ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol. Exp. 1996, 56: 407-421

Development of oscillatory activity in the limbic cortex *in vitro*

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Abstract. The generation of EEG theta rhythms in the mammalian limbic cortex is a prime example of rhythmic activity that involves central mechanisms of oscillations and synchrony. In 1985 for the first time we demonstrated that bath perfusion of the hippocampal formation slices with cholinergic agonists resulted in theta-like oscillations. Since this initial demonstration of the *in vitro* theta activity, we have carried out a number of experiments in an attempt to answer the general question: what are the similarities between the cholinergic-induced *in vitro* theta and theta rhythm which naturally occurs in the *in vivo* preparation. Thus far, our *in vitro* studies provided strong evidence that the *in vitro* recorded theta oscillations replicate many physiological and pharmacological properties of the *in vivo* observed theta rhythm. In addition, our studies validate the *in vitro* maintained limbic cortex as a model for studying central mechanisms of oscillations and synchrony.

Key words: theta rhythm, limbic cortex, *in vitro*, cholinergic receptors, GABA-ergic receptors

Review

INTRODUCTION

Theta rhythms are electroencephalographic (EEG) activity consisting of rhythmical sinusoidal slow waves. They are the largest (several mV), most prominent, and the best synchronized waveforms generated by the mammalian brain. Commonly, theta activity has been associated with the hippocampal formation (HF) since in this structure it is most conspicuous (Bland 1986, Lopes da Silva et al. 1990, Bland and Colom 1993). However, a number of the *in vivo* reports have revealed the hippocampal formation not to be the only limbic cortical region involved in the production of theta. Theta oscillations were also recorded from the entorhinal cortex (EC) and cingular cortex (CC) in freely behaving or anaesthetized animals (Michell and Ranck 1980, Borst et al. 1985, Leung and Borst 1987, Alonso and Garcia--Austt 1987, Dickson et al. 1994).

The physiological significance of theta activity is assigned by the following reports:

1. In humans theta rhythm can be observed in patients with temporal lobe epilepsy (Arnolds et al. 1980).
2. Theta activity appears to modulate synaptic transmission in the HF and in this way it may "gate" the flow of information through this structure (Winson 1986).
3. Theta facilitates the transmission of information from the HF to the nucleus accumbens and the motor circuits, which suggests an important role of the theta band in sensorimotor integration (Bland 1986, Lopes da Silva 1992).
4. Theta rhythm promotes both the induction of long-term potentiation (LTP) and its subsequent reversal, which suggests a strong modulatory effect of this rhythm on neural mechanisms responsible for storage of information (Larson et al. 1993).

In a recent excellent review Bland and Colom (1993) presented arguments that some extrinsic and intrinsic properties of the limbic cortical neurones represented in fact multiple synchronizing systems which underlay theta field potential. In this review I intend to demonstrate that the limbic cortex mechanisms of oscillations and synchrony, which are responsible for theta to appear can also be successfully investigated in complete isolation from the extrinsic input - i.e. in the *in vitro* maintained brain slice preparations obtained from the HF and EC of rats and cats. The value of the slice preparations is related to the extent to which it preserves the physiological phenomena typical for the intact brain. Considering the number of comparisons that have been made for the HF slices, the conclusion is that the *in vitro* preparations are suprisingly normal (Yamamoto and Kurokawa 1970, Schwartzkroin 1975, Lynch and Schubert 1980). Interestingly, more than 10 years ago Lynch and Schubert (1980) pointed out that one of differences in electrophysiological "behaviour" of slice and whole brain "is that the synchronous slow waves characteristic of the hippocampus are not to be found". The results of experiments presented here demonstrate that rhythmic slow waves (theta rhythm) can also be observed in some conditions *in vitro*. In addition, they provide evidence that in many aspects the recorded theta oscillations replicate physiological and pharmacological properties of the *in vivo* recorded theta rhythm.

THETA OSCILLATIONS RECORDED FROM THE HIPPOCAMPAL FORMATION SLICE PREPARATIONS

The idea of recording theta oscillations in the hippocampal formation maintained *in vitro* dates back to the early 1970s when dr. B. Bland made the first *in vitro* observations of cholinergic induced theta-like activity in Per Andersen's laboratory. Fifteen years later we began systematic study of the *in vitro* theta with use of the HF and EC slices obtained from rats and cats, at first in cooperation with my friends dr. B. Bland and dr. B. MacIver in dr. S. Roth's laboratory (The University of Calgary) and from 1989 on with my present team in the Department of Neurobiology, The University of Łódź.

Almost 10 years ago we documented for the first time that the perfusion of the hippocampal forma-

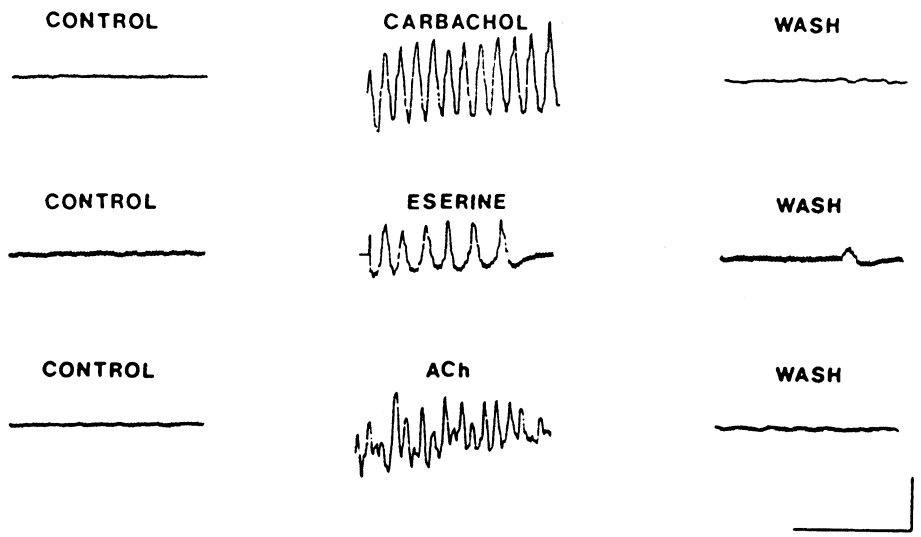


Fig. 1. Comparison of cholinergic agent effects on slow wave activity recorded from the molecular layer of the dentate gyrus: carbachol (0.05 mM), eserine (0.6 mM), and acetylcholine (ACh, 0.8 mM). Theta frequencies from 4 (e.g., eserine) to 9 Hz (e.g., ACh) were produced by each agent and amplitudes of 200 μV to 1.5 mV were observed, consistent with *in vivo* responses. A small residual effect of eserine was seen following washout in the experiment shown, although slow wave activity was usually reversed within 0.5 h. Calibration: 500 μV and 1 s, positive up. (Reprinted with the permission from Brain Res. 1987, 18: 25-27).

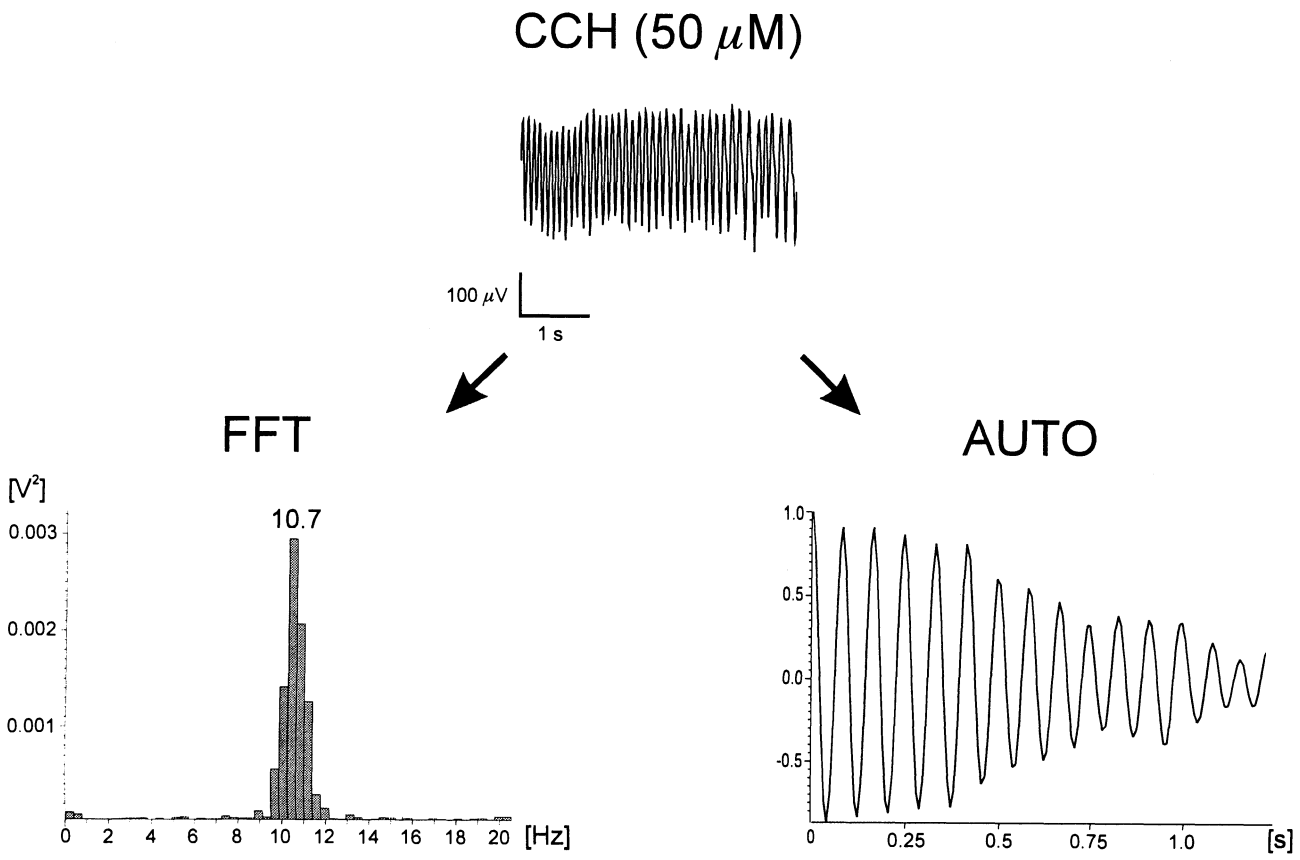


Fig. 2. Example of carbachol (CCH, 50 μM) induced theta oscillations recorded in a separate experiment from the *stratum moleculare* of the dentate gyrus and associated FFT and autocorrelation (AUTO) analysis.

tion slices with carbachol (CCH), acetylcholine (ACh) and eserine resulted in the production of theta-like slow waves (Fig. 1, Konopacki et al. 1987c,d). The cholinergic induced EEG response was found to be reversible after 10-40 min of washout with artificial cerebrospinal fluid (ACSF). Typically, cholinergic (CCH) induced theta ranged in a frequency 3-12 Hz and amplitude 0.2-2.0 mV. Autocorrelation analysis revealed excellent synchronization of the *in vitro* recorded theta rhythm (Fig. 2).

After this initial demonstration of the *in vitro* theta-like oscillations in the hippocampal formation slices obtained from rats the basic question arose as to the similarities between cholinergic-induced *in vitro* theta and theta rhythm that occurs in the *in vivo* preparations. The number of further experiments was specifically designed to answer this question.

In the first series of experiments we demonstrated that, as it is in the case of the rat cholinergic--mediated type 2 theta, CCH-induced *in vitro* theta oscillations can also be antagonized by muscarinic blocker, atropine sulphate, but not by nicotinic antagonist - d-tubocurarine (Fig. 3, Konopacki et al. 1987c). We also, obtained similar results in the experiments with use of the cat HF slices (Konopacki et al. 1992b)

The next experiments addressed the problem of generators of theta, localized in the HF. The earlier *in vivo* studies suggested that neurones in the CA1 area of the HF generated the currents underlying theta field potential (Green and Machne 1955). Subsequent detailed topographic investigations reported two theta amplitude maxima, one in the *stratum oriens* of the CA1 area and the other in the stratum moleculare of the dentate gyrus (DG) (Bland et al. 1975, Winson 1976, Bland et al. 1979). These results indicated that synaptic potentials both in CA1 and DG areas are capable of independent theta generation, as proposed by two generators hypothesis (Bland et al. 1975, Bland 1986). Using the model of transected slice preparations (Fig. 4) in which CA1 and DG regions were completely anatomically separated, we demonstrated that both CA1 and DG regions were capable of independent generation of theta oscillation in the presence of continous cholinergic stimulation (perfusion with CCH) (Konopacki et al. 1987a). This finding was

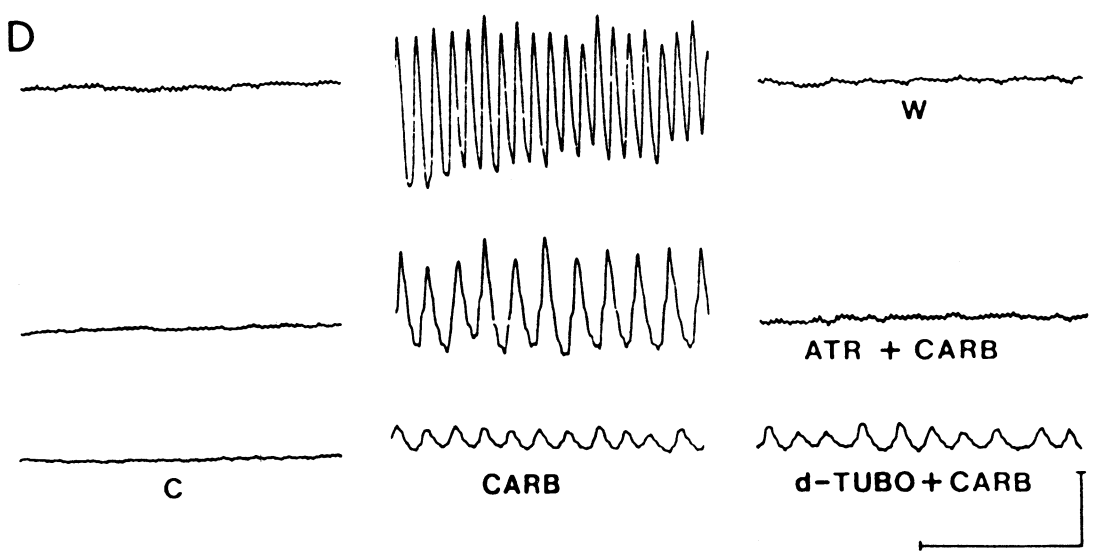


Fig. 3. Theta activity recorded from 3 separate experiments on different slices. The top recordings illustrate carbachol-induced (CARB; 50 μM) theta and reversal with wash (W). Antagonism of carbachol-induced theta by atropine sulphate (ATR, 50 μM) and lack of antagonism by d-tubocurarine (d-TUBO, 50 μM) are also shown. Calibration: 1 s and 0.5 mV.

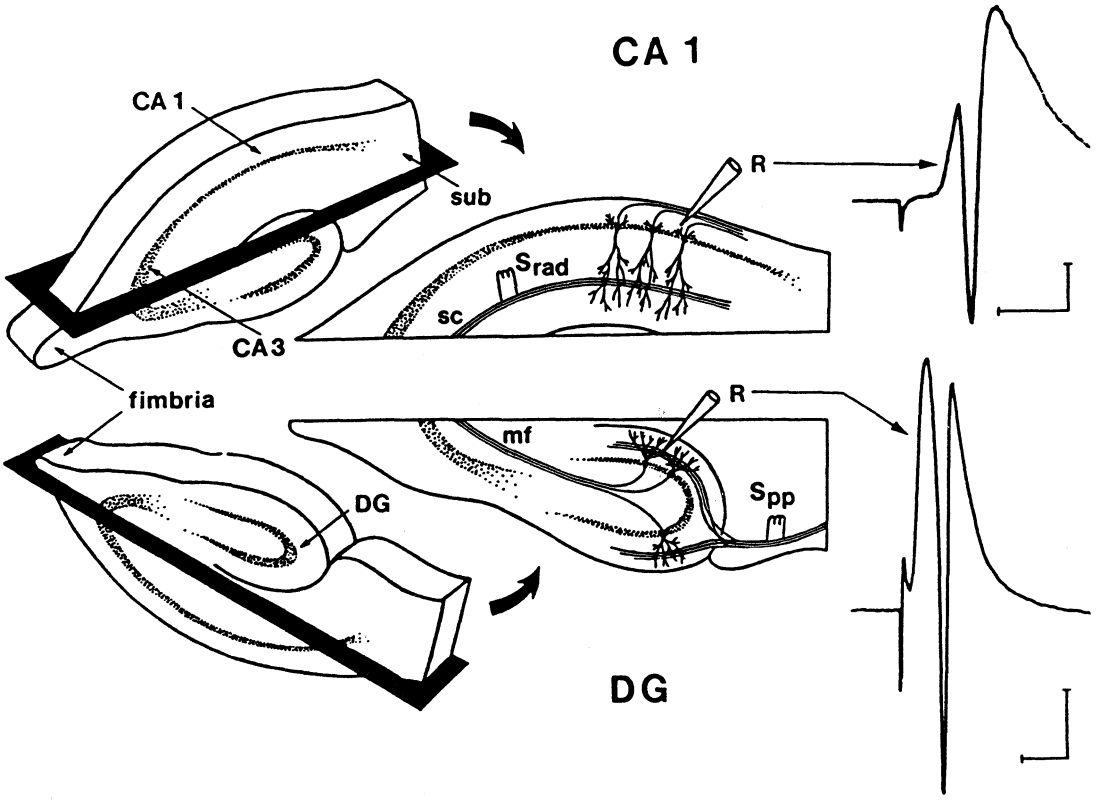


Fig. 4. The preparation of the CA1 and DG transected slices of the rat hippocampal formation. The diagrams in the middle show locations of stimulating (S) electrodes placed in stratum radiatum (rad) or on perforant path fibres (pp). Recording electrodes (R) were placed in the cell body layers of the CA1 or DG areas to record evoked field potentials, shown on the right. Note that evoked field potential responses closely resembled the potentials recorded from the intact slices indicating that the sectioning procedure preserved synaptic integrity in both CA1 and DG area. Calibration: 5 ms and 2 mV, positive up. (Reprinted with the permission from Brain Res. 1987, 436: 217-222).

the first *in vitro* observation supporting two generators hypothesis. Further physiological findings concerning the *in vitro* CA1 and DG theta rhythm were consistent with numerous earlier *in vivo* reports suggesting that the generator producing the largest amplitude of the HF type 2 theta is localized in DG region (Stumpf et al. 1962, Winson 1976, Bland 1986). We demonstrated *in vitro* that in conditions of anatomical separation the CA1 and DG generators could indepedently generate theta of different amplitude as shown in Fig. 5A and C. The results of experiments conducted with use of transected slices also revealed that the integrity of laminar, trisynaptic hippocampal circuit was not required for generation of theta oscillations. Furthermore, the pharmacological profiles for theta recorded from the isolated CA1 and DG areas supported earlier *in vivo* findings that the cholinergic muscarinic receptors mediate this EEG response (Kramis et al. 1975, Bland 1986, Bland and Colom 1993): both CA1 and DG *in vitro* recorded theta oscillations were antagonized by muscarinic blocker, atropine sulphate, and were found to be completely resistant to nicotinic antagonist - d-tubocurarine (Fig. 5B).

The transected slice technique was found to be also very useful in questing for other regions of the HF, 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 Buzsaki et al. (1985). Using our technique of transected slices we demonstrated that CCH-induced *in vitro* theta could

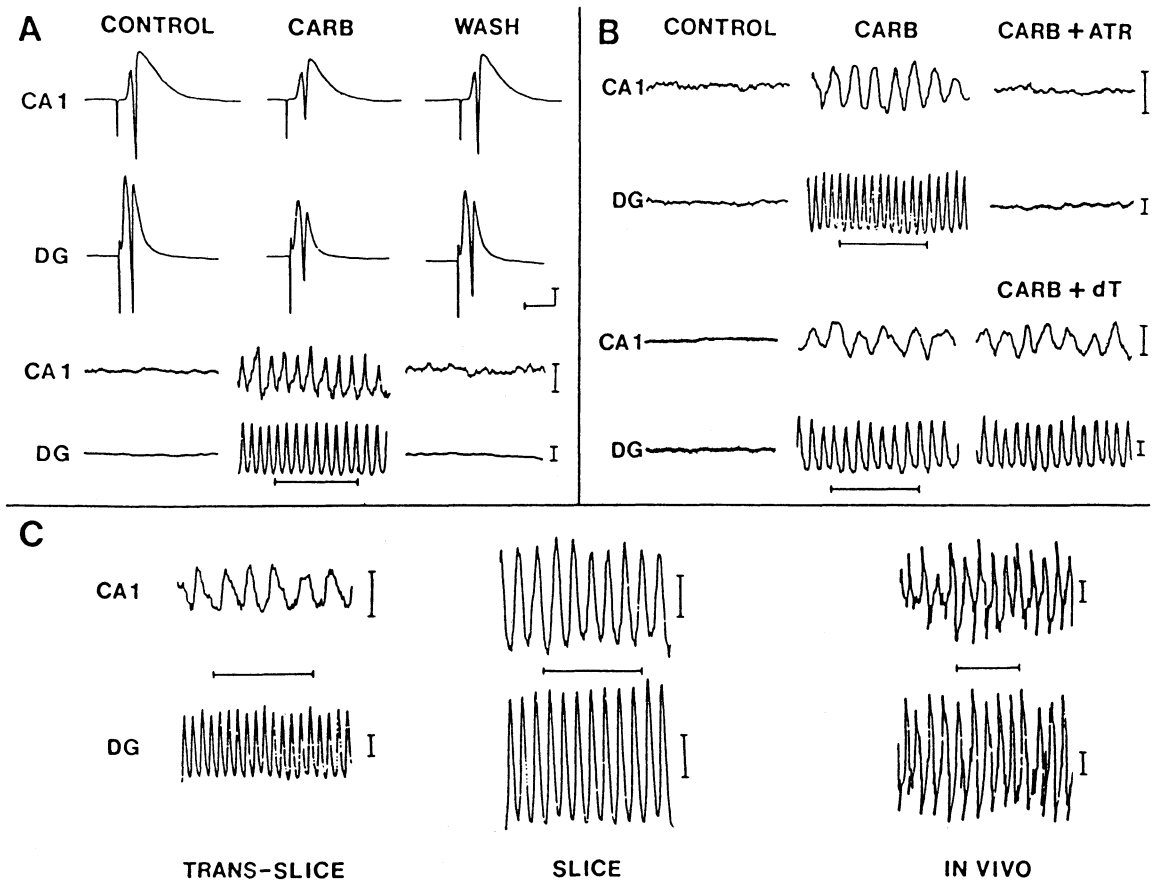


Fig. 5. Carbachol-induced theta-activity and depression of field potentials in the hippocampal transected slices. A, *stratum radiatum* to the CA1 and perforant path to the dentate granule (DG) neurone evoked field potentials were depressed in the presence of 50 μM carbachol (CARB). Partial recovery of field potential amplitudes are shown on the right following 60 min of wash with control solution. The lower recordings are at a higher gain and slower time scale, showing carbachol-induced (50 μM) theta-activity recorded from the *stratum oriens* of the CA1 and stratum moleculare of the dentate area. Recovery following 10 min of wash is shown on the right. Calibration: 10 ms and 2 mV for field potentials; 1 s and 200 μV for theta-activity. B, carbachol-induced (CARB 50 μM)theta -activity from both the CA1 and DG areas was antagonized by 50 μM of atropine (ATR), but unaffected by 50 μM of (d)-tubocurarine (dT). Calibration: 1 s and 200 μV. C, comparison of carbachol-induced theta-activity recorded from the CA1 and DG areas in the hippocampal transected slices (left), intact slices (middle) and from anaesthetized rats (*in vivo*, right). Calibration: 1 s and 200 μV; note that the *in vivo* recordings are curvilinear, all others are linear. (Reprinted with the permission from Brain Res. 1987, 436: 217-222).

be recorded indeed from the isolated population of CA3c pyramidal cells and was also found to be muscarinic mediated (Konopacki et al. 1988a).

Summing up, our studies utilizing transected slices provided strong evidence that there are in fact 3 anatomically separated intrahippocampal generators of cholinergic-induced theta oscillations, one localized in the basal part of the CA1 neurones (*stratum oriens*), the second in the *stratum moleculare* of the dorsal blade of the dentate gyrus and the third in the CA3 region of the hilus. Transected slice preparations revealed that these generator zones could operate independently of each other.

In the intact brain, theta rhythms recorded simultaneously from the CA1 and DG areas were found to be aproximately 180° out of phase (Robinson 1980, Bland 1986). However, this feature of the CA1 and DG theta was found to be hardly replicable *in vitro*. When the *in vitro* induced theta was recorded simultaneously from CA1 and DG regions, the high

variability in phase between these two signals (ranging from 0^{o} to 180^{o}) was observed (Konopacki et al. 1987b). This observation gave rise to our earlier suggestion that the medial septal rhythmic input was responsible for 180^{o} phase shift in the *in vitro* preparations. However, further *in vivo* observation made in B. Bland's laboratory (Colom et al. 1991, Smythe et al. 1992) demonstrated that this suggestion was not correct. The authors observed a 180^{o} phase shift in CA1 and DG theta oscillations induced by infusion of CCH and bicuculline in the septally deafferentated HF.

In the next stage of our *in vitro* study we analysed postnatal development of CCH induced theta and compared it with the pattern of development of spontaneous theta described earlier in neonatal rats. Just briefly, Leblanc and Bland (1979) demonstrated that type 2 theta appeared in rats around 10 days of age (during voluntary movements and during 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 (Konopacki et al. 1988c). Despite the difference in the time course of neurogenesis between CA1 and DG generators (Bayer and Altman 1974), CCH induced theta was observed in these two areas at about the same time (8-10 days after birth) and around 14 days of age reached the frequency and amplitude typical for adult rats (Fig. 6, Konopacki et al. 1988c).

A number of substances have been identified in the HF and those suggested as transmitter candidates include ACh, aromatic amines, γ-aminobutyric acid (GABA) and glutamic acid (Storm-Mathisen 1977). In the next series of experiments we attempted to determine other neurotransmitters which may have contributed to theta production. Specifically, we tested the effectiveness of glutamic acid, GABA, serotonine, noradrenaline, dopamine, nicotine on production of the *in vitro* theta oscillations.

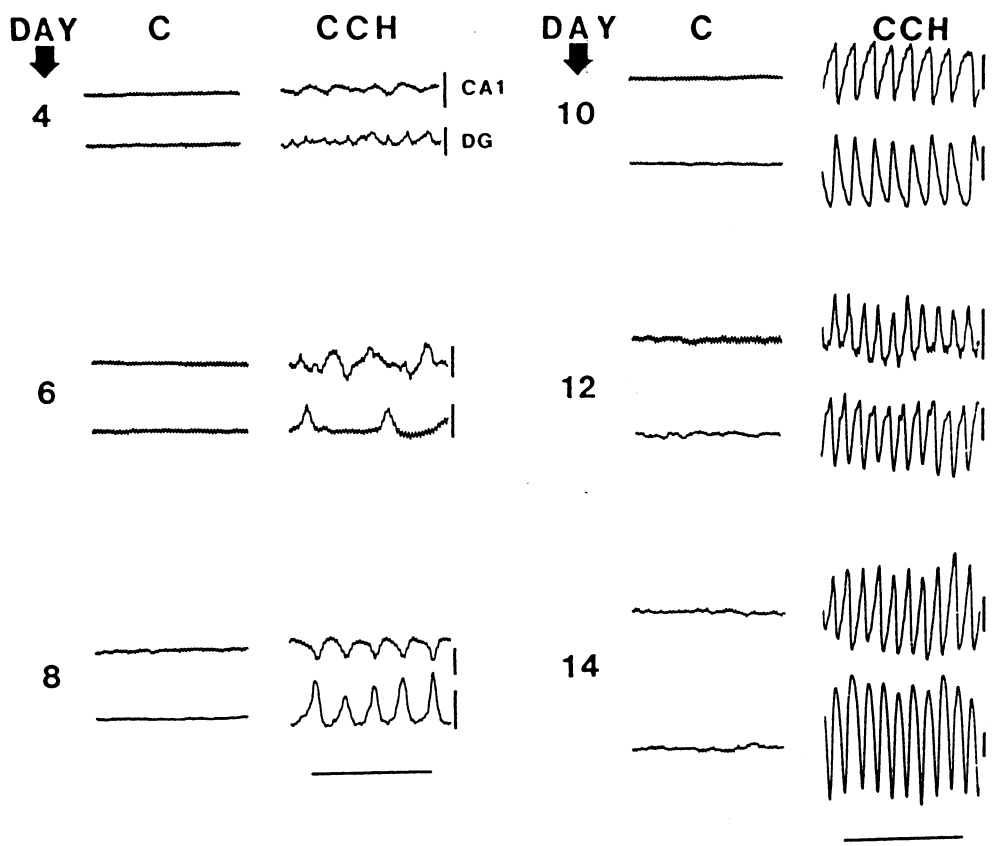


Fig. 6. Development of carbachol (CCH, 50 μM) induced theta in the CA1 and DG regions of hippocampal slices. At 4 and 6 days of age, perfusion of slices with carbachol induced irregular, short-lasting activity. Regular theta-rhythm was induced for short periods in 8 day-old slices. From 8 days onward increases in frequency and amplitude were observed; Calibration 200 μV, 1 s. (Reprinted with the permission from Dev. Brain Res. 1988, 38: 229-232).

None of the agents tested was capable of producing rhythmic slow waves (Konopacki et al. 1988b). The possible neurotransmitter interaction in the mechanism responsible for theta production will be discussed in the last part of this review.

The advantages of the *in vitro* brain slice preparation for intracellular recordings and pharmacological manipulations are well documented. In the next stage of our studies of the *in vitro* recorded theta oscillations we validated that the *in vitro* hippocampal model of CCH-induced theta could be used to analyse cellular mechanisms underlying this rhythm. 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 recognized as theta related (Fig. 7). They exhibited clear membrane potential oscillations (MPO-s, 5-28 mV) and multiple spike discharges occurring close to the peak positivity. MPO-s always had the same frequency as extracellulary recorded theta field potential and disappeared when extracellular theta oscillations were no longer observed (Bland et al. 1988). Similar the *in vitro* observations were also made by other authors (MacVicar and Tse 1989, Leung and Yim 1991, Garcia-Muňoz et al. 1993, Bianchi and Wong 1994). In addition, MacVicar and Tse (1989) demonstrated later that application of tetrodotoxin (TTX) or inorganic calcium channel blockers abolished carbachol induced intracellular theta oscillations. It supposed to be emphasized that MPO-s (intracellular theta rhythm) and rhythmic spike discharges can also be observed *in vivo* (Fujita and Sato 1969, Artemenko 1973, Leung and Yim 1986,

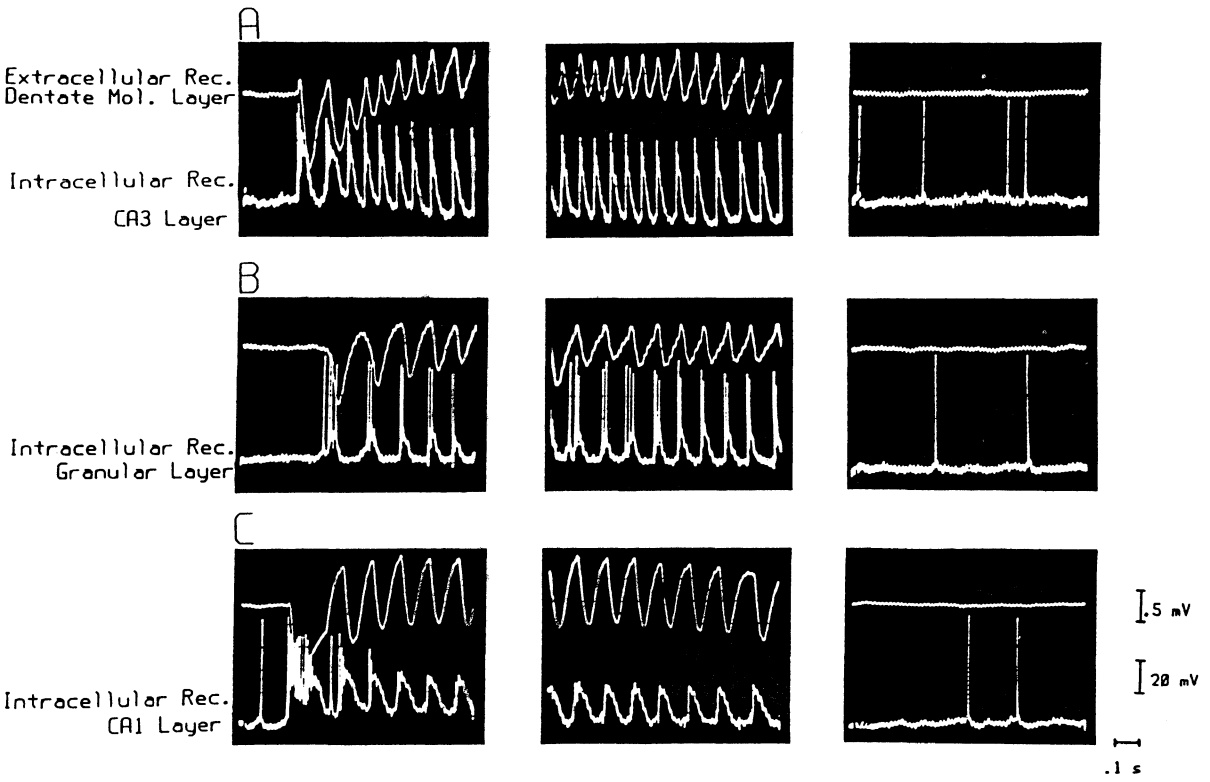


Fig. 7. Relationships between the amplitude of membrane potential oscillations and accompanying spike discharges, in theta-related cells. A, an example of the CA3 layer cell recording. The 3 panels were continuous recordings, from the left to the right. Note that the intracellular oscillations were large (>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. (Reprinted with the permission from Brain Res. 1988, 447: 364-368).

Nuñez et al. 1987, Konopacki et al. 1992a) in the so called phasic theta "on" and "off" cells (Bland et al. 1988, Ford et al. 1989, Bland and Colom 1993) during extracellulary recorded theta.

The above findings clearly demonstrated that CCH-induced *in vitro* theta was not an epiphenomenon without a cellular basis. The model of the *in vitro* recorded theta is valuable for studying cellular processes underlying type 2 theta, offering all the advantages concomitant with the slice preparation.

It has been histochemically demonstrated that aproximately 50% of fibres forming the septohippocampal projection are cholinergic and 30% GABA-ergic (Baisden et al. 1984). In addition, the HF was reported to contain a significant amount of glutamic acid decarboxylase (GAD) immunoreactive cells - i.e. the cells which possess GABA synthesizing enzyme (Ribak et al. 1986). Furthermore, it has been recently demonstrated *in vivo* that bicuculline, GABA-A antagonist, facilitated the effect

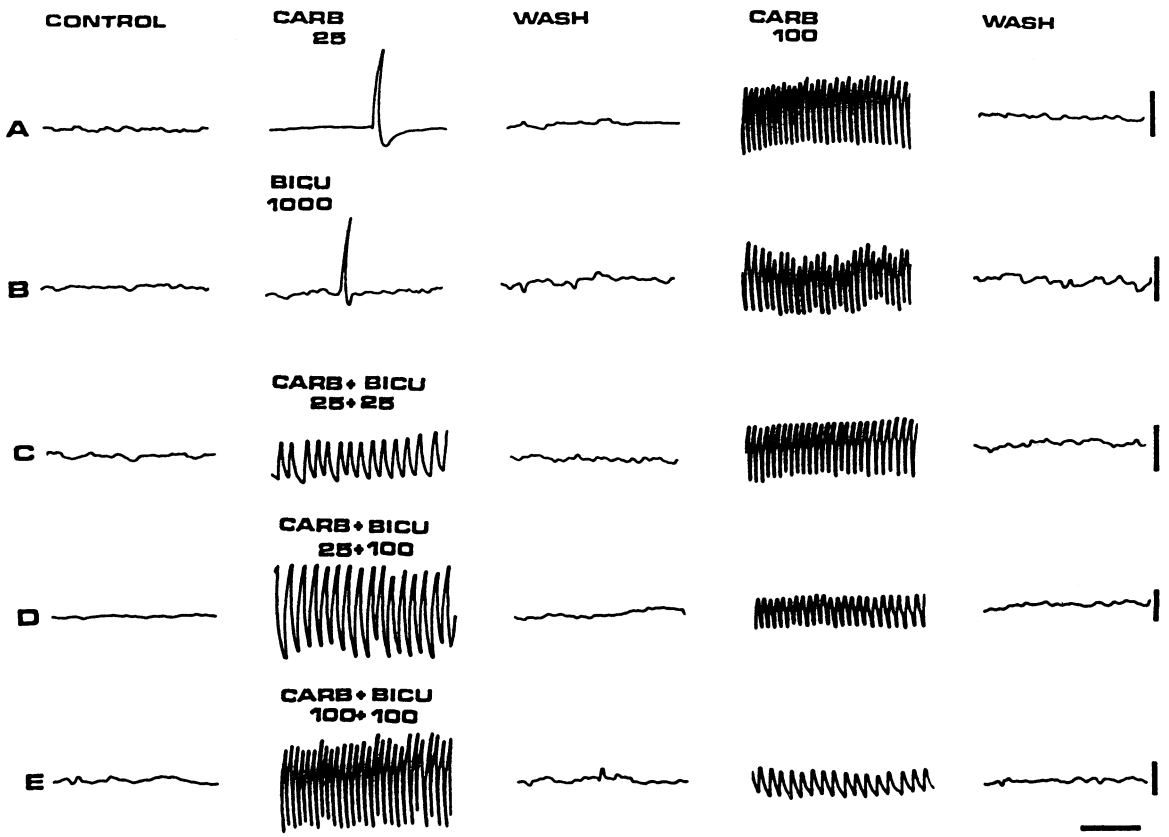


Fig. 8. Cholinergic-GABAergic interaction in generation of theta-like oscillations recorded in the hippocampal slices. In control conditions (perfusion with artificial CSF) the hippocampal slices did not manifest spontaneous EEG activity (CONTROL). A and B, these traces show lack of rhythmical oscillations after perfusion of a low (25 μM) concentration of carbachol (CARB 25) and a high (1,000 μM) concentration of bicuculline (BICU 1000). Note that the slices tested responded with theta-like slow waves to 100 μM of carbachol (CARB 100); C, when 25 μM of carbachol was perfused in the presence of 25 μM bicuculline (CARB 25 + BICU 25), rhythmical slow waves could be observed. Note that perfusion of 100 μM carbachol (CARB 100) produced better synchronized theta-like activity; D and E, these traces show an increase (in comparison with theta-like oscillations induced by perfusion of 100 μM carbachol alone) in the amplitude of carbachol-bicuculline induced theta-like field potentials. This effect was observed with low (25 μM) and high (100 μM) doses of carbachol + 100 μM bicuculline (CARB 25 + BICU 100 and CARB 100 + BICU 100 respectively). Induced field potentials were usually reversible after 10-20 min of wash with artificial CSF, and in case of carbachol-bicuculline induced theta-like activity after 40-60 min. Calibration: 1 s and 500 μV. (Reprinted with the permission from NeuroReport 1993, 4: 963-966)

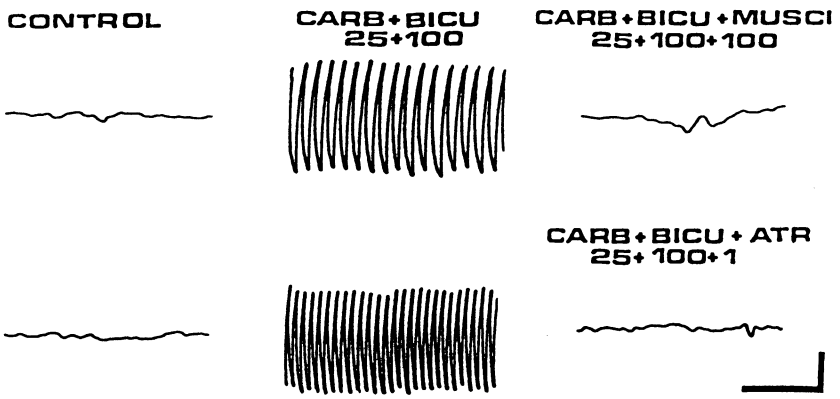


Fig. 9. The effect of muscimol and atropine sulphate on carbachol-bicuculline induced theta-like oscillations. Both 100 μM of muscimol and 1 μM of atropine sulphate antagonized carbachol-bicuculline (25 μM + 100 μM) induced theta-like oscillations (CARB 25 + BICU 100 + MUSCI 100 and CARB 25 + BICU 100 + ATR 1, respectively). Calibration: 1 s and 500 μV. (Reprinted with the permission from NeuroReport 1993, 4: 963-966).

of CCH in inducing theta when the medial septum was reversible suppressed by procaine (Colom et al. 1991, Smythe et al. 1992). The authors suggested that the HF type 2 theta resulted from dynamic interaction between the cholinergic and GABA-ergic systems. This was precisely what we observed *in vitro* (Fig. 8, Konopacki and Gołębiewski 1993): CCH at the low concentration (25 μM) never induced theta oscillations. The overall level of activation of the hippocampal neuronal network in this case was probably insufficient for theta to appear. When the same concentration of CCH was perfused

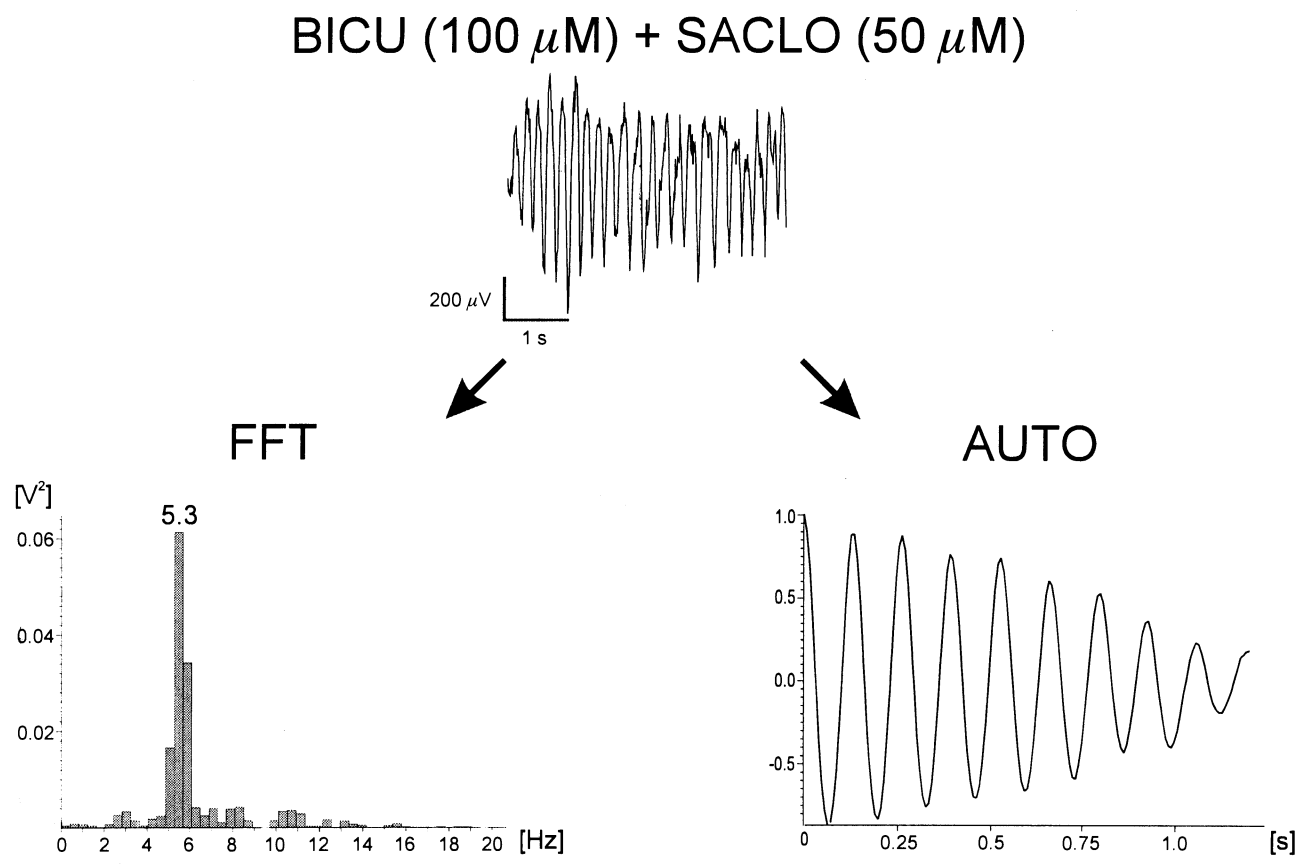


Fig. 10. Example of bicuculline (BICU, 100 μM) + 2-hydroxysaclophen (SACLO, 50 μM) induced theta oscillations recorded in a separate experiment from the *stratum moleculare* of the dentate gyrus and associated FFT and autocorrelation (AUTO) analysis.

simultaneously with bicuculline (25 μM), a well synchronized theta-like activity was observed. By blocking GABA-A receptors bicuculline reduced the hippocampal inhibition and this diminution of GABA-ergic inhibition together with subtreshold excitation of the hippocampal cholinergic network produced the level of activity required for generation of theta oscillations. Further disinhibition of the hippocampal neuronal network by 100 μM bicuculline or increase of the cholinergic excitation (with 100 μM CCH) resulted in pronounced increase of the amplitude of the *in vitro* theta (Fig. 8).

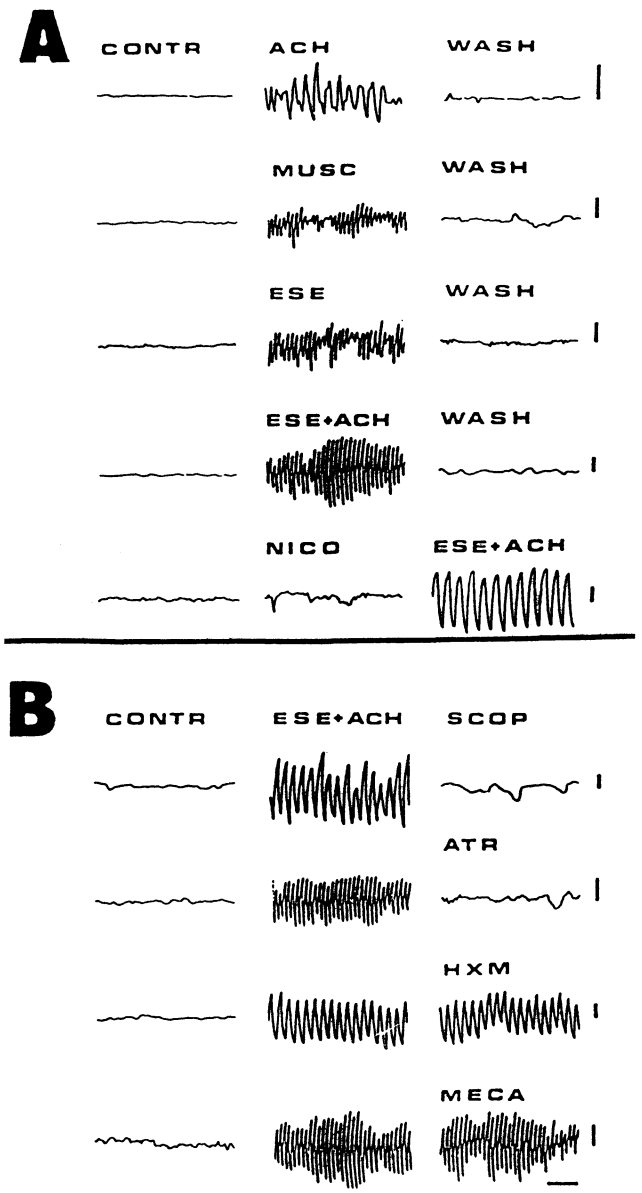
In another set of experiments we provided additional evidence supporting GABA-ergic/cholinergic interaction in the mechanisms responsible for theta production (Konopacki and Gołębiewski 1993). Muscimol, GABA-A agonist, diminishes overall hippocampal excitation (by increasing the level of GABA-ergic inhibition) and resulted in abolition of carbachol-bicuculline induced theta rhythm (Fig. 9). Atropine sulphate blocking the hippocampal muscarinic receptors and thereby decreasing the level of the cholinergic excitation produced a similar effects (Fig. 9).

Thus far we have been presenting theta oscillations resulting from the cholinergic excitation of the HF neuronal network or resulting from simultaneous cholinergic stimulation and GABA-A-ergic disinhibition. The next question arises whether strong diminution of GABA-ergic inhibition *per se* is capable of producing the level of excitation of the HF essential for theta to appear. Just recently this idea has been tested in our laboratory. The HF slice preparations were perfused with different concentrations of GABA-A antagonist - bicuculline and GABA-B antagonist - 2-hydroxysaclophen (2-HS). Well synchronized theta osciliations (3 -8 Hz, 0.5-3 mV) were observed only in the response to simultaneous perfusion of 100 μM bicuculline and 75 μM 2-HS in approximately 50% experiments performed (Fig. 10). The bath perfusion of the HF with bicuculline (100 μM) or 2-HS (1000 μM) alone produced only seizure activity (data not shown). Bicuculline/2-HS induced theta oscillations are the first *in vitro* evidence demonstrating that specific level of excitation of the HF neurones required for theta to appear can also be reached by strong diminution of GABA-A and GABA-B inhibition.

THETA OSCILLATION RECORDED FROM THE ENTORHINAL CORTEX SLICE PREPARATIONS

Increasing attention has been paid to the role of the EC in the mechanisms responsible for generation of theta rhythm. The EC is the main source of afferents to the HF and receives strong multisynaptic projection 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 HF theta (Vanderwolf et al. 1985). In addition, the EC *per se* was suggested to be a source of the *in vivo* recorded theta rhythm (Michell and Ranck 1980, Alonso and Garcia-Austt 1987, Dickson et al. 1994). This suggestion was strongly supported by the experiments we recently conducted on the medial EC slice preparations obtained from rats and cats (Konopacki and Gołębiewski 1992, Konopacki et al. 1992c, Gołębiewski et al. 1994). 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 rhythm when cholinergic agonists were added to the bath (Figs. 11 and 12). Four lines of evidence demonstrate that cholinergic-induced theta oscillations are mediated by muscarinic (M1) receptors: (1) slices containing medial EC have manifested theta slow waves in the presence of muscarine, (2) cholinergically induced theta has been antagonized by atropine sulphate and pirenzepine (specific M1 receptor blocker) but not by gallamine (the M2 receptor antagonist), (3) nicotine perfusion has never induced rhythmic slow waveforms, (4) hexamethonium and mecamylamine (nicotinic antagonists) have been found to be ineffective in blocking cholinergic induced oscillations (Figs. 11 and 12).

The *in vitro* studies disscussed above presented the result delivered from two limbic cortex preparations: the slices obtained from the HF and EC. Although it is hard to exclude a possiblity of inducing theta field potential in other limbic cortex regions (for example slices of the cingular cortex), the experiments we have conducted in our laboratory do not allow to confirm this statement. In addition, I am not aware of any studies investigating the cholinergic induced oscillation in these limbic cortex regions *in vitro*.



CONCLUSIONS

1. The experiments we have been conducting for the last 10 years on slice preparations obtained from the HF and EC demonstrate that a number of properties of the *in vivo* recorded theta rhythm can be replicated and investigated *in vitro*. The experimental model of CCH-induced *in vitro* theta was found to be valuable for studying many aspects of the central mechanism of oscillation and synchrony.

2. The appearance of theta in the limbic cortex requires a specific level of excitation of neuronal network. This level can be attained by direct stimulation of cholinergic muscarinic receptors, cholinergic stimulation and GABA-A-ergic blockade and by strong diminution of GABA-A and GABA-B inhibition.

Fig. 11. Representative examples of the cholinergically induced theta-like activity in the medial entorhinal cortex *in vitro*. A, cholinergically induced theta (middle panel) recorded from separate experiments conducted on different slices in the presence of acetylcholine (ACH, 0.6 mM), muscarine (MUSC, 0.5 mM), eserine (ESE, 0.1 mM), eserine and acetylcholine (ESE + ACH, 0.1 + 0.1 mM). Note that in control bath conditions the slices did not manifest spontaneous EEG activity (CONTR, left panel). At 10-15 min following the washout (WASH, right panel) the recordings returned to the control level. Of the cholinergic agents tested only nicotine (NICO, 1.0 mM) was found to be ineffective in producing slow rhythmical waves; however, the same slices exhibited EEG theta-like activity when perfused with eserine and acetylcholine (ESE + ACH, 0.1 + 0.1 mM, right panel). B, eserine and acetylcholine (ESE + ACH, 0.1 + 0.1 mM) induced theta-like activity recorded from separate experiments conducted on different slices (middle panel). The top two recordings (right panel) show antagonism of cholinergically induced theta by scopolamine (SCOP, 0.001 mM) and atropine sulphate (ATR, 0.001 mM). The lower recordings show lack of antagonism by hexamethonium (HXM, 0.1 mM) and mecamylamine (MECA, 0.1 mM). A, B, the samples of the cholinergic theta-like oscillations were taken 10-15 min from the onset of the perfusion; scopolamine and atropine samples were taken 10 min from the onset of the perfusion; hexamethonium and mecamylamine traces were taken 30 min after the perfusion onset. Calibration: 1 s and 200 μV. (Reprinted with the permission from Neurosci. Lett. 1992, 141: 93-96).

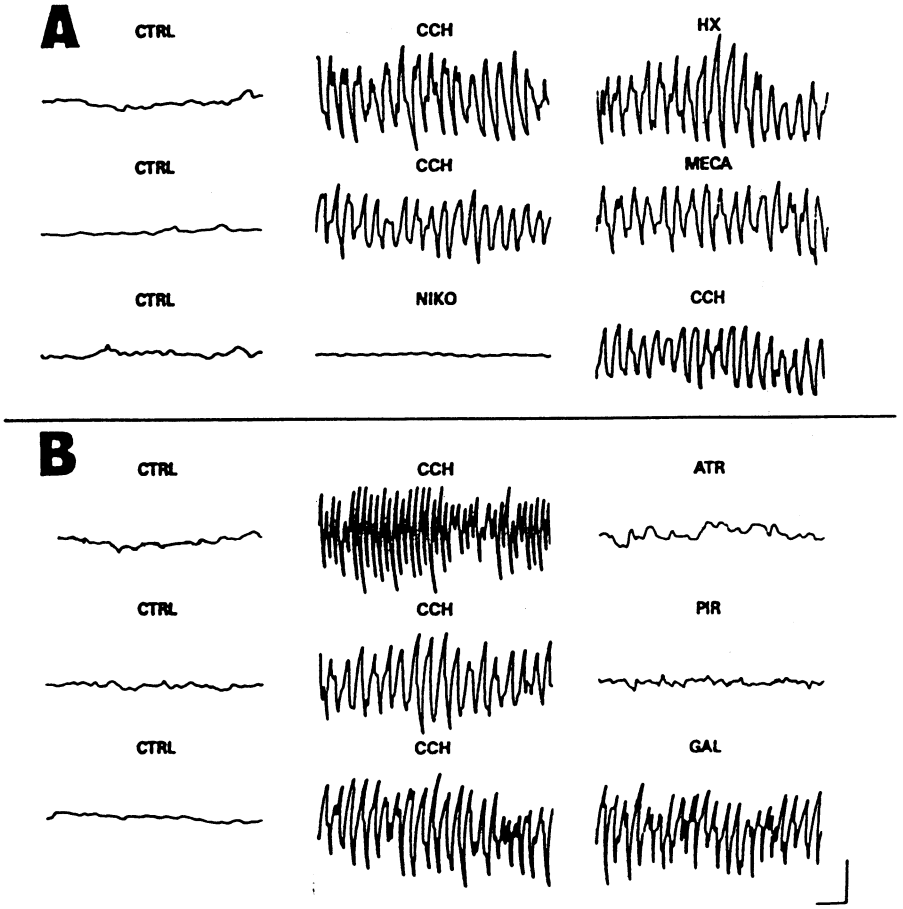


Fig. 12. Representative examples of the carbachol (CCH, 50 μM) - induced theta in the cat medial entorhinal cortex. A, CCH-induced theta activity recorded from three separate experiments on different slices (upper two traces). The recordings show the lack of antagonism of CCH-induced theta by hexamethonium (HX, 100 μM) and mecamylamine (MECA, 100 μM). The bottom recording shows the ineffectiveness of nicotine (NICO, 1,000 μM) in inducing theta oscillations. However, when the same preparations were perfused with CCH, rhythmic activity could be observed (bottom right). B, CCH - induced theta was antagonized by a classic muscarinic blocker, atropine sulphate (ATR, 1 μM) and by M1 receptor antagonist, pirenzepine (PIR, 1 μM) but was unaffected by gallamine (GAL, 100 μM) the M2 receptor antagonist. Calibration: 200 μV and 1 s. (Reprinted with the permission from NeuroReport 1994, 5: 1989-1992).

ACKNOWLEDGEMENTS

The author thanks dr B. Eckersdorf and dr H. Gołębiewski for critical comments and suggestions and Mrs K. Wesołowska for technical assistance. The experiments whose results were partially described in this paper were supported by the State Commitee for Scientific Research (grant PB 0839/p2/94/07).

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Paper presented at the 2nd International Congress of the Polish Neuroscience Society; Session: Mechanisms of synaptic transmission - electrophysiological in vitro studies