# Fast network oscillations in the hippocampus

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N. Maier[1] · A. Draguhn[2] · D. Schmitz[1] · M. Both[2]
[1] Neurowissenschaftliches Forschungszentrum der Charité, Charité-Universitätsmedizin Berlin, Berlin
[2] Institut für Physiologie und Pathophysiologie, Medizinische Fakultät der Universität Heidelberg, Heidelberg

# Fast network oscillations in the hippocampus

## Phenomena, mechanisms and open questions at the interface of cellular and systemic neurosciences

### Hippocampal structure and activity patterns – a short introduction

The hippocampus appears as a bent, C-shaped structure and is embedded in the mammalian temporal lobe. Afferent inputs emerge primarily from neighboring cortical areas, mainly from the entorhinal cortex, but also from the septum, the amygdala, the contralateral hippocampus and other brain regions. In turn, the main efferent fibers target the entorhinal cortex and septum, finally projecting to the frontal cortex. A prominent concept of intrahippocampal connections is the "trisynaptic loop" (**Fig. 1a**), which originates as a glutamatergic fiber tract (*perforant pathway*) from layer II of the entorhinal cortex and terminates in the dentate gyrus. Granule cell axons (*mossy fibers*) synapse onto area CA3 whose output (*Schaffer collateral*) projects to area CA1. Principal neurons from this region send fibers back to the entorhinal cortex (in part with an additional synapse in the subiculum), thereby closing the "loop". The synapses of these fiber tracts have the potential to undergo strong associative plasticity, both towards potentiation and depression. Based on these features the hippocampal trisynaptic loop has virtually become a metaphor for plasticity and learning. However, more recent research emphasizes that the architecture of the hippocampus is more complex than inferred from the above concept: for instance, area CA1 receives direct "temporoammonic" input from layer III of the entorhinal cortex and area CA2, a small region that is anatomically not easily separable from neighboring fields, receives strong synaptic input from CA3 and relays it to CA1 [1].

Within this network of the hippocampus, several sets of prominent population oscillations are generated, which often appear coordinated among the different subregions (**Fig. 1b, c**). In rodents, during phases of motor activity (e.g., during foraging), theta oscillations are observed at frequencies between ~5 and 10 Hz. Theta waves are superimposed by more rapid gamma oscillations whose frequency band is commonly limited to 30 and 100 Hz. However, phases of rest and slow wave sleep are dominated by different patterns of activity; they represent short network bursts of strong synaptic activation that travel along the classic efferent pathway from CA3 via CA1 and finally to the entorhinal cortex. These "sharp waves" are associated with brief trains (~100 ms) of high-frequency oscillations at about 200 Hz that were termed "ripples" by O'Keefe and Nadel [2]. In addition to these rhythms, very slow waves can be observed in the hippocampus (slow oscillations ≤1 Hz; [3]), as well as several coordinated patterns of activity during the early postnatal period, e.g., early network oscillations (ENOs; [4]). So-called fast ripples, very fast oscillations above 300 Hz, are prominent in pathologically altered networks in epileptic animals and humans [5, 6].

At the cellular level, commonly excitatory projection neurons are distinguished from (mostly) inhibitory interneurons. The first group includes the numerous small granule cells of the *area dentata* as well as the pyramidal neurons of the *cornu ammonis* regions and the subiculum. A very prominent feature of the hippocampus is its strict three-layered organization: the principal cells' somata form densely packed layers, i.e., the granuale cell layer and the *stratum pyramidale* that are clearly visible in the slice preparation even without staining. Strong apical dendrites form the *strata moleculare* and *radiatum/lacunosum-moleculare* where they receive thousands of excitatory inputs that terminate on specific parts of the dendrites, depending on their region of origin. Finally, on the opposite side of the cell body layer, the *stratum oriens* is found in the CA regions that also receives specific input on the basal dendrites, e.g. commissural fibers. The corresponding basal part of the area dentata is called *hilus* where in rodents, different from humans, no basal dendrites are located, but multiple types of inhibitory interneurons and excitatory mossy cells. Inhibitory neurons form about 5–10% of the entire neuronal

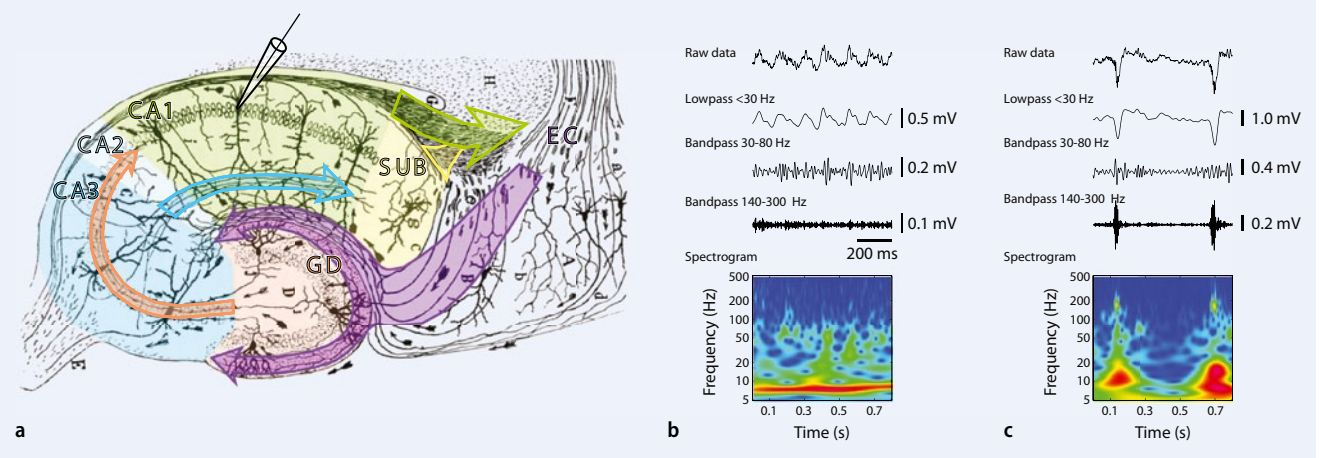


**Fig. 1** ▲ Structure and typical electrical activity of the hippocampus (schematic, modified, after Ramón y Cajal, 1911). **a** Subregions and most important projections in the hippocampal formation. The entorhinal cortex (*EC*) innervates the dentate gyrus (*GD*) via the perforant path (*tractus perforans*). This region sends the mossy fibers to the *cornu ammonis* subfield 3 (*CA3*) whose main output pathway (the Schaffer collateral) innervates the *CA1* region. Output fibers of this region terminate in the entorhinal cortex (partly via an additional synapse in the subiculum). **b** Theta/gamma activity in a recording from area *CA1* during exploration. The recording was conducted in the dorsal hippocampus of a mouse. *Top trace* Raw data. *Middle traces* Digitally filtered data of characteristic frequency bands. The lowpass-filtered trace highlights the theta component, while the bandpass-filtered signal isolates gamma oscillations. *Bottom* Spectral analysis of the raw data trace in **a**. Signal strength ("power") of frequency components at a certain time point is color-coded; higher power values are represented in *reddish*, lower values in *blue colors*. Notice the dominating signal contribution at theta frequency (at ~8 Hz) and at gamma frequency at ~30–80 Hz. **c** Sharp wave–ripple complexes during slow wave sleep. *Sharp waves* appear as negative voltage spikes in the original recording and in the lowpass-filtered version, respectively. The high-frequency *ripples*, superimposed on sharp waves, become clearly visible upon bandpass filtering at a higher frequency band (140–300 Hz). In the spectrogram, both components of SPW-R are displayed at ~10 Hz (*red, sharp wave*) and ~200 Hz (*yellow/reddish, ripples*). (The authors thank J. Brankačk and G. Köhr for providing the in vivo data examples)

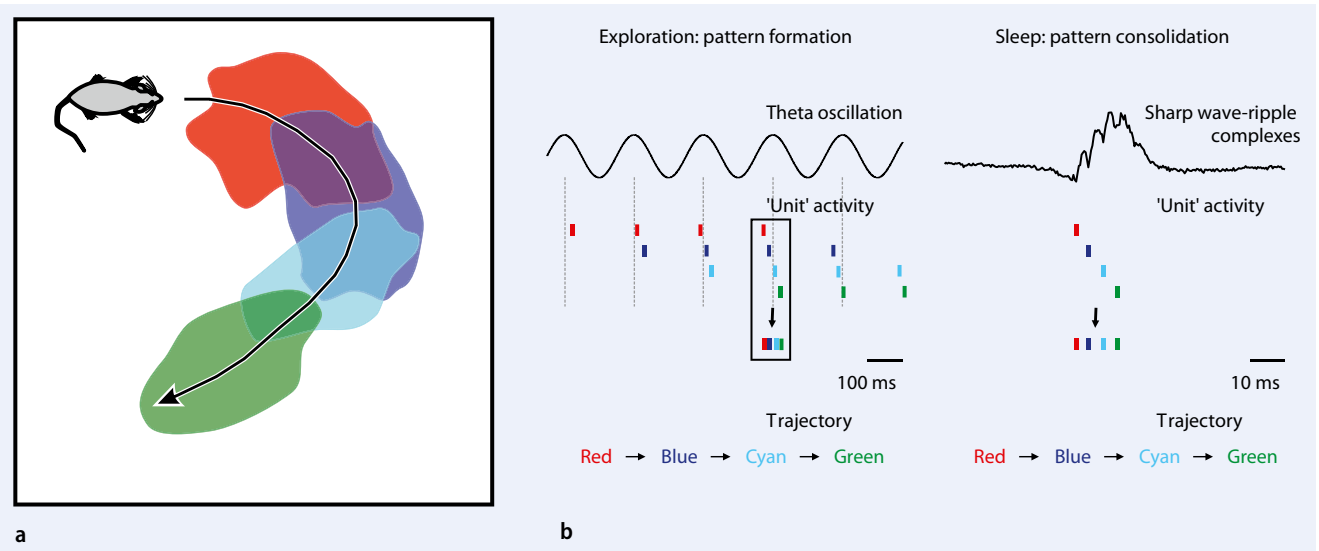


**Fig. 2** ▲ Connection between hippocampal single-cell activity and spatial memory. **a** Schematic representation of four overlapping place fields (*colored*) and trajectory of a mouse (*arrow*). **b** Single-cell activity ("unit"', schematic) of four overlapping place cells assigned to the four place fields; each *colored bar* indicates the discharge of one unit. During exploration (*left*) one place cell sequence is formed due to phase precession occurring during theta/gamma oscillations. In a subsequent period of slow wave sleep the reactivation of the same sequence takes place during sharp wave–ripple activity (*right*). Notice the compression of the duration of the replayed sequence (~10 ms during SPW-R compared with ~100 ms during theta)

population. Due to their structural, molecular and functional heterogeneity they have been subject to intense research in recent years [7]. At present, in the CA1 region alone 20 different subtypes of interneurons have been differentiated. Based on characteristics of dendrite morphology and axonal projection patterns, it is likely that specific interneuron types "control" specific excitatory inputs by synaptic inhibition. An additional and more recent finding is the active and very specific participation of specialized interneurons in certain network patterns. Even more, these neurons seem to participate in shaping these patterns by rhythmic inhibition, and disinhibition of postsynatic pyramidal cells. In this context, the widely collateralized axon trees with their numerous GABAeric terminals play an important role, along with the often large conductances of postsynaptic inhibitory potentials (see below).

## How network oscillations can contribute to the formation of spatial memory

As mentioned above, theta and gamma oscillations are prominent during exploration of an environment, for instance during foraging in rodents. As a result of rhythmic fluctuations of the membrane potential, temporal windows are imposed on pyramidal neurons, in which the probability of action potential (AP) "firing" is high (in response to depolarization) or low (during hyperpolarization). Hence, action potentials become synchronized. However, not random cells, but very specific principal neurons ("place cells") discharge when a rat or mouse stays at a specific location in its environment (**Fig. 2**). This prominent finding by John O'Keefe et al. [8] has shaped the view on hippocampus as a "spatial map" and has been often verified since its discovery.

Stimulated by Györgyi Buzsáki, O'Keefe investigated about 20 years later whether there was a dependency between the organized activity patterns during theta oscillations and place cell firing. And indeed, these cells fire tightly coupled with the theta cycle: preferred AP phase occurs late upon entry into the place field of the respective neuron and shifts to earlier phases of the oscillation when passing the place field [9]. As place fields overlap, this "phase precession" results in sequences of discharge patterns—cells that fire early in these sequences belong to more distant place fields. In principle, with precise knowledge of the place fields, the animal's trajectory can be reconstructed from the firing pattern of different neurons—the principle of place theory and phase precession give rise to the representation of (egocentric) space.

Such "internal reconstruction" from AP patterns seems to take place during the formation of memory. Matthew Wilson, and Bruce McNaughton conducted recordings of action potentials in hippocampi of freely moving rats and discovered that neurons that were co-active during spatial exploration also co-fired during later sleep [10]. Hence, co-activity induced the enduring coupling of cells. Later it was shown by Albert Lee and Matthew Wilson that indeed entire sequences of place cell spikes were repeated ("sequence replay"), which happens during slow wave sleep, now in association with the much faster ripple oscillations [11]. This experimental finding confirmed the two-stage model of memory trace formation, proposed by Buzsáki [12]: during the acquisition of information place cells are activated in theta/gamma periods that have also been shown to foster synaptic plasticity [13]. Defined place-cell sequences are formed that are re-activated in following periods of rest and slow wave sleep during sharp wave–ripple complexes (SPW-R). In association with the sharp waves, these activity patterns propagate to the neocortex, where they as well induce plasticity and generate long-term memory engrams. In agreement with this concept of long-term storage in the neocortex, Siapas and Wilson [14] showed activation of frontal networks (*spindles*) that followed hippocampal ripples.

The two-stage model is also in line with the observation that hippocampal lesions typically result in severely impaired acquisition of novel information compared with the recall of more remotely encoded memories. In humans, as for spatial memory, this is true for the declarative memory (which seems to be mechanistically closely related to the first), but also for autobiographic facts (episodic memory). This has been impressively demonstrated on patient H. M. by Brenda Milner et al. [15]. H. M. had undergone extensive epilepsy surgery where both hippocampi and neighboring brain tissue had been removed. Recently, the technical advance of experimental neurophysiology has enabled the selective suppression of ripple activity in behaving animals. Indeed, this interception specifically impaired the long-term storage of spatial memories, providing not only a correlative but an increasingly causal link between ripples and memory consolida-

## Abstract

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### Fast network oscillations in the hippocampus. Phenomena, mechanisms and open questions at the interface of cellular and systemic neurosciences

**Abstract**
Neuronal networks often express coherent oscillatory activity. These rhythms can provide a temporal reference for the activity of single neurons and allow the formation of spatiotemporal activity patterns with a defined phase relationship of action potentials. In a single brain nucleus, oscillations at different frequencies might be simultaneously generated, but isolated rhythms might also be characteristic for specific functional brain states. During the last two decades the mammalian hippocampus has become an important model system for the study of neuronal network oscillations. In this brain area, cellular mechanisms underlying neuronal synchronization have been described, but also models were developed to explain the contribution of oscillations in encoding, consolidation, and recall of memories. Neuronal rhythmic activities provide an important field of analysis bringing together cellular mechanisms and systemic functions of the brain. Here, we use a particularly fast type of neuronal oscillation, hippocampal "ripples", as an example to outline current knowledge and open questions related with this research field.

**Keywords**
Neuronal network oscillation · Learning and memory · Hippocampus · Cellular mechanisms · Ripples

tion [16, 17, 18]. Equally influential studies by the Norwegian group of May-Britt and Edvard Moser [19] have revealed how the different architectures of hippocampal sub-networks support the processing of different aspects of spatial representation. For instance, the high number of granule cells with their relatively negative resting membrane potential and sparse firing rate enables the representation of small variations of the environment by means of different population patterns. Their axons, the mossy fibers, strongly converge onto a much smaller number of CA3 pyramidal cells (ratio of 1:5) that are recurrently connected in an excitatory and auto-associative network.

Thus, from different patterns represented in the dentate gyrus again identical, but more stereotypical patterns are generated in CA3. This differential processing of patterns might underlie the cognitive ability to realize changes in spatial contexts ("… a new painting is hanging in your room!"), while identifying their similarity ("But the room is still your living room…"). Taken together, at the level of hippocampal networks, the research on spatial memory has already provided tantalizing insights in correlations and mechanisms.

## Extensions and open questions

The above-mentioned, very simplified scenario has been subject to several modifications and evokes questions that are currently under intensive investigation. In the following sections, we will briefly summarize the current problems, before focusing on a specific topic that is central to us working in cellular neuroscience: how is neuronal activity synchronized during ripples and by what mechanisms are selected cells preferentially activated in this high-frequency regime?

First, we should mention several findings that extend the two-stage model:

I. Recent work has demonstrated that place cell sequences are not only reactivated but also replayed in reverse order after passage of a track [20]. This result triggers the question whether sequences are indeed that tightly imprinted into the network as previously assumed. In addition, it should also not be ignored that *replay* is a statistical phenomenon where sequences of activated neurons are reactivated above chance level, without being repeated truly stereotypically.
II. The trisynaptic loop model might overstate the "readout" function from the hippocampus to the neocortex. In that respect, results of multielectrode recordings demonstrate that ripples can be also triggered at certain cortical phase transitions, in particular in the entorhinal cortex [21, 22, 23]. As the hippocampus modulates cortical activity it is apparently likewise influenced by neocortex—hence the flow of information is not unidirectional.
III. A similarly spectacular finding was that ripple-associated reactivation of place cells can represent also place sequences in an environment that has never been explored previously by the investigated rats [24]. It remains unclear whether this 'preplay' represents possible trajectories, whether it is generated merely by chance as a pattern of particularly active neurons, or, finally, whether it represents a necessary transition phase for the completion of sequences.
IV. The biggest mystery—and, actually, a provocation to all researchers working on memory—is the complete ignorance of mechanisms that underlie the long-term storage of spatial memory. Do they really involve hippocampal readout of sequences into the neocortex? Where actually is the engram to be found? After all, experimentally supported models exist to explain the possible contribution of hippocampal synaptic plasticity in the formation of spatial representations in neuronal network oscillations. However, to date these processes are not accessible to any direct investigation. The mechanisms of memory formation in the neocortex, downstream to hippocampal networks, are entirely unresolved.

## Cellular mechanisms of network oscillations

Before we turn to mechanisms underlying the ripple oscillation in particular, we will summarize the most important principles that potentially contribute to coherent oscillations in neuronal networks. These mechanisms should explain the generation of subthreshold membrane potential oscillations in all, or in the majority of cells in the network, i.e., rhythmic de- and hyperpolarizations that do not regularly reach the firing threshold. In addition, following the principle of sparse firing, some neurons should still fire—this is observed in multielectrode recordings and it complies with predictions from information theory (when all cells are silent, or all cells are "chatting" simultaneously, transmission of information is minimal, actually not that different from what often happens in the lecture hall or in the family…). When a neuron is excited above the firing threshold its action potential should be coupled to a particular time point of the oscillation cycle. In addition, a stable balance of inhibition and excitation should always be maintained. In our case, we finally demand the few firing neurons to be those that have been previously activated and plastically modified during spatial exploration. With these requirements fulfilled, the replay phenomenon and potentially memory consolidation might be explained. Some of the aforementioned topics and the latter in particular are still unresolved. However, there is much knowledge on neuronal principles to provide relevant partial responses. The most important principles underlying neuronal network oscillations are described below.

### Intrinsic neuronal properties

Similarly heterogeneous as the diversity of neuronal subtypes are their properties of discharge behavior. From sparse single and fast action potentials (e.g., in granule cells of the dentate gyrus) or short bursts in pyramidal neurons, this diversity extends to complex dendritic and axonal spikes (in Purkinje cells and thalamocortical projection neurons) or endogenous pacemaker properties in neuroendocrine cells. With respect to ripples two prominent patterns are of particular importance: perisomatically or "dendritically-targeting inhibitory" interneurons express high-frequency trains of APs that fall in the ripple frequency band of about

200 Hz, while pyramidal neurons mostly fire once, if at all [25, 26, 27, 28].

## Local inhibition

Research on (mostly) inhibitory interneurons in neuronal networks has developed to an independent chapter in modern neuroscience. Single interneuron types contribute very specifically to particular patterns of activity in neuronal networks. The earliest models to explain fast network oscillations by neuronal inhibition were based on simple feedback loops: A population of active pyramidal cells excites interneurons via local axon collaterals, which in turn inhibit the principal neurons ("feedback inhibition"). Determined by the decay time constant of the inhibitory postsynaptic potential (IPSP) the discharge probability of the pyramidal neuron increases again, which initiates a new cycle. By this, an entire network can be entrained in rhythmic oscillations, provided the interneurons efficiently inhibit an adequate number of excitatory cells [29, 30, 31]. The frequency of this type of network oscillation roughly correlates with the inverse decay time constant of the IPSP (assuming relatively short durations of excitation and local propagation times). From these initial models that were particularly seminal in understanding gamma oscillations, more differentiated models on several specific networks have been derived during the last two decades. The concept of synaptic inhibition representing a mere "braking action" (supporting the balance of excitation and inhibition) has evolved to a central organizational principle of coordinated network activity [32]. It now seems that the "balancing" function is provided by tonic inhibition to large extent, i.e., by a "background" local accumulation of GABA [33], while phasic (transient) inhibition seems to supply the temporal coordination of activity patterns [34, 35, 36]. A neuroscience topic currently under heavy investigation is the research on the remarkable heterogeneity of inhibitory interneurons. Interneuron subtypes are differentially embedded into local networks and are also differentially sensitive to neuromodulators. Due to these differences, these cells participate very selectively in various activity patterns that in turn are shaped by interneuron action. For area CA1 of the hippocampus this was exemplified in the work of Thomas Klausberger and Peter Somogyi [7]. Different inhibitory synapses partially express differing pre- and postsynaptic receptors. These properties can be used in elegant genetic and pharmacological experiments to selectively manipulate the network. During ripples, especially parvalbumin expressing, fast-discharging basket cells seem to be involved, whose strongly diverging axons form an effective perisomatic inhibition of large numbers of pyramidal cells. In addition, "bistratified" interneurons that target proximal compartments of basal and apical pyramidal cell dendrites also seem to be important [25, 26, 28].

## Synaptic excitation

Naturally, excitatory (glutamatergic) connections equally importantly contribute to network activity. Of note, beside the afferents from neighboring structures (e.g., the Schaffer collateral input from CA3 onto CA1) local excitatory connections also exist. We have already mentioned feedback inhibition by local inhibitory interneurons. Similarly, local excitatory feedback connections are also present in particular in area CA3. These connections can produce dynamical instabilities that can result in the synchronous discharge of numerous neurons, a mechanism that is likely to contribute to the initiation of sharp wave–ripple complexes in the CA3 region. It is feasible that the strong recurrent excitation within CA3 is the reason that this region is especially prone to the generation of epileptiform activity.Of interest, the hilus of the dentate gyrus (the initiation zone of the CA3 pyramidal cell layer) contains *excitatory* interneurons (mossy cells). These cells probably play an important role in organizing population activity of the area dentata by providing positive feedback during certain network patterns. Finally, peculiarities in mechanisms of excitation during pre- and early postnatal development should be mentioned. Since the 1980s in meanwhile classic studies, Ben-Ari and coworkers (e.g., [37]) showed that the high chloride concentration of immature neurons is the origin of GABAergic depolarizing responses rendering GABA an excitatory neurotransmitter. Although these results are presently under debate, it seems clear that the delayed maturation of the glutamatergic system during ontogenesis leads to compensatory actions by GABA or other mechanisms. Besides depolarizing GABA responses, the increased electrical coupling of neurons might be involved.

## Electrical coupling

The concept of direct neuronal coupling has a long and checkered history, extending from the dispute between reticularists (Golgi) and supporters of the neuron doctrine (Cajal) to the pioneers of modern synaptology (Eccles, Katz). As is known, the view has been gained ground that neurons are independent cellular entities enclosed by membranes and are communicating primarily by chemical neurotransmitters. Until recently, for historical reasons, the investigation of indeed existing direct electrical contacts was restricted to certain specific research topics, like early postnatal development or brain nuclei as the inferior olive. Methodological complications have contributed, as well: being inter-cellular channels, gap junctions are very poorly accessible to pharmacological manipulations and direct proof of electrical coupling usually requires the paired simultaneous recording from two neurons [38]. Nonetheless, over the last few years we have experienced a renaissance of electrical coupling. The direct connection of interneurons has been convincingly demonstrated in the meantime, also for the hippocampus. It is clear now that electrical coupling contributes to the synchronization of these cells. Interestingly, cells of the same subtype are selectively coupled, which contributes to coordination of activity among these cells, and, as a result their action on the population of downstream pyramidal cells will be boosted, especially with respect to temporal patterns of oscillations. Coupling of excitatory neurons in the hippocampus and neocortex during ontogenesis remains undisputed. However, for mature tissue, the extent, localization, and functional significance of gap junctions have not been finally resolved. As demonstrated below,

ripples are a good example for these uncertainties.

## Interdependence of structure and functions in networks

Beside the functional properties briefly summarized above, the structure of neuronal networks inherently provides essential conditions for their activity. This was, in principle, already acknowledged by Ramón y Cajal and characterized in his specific description of neuronal subtypes and their connections within the various brain nuclei. Today, this concept of functionally oriented anatomy is still highly topical and can now be complemented with direct measurements of electrical functions. Novel techniques, like juxtacellular recordings, high-resolution cellular recordings in vivo, multi-electrode recordings in freely moving animals and "life-imaging" significantly expand our opportunities to analyze cellular functions in native neuronal networks. In addition, molecular biological techniques to label, and increasingly, to manipulate defined neurons will enable for the first time highly selective interventions and foster the analysis of causal mechanisms. With the example of hippocampal ripples, however, it will become clear that the understanding of mechanisms of complex network dynamics will not fall into our laps, in spite of all technical improvements.

# Cellular mechanisms of 200 Hz ripples

As mentioned above, during slow wave sleep or immobility typical patterns of activity occur, namely sharp wave–ripple complexes (SPW-R). The underlying functional condition of "large irregular activity" had been already described by Cornelius Vanderwolf [39]. The explicit demonstration of ripples and sharp waves was later published by John O'Keefe [2]. In particular, sharp waves were then studied in detail by Györgyi Buzsáki, who systematically described in rats the behavioral physiology of sharp waves during different behavioral states, some aspects of their pharmacology, and the "laminar profile", i.e., the occurrence of these events in different somato-dendritic layers [57]. Together with his collaborators, among others, Jozsef Csiscvari, Thomas Klausberger, Aarne Ylinen, and Anatol Bragin, Buzsáki characterized in detail the cellular activity patterns during SPW-R [53, 54, 55, 56]. These complexes emerge within CA3, and spread along the "hippocampal loop" to CA1, the subiculum and the entorhinal cortex. At the neuronal level, it is especially interesting that principal neurons and some subtypes of interneuron not only increase their firing rate, but their APs couple very precisely to specific phases of the fast oscillation (nota bene: the oscillation frequency of 200 Hz is equivalent to a cycle durations of 5 ms corresponding to a 1-ms precision of AP firing!). The work of McNaughton, Wilson, Lee and others demonstrated that previously activated place cells discharge during ripples and contribute to the replay of previously encoded temporal patterns. These properties have fostered the hypothetical role of ripples in processes underlying memory formation (see above).

We and others have focused on the cellular mechanisms of sharp wave–ripple complexes over the past years [27, 40, 41, 42]. We have mostly worked on the experimental model system of hippocampal slices of mice (and rats). These ~400 µm thick slices, cut approximately along the laminar network structure, express activity patterns, entirely independent of external activation and resemble in vivo SPW-R with respect to many aspects: they emerge within CA3, propagate toward CA1 and the entorhinal cortex, display a similar laminar profile as has been described by Buzsáki et al. Finally, the activation patterns of pyramidal cells and interneurons are very similar, as has been shown recently (e.g., see [28, 42, 51, 52]). Obviously, the advantage of the in vitro preparation is the easy access for multiple recording techniques, in particular for high-resolution cellular recording techniques. Furthermore, pharmacological tests can be easily applied without worrying about systemic side effects as for in vivo pharmacological treatments. On the other hand, naturally, the slice preparation is an extremely reduced model system with partly deafferentiated neurons and truly does not reflect the native whole-brain situation—occasionally, reviewers of our manuscripts remind us of this important limitation…. Nevertheless, in several laboratories it was independently verified that hippocampal slices robustly express activity patterns very similar to those observed in vivo and hence slices provide us with a valid and useful model system to study features underlying SPW-Rs. This mere presence of SPW-R in the hippocampal slice also hints at the fact that these events result from concerted activity of the *local* network and do not depend on input from other brain nuclei.

*Which properties of SPW-R should be resolved at the cellular level?* To our understanding the following questions are particularly important:

1. What neuronal signals are the immediate sources of field ripples, i.e., the average extracellular signal of rhythmic potential oscillations at ~200 Hz?
2. Which mechanisms initiate SPW-R and—equally relevant!—how are they terminated after approximately 100 ms?
3. What triggers pyramidal cells and interneurons and what is the explanation to their high temporal firing precision?
4. How do pyramidal neurons 'know' whether to participate in the current SPW-R or not? This question is the key to the explanation of the selective recruitment of previously activated neurons.

Several further questions can be added to those summarized above, with respect to the neuronal subtypes that participate, the modulation by neuromodulators, the activity-dependent plasticity of SPW-R, their "downstream" effects in the entorhinal cortex, among others. However, the questions outlined above are decisive for an extensive model of mechanisms underlying SPW-R. To clarify, we and other colleagues have applied a variety of techniques, in particular different electrophysiological approaches: Recordings of extracellular field potentials represent a weighted average of synaptic and intrinsic membrane currents in proximity to the pipette, hence a kind of local EEG. Particularly well these recordings describe coherent network activity. In addition, APs can be extracellularly registered by glass

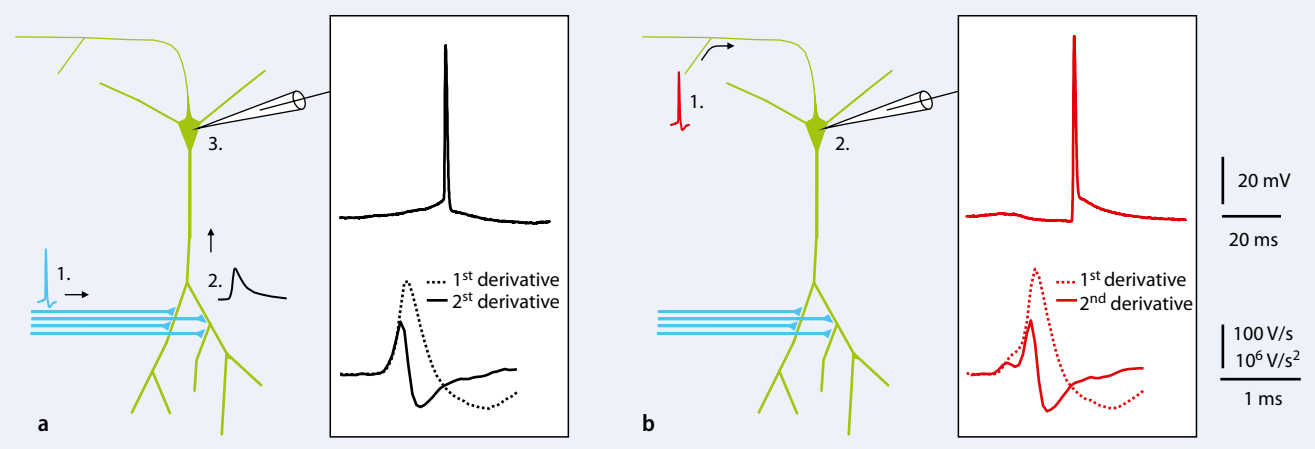


**Fig. 3** ▲ Different modes of neuronal activation during SPW-R. **a** "Classical", orthodromic activation: *1*: A presynaptic AP induces synaptic transmission. *2*: An excitatory postsynaptic potential propagates along the dendrite toward the soma. *3*: Generation of an AP following sufficient somatic depolarization. **b** Antidromic activation. *1*: Generation of an AP at a distal axon collateral with antidromic propagation towards the soma. *2*: Generation of a somatic AP without previous depolarization

electrodes (unsorted spikes), by tetrodes (for single, identified "units") or by juxtacellular recordings, a technique developed by Didier Pinault [43] that allows the post hoc staining and identification of neurons. Furthermore, purely "cellular" recording techniques exist: the "classical" intracellular recording technique that perturbs the intracellular milieu only to a minor extent but offers limited access to biophysical analyses; second, the patch–clamp technique, which allows a detailed dissection of inhibitory, excitatory, and intrinsic conductances. Finally, imaging techniques can be applied, specifically using $Ca^{2+}$-sensitive dyes. Without a doubt, the most influential ideas regarding the functioning of the network were provided by computational neuroscience, i.e., mathematical reconstruction of networks. It is impossible to simply calculate the generation of complex spatiotemporal patterns. The large number of neurons and connections, the bi-directional causal links between synapses or neurons and macroscopic phenomena and the emergence of complex phenomena from the non-linear coupling of simple components can only be achieved in computational models. This is realized at different levels of abstraction, from reduced models of integrate and fire neurons to structurally and functionally highly realistic reconstructions. Strengths and weaknesses of these differing philosophies of models are an interesting topic per se. The work of our collaboration partner Roger D. Traub should be emphasized. His simulations are based on multicompartment models of single neurons rich in details and equipped with experimentally supported intrinsic and synaptic details. With respect to research on memory function of hippocampal networks our collaborations with Richard Kempter and Christian Leibold have been essential [44].

One of the most important and to date controversial findings of our studies is the expression of atypical action potentials of pyramidal neurons during SPW-R [27, 45]. They were first identified in brain slices of rats, but now also from mice, and are characterized by a fast sodium current that is not only activated by excitatory postsynaptic potentials but also by non-synaptic processes that generate aberrant waveforms of APs as observed in somatic recordings. These so-called spikelets (also abortive AP or fast prepotential) were first described in recordings from anesthetized cats by Eric Kandel in the 1960s [46]. They are now regarded as the result of either electrical coupling and/or as an indication of the ectopic origin of spikes, i.e., an origin at locations far away from the soma. Similar potentials were recently observed by Jérôme Epsztein, Michael Brecht et al. [47] in patch–clamp recordings from area CA1 of freely moving rats where they seem to be modulated in a space-dependent way.

Modeling and evidence from the literature suggest that those APs observed in our recordings are likely to be generated in distal compartments of the axon; these areas are usually not directly innervated by excitatory synapses. This matches previous findings which support the idea of electrical coupling between axons playing a major role in the generation of SPW-R-associated APs [27, 45] (◘ **Fig. 3**). Indeed, in paired recordings, ectopic APs are measured first in the axon before reaching the soma. Their initiation shares many functional as well as pharmacological properties compatible with axo-axonal coupling [48]. To date, ultrastructural data directly supporting the presence of gap junctions on hippocampal projection neurons are scarce. However, the hypothesis of axo-axonal electrical coupling implies exciting functional consequences: hypothetically, the AP could be transmitted between axons without the need of somato-dendritic processes of synaptic activation and conventional AP initiation. The connected plexus of axons would become autonomous and—by the selective coupling of specific cells—form a neuronal assembly, i.e., a functional network of co-active neurons. At the same time, the AP, back-propagating into the dendritic tree, could promote Hebbian synaptic plasticity and thereby foster the coupling of neurons with their excitatory inputs. Mechanisms underlying the selection of participating neurons, and their coupling, are still unclear.

However, a detailed analysis of subthreshold potentials, that is to say, of the postsynaptic action of inhibitory and ex-

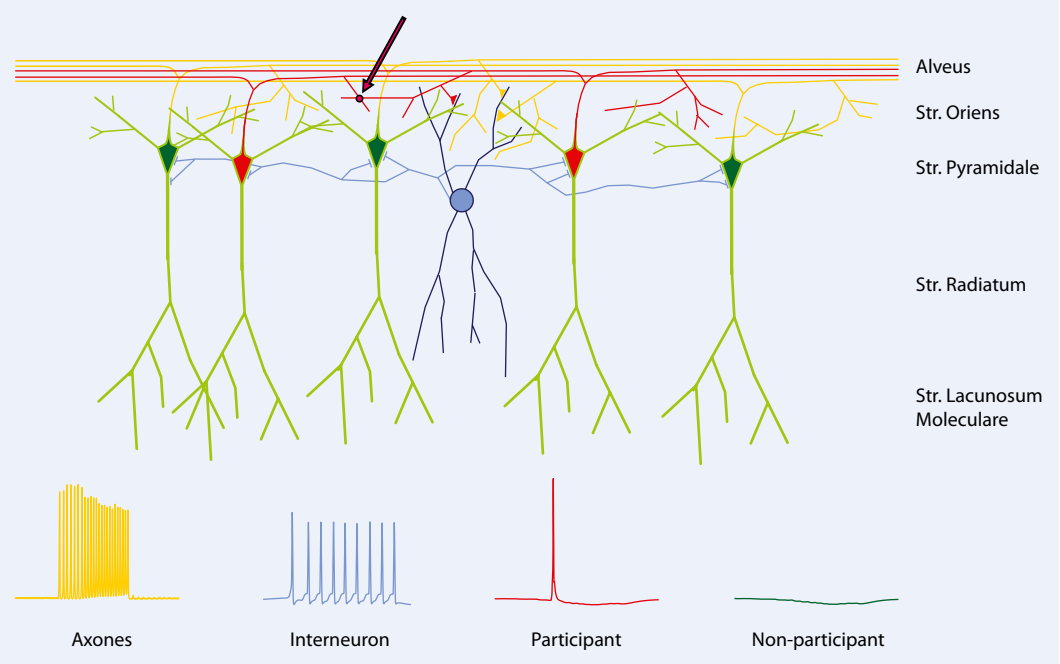


**Fig. 4** ◀ Generation of sharp wave ripple-complexes (R. D. Traub); working hypothesis. Simplified schematic of the local neuronal network in the alveus and the stratum oriens. Pyramidal neurons fall into two distinct classes: participating and non-participating neurons. Participating cells are connected by gap junctions localized at some of their axon collaterals (*red arrow*). Participating as well as non-participating neurons receive strong inhibitory inputs. Axon collaterals with small diameters fire at higher frequency compared with firing rates in the soma or in projection axons

citatory synapses, has demonstrated that CA1 pyramidal cells receive strong and temporally precise synaptic inputs that are likely to influence their behavior during SPW-R. This is also in line with reports demonstrating the origin of SPW-R in CA3 (in vivo and in vitro) and their propagation to CA1. At the same time, perisomatically inhibitory interneurons are recruited, both processes giving rise to an interplay between excitation and inhibition. The dynamics of the temporal increase of inhibition might then give the explanation of how the transiently enhanced activity during the sharp wave finally again ceases. In this scenario, the hypothesis of axo-axonal gap junctions offers the interesting possibility of suppression of "conventional" AP initiation by the strong and rather unspecific perisomatic inhibition during SPW-R. In contrast, ectopic spikes occurring in selected neurons would remain unaffected by this perisomatic inhibitory control. Taken together, axons should probably be attributed a far more active role during neuronal network activity than previously thought. Support comes from a recent report on high firing rates in CA3 axons (during gamma activity) that could not be resolved at the somatic level [49]. The interplay between inhibitory, excitatory and electrical coupling as well as of axonal activity during SPW-R, however, is not conclusively understood so far.

The findings summarized here were elaborated in great detail in the computer models by Roger D. Traub. Due to the current lack of anatomical proof of electrical coupling between axons they are not without controversy and challenge cellular neurophysiologists and -anatomists. A simplified scheme of the postulated generation of SPW-R in CA1 is displayed in **Fig. 4**.

It should be mentioned that events sharing similarity with SPW-R can also be reproduced in adequately structured feed-forward networks with nonlinear excitatory synapses [50]. This alternative emphasizes the significance of glutamatergic connections, which are definitely involved in the generation of SPW-R. The interactive task of experimentalists and theoreticians for the next few years will be to further develop the differing models. It is well feasible that elements of both approaches will finally play a role. After more than 10 years of investigation into the mechanism of the SPW-R phenomenon it has become obvious that the explanation of complex spatiotemporal patterns in neuronal networks is still challenging—in spite of many well-known principles. In turn, insights from cellular neurobiology will better define conditions which should be taken into account in models of cognitive performance related to neuronal network oscillations.

## Corresponding addresses

**N. Maier**
Neurowissenschaftliches Forschungszentrum der Charité,
Charité-Universitätsmedizin Berlin
Charitéplatz 1, 10117 Berlin
Germany
nikolaus.maier@charite.de

N. Maier studied medicine at the Medical Faculty of the Humboldt-University Berlin (Charité), followed by a research internship ('AIP') in 2004. At the Institute for Neurophysiology at the Charité, he finshed his doctoral thesis on cellular mechanisms underlying hippocampal high-frequency network oscillations (Advisor: Prof. Dr. Andreas Draguhn). Since 2004 Nikolaus Maier is researcher at the Charité Neuroscience Research in the group of Prof. Dr. Dietmar Schmitz, where he is particularly interested in the cellular and synaptic bases of high-frequency ripple oscillations.

**A. Draguhn**
Institut für Physiologie und Pathophysiologie,
Medizinische Fakultät der Universität
Heidelberg
Im Neuenheimer Feld 326,
69120 Heidelberg
German
andreas.draguhn@
physiologie.uni-heidelberg.de

A. Draguhn studied medicine, physics, and philosophy in Bonn and completed his doctoral thesis in 1992 at Heidelberg on the function of different molecular isoforms of the GABAA receptor under supervision of Bert Sakmann. Subsequently he went on to work as a postdoctoral researcher with Prof. Dr. Uwe Heinemann at the Institute of Physiology at the University Cologne and followed him in to the Charité (1994). Fast network oscillations were the subject of a residency in 1997 with John G.R. Jefferys and Roger D. Traub in Birmingham (UK). Andreas Draguhn completed his 'Habilitation' after returning to the Charité in 1999. He received the appointment as chair at the Department of Neurophysiology at the Medical Faculty of Heidelberg University in 2001. His group researches the cellular mechanisms of neuronal synchronization, spatio-temporal patterns of network activity, and function and plasticity of GABAergic synapses.

**D. Schmitz**
Neurowissenschaftliches Forschungszentrum
der Charité,
Charité-Universitätsmedizin Berlin
Charitéplatz 1, 10117 Berlin
Germany
dietmar.schmitz@charite.de

D. Schmitz studied medicine at the University of Cologne and the Charité in Berlin. He received his doctoral degree in 1997 at the Department of Neurophysiology, Humboldt University (Prof. Dr. Uwe Heinemann) with a thesis on the basics of serotonergic modulation of cortical networks. Subsequently, he worked as a postdoctoral researcher at the Institute of Neurophysiology of Humboldt-University Berlin followed by a second postodoctorate in the laboratory of Prof. Roger Nicoll at the University of California (San Francisco, USA) on mechanisms of synaptic plasticity. Since 2002 he is professor of neuroscience and since 2005 Director of the Neuroscience Research Center at the Charité-Universitätsmedizin Berlin. His research focuses include not only cellular and synaptic mechanisms of neural networks, but also the molecular basis of synaptic plasticity and neurodegenerative changes in the CNS.

**M. Both**
Institut für Physiologie und Pathophysiologie,
Medizinische Fakultät der Universität
Heidelberg
Im Neuenheimer Feld 326,
69120 Heidelberg
German
mboth@physiologie.uni-heidelberg.de

M. Both studied physics at the University of Heidelberg and the University of Kentucky, USA. He received his PhD in 2004 with Prof. Dr. Rainer H. A. Fink at the University of Heidelberg on the application of nonlinear optical effects for microscopy biologics. He worked then as a postdoctoral researcher in the laboratory of Prof. Dr. Andreas Draguhn at the Institute of Physiology at Heidelberg University where he – since 2008 as a team leader – has been working on memory-related mechanisms in hippocampal network oscillations.

**Conflict of interest.** On behalf of all authors, the corresponding author states that there are no conflicts of interest.

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