General Discussion

The research presented in this thesis aimed to gain more insight into genes and proteins involved in the generation of oscillations and in the spread of rhythmic activity. In chapter 2, we identified the quantitative traits that are potentially useful for identifying genes influencing hippocampal activity, by studying gamma oscillations and spontaneous activity in acute hippocampal slices from 8 distinct common inbred mouse strains. The heritability of more than 200 quantitative traits was derived from this activity. The findings showed that several traits of hippocampal gamma oscillations and spontaneous activity are heritable and, thus, potentially useful in gene-finding strategies. In chapter 3 we aimed to find mechanisms at the cellular/molecular level that might underlie the differences in network oscillations between strains explain differences. We showed that differences in frequency of fast network oscillations within in particular three out of the eight common inbred, mouse strains correlated well with GABAergic decay kinetics. Furthermore gene expression of GABA\(\alpha\)-receptor \(\beta2\) (Gabrb2) and \(\beta3\) (Gabrb2) subunits was higher in mouse strains with faster decay kinetics compared with mouse strains with slower decay kinetics. This indicated that differences in GABA\(\alpha\) receptor subunit composition in the hippocampus might influence the frequency of fast network oscillations in the hippocampus (chapter 3). In chapter 4 we studied which GABA\(\alpha\) receptor subunits contribute to fast network oscillations. We focused on the contribution of the \(\alpha1\) and \(\alpha2\) subunits, since these two subunits determine the kinetics and sub-cellular location of synaptic GABA\(\alpha\) receptor, and showed that the GABA\(\alpha\) receptor \(\alpha2\) subunit on CA3 pyramidal cell synapses is determining the frequency and amplitude of cholinergically-induced fast network oscillations. Therefore it is likely that GABA\(\alpha\) receptors with an \(\alpha2\) subunit provide the perisomatic inhibition essential for generating fast network oscillations. Finally in chapter 5, we show that modulation of GABAergic inhibition affects the frequency dependent spreading of excitation after white matter stimulation. In the current chapter we describe how these findings add to the existing models for the generation and spreading of high frequency oscillations.

**Inhibitory control of fast network oscillations**

Cholinergically induced oscillations are generated by a feedback mechanism between pyramidal cells and interneurons (Figure 6.1)(Fisahn et al., 1998, Mann et al., 2005). By activation of the muscarinic M1 receptor, pyramidal cells are depolarized and start firing action potentials (Fisahn et al., 2002), which activates interneurons. The inhibition
provided by the interneurons in turn suppresses pyramidal cell firing for a short time period. The amplitude and decay time kinetics of the inhibitory feedback determines the frequency and synchronization of the fast network oscillations (Whittington et al., 1995, Fisahn et al., 1998, Traub et al., 2000). Indeed the input to pyramidal cells as well as the field potential itself are largely shaped by inhibitory currents (Oren et al., 2006, Oren et al., 2010a), which in turn are largely being dictated by GABA<sub>A</sub> receptor subunit composition (Brussaard et al. 1997, 1999).

However, it was unknown which GABA<sub>A</sub> receptor subunits are involved in setting the oscillation frequency. In chapter 3 we show that differences in GABA<sub>A</sub> receptor kinetics caused by variations in genetic background are correlated with the frequency of fast network oscillations (Whittington et al., 1995, Fisahn et al., 1998, Traub et al., 2000). Indeed the input to pyramidal cells as well as the field potential itself are largely shaped by inhibitory currents (Oren et al., 2006, Oren et al., 2010a), which in turn are largely being dictated by GABA<sub>A</sub> receptor subunit composition (Brussaard et al. 1997, 1999).

Figure 6.1 Minimal model for the generation of gamma oscillations (A). Schematic diagram of minimal neuronal network (B). Time sequence of firing of CA3 pyramidal cells (CA3 PC) and perisomatic inhibitory cells (BC/AAC, basket and axo-axonic cells) during an oscillatory cycle. Shown in gray (C). Time sequence of the relative peak amplitude distributions of EPSCs (downward) and IPSCs (upward) during an oscillatory cycle. Population discharge of CA3 pyramidal cells drives the firing of perisomatic inhibitory cells, which subsequently temporarily silence the pyramidal cell population. The duration of the inhibitory current determines when the next gamma cycle begins. (Taken from Hajos & Paulsen 2009)

Whether GABA<sub>A</sub> receptor α2 subunits play a similar role in other models for gamma oscillations is not known, but it is likely. DHPG induces oscillations at a higher
frequency than carbachol and respond differently to zolpidem modulation (Palhalmi et al., 2004) suggesting that there might be a different GABA\textsubscript{\textalpha} receptors and/or interneuron involvement between glutamatergic and cholinergic induced oscillations. In contrast we found that zolpidem modulated cholinergically induced oscillations in the same direction as in DHPG induced oscillations described by Palhalmi et al (2004). While carbachol excites mainly pyramidal cells, DHPG also increases the excitability of interneurons (van Hooft et al., 2000), which might explain the higher frequency of DHPG induced oscillations. Both models for oscillations depend on AMPA and GABA\textsubscript{\textalpha} receptors (Palhalmi et al., 2004) and are generated in CA3, indicating that similar neuronal networks might be involved. Kainate-induced oscillations are independent of AMPA receptors and are probably generated by excitations of interneurons (Fisahn et al., 2004). However, kainate-induced oscillations in the presence of AMPA receptor blockers have a lower frequency, indicating that the slower kainate receptors provide the necessary excitatory drive. The fact that the firing of pyramidal cells and different interneuron types during carbachol and kainate-induced oscillations is similar indicates that apart from different activation, the neuronal involvement is also quite similar. As all these models for gamma oscillations depend on inhibition in the CA3 region it is likely that other in vitro models also depend on GABA\textsubscript{\textalpha} receptors with α2 subunits on CA3 pyramidal cells.

Whether this is also the case for in vivo gamma oscillations still has to be determined, although it has been shown that diazepam modulation of beta oscillations in awake and sleeping animals depends on the α2 subunit (Kopp et al., 2004). Gamma oscillations in vivo are generated at two different locations in the hippocampus. One oscillation is generated in CA3-CA1 while the other oscillation is generated in the dentate gyrus which depends on input from the enthorinal cortex (Bragin et al., 1995, Csicsvari et al., 2003). The current sink and source distribution and firing of pyramidal cells and interneurons in CA3 are similar between cholinergic induced oscillations and in vivo oscillations. Therefore, it is not unlikely that similar interneuron types and GABA\textsubscript{\textalpha} receptors are involved in the generation of in vivo gamma oscillation within CA3-CA1 as for the generation of oscillations in slices.

**Inhibitory control in spread of rhythmic cortical activity.**

Why can gamma oscillations only be generated in some brain regions? And how does rhythmic activity spread in a brain region that is not actively involved in rhythm generation? The first question might be answered by comparing the CA3 and CA1 region. Gamma oscillations in vivo and carbachol-induced oscillations in hippocampal slices are both generated in CA3 and not in CA1 (Csicsvari et al., 2003, Mann et al., 2005). By cutting the connections between CA3 and CA1 carbachol induces only oscillations in CA3 (Fisahn et al., 1998). We show in chapter 3 that CA1 pyramidal cells do not fire in phase with the oscillation and blockade of sodium channels or increasing GABA\textsubscript{\textalpha} receptor kinetics in CA1 does not affect the oscillations in CA1 (chapter 4). Therefore, we conclude that CA1 does not actively participate, but rather that the oscillations in CA1 merely reflect synaptic input coming from CA3.

What are the differences between CA3 and CA1 that might explain why CA3 is a
better oscillator? In contrast to CA1, the CA3 region contains extensive recurrent excitatory connections, which may provide the necessary excitation required for gamma oscillations. This idea is supported by the fact that other regions with high recurrent excitatory connections are able to generate oscillations, such as the neocortex and amygdale (Collins et al., 2001, Hajos et al., 2009). However, it might also be possible that the perisomatic feedback to pyramidal cells in CA1 is too short lasting to synchronize pyramidal cell activity. We found in chapters 3 and 4 that IPSC kinetics during fast network oscillations in CA1 were faster then in CA3 and that these currents are mediated via GABA\textsubscript{A} receptors with \(\alpha 1\).

In contrast to the spread of oscillations in the CA1 region, we show in chapter 5 that the spread of rhythmic input into the visual cortex is modulated by inhibitory input. Decreasing GABAergic inhibition has been shown to increase the spread of incoming activity (Contreras and Llinas, 2001), while increasing GABAergic inhibition reduces the spread of incoming activity (chapter 5). However, when the input comes in at high frequencies increased GABAergic activity enhances the spread and adaptation (chapter5). So how can the spread of incoming signals be enhanced by an increase in inhibition? Possible the enhanced inhibition by benzodiazepine modulation can be overcome by high frequency stimulations. This idea is supported by the fact that excitatory input from the thalamus and local pyramidal cells onto fast spiking cells in the cortex is depressing upon high frequency stimulation (Reyes et al., 1998, Gibson et al., 1999). On the other hand the excitatory input to low threshold spiking cells is facilitating and in addition inhibitory input from these cells in pyramidal cells is also facilitating (Reyes et al., 1998, Beierlein et al., 2003). A more detailed study on how benzodiazepine modulates the frequency-dependent adaptation at different synapses might shed more light on how GABAergic inhibition modulates the spread of incoming signals and which interneuron types are involved in this process.

**Cellular GABA\textsubscript{A} receptor distribution in hippocampus**

Although many studies show that most interneurons fire phase locked both to gamma oscillations in vivo and to fast network oscillations in slices (Hajos et al., 2004, Gloveli et al., 2005, Tukker et al., 2007), it is still not known which interneurons are essential for oscillations. With use of optogenetic techniques in the neocortex it has been shown that when spiking of parvalbumin expressing interneurons is largely prevented oscillations are reduced, while driving these interneurons increases oscillations (Cardin et al., 2009, Sohal et al., 2009). However several interneuron types express parvalbumin and in the interneuron involvement in the hippocampus might differ from the neocortex. We show in chapter 4 that GABA\textsubscript{A} receptor \(\alpha 2\) subunit on pyramidal cells determines the frequency of oscillations. As the expression of the \(\alpha\) subunit depends on the presynaptic interneuron, more knowledge on which synapses express the \(\alpha 2\) subunit will give more insight in which interneurons controls the frequency of oscillations. In CA1 synapses from both axo-axonic and CCK cells express \(\alpha 2\) subunits (Nyiri et al., 2001). These cell types could therefore be important for the frequency of fast network oscillations. Suppressing GABA release from CCK cells by activation of cannabinoid receptors (CB1) reduces gamma activity
(Hajos et al., 2000) and axo-axonic cells could effectively synchronize pyramidal cell firing by inhibiting the axon initial segments. Furthermore there might be differences in α subunit expression between CA1 and CA3. Paired recordings between interneurons and pyramidal cell in α2 benzodiazepine insensitive mice might give more information on which synapses express α2 subunits in CA3 and are thereby controlling the frequency of oscillations. More knowledge on which interneurons and GABAergic synapses are involved in neuronal oscillations might in turn help understand the mechanism by which polymorphisms affecting inhibition might underlie heritable variation between humans in oscillations and cognition.

Are polymorphisms affecting GABAergic inhibition involved in properties of neuronal oscillations in humans?
As GABAergic inhibition plays a fundamental role in the generation of many types of oscillations, it is likely that polymorphisms that affect GABA_A receptor-levels, function or -assembly, may have large impact on the occurrence of different oscillations in various brain areas and thereby affect cognition. We found in chapters 3 and 4 that differences in frequency of fast network oscillations in the hippocampus are correlated with GABA_A receptor kinetics. Whether this frequency difference is similar in the hippocampus in vivo and whether this difference in frequency underlies differences in and memory formation is not known. In humans, several polymorphisms in GABA_A receptor functioning have been associated with brain diseases in which neuronal oscillations are affected. Polymorphisms within introns of the gene coding for the α2 subunit, are associated with the power in the beta-band and with alcohol dependence (Edenberg et al., 2004), which is in agreement with our findings that the α2 subunit is important for fast network oscillations. Polymorphisms within other GABA_A receptor subunits, such as α1,α4,α5 and β1-β3 are associated with brain diseases such as schizophrenia and bipolar disorder (Petryshen et al., 2005, Craddock et al., 2010), which are paralleled with altered EEG activity in different bands (Lewis et al., 2005, Ozerdem et al., 2008, Ozerdem et al., 2010). The aggregate of these arguments underline the idea that GABA_A receptor polymorphisms affect GABAergic inhibition, and thereby neuronal oscillations and cognition.

Do GABA_A receptor subunits shape cognitive functioning?
As we observed a differential role for different GABA_A receptor subunits in fast network oscillations it is likely that they are also differentially involved in cognitive functions. Behavioral studies on GABA_A receptor knockouts has given little results on their role in behavior as these mutants are either lethal (γ2-/-)(Gunther et al., 1995) or show little deficits in behavior but show large up or down regulation of other subunits (α1-/-,β3-/-) (Sur et al., 2001, Kralic et al., 2002, Ramadan et al., 2003). From mice lacking the α2 subunit there is little behavioral data published except that they showed less activity than wild type mice (Boehm et al., 2004). However mice lacking the α5 subunit, which is mainly expressed in the hippocampus, improves learning and memory (Collinson et al., 2002). Furthermore, a partial inverse agonist for the α5 subunit improved performance in spatial learning tasks in rats (Chambers et al., 2003). These data suggests that the α5 subunit plays a large role in
memory formation. As this subunit is mainly expressed extrasynaptically by hippocampal pyramidal cells (Fritschy and Brunig, 2003, Prenosil et al., 2006, Glykys et al., 2008) it is not unlikely that they influence memory formation by regulating the power of gamma oscillations. In mice lacking the α3 subunit there was a deficit in pre-pulse inhibition of acoustic startle reflex (Yee et al., 2005), which is also observed in schizophrenia patients and has been suggested to depend on the dopaminergic system, which is controlled mainly by the α3 subunit (Fritschy and Mohler, 1995, Pirker et al., 2000).

The role of α1- and α2 subunits in EEG and cognitive functioning has been more clarified by the study of point mutations in each of these subunits making them insensitive to benzodiazepine modulation. The α1 subunit has been shown to be responsible for the sedative effects of benzodiazepines (Rudolph et al., 1999, McKernan et al., 2000), however the diazepam modulation of the EEG spectrum in the delta, theta and beta bands during sleep and the beta modulation in awake states were mediated by the α2 subunit (Tobler et al., 2001, Kopp et al., 2004). Other studies showed that the anxiolytic effects of benzodiazepine are mediated by the α2 subunit (Low et al., 2000). The role of different beta subunits is less well studied although there are indications that the β2 preferentially assembles in receptors with an α1 subunit, while the β3 subunit co-assembles with the α2 subunit (Mohler, 2006).

Although behavioral studies on α2 subunit involvement in cognition are largely lacking, there are many studies indicating that α2 containing GABA_A receptors are the most important synaptic inputs involved in synchronizing neurons at different frequencies and are likely to play a large role in many cognitive functions. It is especially striking that the role of the α1 subunit - although being the most abundant α receptor in the brain - in cognitive processes and synchronization of neuronal populations seems rather limited. A better understanding of different GABA_A receptor involvement in EEG activity during different behaviors might therefore help understanding how large populations of neurons synchronize their activity and how this affects cognition.