The use of transgenic techniques to study oscillatory brain activity

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Developmental profile of oscillatory network activity

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We have examined the molecular and cellular mechanisms that lead to the emergence of synchronous firing of CA1 pyramidal neurons in the developing rat hippocampus in response to high-frequency stimulation (HFS). The experiments were done on isolated CA1 pyramidal neurons (microfluorescence measurements of pH_i) and hippocampal slices (both electrophysiological and microfluorescence measurements). High-frequency stimulation in s. radiatum evoked synchronous gamma-frequency (20–80 Hz) firing only after postnatal day 12. This was attributable to an abrupt developmental upregulation of intrapyramidal carbonic anhydrase (CA). The data obtained show that intrapyramidal CA is a key molecule in the generation of HFS-induced non-synaptic GABAergic excitation which depends on the presence of CO_2/HCO_3 and is mediated by an increase in extracellular $[K^+]$. These results point to a crucial role for the developmental expression of intrapyramidal carbonic anhydrase in shaping hippocampal integrative functions, long-term plasticity, and susceptibility to epileptogenesis. Further studies are needed to find out whether neuronal CA has a similar role in other brain areas in the control of large-scale population activity.

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Cellular mechanisms of induced '40 Hz' rhythms

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Neuronal pacemaker activity and network oscillations

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Network oscillations are a prominent phenomenon in neuronal signal processing, yet their functional role remains elusive. Three main classes of cellular mechanism have been suggested to contribute to network oscillations: (1) rhythmic activity in external pacemaker neurones could drive the network oscillation (Petsche *et al.* 1962). (2) Network oscillations could be generated by a synaptic feedback loop between excitatory and inhibitory neurones (Freeman, 1968). Alternatively, or additionally, a subnetwork of synaptically interconnected neurones could express a frequency preference based on the kinetic properties of the synaptic connections (Whittington *et al.* 1995). (3) Intrinsic frequency properties of neurones within the network could support the emergence of population oscillations when the network is activated (Llinas, 1988). The pacemaker current (I_h) is a mechanism that has been implicated in rhythmic activity in excitable cells including neurones. The aim of the present project was to investigate the contribution by I_h to frequency preference at physiologically relevant frequencies in hippocampal CA1 pyramidal neurones.

Transverse hippocampal slices were made from P12–P18 Wistar rats, decapitated under isoflurane anaesthesia in accordance with the UK Animals (Scientific Procedures) Act, 1986. Following a recovery period of at least 1 h, whole-cell patch-clamp recordings were obtained from individual neurones at room temperature (23–25 °C) under visual guidance by infra-red video-microscopy using standard procedures. The patch pipette solution contained (mM): potassium gluconate (100), NaCl (4), Hepes (40), Mg-ATP (4), GTP (0.3) and biocytin (5 mg ml^{-1}); pH 7.2–7.4.

To investigate the intrinsic resonance properties at subthreshold membrane potentials, a sinusoidal current with linearly increasing frequency (0–30 Hz) was applied. The impedance magnitude, estimated as the magnitude of the ratio of the fast fourier transform (FFT) of the voltage response to the FFT of input current, showed a maximal response around 2–4 Hz ($n = 14$) at resting membrane potential. The peak frequency increased with hyperpolarisation (0.03 Hz mV^{-1}) along with a reduction of the peak impedance magnitude (−3.3 MΩ mV^{-1}), suggesting involvement of h conductance. Consistent with this hypothesis, the resonance peak was completely abolished by a blocker of the h conductance, ZD7288 (10 μM; $n = 6$). Dynamic clamp experiments, mimicking electronically the h conductance, whose properties were determined in independent voltage-clamp experiments, confirmed that this conductance could generate resonance ($n = 3$).

To study to what extent the h conductance could explain spike frequency preference in these neurones, we determined the effect of oscillation frequency on spike generation using sinusoidal current at discrete frequencies superposed on a current step. In pyramidal cells, at low rates of firing (< 1 Hz), input frequency preference was demonstrated at ~2 Hz. The input frequency at peak firing rate increased steeply with depolarization. The addition of 10 μM ZD7288 abolished the input frequency preference only at the lowest firing rates, leaving the frequency preference at higher rates largely unaffected ($n = 4$). Conversely, blocking a component of the spike after-hyperpolarisation (AHP) with apamin (50 nM), altered the input frequency preference only at higher firing rates ($n = 6$). Thus the control of subthreshold resonance and suprathreshold firing entrainment can be experimentally disassociated and is mediated by distinct mechanisms.

These results suggest that distinct intrinsic membrane properties support resonance in physiologically relevant frequency ranges, and that these may contribute to signal processing in individual neurones.

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Salient mechanisms underlying the generation of hippocampal network oscillations

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Gamma and theta frequency oscillations are a predominant feature of rhythmic activity in the hippocampus. We have previously demonstrated that gamma oscillations in the hippocampus may manifest as a transient (*ca* 1 s duration) network event or as a persistent network phenomenon. In both cases tonic network drive via activation of metabotropic glutamate or acetylcholine receptors plays a critical role in rhythmogenesis. However, the specific mechanisms involved in these two forms of network behaviour appear different. Transient episodes of gamma oscillation may occur in the absence of phasic excitatory synaptic outputs from principal cells, with large tonic depolarisations of fast-spiking interneurons alone being sufficient to elicit a gamma frequency oscillation (interneuron network gamma, ING). In contrast, persistent gamma oscillations are characterised by rhythmic trains of AMPA receptor-mediated excitatory synaptic potentials onto interneurons (pyramidal interneuron network gamma, PING). Blockade of these synaptic events leads to collapse of persistent population gamma oscillations. In each case the gamma oscillation is characterised by trains of $GABA_A$ receptor-mediated inhibitory postsynaptic potentials in principal neurons.

Here we demonstrate that, in hippocampal area CA1, a transition from gamma to theta frequency population oscillations was seen in response to metabotropic glutamate receptor activation when AMPA receptor activation was reduced. This theta frequency activity occurred in the absence of inputs from area CA3 and extra-ammonic areas and was resistant to atropine. Field theta oscillations were expressed via pyramidal distal apical dendritic burst spiking and were temporally related to trains of IPSPs with slower kinetics than those seen during gamma oscillations. Pyramidal somatic responses showed theta oscillations consisted of compound inhibitory synaptic potentials with initial IPSPs with slow kinetics followed by trains of smaller, faster IPSPs. Pharmacological modulation of IPSPs altered the theta oscillation, suggesting an inhibitory network origin. Somatic IPSPs, dendritic burst firing and stratum pyramidale interneuron activity were all temporally correlated with spiking in stratum oriens interneurons demonstrating intrinsic theta-frequency oscillations. Disruption of spiking in these interneurons abolished both field theta and theta frequency IPSP trains.

These data indicate that the magnitude of AMPA-receptor mediated excitatory synaptic responses governs the degree of gamma and theta frequency oscillations expressed in area CA1 *in vitro*. We suggest that low levels of AMPA-mediated drive to interneurons favours control of local network activity via slow spiking, stratum oriens interneurons (theta), whereas high levels of AMPA-mediated drive to interneurons favours control of local network activity via fast spiking, predominantly stratum radiatum interneurons (gamma). Thus factors influencing fast excitatory synaptic transmission (plasticity, neuromodulators) may consequently modify the pattern of rhythmogenesis expressed in hippocampal area CA1.

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Glutamate receptors, synaptic plasticity and learning in mice

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Fast (~200 Hz) field potential oscillations in the rodent hippocampus *in vitro*: cellular and network mechanisms

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Coherent membrane potential oscillations in neuronal networks provide a common 'clock' for individual neurons which can discharge at defined time points within the cycle. In the mammalian hippocampus, this 'phase coding' seems to be of particular importance during combined theta/gamma oscillations (5–10 and 30–100 Hz, respectively) and during very fast (~200 Hz) oscillations called 'ripples'. Fixed temporal sequences of consecutively firing place-encoding neurons are established during theta/gamma activity when a rat explores a new environment and are replayed at a faster time scale during ripples at rest or sleep. These observations have given rise to the idea that ripples are involved in memory consolidation (reviewed in Draguhn *et al.* 2000).

While the cognitive function of ripples can be best studied in the living animal, the underlying mechanisms of synchronisation are most easily analysed in *in vitro* preparations. We have therefore studied high-frequency (~200 Hz) oscillations in rat and mouse hippocampal slices. Hippocampi were dissected and sliced after brain removal in deep ether anaesthesia (as permitted by the Berlin local government). Extracellularly recorded field potentials present brief periods of spontaneously occurring fast potential oscillations in the principal cell layers. The waveforms, however, are strikingly different between rat and mouse tissue: in slices from juvenile or adult rats, we regularly observed series of 3–10 negative-going deflections reminiscent of small (0.02–0.4 mV) population spikes (Draguhn *et al.* 1998). In slices prepared from mice, the fast negative deflections are superimposed on positive-going waves of 20–80 ms duration (Fig. 1). The global pattern and frequency content of these potential fluctuations is very similar to sharp wave-ripple complexes *in vivo* (Buzsáki *et al.* 1992) and therefore we term the spontaneously occurring network activity in mouse hippocampal slices 'sharp wave-ripple complexes *in vitro*' (SW-R).

Recordings with multiple electrodes revealed that SW-R propagate from CA3 towards CA1 and to the subiculum. Besides this propagation along the hippocampal output loop, we also found a 'backward' propagation towards the hilus and the dentate gyrus. Propagation velocity was around 2 cm s^{-1}, far below the axonal conductance velocity in the hippocampus. The laminar profile revealed most prominent signals in the pyramidal cell layer and a phase reversal of sharp waves and ripples between stratum pyramidale and stratum radiatum. These spatial properties are very similar to the behaviour of sharp wave-ripple complexes *in vivo*.

SW-R do depend on fast chemical synaptic transmission: CNQX completely blocked SW-R activity while the NMDA-receptor antagonist DL-APV had no visible effect. Likewise, SW-R were completely blocked when we suppressed GABAergic inhibition with bicuculline or gabazine. In these disinhibited slices we

regularly observed large interictal-like discharges with superimposed oscillations at ~200 Hz.

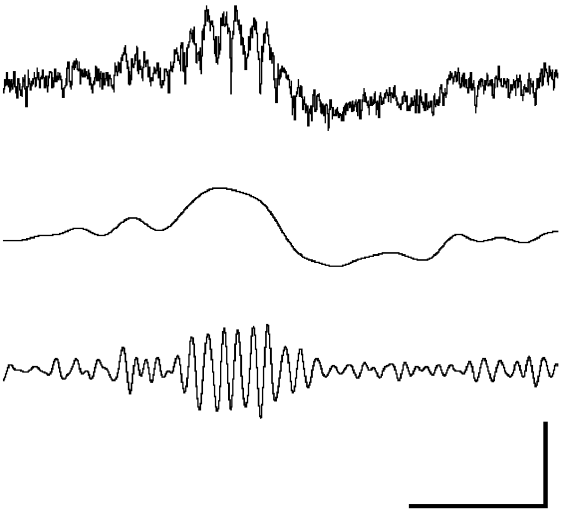

Figure 1. Sharp wave-ripple complex recorded in CA3 pyramidal cell layer of a mouse hippocampal slice. Top trace shows raw data (0–3000 Hz bandwidth), middle trace shows low-pass filtered sharp wave (< 50 Hz) and bottom trace shows isolated ripple oscillation (150–300 Hz). Calibration 50 ms, 0.2 mV (top and middle) and 0.1 mV (bottom).

We had previously suggested that ripples are synchronised by electrical coupling via gap junctions. We therefore applied three different uncoupling agents (carbenoxolone, octanol and quinine) and found that all of them strongly reduced or even abolished the SW-R activity. Interestingly, ripples were more strongly suppressed than the underlying sharp waves, indicating that 200 Hz oscillations depend more critically on gap junctions than the slow excitatory waves.

We finally recorded the intracellular potential of CA1 pyramidal neurons ($n = 13$) together with the nearby field potential. Sharp waves were accompanied by depolarising, hyperpolarising or biphasic potential fluctuations in all pyramidal cells. However, even during depolarising responses membrane potential never reached threshold for action potential generation. Voltage dependence of the signals revealed a negative (< −60 mV) reversal potential, consistent with a dominant $GABA_A$ receptor-mediated chloride conductance. When we depolarised the cells sufficiently to generate regular firing, action potentials usually ceased during SW-R, again indicating that the net effect of SW-R on most pyramidal cells is inhibitory.

In summary, sharp wave-ripple complexes can be well studied in mouse hippocampal slices and show a spatial distribution, propagation and frequency content which is very similar to sharp wave-ripple complexes *in vivo*. The signals are generated by chemical as well as by electrical coupling of neurons and result in a strong inhibition of most local pyramidal cells.

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Gap junctions between the axons of principal neurons, and the generation of fast oscillations in neuronal populations

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In 1998, it was hypothesized that gap junctions existed between the axons of hippocampal pyramidal cells (Draguhn *et al.* 1998). This hypothesis was suggested by two experimental observations: the occurrence of 200 Hz population oscillations in neuronal networks in which synaptic transmission was blocked, but where the oscillations required gap junctions; and the shape of putative coupling potentials in principal neurons, which were too fast to be generated by gap junctions located on somata or dendrites. There is now electrophysiological and dye-coupling evidence that such gap junctions exist, and are located roughly 100 μm from the soma (Schmitz *et al.* 2001). Modelling shows that gap junctions in this location can give rise to very fast oscillations in networks of principal neurons, as well as to 200 Hz 'ripples' (as seen *in vivo*, and consisting of IPSPs), when interneurons are also in the circuit (Traub *et al.* 1999; Traub & Bibbig, 2000). In addition, axonal gap junctions can underlie the generation of 40 Hz oscillations, in the presence of cholinergic agonists or of kainate (Traub *et al.* 2000). Modelling predicts, and experiments confirm, that in such conditions, the oscillation spectrum contains both 40 Hz and also very fast (> 80 Hz) components.

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The cellular bases of goal-directed locomotion in lamprey – from ion channels to neuronal networks

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The neuronal networks underlying vertebrate locomotion have been studied in considerable detail using a lower vertebrate model system, the lamprey. The segmental network consists of ipsilateral excitatory glutamatergic and inhibitory crossed glycinergic interneurons. In addition, there is a sensory movement related input to the network from ipsilateral excitatory and crossed inhibitory stretch receptor neurons that help adapt the movements to external events (see Grillner *et al.* 2001). The network is activated from the brainstem via reticulospinal neurons, which in turn can be driven from mes- and diencephalic glutamatergic pathways. Visual and olfactory stimuli elicit goal-directed behaviour, most likely via the direct projections from the olfactory bulb and the optic tract to the diencephalic locomotor centre.

For the segmental pattern generation the intrinsic properties of the different network neurons play a critical role. One focus will be on the role of different subtypes of Ca^{2+} and Ca^{2+}-dependent K^+ channels for neuronal network function. The modulation of different ion channel subtypes affects neuronal function and

causes thereby characteristic changes at the network level. Different modulators like aminergic and peptidergic transmitters often exert neuron- and synapse-specific effects. Modulators like tachykinins, in addition to short-term effects, also have effects on the cellular and network levels that are dependent on protein synthesis and last more than 24 h. In the lamprey network it is possible to bridge from the molecular and cellular to the behavioural level and predict what changes a modulation of a given type of ion channel in a given cell type will have on the network level.

In the analysis of this system, we have investigated the cellular and network properties experimentally, and explored their potential role by detailed mathematical modelling using model neurons with projection similar to their natural counterparts. These model neurons are connected as in the spinal cord. The simulation has greatly enhanced our insights into the mode of operation of the spinal locomotor system (Ekeberg *et al.* 1995).

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Role of hippocampal theta in temporal coding for location

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Conversion of firing rate to timing in the hippocampus

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The temporal coding hypothesis states that neurons encode information by the exact timing of spikes. An example of temporal coding is the hippocampal phase precession phenomenon, by which the timing of pyramidal cell spikes relative to the theta rhythm shows a unidirectional forward precession during spatial behaviour. Here we show that phase precession occurs in both spatial and non-spatial behaviours. Spike phase correlated with instantaneous discharge rate, and precessed unidirectionally at high rates, regardless of behaviour. The spatial phase precession phenomenon is therefore a manifestation of a more fundamental principle governing the timing of pyramidal cell discharge. We suggest that intrinsic properties of pyramidal cells play a key role in determining spike times, and that the interplay between the magnitude of dendritic excitation and rhythmic inhibition of the somatic region is responsible for the phase assignment of spikes.

Functional role of fast oscillations for perception and behaviour

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Cognitive functions like perception, attention, memory or language are based on highly parallel and distributed information processing by the brain. One of the major unresolved questions is how information can be integrated and how coherent representational states can be established in the distributed neuronal systems subserving these functions. It has been suggested that this so-called 'binding problem' may be solved in the temporal domain. The hypothesis is that synchronization of neuronal discharges can serve for the integration of distributed neurons into cell assemblies and that this process may underlie the selection of perceptually and behaviourally relevant information. Moreover, it has been suggested that fast oscillations at frequencies in the so-called gamma range (> 30 Hz) may help to entrain spatially separate neurons into synchrony and thus may indirectly promote the dynamic binding of neuronal populations. In accordance with these predictions, states characterized by synchronized gamma activity have been shown to be associated with functions like processing of coherent stimuli, perceptual discrimination, focused attention, short-term memory, or sensorimotor integration. Typically, the observed magnitude of gamma activity is positively correlated with increased 'processing load' and thus with the level of vigilance and attention, as well as with the difficulty or integrative nature of the processing. The talk will focus on the potential functional relevance of gamma oscillations for dynamic binding operations in sensory systems. The presentation will review experimental results, obtained in cats and humans, which support the notion that synchrony may indeed implement temporal binding and response selection. Moreover, data obtained in the mouse are discussed that shed light on potential mechanisms involved in the generation of synchronized gamma-band activity.