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The evolving concept of the intrinsic hippocampal theta/gamma oscillator

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1. ABSTRACT

Three main types of electrical oscillations are recorded from the hippocampus *in vivo*: theta (θ), gamma (γ) and sharp wave ripples with frequency bands of 4–12, 25–100 and 110–250 Hz, respectively. Theta activity is the more robust of them, and has important physiological roles because it is involved in spatial navigation, memory formation and memory retrieval. Classical lesion studies *in vivo* have suggested that the hippocampus passively follows the θ rhythm generated in the septum by neurons that are synaptically connected with hippocampal neurons though septo-hippocampal connections. This view has been questioned since several studies have shown that oscillations in the θ range can be recorded in *in vitro* hippocampal preparations thus indicating that the hippocampus itself can act as a θ oscillator. In this review, we will describe how the paradigm of the intrinsic θ oscillator has been changing over the years from simple models that have proposed single hippocampal lamellae to contain the θ oscillator to the current models that include some degree of septo-temporal integration.

2. INTRODUCTION

Spontaneous field potential oscillations are known to dominate the EEG activity; they act as carrier waves that provide neuronal inputs with the temporal and spatial codes for signal discrimination, and play a role in the regulation of synaptic plasticity (1). The hippocampus is presumably the brain region where oscillatory activity has been more extensively investigated, and three main types of oscillations have been identified: theta (θ), gamma (γ), and sharp wave ripples (SWRs) with frequency bands of 4–12, 25–100 and 110–250 Hz, respectively (2, 3). Among these

three hippocampal oscillations θ rhythm is the most extensively studied as it appears to play prominent roles in memory formation, in sensimotor integration and in spatial navigation (4–7).

Over sixty years ago, the classical study by Green and Arduini (1954) (8) found that the θ rhythm is imposed on the hippocampus by an oscillator located in the septum that is synaptically connected with the hippocampus through the fibers of the septo-hippocampal pathway. These fibers run along the mediolateral and rostro-caudal axes in a highly topographically organized manner. Since the medial septum receives incoming fibers from other brain regions such as the brain stem or the hypothalamus, it was proposed that this structure could play a role in integrating signals that control hippocampal θ activity (9–11).

Different classes of septal neurons (such as cholinergic, GABAergic and glutamatergic cells) project to the hippocampus and entrain hippocampal θ rhythm. Septal cholinergic neurons project extensively through the hippocampus making synaptic connections with the dendrites of pyramidal cells as well as with the somas and dendrites of GABA- and somatostatin-containing interneurons (12,13); in contrast, GABAergic septal cells preferentially make synapses with basket cells that represent the main GABAergic interneuron subtype in the hippocampus (10, 14, 15). Finally, glutamatergic neurons project to a restricted number of principal and inhibitory cells of the hippocampus (16, 17).

It was initially proposed that these different septal neurotransmitter inputs control different forms of hippocampal θ rhythm. Indeed, two different types of θ rhythm were identified, type I and type II. Type I θ rhythm has a frequency in the 7–12 Hz band and occurs during explorative movements, whereas type II θ has a slower frequency (4–6 Hz) and is recorded during REM sleep or urethane anesthesia (19). Early studies showed that atropine and selective lesions of cholinergic projecting septal neurons abolished type II but not type I θ rhythm whereas urethane anesthesia suppresses type I θ rhythm leaving type II unaltered (19,20). It was, therefore, concluded that type I and type II θ rhythm are controlled by non-cholinergic and by cholinergic neurotransmission, respectively. However, this distinction is far from being unequivocal because an atropine-resistant component was found in type II θ rhythm (21–23) due to non-cholinergic mechanisms that are mainly contributed for by septo-hippocampal GABAergic (as reviewed by Teles-Ruivo and Mellor (13)) and, as more recently demonstrated, glutamatergic inputs (24). Moreover, a cholinergic component was also observed in type I θ rhythm (25).

Overall, the aforementioned data suggested that the septum generates rhythmic θ activity and the hippocampus simply acts as a transducer of rhythmic septal inputs. Contradicting this hypothesis, a wealth of data indicate that θ rhythm is instead actively generated in the hippocampus in response to septal inputs and that, under specific conditions, the hippocampus can also autonomously generate θ oscillations in the absence of extra-hippocampal synaptic inputs. Therefore, the concept that the hippocampus contains one or more intrinsic oscillators was formulated and became widely accepted. In this review we will go through the different experimental models *in vitro* that first provided the evidence of the existence of these oscillators and, then, were employed to dissect and characterize their mechanism of operation. We will thus examine the contribution to the knowledge of the intrinsic hippocampal oscillators brought by acute hippocampal slices, acute septo-hippocampal slices, and hippocampal and septo-hippocampal slice cultures and by the isolated intact hippocampus preparation. The present paper does not intend to systematically review the enormous amount of work that has been done on hippocampal oscillations but to highlight how the concept itself of the intrinsic hippocampal oscillator has been evolving in parallel with the experimental models employed for its study.

3. ACUTE HIPPOCAMPAL SLICES PROVIDE THE FIRST EVIDENCE OF THE INTRINSIC HIPPOCAMPAL OSCILLATOR

3.1. The birth of the paradigm of the hippocampal θ oscillator

It was first shown in the 1980s that the hippocampus can act as an autonomous θ oscillator. This evidence came from experiments performed by using the *in vitro* acute hippocampal slice preparation. This powerful experimental tool, which was developed by McIlwain in the 1950s (see Collingridge (1995) (26) for a historical review), has greatly contributed to advancing our understanding of hippocampal neurophysiology. It usually employs transverse slices, which are cut orthogonally respect to the longitudinal axis of the hippocampus, or pseudo-transverse slices such as coronal slices, which are not perfectly orthogonal but have a certain degree of angulation in respect to this axis (Figure 1). On the contrary, longitudinal slices, which are cut along the septo-temporal axis of the hippocampus, are only rarely used. Hippocampal slices can be used to perform electrophysiological recordings for 6–10 hours after their isolation. In a significant proportion of properly cut transversal, coronal or horizontal hippocampal slices the trisynaptic circuit that connects the dentate gyrus (DG) with CA3 and CA1 remains intact because of the lamellar organization of the hippocampus. According to this microanatomy model that was described in the 1970s by Andersen *et al*. (27, 28), the hippocampus is a complex structure made by the juxtaposition of a

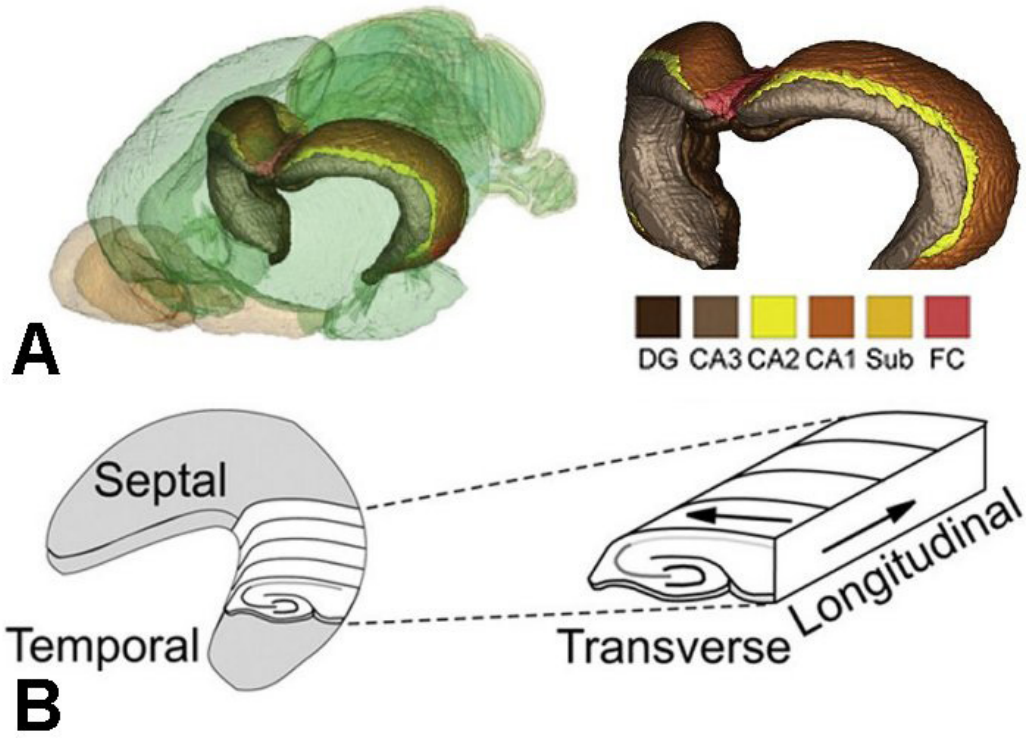

Figure 1. Tridimensional structure of the hippocampus and orientation of the hippocampal slices employed in electrophysiological recordings. A. Tridimensional reconstruction of the hippocampal structure from the Waxholm Space atlas (v2.0.) of the rat brain. On the left, the hippocampus is shown (in brown) in transparency *in situ* inside the rat brain. In the right side of the panel the hippocampus is shown in isolation to illustrate its complex tridimensional structure. The color palette reports the codes for the single subregions of the hippocampus as depicted in the panel. B. Schematic drawing illustrating how the hippocampal slices can be obtained by cutting the hippocampus along its transversal or its septotemporal axis. Notice that because of the spatial orientation of the hippocampus, slices are obtained by horizontal or by coronal cuts are not perfectly orthogonal respect to the septo-temporal axis. A is reproduced under creative common license from Kjonigsen LJ, Lillehaug S, Bjaalie JG, Witter MP, Leergaard TB. Waxholm Space atlas of the rat brain hippocampal region: three-dimensional delineations based on magnetic resonance and diffusion tensor imaging. Reproduced with permission from (112).

series of lamellae or “elementary slices” arranged in parallel along its septo-temporal axis (29). Each lamella is a functionally and anatomically partially independent structure with its own input and output being connected with the entorhinal cortex (EC) in a laminar way and with a precise orthotopic arrangement. Therefore, properly cut hippocampal slices include one or more intact lamellae whose activity can be recorded *in vitro*. Depending on how the slice is prepared, the connections with parahippocampal regions, the entorhinal and the perirhinal cortices, can be maintained or not, whereas the connections with the septum and with the rest of the brain are lost. Therefore, any rhythmic activity recorded in acute hippocampal slices could be considered as: i) autonomously generated in the hippocampus; ii) independent from synaptic inputs from extrahippocampal brain structures; and iii) generated by mechanisms totally included in elementary laminar structures of the hippocampus itself. This explains why the evidence that θ oscillations are recorded in isolated hippocampal slices has been so relevant for the development of the concept of the hippocampal intrinsic θ oscillator.

As reported by Konopacki (30), the first unpublished observation of θ activity in the *in vitro* isolated hippocampal slice was made by Brian H. Bland in the laboratory of Per Andersen in the 1970s. It was, however, only in the middle 1980s that several publications (most of which authored by Konopacki himself, first in Roth’s laboratory at Calgary and then in his own lab at Lodz) systematically explored the hypothesis that θ activity could be autonomously generated in hippocampal slices. In these experiments hippocampal slices were exposed to direct or indirect acting cholinergic agonist to reproduce *in vitro* the effect of septal activation *in vivo* (31, 32). In 1986, MacIver *et al*. (33) showed that the direct cholinergic agonist carbachol (CCh) and the cholinesterase inhibitor eserine do induce the appearance of rhythmic discharges in the DG of isolated hippocampal slices. These discharges were organized in trains 1 to 10 second in duration and Fast Fourier transform (FFT) analysis showed that their main frequency component was in the θ range (Figure 2A). Although CCh also stimulates nicotinic receptors (http://www.guidetopharmacology.org/GRAC/LigandDisplayForwa

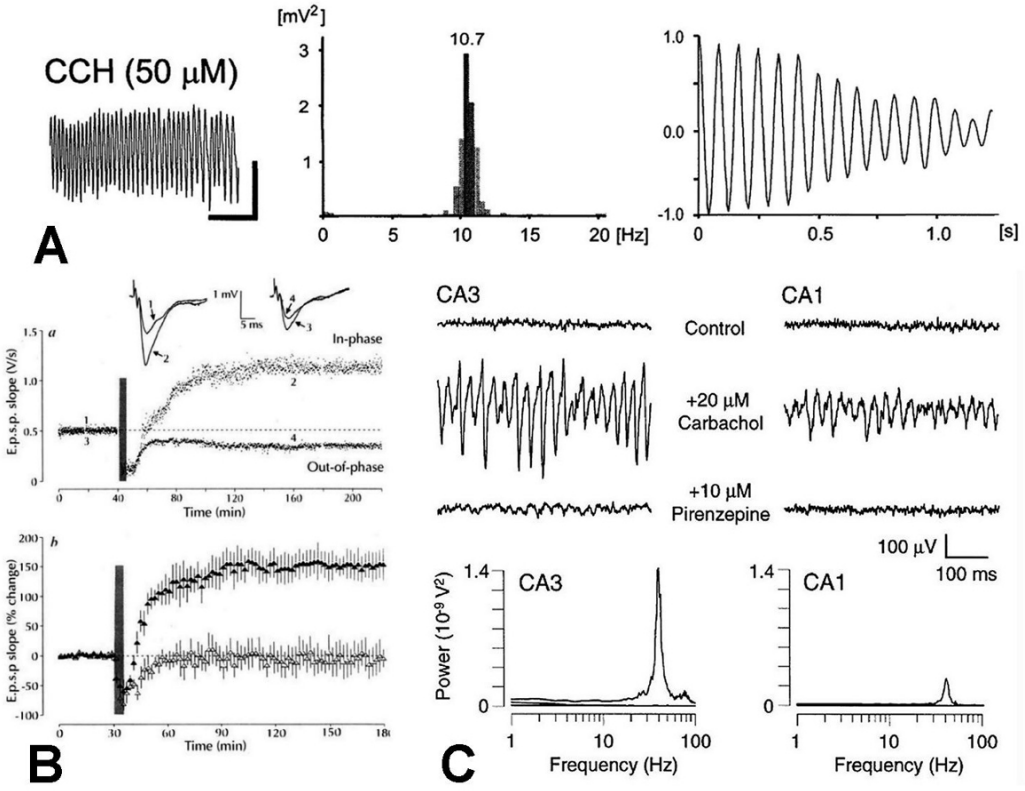

Figure 2. Carbachol-induced oscillations in acute hippocampal slices. A. The pales shows a representative trace (on the left), the spectrogram (on the middle) and the autocorreletaion plot (on the right) of θ oscillations recorded in the CA3 in acute rat hippocampal slices upon exposure to 50 μM CCh. B. Selective enhancement of Long Term Potentiation by CCh-induced θ-rhythm. The panel compares the EPSP slopes expressed either as V/S (top) or as percent change (bottom) in slices that were electrically stimulated in phase or out of phase with CCh-induced oscillations (as indicated). The inset shows representative traces obtained in these two different conditions. C. Representative traces (top) and power spectra of γ oscillations induced by 20 μM CCh in the CA1 and CA3 (as indicated) of acute rat hippocampal slices. The images were reproduced with permission from (30, 47, 68).

rd?ligandId=298) and selective agonists of α7 neuronal nicotinic receptors induce neuronal oscillations in the hippocampus (34), it was demonstrated that CCh-induced activity was primarily mediated by the stimulation of muscarinic receptors because it was suppressed by atropine but not by d-tubocurarine (35).

3.2. CCh-induced and physiological θ do share similar features

Based on its spectral characteristics and atropine sensitivity, the CCh-induced activity was proposed to be the *in vitro* correlate of septal driven θ *in vivo* and these observations were considered the formal demonstration that the hippocampus could generate *per se*, independently from septal innervation, a *bona fide* θ activity. These findings also suggested that the cholinergic stimulation required to elicit the θ rhythm did not have to be patterned at a θ frequency by a septal pacemaker. Hence, the rhythm generator was believed to reside in the hippocampus itself. From then on, CCh has been often used as a pharmacological tool to evoke hippocampal "θ" oscillations *in vitro* even though the real identity of the CCh-induced activity and of the "true" θ rhythm remains a matter of controversy. Following the paper of MacIver *et al*. (33), a thorough investigation of the properties of CCh-induced activity was performed in several laboratories. First, it was demonstrated that CCh-induced θ activity can be recorded only if hippocampal slices are obtained from rats older than 8–10 days, and this age-dependence could represent a further similarity with physiological θ that, in the rat, appears around 8–10 days of age (36). Then, a detailed topographic study of the distribution of CCh-induced activity in the hippocampus and in para-hippocampal areas was performed and θ-like oscillations were reported to occur not only in CA1 and CA3 but also in the subiculum and in the EC (37–43). Moreover, studies performed by employing the *in vitro* brain slice preparation demonstrated that CCh-induced oscillations occur also in the hypothalamus and in the neocortex (44, 45). Finally, it was shown that the *in vitro* CCh-induced activity also resembled physiological θ (46) in its ability to heighten synaptic plasticity and potentiate Long Term Potentiation (LTP) (47). Accordingly, it was shown that the increase in the field excitatory postsynaptic potential (fEPSP) slope attained with an LTP protocol in hippocampal slices in

the presence of CCh was significantly higher when the electrical stimulation was in phase than when it was out of phase with the θ oscillations (47) (Figure 2B).

The results of these early studies led to the wide perception that there was indeed a θ oscillator in the hippocampus and that it could possibly have something to do with the mechanism generating "real" physiological θ rhythm *in vivo*. Importantly, evidence was reported of the existence of intrinsic hippocampal oscillators also *in vivo* (48) and it was shown that CCh induces θ oscillations in septally deafferented hippocampus of the anesthetized rat (49). To establish whether there were a single oscillator or multiple oscillators in acute hippocampal slices, experiments were performed on transected slices in which the connections among different regions of the hippocampus were knife cut. The rationale underlying these experiments was that if a certain region of the hippocampus was a rhythm generator than it should continue to discharge at a θ frequency when disconnected by the rest of the hippocampus. The results of these experiments showed that at least two or three different oscillators do exist in the hippocampus. One or two oscillators were identified in the CA1 and an additional oscillator was located in DG (40, 50). These findings were considered a further demonstration of the similarities between *in vitro* and *in vivo* θ rhythm because θ oscillations can be recorded in living animals in both of these regions (50). Later investigation established that the second oscillator was actually located in the CA3 that was indeed partially included in the original "DG" slices (51). Collectively, the studies performed with surgically disconnected hippocampal slices suggested that multiple oscillators coexist in the hippocampus whose activity gets presumably synchronized when their synaptic connections are intact.

4. CRITICISMS ARE RAISED ON THE RELIABILITY OF ACUTE HIPPOCAMPAL SLICES AS AN EXPERIMENTAL MODEL OF THE θ HIPPOCAMPAL OSCILLATOR.

4.1. CCh-induced oscillations have electrophysiological characteristics different from physiological θ

In order to characterize the electrophysiological mechanism responsible for CCh-induced oscillations in acute hippocampal slices, intracellular recordings with sharp microelectrodes were performed. Using this approach, Bland *et al*. (1988) (52) showed that both in CA1 and in CA3 hippocampal subfields only 50% of the recorded neurons generated action potential discharges that are synchronous with the θ oscillations recorded extracellularly. This rhythmic firing was preceded by an increased percentage of spontaneously discharging neurons with progressive membrane depolarization. Shortly after, Mac Vicar and Tse (1989) (53) performed simultaneous intracellular and extracellular recordings in the CA3 subfield of transverse isolated hippocampal slices from 21–28 day-old rats and found that the initial response to CCh was not oscillatory and included: i) a gradual reduction of the AHP that follows spontaneous action potentials, ii) a progressive increase in the frequency of EPSPs ultimately leading to their summation and to the appearance of single or burst action potentials, and iii) a gradual membrane depolarization that developed independent from action potentials generation. Stable, well defined θ-like activity appeared later and consisted of membrane potential oscillations that were often, but not always, crowned by burst of action potentials. During the time between oscillations membrane repolarized and neurons remained quiescent for about 30–70 seconds. The kinetics of membrane potential oscillations was better appreciated in neurons not showing action potential bursts on the top of it, and resulted clearly slower than conventional EPSPs. These results were similar to those obtained by Fujita and Sato (1964) (54) and by Nunez *et al*. (1987) (55) who performed intracellular recordings in CA1-CA3 hippocampal pyramidal neurons in behaving animals during θ rhythm. This similarity was considered an additional argument in support of the hypothesis that an authentic θ rhythm was elicited by CCh in acute hippocampal slices. However, important differences in single neuron electrophysiological behavior *in vivo* and *in vitro* were later noticed. Specifically, in 1992, Traub *et al*. (56) pointed out that the CCh-induced activity differed from "true" θ activity because the majority of the pyramidal cells fired action potential very often in bursts, whereas most of them are silent in physiological θ rhythm (57). In addition, they noticed, that CCh response in slices was dominated by EPSPs whereas in most of the recordings *in vivo* IPSPs predominate in physiological θ (58). Intriguingly, these basic functional features, i.e. pyramidal cell bursting and EPSPs, had to be both included in computer neuronal models to accurately reproduce the electrical activity evoked by CCh in hippocampal slices *in vitro* (56).

Another study that seriously questioned the identity of *in vivo* and *in vitro* θ-activity was published by Williams and Kauer in 1997 (59). Working in coronal hippocampal slices they found that CCh-induced activity was driven by CA3 and disappeared in CA1 when it was surgically disconnected from CA3. According to these data not only CA3 generated its own θ-activity but it was actually the primary engine of this kind of oscillations in intact slices. This was interpreted as an important difference from physiological θ rhythm because, at that time, CA3 was believed to be minimally involved in physiologic θ (60, 61). Moreover, intracellular recordings showed that upon CCh exposure pyramidal cells displayed prominent phasic discharges whereas, *in vivo*, in

physiological θ rhythm, there is a predominant phasic discharge of interneurons (62, 63). On the basis of these observations, it was concluded that the activity elicited by CCh was more similar to the epileptiform activity induced by the cholinergic agonist pilocarpine than to θ rhythm (59). It should be emphasized that the activity induced by CCh in the EC is usually epileptiform-like (64–66) and it is has been shown to be sensitive to antiepileptic drugs (67). The ability of cholinergic agonists to elicit epileptiform discharges besides θ oscillations raises the important and still unresolved question of how these two phenomena could be related (68).

4.2. In acute hippocampal slices the intrinsic oscillators generate oscillations in frequency ranges other than θ

The idea that CCh induced an authentic θ rhythm *in vitro* in acute hippocampal slices was further questioned by the evidence that upon exposure to this cholinergic agonist also oscillations in other band ranges could be observed. Indeed, Fishan *et al*. (1998) (69) reported that in horizontal hippocampal slices, CCh predominantly elicited oscillations faster than θ with frequency components around 40 Hz, hence in the γ range. This activity often occurred nested in θ frequency oscillations. CCh-induced activity was abolished upon pharmacological blockade of muscarinic receptors, and also disappeared when either $GABA_A$ or AMPA receptors were antagonized. Several observations suggested that the θ/γ rhythm generator was located in CA3. Specifically, although CCh-induced oscillations were highly coherent in CA3 and CA1, discharges in CA3 systematically anticipated those in CA1. Moreover, surgical disconnection of CA3 and CA1 caused the loss of CCh-induced activity in CA1 but not in CA3 (69). Results consistent with the hypothesis that an intrinsic γ oscillator was located in CA3 were also obtained by Fellous *et al*. (2000) (70) who showed that CCh-induced γ oscillations could be observed in transversal mini-slices including the isolated CA3 but not in those containing only CA1. The mechanism responsible for γ activity propagation from CA3 to CA1 has been identified by later investigations with feedforward inhibition and consists in the phasic activation of CA1 GABAergic interneurons by the glutamaterigic projections of CA3 pyramidal neurons (71). These results *in vitro* matched well with the evidence showing that *in vivo* two main oscillators are responsible for hippocampal γ rhythm generation, one slower (≈40 Hz) and intrinsic to the hippocampus proper being located in CA3 and the other faster (≈90 Hz) and extrinsic to this structure located in the EC (72, 73). Interestingly, *in vivo*, these two oscillators could subserve independent physiological activities such as memory retrieval and encoding (74). More recently, the existence of a second intrinsic hippocampal CCh-activated fast γ oscillator was demonstrated in CA1 mini-slices (75). In intact hippocampal slices this oscillator is overridden by the CA3 oscillator that discharges at a significantly lower frequency (75).

The evidence that CCh elicited γ or θ/γ nested oscillations in hippocampal slices and not the pure θ rhythm reported by earlier studies raised the question of how to explain these differences. It was proposed that slice orientation could matter (69). Indeed, Fisher *et al*. (2002) (76) stated that muscarinic agonists elicit epileptiform activity in transverse and prevalently γ oscillations in horizontal slices with a significant θ component only in about 4% of the slices. Other authors reported evidence that the major determinant of the frequency of the oscillations evoked by CCh was the concentration at which this muscarinic agonist was applied. For instance, Fellous *et al*. (2000) (70) observed that CCh induced γ, or θ oscillations when used at concentrations of 4–13, 8–25 and 13–60 μM, respectively.

Because of these new data obtained in acute hippocampal slices the intrinsic hippocampal oscillator started to be viewed not more as a pure θ oscillator but as a γ or a θ/γ oscillator. Whereas these findings appeared somehow disturbing at the time they were obtained, the discovery that the intrinsic hippocampal oscillator generates γ and θ/γ nested activity in response to cholinergic stimulation appears nowadays intriguing. It has been clearly demonstrated, indeed, that γ and θ oscillations do coexist in the hippocampus *in vivo* and that their functional interaction has specific physiological roles. Discharges in the γ frequency range at specific times on a θ carrier wave could, for instance, provide the key for spatial navigation decoding by place cells or represent a mechanism for controlling memory formation and retrieval (77–79). Moreover, it has been shown that because of the filtering properties of their dendrites, CA1 pyramidal cells preferentially respond to inputs in the γ range maximizing the oscillatory response in θ range (80).

A few reports were also published showing that CCh can also evoke β activity in hippocampal slices. For instance, using a multi-electrode array Shimono *et al*. (2000) (81) showed that CCh (50 mM) elicited in CA1 and in CA3 oscillations a rhythmic activity whose predominant frequency component was in the β range in 83% of the slices and in θ range only in the 17% of the slices. The authors suggested that β and γ rhythm could be part of a continuum spectrum and that transition from β to γ activity could occur in specific circumstances as reported by Traub *et al*. in 1999 (82) for the oscillatory activity elicited by tetanic stimulation in hippocampal slices. In agreement with this hypothesis, Arai and Natsume (2006) (83) were successful in converting CCh-induced β into θ activity by blocking $GABA_A$ receptors with 1–10 μM bicuculline. They concluded that a higher GABAergic activity was

required to generate β activity whereas θ activity appears at lower levels of activation of the GABAergic system.

The results reviewed so far show that in acute hippocampal slices cholinergic stimulation does not elicit only θ rhythm but also oscillations with different frequency components. Similar results were obtained also with oscillation evoked by non-cholinergic pharmacological stimuli. In 1995 Whittington *et al*. (84) reported that an oscillatory response in the γ range could be evoked in acute hippocampal slices by delivering an electrical stimulation two seconds after a weak conditioning train. These oscillations, which consisted in rhythmic IPSPs, persisted in the presence of blockers of the NMDA and $GABA_A$ receptors but were abolished by the wide spectrum metabotropic receptor blocker MCPG. A similar response was elicited by pressure injection of the glutamate metabotropic receptor agonist (1S, 3R)-ACPD. Therefore, the intrinsic hippocampal oscillator can be activated to generate a rhythm in the γ frequency range in a non-cholinergic manner through the activation of metabotropic glutamate receptors. Intriguingly, the same kind of pharmacological stimulus can also elicit θ oscillations. This was shown by Cobb *et al*. (2000) (85) who observed that in CA3, ACPD elicit intermittent episodes of θ activity when used at concentrations between 100 and 400 μM. These data indicated that, depending on the conditions, either θ or γ activity could be evoked in acute hippocampal slices by the activation of metabotropic glutamate receptors: a story strangely similar to that of CCh. If a common mechanism does exist for θ and γ oscillations in the hippocampus then it would be possible to switch from one to the other of these oscillatory modes and vice versa. This is what was demonstrated by Gillies *et al*. (2002) (86) who showed that the γ activity elicited by the metabotropic glutamate receptor agonist DHPG in CA1 transformed into θ rhythm when ionotropic glutamate AMPA receptors were blocked with NBQX.

In conclusion, at the end of the 1990s the initial enthusiasm about acute hippocampal slices as a reliable model *in vitro* of θ activity was mitigated by the evidence that: i) they do not display any spontaneous activity in the absence of pharmacological stimuli, ii) they do not generate a pure θ activity but a γ or a θ/γ nested rhythm and iii) the electrophysiological characteristics of the oscillations elicited by their pharmacological stimulation are quite different from those of θ oscillations *in vivo*.

5. SEPTO-HIPPOCAMPAL SLICES, SEPTO-HIPPOCAMPAL COCULTURES, AND HIPPOCAMPAL SLICE CULTURES

The evidence that in response to cholinergic stimulation acute hippocampal slices do not generate a pure θ but either γ or θ/γ nested rhythm and, sometimes, a frank epileptiform activity, suggested that they were not a good experimental model of the hippocampal θ oscillator and gave new strength to the old hypothesis that the presence of septo-hippocampal connections was essential for θ rhythm generation by the hippocampal oscillator. Therefore, efforts were produced to develop the combined septo-hippocampal preparations that we describe in the following paragraphs to test the hypothesis that in the presence of septal inputs the hippocampus could generate a θ rhythm *in vitro*.

5.1. Septo-hippocampal slices

The first of these preparations, the *septo-hippocampal slices*, was introduced in 1997 by Toth *et al*. (87). This complex preparation was designed to include in a single slice the hippocampus, the septum and the entire course of septo-hippocampal fibers. To this aim the brain was cut in a complex way by using multiple cutting angles to follows the direction of septo-hippocampal fibers so that they would not be severed during the slicing process. Because the septo-hippocampal fibers originate in the septum that is a midline structure and, then, bend to reach the hippocampus that has a more lateral location, three different cuts were made to prepare a block of brain tissue containing the intact septo-hippocampal fibers (Figure 3A). The first was a parasagittal cut made along the entire length of one of the two hemispheres, the second was a parasagittal cut made in the other hemisphere from the frontal lobe back to the point of origin of the fimbria whereas the last cut was made with a 120° angle orientation towards the posterior part of the temporal lobe to go parallel to the septo-hippocampal fibers. The angulated brain block that was obtained contained the intact septo-hippocampal fibers of one hemisphere. To obtain recordable brain slices this block was then flattened onto the stage of the vibratome and cut in slices. Using this approach slices containing the septum, the hippocampus and their synaptic connections were obtained (Figure 3A). Toth *et al* (1997) (87) used this preparation to investigate whether the septum could drive a θ activity in the hippocampus *in vitro*. To this aim they delivered a patterned electrical stimulation at a frequency of 5 Hz, hence in the θ range, to the septum and recorded the electrical response in the CA3 subfield of the hippocampus. They found that in response to the stimulation of the septum a rhythmic response with a θ frequency was elicited in CA3 consisting of out of phase oscillations of pyramidal cell discharge and inhibitory cell firing. The authors reported arguments to suggest that this response was dependent on the periodic disinhibition of pyramidal cells due to the periodic removal of their inhibition by interneurons. The periodic shutting down of interneurons could be explained by the activation of GABAergic septal fibers

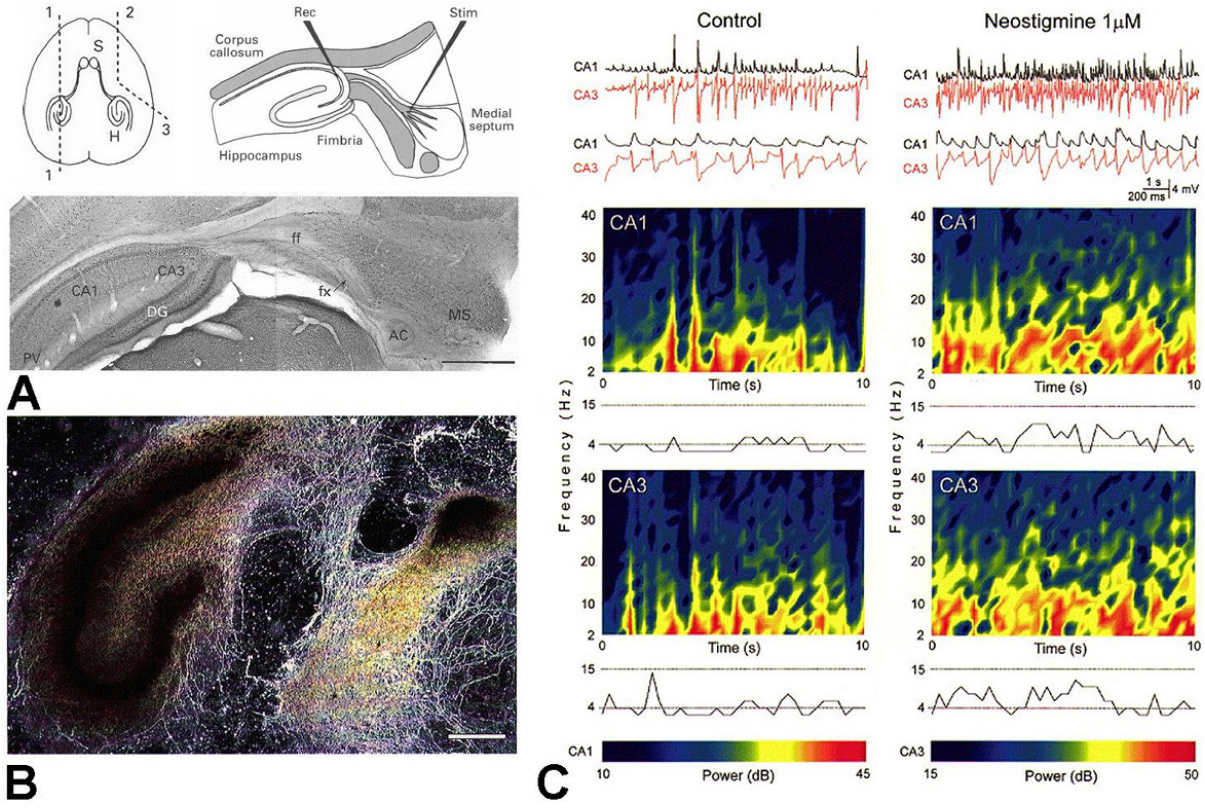

Figure 3. Septo-hippocampal slices and septo-hippocampal slice cocultures. A. Technique for obtaining connected acute septo-hippocampal slices. The schematic drawing on the top left side of the panel shows the three different cuts that are done to isolate the septum, the hippocampus and the septo-hippocampal fibers: 1. a parasagittal cut made along the entire length of one of the two hemispheres, 2. a parasagittal cut in the other hemisphere from the frontal lobe back to the point of origin of the fimbria and 3. a cut with 120° angle orientation towards the posterior part of the temporal lobe to go parallel to the septo-hippocampal fibers. A schematic drawing and a microphotograph of the slices obtained by cutting the block of brain tissue obtained with the aforementioned cuts are also shown in the top right side and in the bottom of the panel, respectively. B. Microphotograph of a septo-hippocampal slice coculture. The slice was stained for acethylcholinesterase and the image was obtained by dark field microscopy. Notice the cholinergic neurons in the septum (on the right) and their sprouted fibers reaching the hippocampus (on the left). C. Representative traces (on the top), spectrogram and frequency plot (on the bottom) of spontaneous (on the left) and neostigmine-induced (on the right) oscillations in septo-hippocampal slice cocultures. The pseudocolor palette reports the color code of oscillation power in dB. The images were reproduced with permission from (86, 89).

that make synapses with them in the hippocampus. Collectively, these experiments provided a mechanistic explanation of how the septum could make the hippocampus oscillate in the θ range by inducing a GABAergic disinhibition of hippocampal interneurons, but they did not conclusively excluded that the hippocampus could act as an oscillator by itself.

5.2. Septo-hippocampal slice cocultures and hippocampal slice cultures

Septo-hippocampal cocultures were developed by Gähwiler *et al*. (88, 89) to investigate *in vitro* the effect of septal cholinergic innervation on the hippocampus and its regulation by NGF. They showed that when septal and hippocampal organotypic slices from 7 week old rats were cocultured *in vitro* for 3–5 weeks *in vitro* septal fibers sprouted into the hippocampal slice and made acethylcholinesterase positive functional synapses with hippocampal neurons, especially in the CA3 and dentate area (88) (Figure 3B). Importantly, this process was greatly enhanced in the presence of NGF (89). The results obtained demonstrated that functional a septo-hippocampal synaptic circuitry could be reproduced *in vitro*. More than 10 years after the original description of this technique, Fischer *et al*. (1999) (90) showed that synchronous rhythmic discharges could be recorded in the absence of any pharmacological or electrical stimulation in CA3 and CA1 of these organotypic cocultures. Importantly, FFT analysis showed that this activity, which occurred as trains lasting several minutes and separated by periods of electrical inactivity, consisted of a virtually pure θ rhythm without any appreciable g component (90) (Figure 3C). Only 22% of the slices showed spontaneous θ-like activity but this percentage increased up to 64% in cocultures treated with NGF suggesting that cholinergic innervations from the septum was required for this kind of events. This hypothesis was further supported by the evidence that atropine caused its disappearance whereas in the presence of the acethylcholinesterase inhibitor eserine it was enhanced and took the form of continuous oscillations lasting up to one hour. Therefore, in

the presence of septal innervations hippocampal organotypic slices produced an acethylcholine (ACh)-dependent θ rhythm. These experiments did not prove, however, that the rhythm generator was in the septum that, instead, could have just played the role of a trigger for an intrinsic hippocampal oscillator. To investigate this point the effect of methacholine, a cholinergic agonist that should act directly on hippocampal neurons was tested. This drug at very low concentrations (5–20 nM) induced the appearance of θ oscillations whose single cell electrophysiological characteristics were similar to physiological θ rhythm because they mainly consisted of IPSPs and EPSPs. Epileptiform discharges were never observed at low methacholine concentrations and only appeared when this drug was used at micromolar concentrations. Not differently from what described in acute hippocampal slices also in *septo-hippocampal cocultures* θ-like activity probably originated in CA3. This was indicated by the evidence that in the CA3 it occurred 5–10 ms before than in CA1. The results of the experiments performed with methacholine showed that the hippocampus proper was able to independently generate θ rhythm independently from septal inputs. If this holds true then cholinergic agonists should be able to elicit θ oscillations in hippocampal organotypic slices even in the absence of septum. This was demonstrated in a paper from the same group published in 2002 (76). Interestingly, by the using of a specific procedure to remove θ component during signal analysis, it was shown that methacoline elicited oscillations in the CA3 region of organotypic hippocampal slices were not a pure θ rhythm but also included a prominent γ component that was predominantly observed in interneurons. Gamma (γ) oscillations were accompanied by action potential discharges in interneurons. This type of oscillations was recorded in organotypic hippocampal slices also upon exposure to ACh, physostigmine or kainic acid (91).

In summary, studies performed in organotypic slices lead to conclusions somehow similar to those obtained in acute hippocampal slices: the hippocampus proper contains an intrinsic θ/γ oscillator that is presumably located in the CA3 and is activated by cholinergic or glutamatergic stimuli. Nevertheless, it has been proposed that hippocampal slice cultures could represent a more physiological experimental model than acute hippocampal slices to study *in vitro* the activity of the intrinsic hippocampal oscillator. Indeed, it has been observed that, because of their thickness, unphysiological conditions such as high glucose concentration and a high partial oxygen tension have to be used to observe an oscillatory in activity acute brain slices (92). Conversely, ACh- and physostigmine-induced γ oscillations also occur in hippocampal slice culture when a physiological concentration of 5 mM glucose is used presumably because during culturing *in vitro* the slices flatten till a thickness of about 200 μM (vs 300–400 μM in acute slices) (91) (Figure 4). In addition, when brain slices are obtained the cutting procedure does induce a tissue damage that could affect network activity. This damage cannot be repaired in acute slices but it could be healed in organotypic slices as showed by the appearance of a robust fiber sprouting upon slice culturing *in vitro* (93, 94). However, during this repair process, aberrant recurrent synaptic connections may be formed by the fiber that sprout during culturing explaining why spontaneous epileptiform events are often observed in this hippocampal preparation. Interictal events are, indeed, observed in the majority of the slice cultured *in vitro* for 14 days and, after 21 days *in vitro*, ictal discharges occur in 50% of them (95). This is a serious concern for the use in studying hippocampal oscillations of slice cultures that, instead, are considered, a reliable experimental model *in vitro* of post-traumatic epilepsy (95).

6. EXPERIMENTAL STUDIES IN ACUTE SLICES IDENTIFY MICROSCOPIC OSCILLATORS IN THE HIPPOCAMPUS AND PROVIDE ELEMENTS TO FORMULATE MODELS OF THEIR FUNCTIONING

Despite the aforementioned concerns about their metabolic asset and in the face of their inability to generate spontaneous oscillations in the absence of pharmacological or electrical stimuli, acute hippocampal slices have been used and continue to be used as a preferential experimental model to study the electrophysiological basis of γ or θ neuronal oscillations. The analysis of the enormous amount of work on hippocampal oscillations that has been done with acute hippocampal slices during the last twenty years goes well beyond the aim of this review that is focused on experimental models. In this perspective we will only underline that experiments performed in acute slices provided not only the evidence for the existence of intrinsic hippocampal oscillators but also rational mechanistic explanations on how hippocampal tissue disconnected from the septum could autonomously generate oscillations. These studies showed that the hippocampus contains elementary microscopic oscillators that are primarily operated by interneurons (96). It was proposed that at least two different types of micro-oscillators could exist in the hippocampus (97). The first is purely GABAergic and involves the reciprocal inhibition of mutually interconnected interneurons; it accounts for the γ oscillations evoked by mGluR agonists or kainate -that are sensitive only to GABAergic and not to glutamatergic or cholinergic antagonists. The second model is GABAergic and glutamaterigic and is based on tightly coupled pyramidal cells and parvalbumin positive (PV+) interneurons (97, 99–102); it accounts for the oscillations elicited by cholinergic stimulation -that are blocked both by inhibitors of GABAergic and

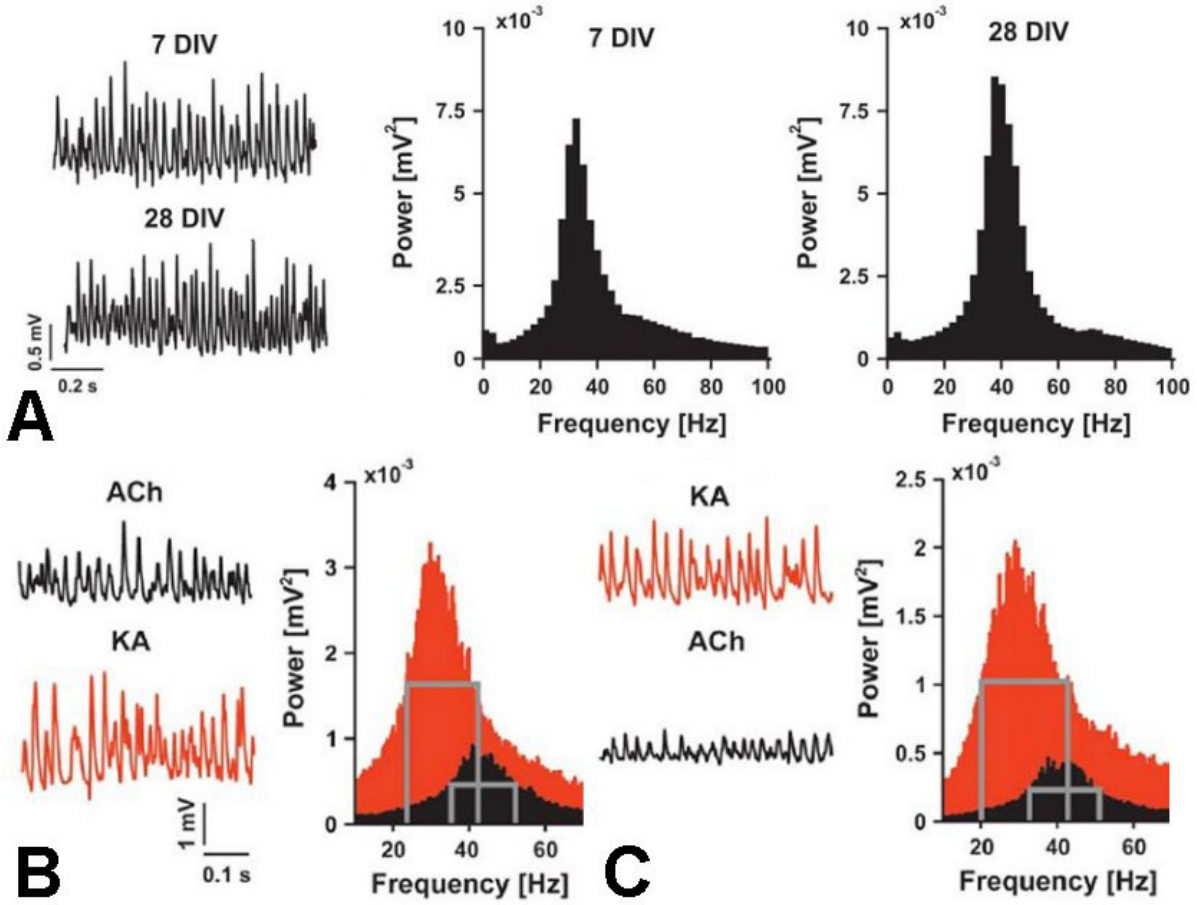

Figure 4. Acetylcholine and Kainate-induced oscillations in hippocampal slice cultures. A The left side of the panel shows two representative traces of γ oscillations elicited in hippocampal slice cultures by ACh (2 mM) plus physostigmine (400 nM) in 7 and 28 DIV hippocampal slice cultures, as indicated. On the right side of the panel the corresponding power spectra are reported. B and C. Representative traces and respective power spectra of γ oscillations (in red) induced by kainic acid (100 nM) given after (B) or before (C) exposure to ACh (2 mM) plus physostigmine (400 nM). The oscillations induced by ACh plus physostigmine are depicted in black. ACh and physostigmine were washed out before exposing the slice cultures to kainate in B and kainate was washed out before starting ACh-physostigmine perfusion in C. Reproduced with permission from (90).

of ionotropic glutamaterigic neurotransmission. The (PV+) family of interneurons includes three main types of cells, basket cells, axo-axonic cells (AACs), and bistratified cells (BiCs) (103). Originally the interest was totally focused on basket cells but the relevance of other members of the PV+ interneuron subfamily is now emerging from more recent studies. Basket cells, whose cell bodies are located close to the cell bodies of pyramidal neurons and whose terminations target the somatic region of pyramidal cells, are extremely well suited to be part of γ oscillator because they resonate in the γ range and are richly interconnected both with neighbor basket cells and pyramidal neurons (99, 104, 105). Therefore, the hippocampus proper contains microcircuits made by the simple functional coupling of interneurons with other interneurons or with pyramidal cells that may work as elementary oscillators. Modeling studies showed that although these oscillators mainly discharge in the γ range they may also oscillate in the θ range hence working as θ/γ oscillators. Classical studies provided evidence that this property is conferred to the elementary hippocampal oscillators by a second class of interneurons known as stratum oriens/lacunosum moleculare (OLM) interneurons. These interneurons are somatostatin-positive and have a different anatomical location respect to basket cells because their cell bodies are in the stratum oriens, where they receive inputs from pyramidal cells, and their axons terminate in stratum lacunosum moleculare where they establish synaptic contacts with the apical dendrites of pyramidal cells and where also the inputs from the enthorinal cortex are directed (106, 107). Because they are slow spiking, show a specific resonance in the θ range, spontaneously discharge at θ frequency *in vitro* (108) (but see also (109)) and are phase locked to θ oscillations *in vivo* (110) OML interneurons have been considered the key factor making the hippocampal oscillator discharge in the θ range (106). However, new evidence is questioning this concept. It has been shown, for instance, that the optogenetic stimulation of PV basket cells induced θ resonance in pyramidal neurons (110). Interestingly, different from other hippocampal interneurons, OLM cells arborize along the longitudinal, temporo-septal

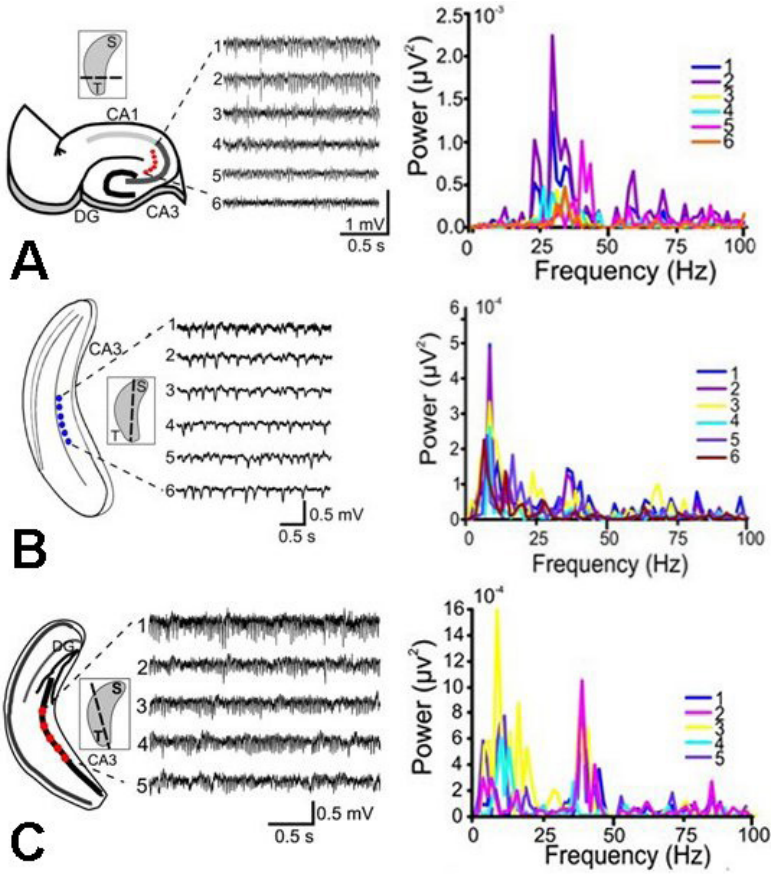

Figure 5. Difference in spectral composition of the oscillations induced by Kainate in transversal, longitudinal and coronal hippocampal slices. Electrode position in CA3 (on the right) representative traces (in the middle) and power spectrum (on the left) of kainate induced oscillations in transversal (A), longitudinal (B) and coronal (C) mouse hippocampal slices. Notice that γ frequency dominates in transversal slices, whereas the main frequency component in longitudinal slices is θ and a mixed θ/γ pattern is observed in coronal slices. Reproduced with permission from (112).

axis of the hippocampus (113). This observation could explain why when Gloveli *et al*. (113) compared the spectrograms of the oscillations elicited by kainate in transversal and in longitudinal slices of the hippocampus they found a γ pattern in the former and a θ pattern in the latter. Intriguingly, a mixed θ/γ pattern was observed in coronal slices that, because of the complex spatial orientation of the hippocampus, are not perfectly orthogonal as the transverse slices but show a certain degree of angulation respect to the septo-temporal axis (113) (Figure 5). Therefore, some longitudinal connectivity along the temporo-septal axis seems to be required to preserve the ability of generating θ oscillations in hippocampal slices. This observation could potentially explain part of the variability in the pattern of oscillatory discharges reported in classical hippocampal slice studies and the results obtained with the new model of the whole hippocampus *in vitro* that we will describe in the next section.

Evidence has been reported that pyramidal cells, interconnected through recurrent synaptic connections, may work as autonomous oscillators (114, 115). However, computational models of pyramidal cell-based oscillators are very unstable and highly susceptible to small perturbations (116). It has been recently demonstrated that the stability of the system can be highly enhanced by incorporating in the model a ICAN conductance that in real neurons is contributed for by TRPC channels (117, 118). Importantly, pyramidal cells are well suited to oscillate at a θ frequency because they preferentially resonate in this frequency range. This electrophysiological property can be explained considering that the repertoire of ion channels that pyramidal cells express in their dendrites including HCN and Kv7 channels confer them a specific adaptive inductance (119, 120). Recently Giovannini *et al*. (2017) (121) showed that a network of pyramidal cell CAN neurons may work as a θ/γ oscillator. More specifically, it can work in three different modes of operation: a slow asynchronous mode, a synchronized θ mode and a synchronized fast γ mode. When included in the model GABAergic inputs from interneurons enhance θ range synchronization.

The studies performed in acute hippocampal slices showed that hippocampal oscillations are

generated by elementary microscopic oscillators. Because both θ and γ oscillations involve much larger portions of the hippocampal slices this raises the question of how the activity of these elementary oscillators could propagate and eventually become synchronous. A possible explanation relies in the structural characteristics of basket cells that receive inputs from multiple neurons and send their outputs to many other neurons hence showing a high degree of divergence (104, 122). In the perspective of signal synchronization, it is also important that pyramidal cells respond synchronously to stimuli in the θ range along all their arborization no matter how far from signal origin (80). This unusual property can be explained considering that there is a gradient in the expression of HCN channels in dendrites of pyramidal cells (80).

In conclusion, acute hippocampal slices have been an extremely valuable tool to develop models of functioning of the intrinsic hippocampal oscillator. In the last few years the old, "sprightly" hippocampal slices have been living a "second youth" because they proved to be extremely well suited for the implementation of optogenetic studies. Indeed, in hippocampal slices from animals that express channelrhodopsins (ChR) in selected neuronal populations a precise focusing of the optogenetic stimulation to restricted anatomical subregions of the slice can be obtained by finely positioning the light source. Moreover by finely moving the light beam over the slice with the help of digital micro-mirror devices, differences in the responses of different regions of the same slice can be monitored in real time. Therefore, hippocampal slices confer an additional micro-anatomical selectivity to the selectivity of optogenetic stimulation due to cell specific expression of channelrhodopsin. The combination of electrophysiology and optogenetics in acute slices has been used as a powerful experimental approach to dissect the mechanisms of hippocampal oscillations (123). This is well exemplified in a paper by Butler *et al*. (124) who were successful in inducing θ/γ nested oscillations in CA1 by delivering sinusoidal light train stimulations with a frequency in the θ band to hippocampal slices of mice expressing ChR2 under the control of the CaMKII-promoter. These oscillations originated autonomously in CA1 as shown by cut lesion experiments and were dependent on $GABA_A$ and glutamatergic AMPA mechanisms. By finely moving the light along the different layers of CA1 Butler *et al*. (2015) (124) also showed that the power of the elicited γ rhythm was maximal when it was delivered to the pyramidal cell layer and minimal in the stratum lacunosum-moleculare suggesting a somatic origin of the oscillations. These experiments provided additional evidence for a pyramidal cell oscillator working as a combined θ/γ and not as a pure θ generator.

7. THE INTACT ISOLATED HIPPOCAMPUS

7.1. The intact isolated hippocampus: the experimental model

A breakthrough in the field occurred when the group of Sylvain Williams in Montreal developed a new experimental model for the study of the intrinsic hippocampal oscillators based on use of the isolated intact hippocampus. More specifically, they adapted for the study of hippocampal oscillations an old technique originally developed by Khalilov *et al*. (1998) (125) to perform electrophysiological recordings in the whole hippocampus *in vitro*. To briefly summarize this method, the whole brain is collected from P15–28 rats, the two hemispheres are rapidly separated after removing the dura mater and cutting away the frontal cortex and the cerebellum. The brainstem and thalamus are, then, separated from cortical mantle with the help of a spatula and the hippocampus is gently removed from the cortex. The entire procedure has to be performed quickly on ice. The isolated hippocampus is then let to rest in oxygenated ACSF for 45–180 minutes before being transferred into an ad hoc made recording chamber (Figure 6A). Using this approach all the intrinsic hippocampal connections are preserved whereas the connections with neighbor brain regions, including the septum, are lost. Therefore, the whole hippocampus preparation does represent a *bona fide* model to test the intrinsic physiological properties of the hippocampal circuit in isolation from any external synaptic input.

7.2. Theta oscillations develop spontaneously in the intact hippocampus

At the end of 2009 Goutagny *et al*. (126) showed that a spontaneous field activity does appear in the isolated hippocampus *in vitro* after 10–50 min of perfusion with artificial cerebrospinal fluid. The main frequency component of this spontaneous activity is between 3 and 10 Hz, hence in the θ range (Figure 6B and 6C). At the single cell level, the θ activity recorded from the whole hippocampus displays the main electrophysiological characteristics of a "true" θ rhythm. Indeed, patch clamp recordings showed that at resting membrane potential pyramidal cells only sparsely fired action potentials and their activity was essentially driven by IPSPs whereas interneurons frequently discharged action potentials and were driven by periodic EPSPs. As emphasized before, the beauty of the whole hippocampus preparation is that it offers the opportunity to study the physiology of hippocampus in its entirety made of lamellar units organized in a complex tridimensional structure. Taking advantage of this opportunity, the regional distribution of θ activity was studied by moving the recording electrodes along the temporal-septal axis. Using this approach θ activity was recorded from the CA1 region along the whole hippocampus with a high

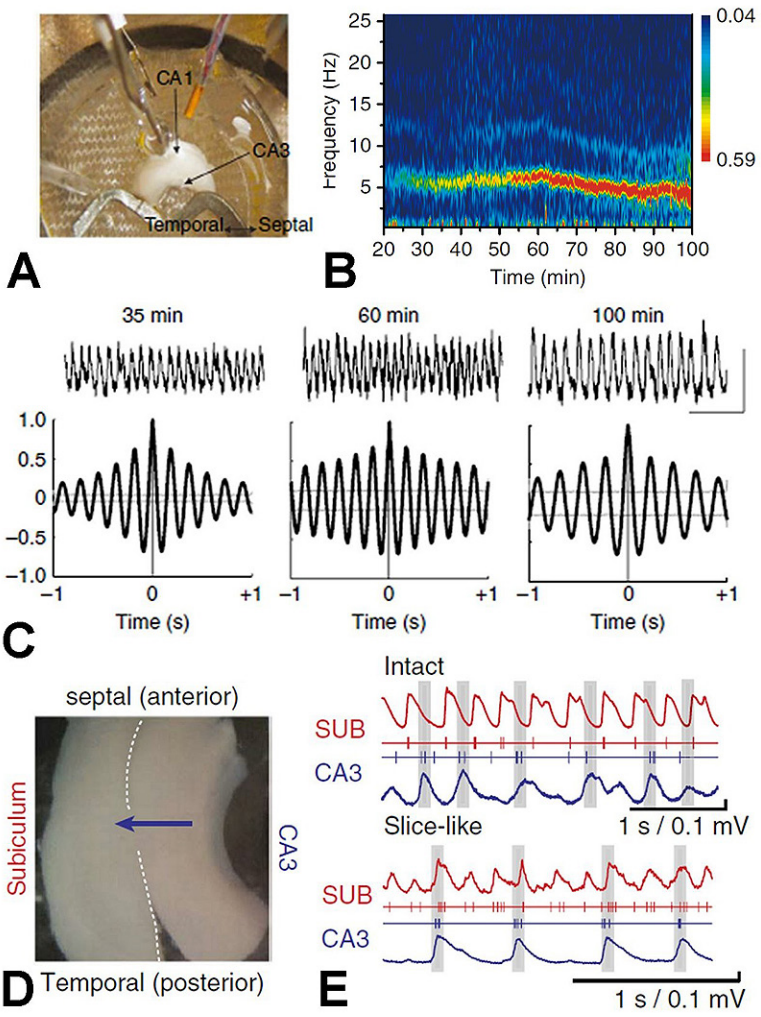

Figure 6. Theta oscillations in the intact isolated hippocampus. A. The intact isolated hippocampus in a slice recording chamber. B. Spectrogram of the spontaneous oscillations recorded from the CA1 region of the hippocampus. Notice that the main spectral component is in the θ range. C. Representative traces and respective autocorrelations of the spontaneous oscillations recorded in the CA1 35, 60 and 100 min after the beginning of the perfusion with ACSF. D. Microphotograph of a isolated hippocampus that has been lesioned to make its connections similar to those of a conventional acute hippocampal slice. As detailed in the text, two different longitudinal cuts were made the first going from the temporal to the septal pole and the other in the opposite direction to separate the CA3 from the subiculum. Only a small (0.5.-1 mm wide) portion of hippocampal tissue was left intact in between the proximal ends of these two cuts to act as a conduit connecting CA3 and subiculum. E. Representative traces of the spontaneous oscillations recorded in the subiculum and in CA3 of the intact isolated hippocampus and in the lesioned hippocampus as indicated. Notice that in the subiculum, spontaneous discharges occur earlier than in CA3 in the intact and later in the lesioned hippocampus. The images were reproduced with permission from (123, 125).

degree of coherence. Theta activity persisted also when the electrodes were moved along the transverse axis from the CA1 towards the CA3 region but, in this case, the degree of coherence was significantly lower. Importantly, θ rhythm was recorded from the CA1 region after its disconnection from CA3 either with knife cut or with the focal application of procaine. This finding was in agreement with many experimental reports showing the existence of θ wave generators in CA1 but clearly in contrast with the data obtained by Fischer *et al*. (1999) (90) in organotypic slices that located the θ rhythm generator in CA3.

The presence of a γ nested component in the spontaneous, not pharmacologically stimulated θ activity occurring in the whole intact hippocampus was investigated as well by Jackson *et al*. (127). The interesting finding of this study was that whereas the remaining regions of the whole intact hippocampus do not seem to generate a prominent γ oscillator both fast and slow γ activity is generated in the subiculum with mechanisms that involve fast GABAergic inhibition for fast γ and rhythmic excitation and inhibition for slow γ (127).

With the aim of understanding whether also in the intact hippocampus multiple θ oscillators do coexist as in acute slices, Jackson *et al*. (2014) (128) performed multi-electrode recordings to simultaneously measure local field potential in different regions of the isolated

hippocampus. Two different oscillators were identified, the first in distal CA1/Subiculum and the second in CA3. Frequency and power of θ oscillations were higher in the subicular than in the CA3 oscillator. Very unexpectedly, cross correlation analysis, spike phase modulation and time lags showed that the CA3 oscillator was actually driven by the subicular oscillator. This was confirmed at a sophisticated quantitative analysis performed by calculating the offset between CA3 and subiculum at which the spike phase modulation was stronger. These results contradicted the most consolidated dogma of hippocampal physiology, i.e. the unidirectional propagation along the trisynaptic circuit and from CA1 to subiculum. Contrary to this dogma, θ oscillations were found, indeed, to propagate in a reverse mode from subiculum back to CA3. The reverse propagation was further demonstrated by the evidence that the local application of procaine in the subiculum reduced θ activity in CA3 whereas when this drug was applied in the CA3 no effect was observed in the subiculum. GABAergic mechanisms were involved in the synchronization of CA3 by subiculum because it was abolished by the $GABA_A$ receptor antagonist gabazine in the presence of blockers of the glutamatergic neurotransmission (128). Moreover, optogenetic stimulation at θ frequency of parvalbumin interneurons in the subiculum induced synchronized activity in CA3. These findings could be explained in light of the documented existence of a subpopulation of interneurons retrogradely projecting from the CA1/subicular region to CA3 (129).

7.3. The intact isolated hippocampus highlights the relevance of the tridimensional structure of the hippocampus in the genesis of spontaneous oscillations

As we mentioned before, the tridimensional structure of the hippocampus, which is lost in the classical acute hippocampal slice preparation, is maintained in the intact isolated hippocampus *in vitro*. Therefore, the intact isolated hippocampus can be used to assess the relevance of the tridimensional multi-lamellar arrangement of the hippocampus in the genesis of spontaneous oscillations. This was made using a "lesional" approach. Specifically, two longitudinal cuts, the first going from the temporal to the septal pole and the other in the opposite direction, were made to separate the CA3 from the subiculum along the whole length of the hippocampus with the only exception of a small (0.5.-1 mm wide) portion that was left intact to act as a conduit connecting these two structures (Figure 6D). It was reasoned, indeed, that by this procedure the whole hippocampus could be made very similar to an acute hippocampal slice. When the synchronization between CA3 and subiculum was tested in the lesioned whole hippocampus it was found that spontaneous spiking was not led by the subiculum as in the intact whole hippocampus, but by CA3 exactly as in acute hippocampal slices (128) (Figure 6E). This finding indicated that the regulation of CA3 by subiculum through back-projecting interneurons is not laminar but requires the preservation of the tridimensional septo-temporal structure of the hippocampus. It was concluded that the concept of the unidirectional signal propagation from CA3 to CA1 and subiculum was probably consolidated because the majority of the classical studies on hippocampal physiology were performed with laminar acute slices lacking of longitudinal connections (128). Therefore, the replacement of acute hippocampal slices with the intact whole hippocampus as an experimental model to study the intrinsic hippocampal oscillators does imply an important change in perspective from a two-dimensional, purely laminar, to a more integrated tridimensional multi-lamellar interpretation of their physiology. This fits well with the ongoing significant revision of the old Andersen (1969, 1971) lamellar theory (130). Evidence has been accumulated, indeed, of a higher than expected longitudinal divergence of hippocampal and EC neuronal outputs along the septo-temporal axis of the hippocampus (130). Intriguingly, studies performed *in vivo* with the help of microarrays of tetrodes implanted in the stratum oriens of freely behaving rats showed that θ rhythm is made by waves traveling along septo-temporal axis of the hippocampus and not, as traditionally believed, the result of synchronized discharge of the whole hippocampus (131). This finding suggests that θ rhythm does not originate in independently activated "lamellar" rhythm generators but is a wave that propagates longitudinally along the hippocampus hence progressively recruiting consecutive lamellar oscillators. Arguments have been also reported that the monotonical phase shifting of θ waves that is observed going from the septal to the temporal pole of the hippocampus can be explained by assuming that a network of weakly connected oscillators in the hippocampus and in the entorhinal cortex produce traveling θ waves (132). An important point that remains to be established is whether the same also occurs with the isolated hippocampus *in vitro*. However, as we reported already in section 6 it is intriguing that by comparing the oscillatory pattern of longitudinal and transversal hippocampal slices Gloveli *et al*. (113) proposed that θ rhythm generation could require some form of multi-lamellar integration along the septo-temporal axis.

7.4. The intact isolated hippocampus is well suited for optogenetic investigations

The intact hippocampus is well suited for optogenetics that can be used to assess the involvement of specific neuronal populations in the activity of the intact intrinsic hippocampal oscillator by selectively modulating with light their activity in mice specifically expressing engineered forms of rhodopsin. By this approach the mechanistic models of functioning of the intrinsic hippocampal

micro-oscillators that have been formulated on the basis of data obtained in acute slices (see section 6) can be tested in the more physiological conditions of the intact hippocampus. While we are just at the beginning of this new story, it is likely that it will yield some surprise and rewrite some pages of the physiology of the intrinsic hippocampal oscillators. For instance, recently, Amilhon *et al*. (2015) (133) revisited the theory that the generation of θ oscillations was dependent on the resonant properties of a subset of somatostatin-positive O-LM interneurons (see section 6). To this aim they tested the effect of light stimulation in the isolated hippocampus of mice expressing the stimulating opsin ChETA or the silencing opsin ArchT in either parvalbumin or in the O-LM interneurons. The results of this study contradicted the hypothesis of a dominant role of O-LM cells in θ rhythm generation because θ oscillations were only marginally affected either by the optogenetic stimulation or inhibition of these neurons. Conversely, dramatic effects on spontaneous θ were observed when the activity of parvalbumin neurons was optogenetically modulated. These findings showed that, contrary to what expected, parvalbumin interneurons control the intrinsic hippocampal θ oscillator with a negligible contribution of somatostatin positive O-LM cells. Instead, because they preferentially make synapses with the distal dendrites of pyramidal cells, where also the fibers of the temporo-ammonic pathway terminate, O-LM interneurons could have a preferential role in regulating EC-driven θ rhythm. This hypothesis was comfirmed by additional experiments in combined EC-hippocampal slices from mice expressing ChETA in the temporo-ammonic fibers and ArchT in O-LM interneurons that showed that the θ rhythm elicited by patterned optical stimulation of the temporo-ammonic fibers was significantly attenuated by the simultaneous silencing of O-LM interneuron activity (133). These new findings have been incorporated in new models of hippocampal θ micro-oscillators (134).

In conclusion, the mechanistic basis of θ oscillations seems to be different in acute slices and in the intact hippocampus. The specific reasons of these differences are currently unknown as it remains to be established whether parvalbumin neurons also have a primary role in controlling the generation of θ rhythm in the hippocampus of living animals.

7.5. Combined preparation of the intact hippocampus and septum give new opportunity to investigate septal regulation of θ rhythm generation

To conclude this section on the isolated whole hippocampus we would like to mention that by a slightly modification of this technique combined preparations of the hippocampus, the septum and their connections can be obtained. We will not review in detail the studies performed with this combined septo-hippocampal preparation because they are more related to the understanding of septal-driven θ rhythm than to the analysis of the intrinsic hippocampal oscillator. However it is important to remind here that convincing evidence of the relevance of glutamaterigic mechanisms in the genesis of non-cholinergic septal-driven θ rhythm was obtained using this preparation and the optogenetic activation of septal glutamatergic neurons (135).

8. CONCLUSIONS

We reviewed the history of the experimental work that led to the idea that the hippocampus could be an autonomous θ oscillator independently from septal inputs. This history parallels that of the development of more and more complex experimental models to study hippocampal electrophysiology *in vitro*. Intriguingly the paradigm itself of the hippocampal oscillator has been changing with these experimental models. Originally it was conceived as something that could be dependent only on the integrity of the laminar structure of hippocampal slices a few hundred micrometers thick whereas nowadays it is believed to be the resultant of a higher degree of functional integration along the septo-temporal axis that can be reproduced only in the whole isolated hippocampus. Many questions remain still open. For instance it is unclear how physiological the spontaneous θ observed in the isolated hippocampus *in vitro* can be considered. Also, it is still to be established whether and how the intrinsic hippocampal oscillator could autonomously take part to the physiological functions of the brain. The study of the intrinsic hippocampal θ oscillator has not only a theoretical interest in the understanding the neuronal mechanisms responsible for rhythm generation and propagation but it could also have practical implications in identifying strategies to (re)generate the θ/γ rhythm even in the presence of septal degeneration and cholinergic dysfunction as it happens in Alzheimer's disease and in other clinical conditions associated with cognitive deficits (136). Indeed, a disruption of the θ/g rhythm is considered to potentially have a pathogenetic role in a wide range of neurological and psychiatric diseases including, for instance schizophrenia (137) and Alzheimer's disease (138, 139).

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Abbreviations: ACh, acetylcholine; CCh, carbachol; DG, dentate gyrus; EC, entorhinal cortex; EPSP: excitatory postsynaptic potential; FFT, Fast Fourier transform; IPSP: inhibitory postsynaptic potential; LTP, Long Term Potentiation; mGluR, metabotropic glutamate receptors; OLM, stratum oriens/lacunosum molecular; PV, parvalbumin.

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