The cellular basis of rhythmic activity patterns occurring in the CA1 region of the hippocampus

Ph.D. thesis

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INTRODUCTION

The hippocampal formation is known to be involved in many higher order cognitive functions, such as encoding and retrieval of memory or spatial navigation. It is also well known that hippocampal circuitry exhibit a wide variety of population patterns, including oscillations at theta (4-7 Hz) or gamma (30-100 Hz) frequencies, or sharp-wave-ripple oscillations (100-200 Hz) under different behavioral states. Several lines of studies show that the occurrence of these special behavior-dependent activity patterns strongly correlates with different cognitive functions. There are many theories related to these observations that suggest that the complex dynamics presumably reflects specific information processing states of the networks. However, the exact role of these complex activity patterns in different operations of the brain remains elusive.

One way to get closer to understand the involvement of rhythmic oscillations occurring at different frequencies in certain brain functions is to reveal the underlying cellular mechanisms. Unfolding the contribution each component of the network makes to network oscillations will produce mechanistic as well as functional insights into their generation and role. In this thesis I addressed the question what mechanisms tune the network to operate at certain frequencies in the hippocampus. To answer this we have to investigate the intrinsic properties of the individual elements of the network as well as the complex properties of the circuitry that makes the system being capable of generating or following rhythmic activity.

AIMS

The main goal of this thesis was to reveal cellular mechanisms that may underlie rhythmic activity patterns in the CA1 region of the hippocampus. To this end, two objectives were identified.

The first objective was to identify intrinsic properties of the single neurons of the network that might contribute to rhythmic activity patterns occurring at population levels. We wanted to determine the subthreshold resonance properties of different types of hippocampal neurons and the underlying mechanisms. We investigated the impedance profiles of distinct types of anatomically identified neurons in the CA1 region of rat hippocampal slices. We focused on the dissimilarities in the voltage response of the cells to sinusoidal current inputs and wanted to determine the role of the hyperpolarization-activated cyclic nucleotide-gated current (I_h) in producing these differences. We also tried to give a quantitative account for the observed differences by using a computational model.

The second objective was to reveal the synaptic mechanisms within the network that may underlie oscillatory activity in the CA1 region, particularly in the gamma frequency range. We were primarily interested in the mechanisms of propagation of intrinsically generated gamma oscillation from the CA3 to the CA1 area of the hippocampus. We presumed two possible mechanisms: "the feed-forward excitation" and the "feed-forward inhibition model". In the feed-forward excitation model CA3 pyramidal cells directly discharge CA1 pyramidal cells and then a recurrent network between

CA1 pyramidal cells and local interneurons produce the local field potential oscillation in CA1, similarly to CA3. In the feed-forward inhibition model CA3 pyramidal cells excite primarily CA1 interneurons instead of CA1 pyramidal cells, and the local field potential oscillation arise from the rhythmic activity of interneurons driven by the rhythmic excitation via the Schaffer-collaterals of CA3 pyramidal cells.

To be able to decide which model is valid, we aimed to reveal what determines the behavior of the different cell types during gamma oscillation in CA1. To this end, we investigated the relationship between firing activity and synaptic inputs of different hippocampal cell types during cholinergically induced fast network oscillations in hippocampal slices.

METHODS

Animals were kept and used according to the regulations of the European Community's Council Directive of 24 November 1986 (86/609/EEC), and experimental procedures were reviewed and approved by the Animal Welfare Committee of the Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest. Horizontal hippocampal slices (400-450 μm) were prepared from young animals (P14-21), after decapitation under isoflurane-anesthesia. Wistar rats were used in study I and CD1 mice in the study II. The slices were incubated in an interface chamber in oxygenated artificial cerebrospinal fluid at room temperature at least 1 h before use. During the recordings the slices were kept submerged

in a chamber perfused with artificial cerebrospinal fluid. In the second study a special recording chamber was used with dual superfusion system to provide better metabolic supply for the slices. Recordings were made at 36±1°C in study I and at 32±1°C in study II.

In the first study we performed whole-cell patch-clamp recordings. A K-gluconate based intrapipette solution was used. To determine the frequency preferences of the different types of hippocampal neurons, we injected 3 s long sinusoidal currents at fixed frequencies (between 0.5 and 40 Hz) into the cells at different membrane potentials. The impedance values were calculated by dividing the Fast Fourier Transform of the voltage response of the cells by that of the input current. The impedance magnitude and phase profiles were calculated and characterized. Whole-cell voltage clamp experiments were performed to verify the presence of I_h and to characterize its basic properties. I_h was recorded by giving 800 ms long voltage-clamp steps in -10 mV increments up to -120 mV from a holding potential of -40 mV. The amount of I_h was calculated by subtracting the current traces before and after the application of 10 μM ZD7288, a specific blocker of the HCN channels. The current-voltage (I-V) relationship was measured at the end of the voltage step. The activation curve was computed from tail current and was fitted with Boltzmann-equation. The activation kinetics were fitted by exponential functions. To give a quantitative explanation of how the voltage-gated conductances interacted with the passive membrane

properties in the different cell types, we also introduced a computational model to our study.

In the second study fast network oscillations were induced by bath application of the cholinergic agonist carbachol (10 μM). Extracellular field potentials and unit activity of neurons were recorded in the hippocampus using patch pipettes filled with ACSF. After measuring the spiking of individual cells, whole-cell patch-clamp recordings were performed to examine the properties of synaptic currents. The intrapipette solution was a K-gluconate based solution. Cells were voltage clamped at the estimated inhibitory (~-70 mV) and excitatory (~ 0 mV) reversal potentials to record EPSCs and IPSCs, respectively. To characterize the field oscillations, extracellular recordings of ~ 1 min were acquired and used to obtain a power spectrum. Wavelet analysis using a Morlet wavelet basis was used to extract the magnitude and phase of different frequency components of the field oscillation. To validate that wavelet analysis provided an appropriate definition of phase, the cycle-averaged field potential was calculated. The firing properties of the cells were quantified using three parameters: mean firing rate, action potential coupling strength (r_{AP}) and mean action potential phase (Φ_{AP}). The balance of excitation and inhibition was investigated by comparison of the ratio of phasic excitatory to inhibitory charge (Q_e/Q_i) between pyramidal cells and interneurons.

In both studies the intrapipette solution contained also 0.3-0.5 % biocytin, and the different cell types were identified post-hoc based on their morphological characteristics.

RESULTS

Part I. Single cell resonances in hippocampal CA1 neurons produced by intrinsic membrane characteristics

The intrinsic properties of distinct types of neuron play important roles in cortical network dynamics. One crucial determinant of neuronal behavior is the cell's response to rhythmic subthreshold input, characterized by the input impedance, which can be determined by measuring the amplitude and phase of the membrane potential response to sinusoidal currents as a function of input frequency.

In this study, we determined the impedance profiles of anatomically identified neurons in the CA1 region of the rat hippocampus. Data were collected from pyramidal cells (PC) as well as interneurons located in the stratum oriens, including oriens-lacunosum-moleculare cells (OLM), fast-spiking perisomatic region-targeting interneurons (FS PTI) and cells with axonal arbor in strata oriens and radiatum (O-R).

We found that the basic features of the impedance profiles, as well as the passive membrane characteristics and the properties of the sag in the voltage response to negative current steps, were cell-type specific.

When comparing the passive membrane characteristics of the cells we found that OLM cells had significantly slower membrane time constant at rest than the other cell types. OLM cells also tended to have higher input resistance and larger membrane capacitance than the other neurons.

We observed a *sag* in the voltage response of PCs, O-Rs and OLM cells to negative current steps of suitable magnitudes. This sag could be eliminated by bath application of the specific I_h blocker, ZD7288 (10 μM). The properties of the sag varied substantially between the cell types. PCs (n=18 out of 19) displayed a small but rather fast sag, O-Rs (n=11) had the largest and fastest sag, while OLM cells (n=12) usually showed a large but relatively slow sag.

With the exception of fast-spiking interneurons, all cell types showed subthreshold resonance, albeit with distinct features. We found that all PCs showed resonance (n=9), indicated as a clear peak in the impedance curve. Resonance was most prominent at hyperpolarized potentials (at -70 and -80 mV) and occurred at theta frequencies (4-6 Hz) Almost all O-Rs also exhibited resonance between 2 and 6 Hz (n=15 out of 16), although a rather large variance could be seen in Q values. Ten out of 15 OLM cells also showed resonance, however, the resonance frequency fell in the range of 1 to 3 Hz.

The HCN channel blocker ZD7288 (10 μM) eliminated the resonance and changed the shape of the impedance curves, indicating the involvement of the hyperpolarisation-activated cation current I_h. Whole-cell voltage-clamp recordings uncovered differences in the voltage-dependent activation and kinetics of I_h between different cell types. We found that I_h was significantly more activated in PCs than in interneurons at all potentials between -60 and -100 mV; the potential of half-maximal I_h activation ($V_{1/2}$) was -82.9 ± 4.9 mV in pyramidal cells (n=6), -97.3 ± 4.7 mV in O-Rs (n=7) and -97.7 ± 5.0

mV in OLM cells (n=7). The activation of I_h was significantly faster at all membrane potentials between -80 and -120 mV in PCs than in O-Rs and OLM cells. No significant difference was seen in the properties of I_h activation between O-Rs and OLM cells.

Biophysical modeling demonstrated that the cell-type specificity of the impedance profiles can be largely explained by the properties of I_h in combination with the passive membrane characteristics.

We conclude that differences in I_h and passive membrane properties result in a cell-type-specific response to inputs at given frequencies, and may explain, at least in part, the differential involvement of distinct types of neuron in various network oscillations.

Part II. Network resonances of hippocampal CA1 produced by synaptic synchronization

Gamma-frequency (30-70 Hz) oscillations are a prominent feature of hippocampal network activity and have been implicated in encoding and retrieval of memory. Data recorded both *in vivo* and *in vitro* suggests that CA3 gamma frequency oscillations drive the synchronous activity in CA1, however, very little is known about the precise cellular mechanisms underlying network oscillations in the CA1 region. This study is aimed to explore how the rhythmic excitatory output of CA3 pyramidal cells generates fast oscillations in CA1. To distinguish between the two possible mechanisms ("feed-forward excitation or inhibition") we investigated the firing properties and synaptic inputs of different types of anatomically-

identified hippocampal neurons during cholinergically-induced gamma oscillations.

Oscillatory activity was induced by bath application of carbachol in mouse hippocampal slices. Oscillations occurred basically at the same frequency in both CA3 and CA1 (~ 31 Hz), however, the power of the oscillation was usually much smaller in CA1 than in CA3. Extracellularly recorded action potentials and intracellularly recorded synaptic currents were obtained from CA1 and CA3 neurons. Two-thirds of the CA1 pyramidal cells (PCs) (n=15 out of 21) and most of the CA1 interneurons (INs) (n=37 out of 40) were phase-coupled to the local field potential oscillation recorded in the stratum pyramidale of CA1, though the average phase-coupling strength was smaller in CA1 PCs (r_{AP}=0.21±0.02; mean ± s.e.m) than in CA1 INs (r_{AP}=0.57±0.06). The firing of both CA3 PCs (n=22) and INs (n=8) was strongly phase-coupled to the oscillation (r_{AP}=0.54±0.03 and 0.69±0.08, respectively). Phase-coupled CA1 PCs fired near the minimum of the field oscillation (Φ_{AP}=-2.25 ± 0.23; phase measured in radians), somewhat earlier within a cycle than CA3 PCs (Φ_{AP}=-1.72±0.04), whereas phase-coupled CA1 INs tended to fire later in the cycle, at the ascending phase of the oscillation (Φ_{AP}=-1.15±0.09), similarly to CA3 INs (Φ_{AP}= -0.97±0.13).

The dominant phasic input to phase-coupled PCs was inhibitory, whereas phase-coupled INs received strong phasic excitation. Correlation analyses showed that the more precise and robust excitatory input a CA1 IN had, the more precisely it fired during a

cycle, while no such correlation could be observed in the case of CA1 PCs. When comparing the phase of the events we noticed that the phase of peak excitation always preceded the phase of peak inhibition in each cell in both CA3 and CA1. The peak of phasic excitation occurred in the ascending phase of the field potential oscillation, while the peak of phasic inhibition could be observed close to the peak of the field potential oscillation. The phase of action potentials was slightly before or after the phase of the peak EPSC in the case of INs in both regions (on average these phase differences translate to a time difference of 1-2 ms between the peak excitation and the action potentials of these cells according to the a mean oscillation frequency of 31 Hz), while the phase of action potentials occurred much earlier in CA1 PCs than the phase of peak excitation (more than 8 ms earlier). CA3 PCs also fired earlier than their peak excitatory input, but still later in the cycle than CA1 PCs (2-3 ms later).

These data indicate that while the spiking activity of CA1 interneurons was driven mainly by their strongly phase-coupled excitatory inputs presumably arriving from CA3 pyramidal cells, the dominant input of CA1 pyramidal cells was inhibitory and their firing activity was less precisely synchronized. Our results suggest that the oscillation generated intrinsically in the CA3 area propagated to the CA1 region via feed-forward inhibition, i.e. CA3 pyramidal cells excite CA1 interneurons and their rhythmic, phase-locked firing induces oscillation in CA1.

These findings are consistent with *in vivo* recordings, suggesting that similar functional networks may underlie carbachol-induced oscillations *in vitro* and gamma frequency oscillations in the hippocampus of the behaving animal.

CONCLUSION

The main issue of this thesis was to reveal particular mechanisms in the hippocampus that are able to tune the network to operate at certain frequencies. In the first part of the dissertation we could see that some hippocampal CA1 neurons are set to operate at theta frequencies by their intrinsic membrane characteristics, while the second part showed how the circuitry of hippocampal CA1 can be synchronized by synaptic mechanisms to produce gamma frequency oscillations. These results imply that the frequency of the network oscillations could be affected by both the cellular and the synaptic dynamics of single neurons. The resonance properties of single cells might promote synchronized membrane potential oscillations in certain elements of the network, which could produce a field potential oscillation in the extracellular space. However, even though most of the neurons in hippocampal CA1 are prompted to operate at relatively low frequencies by their intrinsic membrane properties, the whole network at a population level can be tuned to produce higher frequency oscillations simply by shifting the balance between synaptic excitation and inhibition on the different elements of the circuitry.

LIST OF PUBLICATIONS

Publications related to the dissertation

Zemankovics R, Káli S, Paulsen O, Freund TF, Hájos N (2010) Differences in subthreshold resonance of hippocampal pyramidal cells and interneurons: the role of h-current and passive membrane characteristics. J Physiol 588:2109-2132.

Hájos N, Ellender TJ, Zemankovics R, Mann EO, Exley R, Cragg SJ, Freund TF, Paulsen O (2009) Maintaining network activity in submerged hippocampal slices: importance of oxygen supply. Eur J Neurosci 29:319-327.

Other publications

Varga V, Hangya B, Kránitz K, Ludányi A, Zemankovics R, Katona I, Shigemoto R, Freund TF, Borhegyi Z (2008) The presence of pacemaker HCN channels identifies theta rhythmic GABAergic neurons in the medial septum. J Physiol 586:3893-3915.

Hájos N, Holderith N, Németh B, Papp OI, Szabó GG, Zemankovics R, Freund TF, Haller J (2011) The Effects of an Echinacea Preparation on Synaptic Transmission and the Firing Properties of CA1 Pyramidal Cells in the Hippocampus. Phytother Res. doi: 10.1002/ptr.3556. (*In press*)