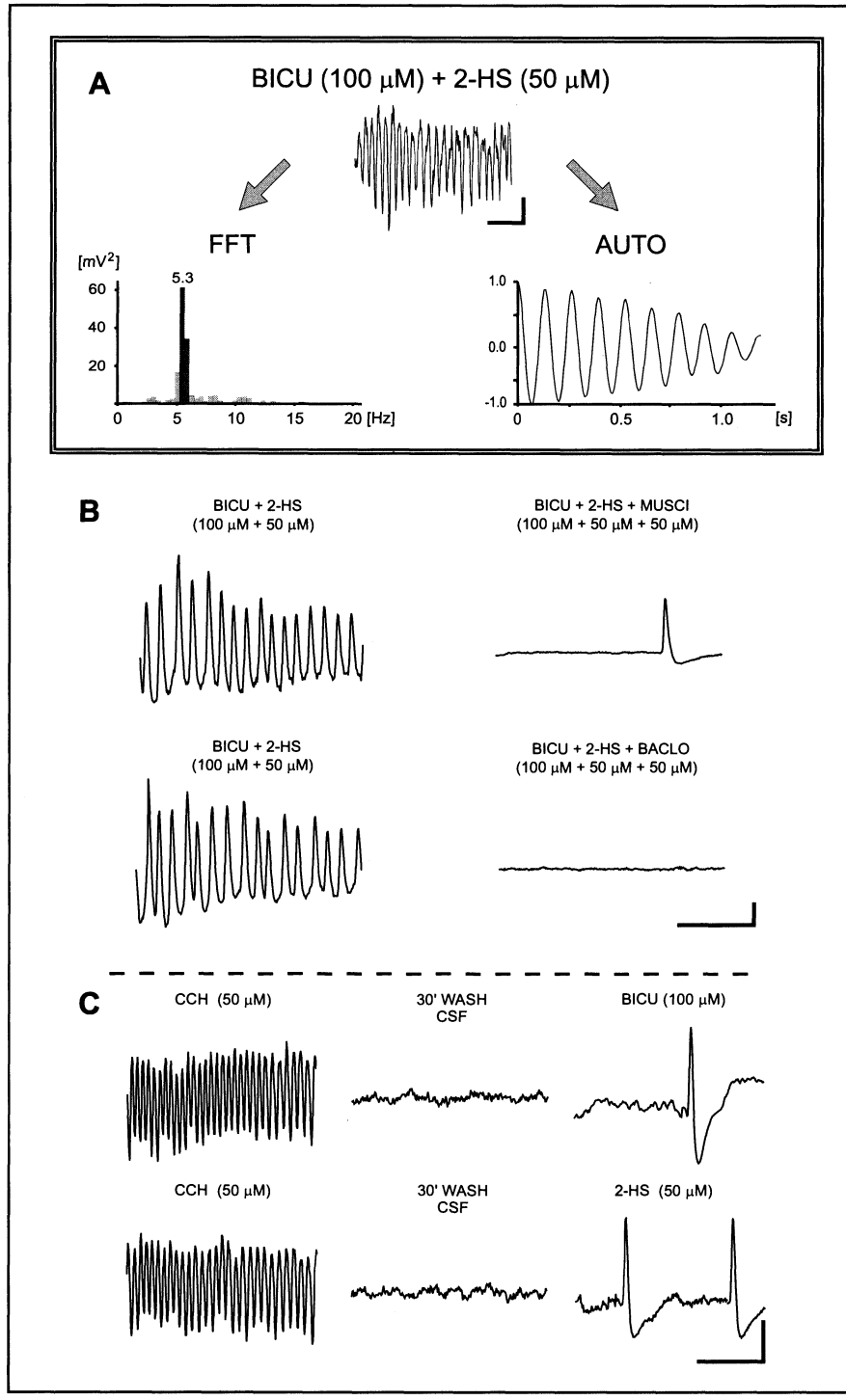

Fig. 11. Bicuculline/2-hydroxysaclophen (BICU, 100 μM + 2HS, 50 μM) induced theta-like activity in the hippocampal slice and the effect of muscimol (MUSCI, 50 μM) and baclophen (BACLO, 50 μM). A, analogue example, power frequency (FFT), and autocorrelation (AUTO) analysis of theta-like oscillations recorded in the region of CA3 pyramidal cells in the presence of bicuculline and 2-hydroxysaclophen. B, the *in vitro* induced theta-like activity was antagonized both by muscimol and baclophen. C, the hippocampal slices which responded with theta-like oscillations in control (perfusion of 50 μM carbachol: CCH, 50 μM) did not manifest rhythmical slow waves when perfused either with bicuculline or 2- hydroxysaclophen; only epileptic discharges were observed. Calibration: for A, B and C: 1 s and 200 μV.

resulted in the abolition of carbachol/bicuculline-induced theta-like activity (Fig. 10A). A similar effect was also produced by atropine sulphate. By blocking hippocampal muscarinic receptors this agent decreased the overall level of cholinergic excitation (Fig. 10B).

Thus far we have presented evidence regarding theta-like oscillations resulting from the cholinergic excitation of the HPC neuronal network or resulting from simultaneous cholinergic stimulation and GABA-Aergic disinhibition. The question arises whether strong diminution of GABAergic inhibition *per se* is capable of producing a level of the HPC excitation essential for theta-like activity to appear. This idea has recently been tested in our laboratory. The HPC slice preparations were perfused

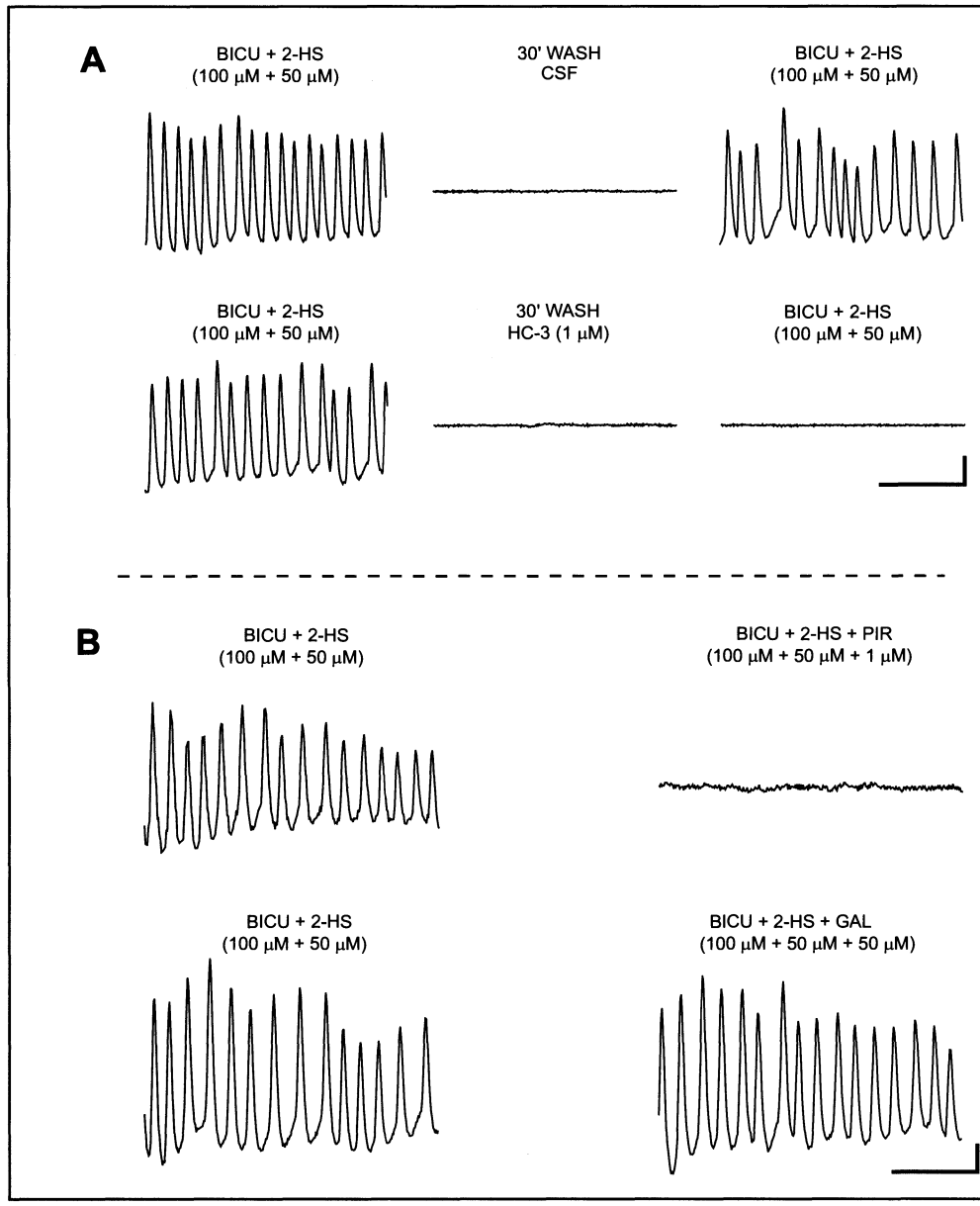

Fig. 12. Bicuculline/2-hydroxysaclophen (BICU, 100 μM + 2HS, 50 μM) induced theta-like activity and the effect of hemicholinium (HC-3, 1μM), pirenzepine (PIR, 1 μM) and gallamine (GAL, 50 μM). A, BICU + 2HS induced theta-like activity was reversed after 10-30 min wash with artificial cerebro-spinal fluid (CSF) or CSF containing HC-3. Note that a wash with CSF alone did not prevent the appearance of theta-like activity after a secondary bath perfusion of BICU + 2HS. B, BICU + 2HS induced theta-like activity was antagonized by pirenzepine (PIR) but was resistant to perfusion with gallamine (GAL). Calibration for A and B: 1 s and 200 μM.

with different concentrations of bicuculline and the GABA-B antagonist, 2-hydroxysaclophen (2-HS). Well synchronized theta-like oscillations were observed only in response to the simultaneous perfusion of 100 μM bicuculine and 100 μM 2-hydroxysaclophen in approximately 50% of the experiments performed (Fig. 11A, Konopacki et al. 1995). Both muscimol and baclophen were found to be effective in antagonizing bicuculline/2-hydroxysaclophen-induced oscillations (Fig. 11B). The bath perfusion of HPC slices with bicuculline or 2-hydroxysaclophen produced only seizure activity (Fig. 11C). Bicuculline/ 2-hydroxysaclophen-induced theta-like activity is the first *in vitro* evidence demonstrating that specific levels of excitation of hippocampal neurons required for theta to appear can also be produced by the strong diminution of GABA-A and GABA-B inhibition.

In the next series of experiments we extended our observations concerning the pharmacological profile of bicuculline/2-hydroxysaclophen-induced theta-like activity. The *in vitro* induced response was studied in the presence of hemicholinium-3 (HC-3). This agent blocks choline transport accross the membrane, thus diminishing acetylcholine content in the slices; (Birks and Mac Intosh 1957, Friedman and Wikler 1970), and the cholinergic M1 and M2 receptor antagonists, pirenzepine and gallamine, respectively. The slices pretreated for 30 min with hemicholinium were found to be completely resistant to bicuculline and 2-hydroxysaclophen when these agents were added to the bath (Fig. 12A). As it was shown in Fig. 12B, bicuculline/2-hydroxysaclophen-induced theta-like oscillations were also antagonized by the M1 blocker, pirenzepine. Gallamine, was completely ineffective in abolishing previously induced theta-like oscilations (Fig. 12B). These results provide evidence which strongly suggests that bicuculline/2-hydroxysaclophen-induced theta-like activity also has a significant cholinergic M1 involvement (Konopacki et al. 1997).

THETA-LIKE ACTIVITY RECORDED FROM ENTORHINAL CORTEX SLICE PREPARATIONS

Increasing attention has been paid to the role of the EC in mechanisms responsible for the generation of theta rhythm. The EC is the main source of afferents to the HPC and receives strong multisynaptic projections from the Ammon's horn field of the hippocampus (Witter et al. 1989, Lopes da Silva et al. 1990). The medial part of the EC has been postulated to play a role in the generation of HPC theta (Mitchell and Ranck 1980, Vanderwolf and Leung 1983). In addition, the EC *per se*, was suggested to be a source of the *in vivo* recorded theta rhythm (Mitchell and Ranck 1980, Alonso and Garcia-Austt 1987, Dickson et al. 1994, Gołębiewski et al. 1994). This suggestion was strongly supported by experiments we recently conducted on medial EC slice preparations obtained from rats and cats (Konopacki et al. 1992a,c, Konopacki and Gołębiewski 1992). Specifically, we demonstrated that in the *in vitro* conditions (i.e., deafferentiation from the hippocampal formation and medial septum) the EC neuronal network was capable of producing theta-like activity when CCH was added to the bath (Fig. 13A and B).

Three lines of evidence demonstrate that CCH-induced theta-like oscillations were mediated by muscarinic (M1) receptors (Fig. 13A and B): (1) nicotine perfusion did not induce rhythmic slow waveforms; (2) cholinergically induced theta-like activity is antagonized by atropine sulphate and pirenzepine (M1 receptor blocker), but not by gallamine (M2 receptor antagonist); (3) hexamethonium and mecamylamine (nicotinic antagonists) have been found to be ineffective in blocking cholinergic induced theta-like activity.

The *in vitro* studies discussed up to now have focussed on two limbic cortex preparations: slices obtained from the HPC and EC. One can hypothesize that theta-like field potential could also be induced in other regions of the brain maintained *in vitro*. Indeed, just recently Lukatch and MacIver (1996) demonstrated theta-like activity in coronal neocortical slices perfused with CCH and bicuculine. However, the experiments we have performed recently on slices obtained from the medial septum, posterior hypothalamus and brain stem do not provide further confirmation of this hypothesis. Much more *in vitro* experiments with the use of brain slices dissected in different planes remain to be done.

COMPARISON OF *IN VIVO* AND *IN VITRO* RECORDED THETA RHYTHM PROPERTIES

The experiments we have been conducting for the last 13 years on slice preparations from the HPC and EC demonstrate that a number of properties of the *in vivo* recorded theta rhythm can be successfully studied *in vitro* (Table I).

Specifically: (1) the frequency and amplitude of the *in vitro* recorded theta-like activity ranges in the frequency

and amplitude of the *in vivo* theta rhythm; (2) the time course analysis reveals that, as is the case for spontaneous theta in the rat, CCH-induced theta-like activity appears typically in a few second trains; (3) the pharmacological profile shows that both in vivo theta rhythm and *in vitro* recorded theta-like activity are muscarinically mediated. Furthermore, both the production of the *in vivo* theta and *in vitro* theta-like activity require a dynamic balance between cholinergic and GABAergic systems; (4) both rhythms have the same loci of the

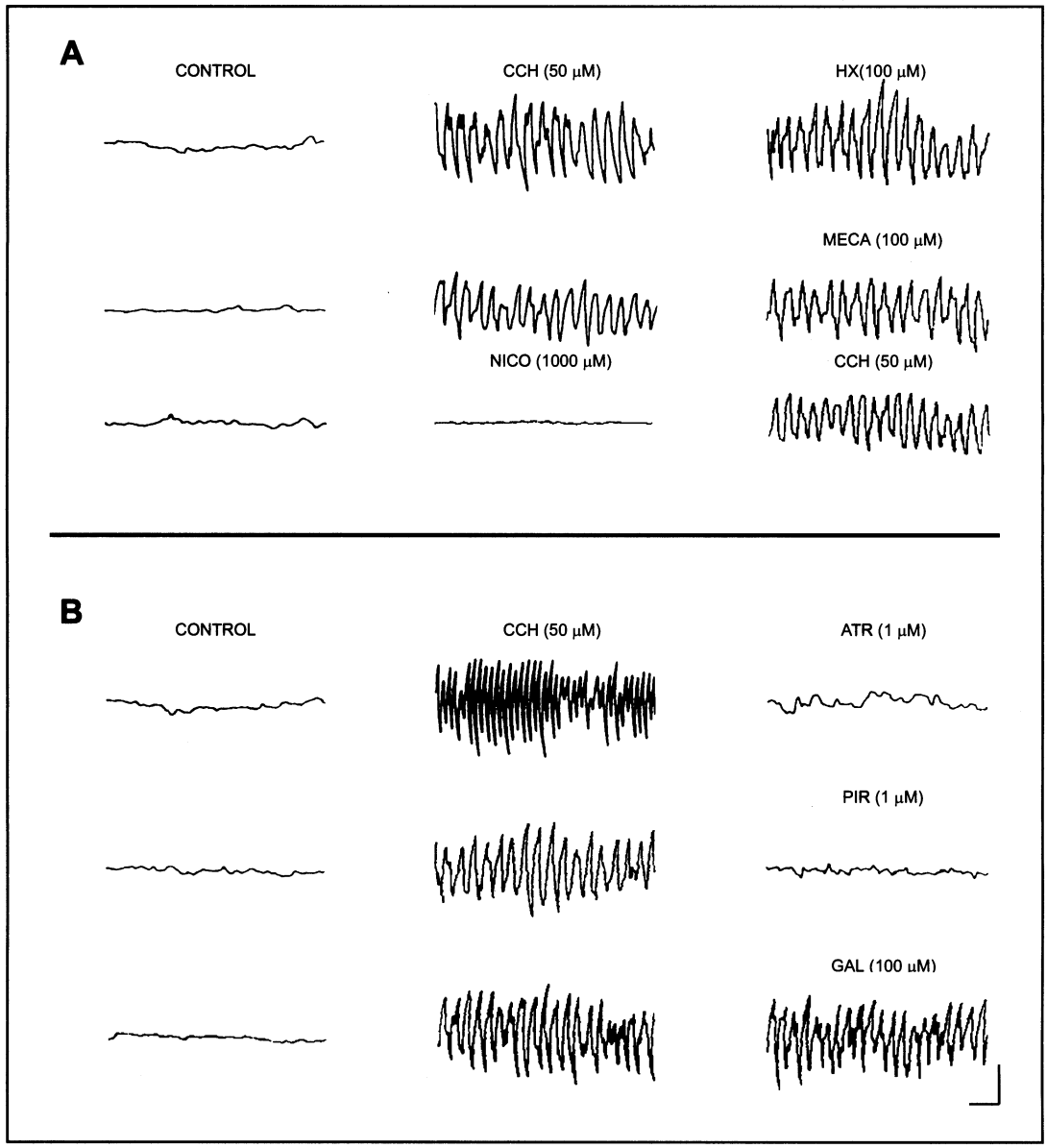

Fig. 13. Representative examples of carbachol (CCH, 50 μM) induced theta-like activity in the cat medial entorhinal cortex slices. A, CCH induced theta-like activity recorded from three separate experiments on different slices. The recordings show the lack of antagonism of CCH-induced theta-like slow waves by hexamethonium (HX, 100 μM) and mecamylamine (MECA, 100 μM). The bottom recording shows the ineffectiveness of nicotine (NICO, 1000 μM) in inducing theta-like rhythm. However, when the same preparations were perfused with CCH, theta-like activity could be observed. B, CCH induced theta-like slowe waves were antagonized by a classic muscarinic blocker, atropine sulphate (ATR, 1 μM), by M1 receptor antagonist, pirenzepine (PIR, 1μM), but were unaffected by M2 receptor antagonist, gallamine (GAL, 100 μM). Calibration for A and B: 1 s and 200 μV.

amplitude maxima, suggesting an overlapping topography of the intrinsic hippocampal generators; (5) the pattern of development of *in vitro* theta-like activity closely resembles postnatal development of the *in vivo* theta rhythm; (6) both the *in vivo* theta rhythm and *in vitro* induced theta-like activity are accompanied by MPOs of "theta -on" and "theta -off" cells; (7) Both, spontaneous theta and *in vitro* recorded theta-like activity have similar consequences for synaptic plasticity. In these oscillatory states the phase relations between incoming stimuli and the oscillation strongly affect the resulting change in synaptic efficacy. If incoming activity to the hippocampus is at theta frequency, only information of appropriate phase will be stored. These results suggest that *in vivo* and *in vitro* theta oscillations may be a stimulus for inducing LTP/LTD (Huerta and Lisman 1995, Natsume and Kometani 1997).

These coincidences in properties of the *in vivo* and *in vitro* recorded rhythmic slow activity leads to a general conclusion that the generation of theta in both these preparations share common mechanisms.

COMPARISON OF THE PROPERTIES OF *IN VITRO* INDUCED THETA-LIKE ACTIVITY AND EPILEPTIFORM DISCHARGES

One more issue should be addressed. The known ability of CCH to induce epileptiform activity (when administered in an appropriate concentration) would suggest that theta-like activity also has an epileptiform component. The theoretical implication of this suggestion would be that theta-like activity reflects the physiological and pharmacological properties of epileptiform discharges. Does it really? (Table II) (1) the typical intracellular correlate of the interictal epileptiform activity is the paroxysmal depolarization shift (PDS) (Bradford 1995). We have never observed a typical PDS in cells during extracellularly recorded *in vitro* theta-like activity. Instead, in a number of intracellularly recorded theta related cells, MPOs and multiple spike discharges developed. In some intracellularly recorded cells ("theta-on") an initial depolarizing shift with spike discharges was observed only at the onset of extracellular theta-like

TABLE I

Properties of the *in vivo* and *in vitro* recorded theta rhythms

Properties of theta rhythm	*in vivo*	*in vitro*
Frequency	3-12 Hz	3-12 Hz
Amplitude	0.1-2 mV	0.1-2 mV
Lenght of trains	0.5-10 s	1-10 s
Pharmacological profile	Cholinergic (Muscarinic M1) GABAergic (GABA-A and GABA-B)	Cholinergic (Muscarinic M1) GABAergic (GABA-A and GABA-B)
Loci of the amplitude maxima in the hippocampal formation	Stratum oriens of CA1 Stratum moleculare of the dentate gyrus CA3c region of the hilus	Stratum oriens of CA1 Stratum moleculare of the dentate gyrus CA3c region of the hilus
Postnatal development	Theta appears around 10 days of age	Theta appears 8-10 days after birth
Intracellular potentials	MPOs of theta - on and theta - off cells	MPOs of theta - on and theta - off cells
The effect on synaptic plasticity	LTP/LTD	LTP/LTD

oscillations. However, the other cells ("theta-off") manifested a hyperpolarizing shift at the onset of *in vitro* theta-like activity; (2) the amplitude of epileptiform discharges was usually 5 to 10 times higher than the amplitude of the *in vivo* theta and *in vitro* theta-like activity and the frequency was typically lower than the range for *in vivo* theta and *in vitro* theta-like activity (3-12 Hz); (3) typically in contrast to epileptiform activity theta-like oscillations appears in trains separated with quiescent periods, when no theta is observed (Fig. 14); (4) although some brain regions are recognized as epileptogenic, generally epileptiform activity is not restricted to specific regions of the brain (except cerebellum). Theta-like activity, in contrast, was observed only in the neocortex, HPC and EC, regions known to produce physiological theta *in vivo*; (5) in contrast to *in vivo* theta and *in vitro* recorded theta-like oscillations, *in vivo* and *in vitro* recorded epileptiform discharges can be induced at all stages of postnatal brain development and even prenatally (Guy et al. 1995); (6) while the appearance of the *in vivo* theta and *in vitro* theta-like activity results from the cholinergic excitation and simultaneous GABAergic disinhibition, both *in vivo* and *in vitro* epileptiform discharges appear mainly in response to disinhibition of the GABAergic system (Bradford 1995). In addition, in contrast to theta-like activity, epileptiform discharges can also be mediated by NMDA type glutamate receptors (Lothman et al. 1991, Bradford 1995). Hence, the epilepsy is usually successfully abolished by glutamate receptor antagonists and GABA agonists but not by the muscarinic receptor blockers, atropine or scopolamine; (7) it is known that orthodromic stimulation of CA1 afferent fibers *in vitro* normally gives one population spike when recorded extracellulary from the pyramidal cell body layer. The bath perfusion of HPC slices with CCH at concentrations sufficient to induce theta-like activity does not change this pattern of the evoked response. However, GABAergic antagonists (penicillin, picrotoxin, bicuculline), which are used to induce epilepsy, typically produce 3 to 10 population spikes, as an effect of disinhibition of pyramidal cells (MacIver et al. 1986).

The answer to the question asked above is that the *in vitro* induced theta-like activity does not reflect the physiological and pharmacological properties of the epileptiform discharges. Since it has much more in common with the naturally occurring theta than with epilepsy, we have adapted the term "theta-like" activity.

In conclusion, there is a body of corroboratory evidence which shows that: (1) *in vitro* recorded theta-like activity replicates physiological and pharmacological

TABLE II

Characteristic features of the *in vitro* induced theta-like activity and the epileptiform discharges

Theta-like activity	Epileptiform discharges
1. Membrane potential oscillations	1. Typical intracellular correlate of epilepsy is paroxysmal depolarization shift (PDS)
2. Amplitude: 0.1 - 1 mV Frequency: 3-12 Hz	2. Amplitude: 1-10 mV or more Frequency: typically lower (2 Hz)
3. Time-course - trains	3. Time-course - continuous discharges
4. Topography: only in regions known to produce physiological theta (hippocampal formation, entorhinal cortex, cingulate cortex)	4. Generally epileptiform activity is not restricted to specific region of the brain (except cerebellum)
5. Theta-induced *in vitro* develops at 8-10 days of age	5. It can be induced in all stages of postnatal brain development and even prenatally
6. Mediated by cholinergic and GABAergic receptors	6. Mediated by NMDA type of glutamate receptors, GABAergic receptors
7. Orthodromic stimulation of CA1 cells, applied during carbachol-induced theta-like activity gives only one population spike	7. Orthodromic stimulation of CA1 cells, applied during perfusion of penicillin, picrotoxin, bicuculline typically produse 3-10 population spikes as an effect of disinhibition of pyramidal cells

properties of the *in vivo* theta. (2) Physiological and pharmacological features of *in vitro* theta-like activity substantially differ from epileptiform discharges.

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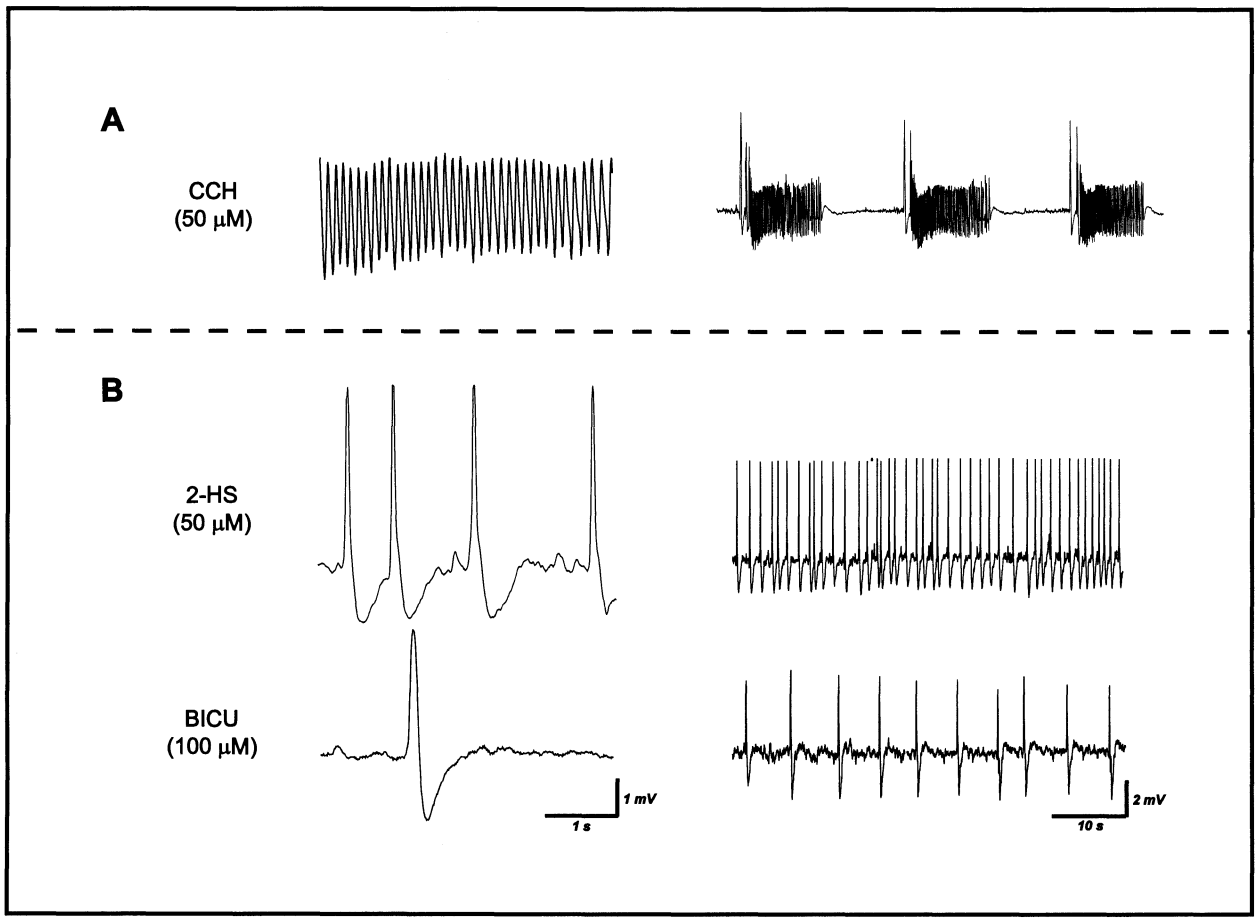

Fig. 14. Time-course of theta-like activity A and epileptiform discharges B recorded from separate hippocampal slices perfused with carbachol (CCH, 50 μM), 2-hydroxysaclophen (2-HS, 50 μM) and bicuculline (BICU, 100 μM). Note that only CCH-induced field potentials appeared in trains. Calibration scale on the left panel is 1 s and on the right panel is 10 s.

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