**ACADEMIC DISSERTATION**

# $GABA_A$ Receptor-Mediated Excitation in the Hippocampus of Adult and Newborn rats

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**Academic Dissertation**

*To be presented for public criticism, with the permission of the Faculty of Science, University of Helsinki, in the auditorium of the Division of Animal Physiology, Arkadiankatu 7, Helsinki, on October 7th , 2000 at 12 o'clock noon.*

**Helsinki 2000**

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## LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles, which are referred to in the text by their Roman numerals, and on unpublished results presented in the text.

(I)

Taira T., Lamsa K. & Kaila K. (1997) Posttetanic excitation mediated by $GABA_A$ receptors in rat CA1 pyramidal neurons. *Journal of Neurophysiology* 77: 2213-2218. http://jn.physiology.org/cgi/reprint/77/4/2213.pdf

(II)

Autere A.-M., Lamsa K., Kaila K. & Taira T. (1999) Synaptic activation of $GABA_A$ receptors induces neuronal uptake of $Ca^{2+}$ in adult rat hippocampal slices. *Journal of Neurophysiology* 81: 811-816. http://jn.physiology.org/cgi/reprint/81/2/811.pdf

(III)

Lamsa K. & Taira T. (2000) Use-dependent shift from inhibitory to excitatory $GABA_A$ action in interneurons induces 20-40 Hz oscillations in the hippocampus (manuscript).

(IV)

Lamsa K. & Kaila K. (1997) Ionic mechanisms of spontaneous GABAergic events in rat hippocampal slices exposed to 4-aminopyridine. *Journal of Neurophysiology* 78: 2582-2591. http://jn.physiology.org/cgi/reprint/78/5/2582.pdf

(V)

Palva J.M.*, Lamsa K.*, Lauri S., Rauvala H., Kaila K. & Taira T. (2000) Fast network oscillations in the newborn rat hippocampus in vitro. *Journal of Neuroscience* 20(3): 1170-1178. http://www.jneurosci.org/cgi/reprint/20/3/1170.pdf

(VI)

Lamsa K., Palva J.M., Ruusuvuori E., Kaila K. & Taira T. (2000) Synaptic $GABA_A$ activation inhibits AMPA/kainate receptor-mediated bursting in the newborn (P0-P2) rat hippocampus. *Journal of Neurophysiology* 83: 359-366. http://jn.physiology.org/cgi/reprint/83/1/359.pdf

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*I do not really consider this to be a masterpiece. I find it clumsy. I find it raw. Nevertheless, it has immeasurable value, I think, as a confrontation between a grad student and his own stubborn simplicity. I was dumb at school. Whatever the nature of that dumbness, it is with me still.*

Adapted from Kurt Vonnegut's "Palm Sunday".

**ABBREVIATIONS**

| | |
|---|---|
| AMPA | α-amino-3-OH-5-methylioxazolate-4-propionic acid |
| AMPA-R | AMPA-gated glutamate receptor channel |
| 4-AP | 4-aminopyridine |
| AP5 | DL-2-amino-5-phosphonopentanoic acid |
| BA | benzolamide |
| CA | carbonic anhydrase |
| CA3-CA1 | *cornu ammonis*, areas 1-3 |
| CNQX | 6-cyano-2,3-dihydroxy-7-nitroquinoxaline |
| CNS | central nervous system |
| ECS | extracellular space |
| EZA | ethoxyzolamide |
| EPSC, EPSP | excitatory postsynaptic current, potential |
| GABA | γ-aminobutyric acid |
| $GABA_AR$ | GABA-gated receptor channel (type A) |
| GDP | GABAergic depolarizing potential (in neonate) |
| GDPSP | GABAergic depolarizing postsynaptic potential (in adult) |
| HEPES | *N*-hydroxyethylpiperazine-*N´*-2-ethanosulfonic acid |
| IPSC, IPSP | inhibitory postsynaptic current, potential |
| LLD | long-lasting depolarization |
| LTD | long-term depression |
| LTP | long-term potentiation |
| LVA $Ca^{2+}$ channels | low-voltage activated calcium channels |
| NBQX | 6-nitro-7-sulfamoylbenzo[f]quinoxaline -2,3-dione |
| NMDA | *N*-methyl-d-aspartate |
| NMDA-R | NMDA-gated glutamate receptor channel |
| PB | pentobarbital |
| PiTX | picrotoxin |
| PSC | postsynaptic current |
| SGE | spontaneous GABAergic event |
| TMA | tetramethylammonium |
| VGCC | voltage-gated calcium channels |

## 1. ABSTRACT

$GABA_A$ receptor ($GABA_AR$) -mediated transmission is the major inhibitory transmitter mechanism in the mammalian brain. The solely inhibitory role of $GABA_A$-type transmission has, however, been challenged by recent findings showing that selective activation of GABAergic interneurons can promote rather than attenuate neuronal activity both in the adult and in the newborn rat hippocampus, where GABA is in fact assumed to be the major excitatory transmitter. The ionic mechanisms underlying the paradoxical excitatory actions of GABA in the pyramidal neurons are relatively well known. However, despite their importance to neuronal functioning, the physiological implications of GABAergic depolarization and subsequent excitation are poorly understood, giving rise to the following studies.

First, the role of $GABA_AR$-mediated depolarization in neuronal transmission was studied in pyramidal neurons in adult rat hippocampal slices using conventional extra- and intracellular recording techniques and ion-selective microelectrodes. Targeted high-frequency stimulation of dendritic GABAergic and glutamatergic afferents resulted in a triphasic depolarization/ hyperpolarization/sustained depolarization sequence in CA1 pyramidal cells. The late depolarization and the associated spike firing were accompanied by neuronal calcium uptake. These were blocked by the $GABA_AR$ antagonist picrotoxin and attenuated by manipulations of transmembrane ion gradients known to decrease GABAergic depolarization. These results suggest that GABAergic and glutamatergic mechanisms can act in concert to enhance neuronal excitation.

Next, the mechanisms and consequences of GABAergic depolarization were studied separately in CA3-CA1 interneurons and in pyramidal neurons. Here, spontaneous bursts of GABAergic interneuron network were pharmacologically induced in the continuous presence of glutamate receptor antagonists. Gramicidin-perforated patch recordings revealed that postsynaptic $GABA_A$ conductance easily gave rise to a positive shift in $GABA_A$ reversal potential and excitatory $GABA_A$ responses in CA3 interneurons. The inward current carried by $HCO_3^-$ was found to be necessary for the shift of $GABA_A$ responses to excitatory. Dependence on $GABA_A$ conductance and on availability of $HCO_3^-$ was also seen in the depolarizing shift of $GABA_A$ responses in pyramidal cells, although the depolarization was below firing threshold. In the interneuron network, GABAergic excitation was sufficient to generate and maintain intrinsic gamma (20-100 Hz) oscillations, thus providing a novel mechanism for synchronous interneuronal oscillations.

Finally, the role of $GABA_AR$-mediated transmission during the characteristic spontaneous neuronal bursting in the newborn hippocampus was examined. Despite its depolarizing nature, $GABA_AR$-mediated transmission was also shown to be a major inhibitory mechanism in pyramidal cells in the newborn hippocampus, while in interneurons GABA is likely to have an excitatory action. During the spontaneous bursts synchronous gamma frequency oscillation was seen in the CA3 interneuron network. The $GABA_AR$-mediated inhibition was also important in synchronizing the CA3 pyramidal cell firing into a coherent population oscillation at gamma frequencies. The fast glutamatergic (AMPA/kainate) transmission was found to provide most of the excitatory drive to pyramidal cells already at birth.

It is concluded here that in some widely used experimental models in adult hippocampus (e.g. tetanic afferent stimulation) $GABA_A$ depolarization generated in dendrites can provide pyramidal cell excitation, a finding that is of much relevance in studies on activity-induced neuronal plasticity and epileptogenesis. However, spontaneous $GABA_A$ depolarization only rarely induces spiking in pyramidal cells. In contrast, in the interneuronal network, $GABA_A$ excitation provides a mechanism for self-sustained synchronous oscillations (e.g. at gamma frequency). In the newborn hippocampus, $GABA_AR$-mediated inhibition is present already at birth, and the excitatory actions of GABA may mainly be restricted to interneurons. The synchronous network oscillations driven by glutamatergic excitation and restricted by $GABA_A$-type inhibition may be an important mechanism in shaping the developing synaptic contacts in the newborn hippocampus.

## 2. INTRODUCTION

In the adult mammalian brain cortex, as in the hippocampus, synaptic inhibition is mainly carried out by GABA- (i.e. γ-aminobutyric acid) mediated transmission. In principle, GABA activates two different classes of receptors: ionotropic and metabotropic. The ionotropic receptor is an anion (chloride-bicarbonate) channel, the dominant type of which is referred to as $GABA_A$. $GABA_B$ is a metabotropic G-protein coupled receptor (Bormann, 1988; Kaila, 1994; Barnard et al., 1998). In this study, the focus is on the ionophores that mediate most of the GABAergic transmission. Postsynaptic inhibition via $GABA_A$ receptors ($GABA_A$Rs) is mainly based on the decrease of membrane resistance that decreases excitability of the neuron. Concurrently, a slight hyperpolarization is generally seen in hippocampal neurons.

Recent studies have shown that $GABA_A$R-mediated hyperpolarizations may turn into depolarizations during intense receptor activation. In certain situations, the GABAergic depolarizations in hippocampal neurons have even been found to promote action potential firing. The shift of $GABA_A$ response from inhibitory to excitatory is produced by changes in the transmembrane anion gradients during strong activation of $GABA_A$ receptor channels (Thompson and Gähwiler, 1989a, 1989b; Staley et al., 1995; Kaila et al., 1997; Perkins, 1999). Studies in pyramidal cells have shown that depolarizations are most likely generated in dendritic arbors where density of $GABA_A$ receptors is thought to be high as compared with cytoplasmic volume (Alger and Nicoll, 1979, 1982; Grover et al., 1993; Staley et al., 1995; Jackson et al., 1999a), suggesting that morphology of the neuron and location of synaptic inputs may be critical factors for the change in GABA response. GABAergic excitation is known to occur in CA3-CA1 pyramidal cells as well as in a subset of hilar interneurons (Michelson and Wong, 1991, 1994; Grover et al., 1993; Forti and Michelson, 1998). While ionic mechanisms underlying $GABA_A$ depolarization have been extensively studied, significance of the GABA response lability in neuronal networks of *cornu ammonis* remains unclear (Grover et al., 1993; Staley et al., 1995; Kaila et al., 1997; Perkins, 1999; Smirnov et al., 1999, Staley and Proctor, 1999). However, it seems *a priori* evident that dynamic changes in the $GABA_A$R-mediated inhibition have important physiological consequences. In particular, use-dependent weakening of postsynaptic inhibition is likely to play a role in the induction of changes in synaptic strength (e.g. long-term potentiation; LTP) and during epileptic activity (Wigström and Gustafsson, 1985; Merlin and Wong, 1993). Besides its fundamental inhibitory function, $GABA_A$R-mediated hyperpolarization may be essential for synchronization of the neuronal network oscillations at gamma (20-100 Hz) frequency range. Gamma oscillations in the adult brain may be used for the timing and co-ordination of neural activity, and they have been implicated in various cognitive functions (for review Freund and Buzsaki, 1996; Traub et al., 1998). Given the recent excitement about the mechanisms of hippocampal gamma oscillations, surprisingly little attention has been paid to the possible role of excitatory GABA action in this phenomenon (see Bracci et al., 1999).

At an early postnatal age, interneurons and pyramidal cells in the rat hippocampus show solely depolarizing responses to $GABA_A$ receptor activation (Fiszman et al., 1990; Leinekugel et al., 1995; Rivera et al., 1999). This is due to fundamentally different regulation of intracellular anion content in neonatal neurons as compared with mature nervous cells (Rivera et al., 1999). Yet, the role of $GABA_A$-type transmission in the newborn hippocampal networks is enigmatic. Both excitatory and inhibitory effects of GABA have been reported in newborn rat hippocampal neurons (Garaschuk et al., 1997; Khazipov et al., 1997; Lei-

nekugel et al., 1997; but see Daval and Sarfati, 1987; Dailey and Smith, 1994; Hollrigel et al., 1998; Psarropoulou and Avoli, 1999). However, it is generally suggested that while in the adult hippocampus GABA carries out critical inhibition, at neonatal age it may be the major excitatory transmitter (for review Ben-Ari et al., 1997) and the neuronal circuits have been thought to operate without fast synaptic inhibition (Holmes and Ben-Ari, 1998; see O`Donovan, 1999). Despite the obvious importance of fast synchronous network oscillations for brain functioning, no attempts have been made to elucidate their early history and developmental mechanisms. Apart from their role in the adult brain, synchronous neuronal oscillations are widely suggested to be important for the refinement of synaptic contacts during development (Hanse et al., 1997). Therefore, the last part of the study focused on the spontaneous rhythmic activity during the early postnatal period when GABA has been proposed to be an excitatory transmitter.

To understand the role of depolarizing GABA responses in the functioning of hippocampal networks, studies were done using three experimental models. First, GABAergic afferents of CA1 pyramidal cells were electrically stimulated by tetanic high-frequency (100-200 Hz) trains. Most studies on depolarizing GABA responses in mature hippocampus have been done in this way. Further, this procedure is generally used in studies of hippocampal plasticity to induce long-term changes in transmission efficacy.

In the second model, $GABA_A$R-mediated depolarizing responses were evoked by application of a convulsant compound, 4-aminopyridine (4-AP). The action of 4-AP is based on generally enhanced transmitter release from presynaptic terminals (Tapia et al., 1985) that results in spontaneous population bursts in the GABAergic network (Michelson and Wong, 1991). In these studies, particular attention was paid to the function of interneurons during network-driven GABAergic events.

Finally, the properties of GABAergic transmission were studied in the newborn rat hippocampus, where the $GABA_A$R-mediated responses are known to be depolarizing until the second postnatal week (Gaiarsa et al., 1991; Rivera et al., 1999). This thus offered a truly physiological context for examining the role of depolarizing GABA responses.

## 3. REVIEW OF LITERATURE

### 3.1. Basic synaptic connectivity in the hippocampal CA3-CA1 area

Ramon y Cajal noticed over a century ago (1893) that neurons constituting any cortical area are far from being uniform with regard to their morphology and connectivity, suggesting that they possess the capacity to interact with each other in a complex and diverse manner. A century after the anatomical classification of “pyramidal” and “nonpyramidal” cells, we have a rather detailed picture about chemical signaling between hippocampal neurons and much knowledge on the functioning of hippocampal networks *in vitro* as well as *in vivo*.

#### *3.1.1. Excitatory glutamatergic synapses*

Pyramidal cells are the principal excitatory neurons in the hippocampal CA3-CA1 region. These glutamatergic neurons form projective intrahippocampal pathways as well as outputs from the hippocampus to e.g. subcortical structures and the entorhinal cortex. The pyramidal cell somas are arranged in a layer called the *stratum pyramidale*, which is a 2- to 3-cell-thick sheet of cell bodies. In the CA3 area, pyramidal cell axons give rise to extensive recurrent arborizations to other pyramidal cells of the same region, which makes the CA3 area prone to epileptic seizures. However, the major output of CA3 pyramidal cells, Schaffer collaterals, projects onto CA1 pyramidal cells and interneurons.

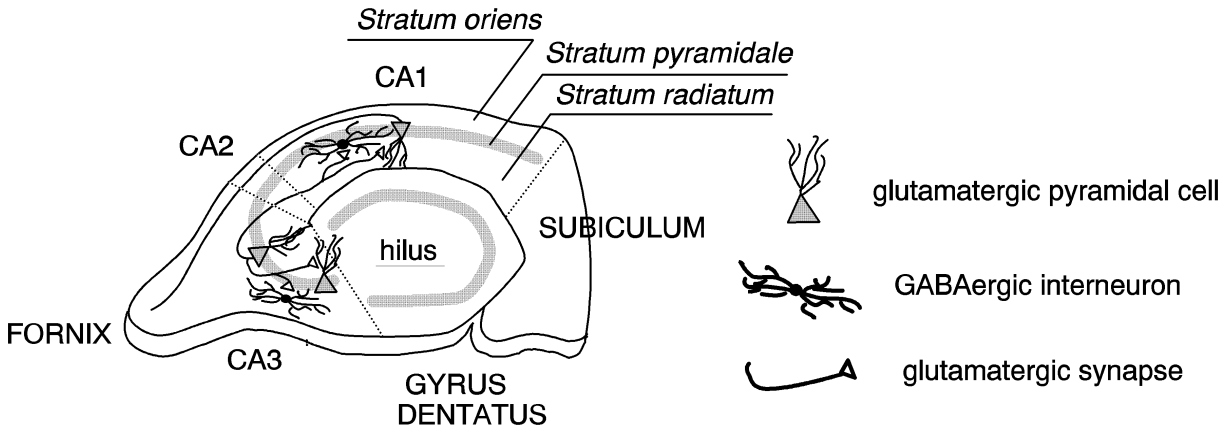



**Figure 1:** Transverse section throughout the hippocampus and schematic representation of basic CA3-CA1 circuitry. Hippocampal formation consists of different sections; *subiculum*, *gyrus dentatus* and *cornu ammonis* (CA). The *cornu ammonis* is divided further into three different regions CA3-CA1. Laminar organization of glutamatergic principal cell somas to the *stratum pyramidale* layer in CA3-CA1 area is depicted as gray. Apical dendrites of the principal neurons (pyramidal cells) ramify in the *s.radiatum* and their basal dendrites as well as axonal arbors project mainly to the *s.oriens*. Connections from CA3 pyramidal cells to other neurons of the same region as well as projections to CA1 pyramidal cells and interneurons (Schaffer collaterals) are schematically illustrated.

Transmission in glutamatergic synapses uses basically two types of ionotropic receptors, AMPA-Rs and NMDA-Rs, which are distinguishable by their pharmacological and biophysical properties. The AMPA receptors mediate rapid excitatory signaling and the fast excitatory postsynaptic potential (EPSP) in hippocampal neurons. AMPA receptors are cation channels mostly permeable to $Na^+$ and $K^+$, which have a rapid desensitization that practically determinates fast decay of excitatory postsynaptic currents (EPSCs) (Sarantis et al., 1993). $Ca^{2+}$ permeability of AMPA receptors varies by receptor subunit composition, but is generally low (Hollmann et al., 1991). NMDA-Rs are mainly calcium channels, since their permeability is 5-10 times higher for $Ca^{2+}$ ions than for $Na^+$ and $K^+$ (Mayer and Westbrook, 1987). Further, in physiological solution conductance of the channel is strongly voltage-dependent. At hyperpolarized potentials, NMDA receptor channels are to a large extent blocked by extracellular $Mg^{2+}$. At resting membrane potential, NMDA-Rs conduct poorly despite the presence of glutamate, but when the cell is depolarized, the $Mg^{2+}$ block ceases and conductance of the receptor channel increases (Nowak et al., 1984). Although a single synapse may express both types of transmission, previous studies have suggested that most connections in early postnatal hippocampus have functional NMDA-type receptors only (Durand et al., 1996; see Isaac et al., 1997).

### *3.1.2. Inhibitory GABAergic interneurons*

Towards the end of the 1950`s, γ-aminobutyric acid (GABA) was suggested to act as a physiological inhibitory substance in the brain. The GABA-mediated inhibition theory was based on two findings: application of GABA strongly suppressed electrical activity in the mammalian nervous system, and large amounts of GABA were found only in the inhibitory axons (Hayashi, 1959; Kravitz et al., 1963). The role of GABA as a classical transmitter in the nervous system was established by experiments showing that GABA perfectly mimicked the effect of stimulus-evoked inhibition (Bazemore et al., 1957;

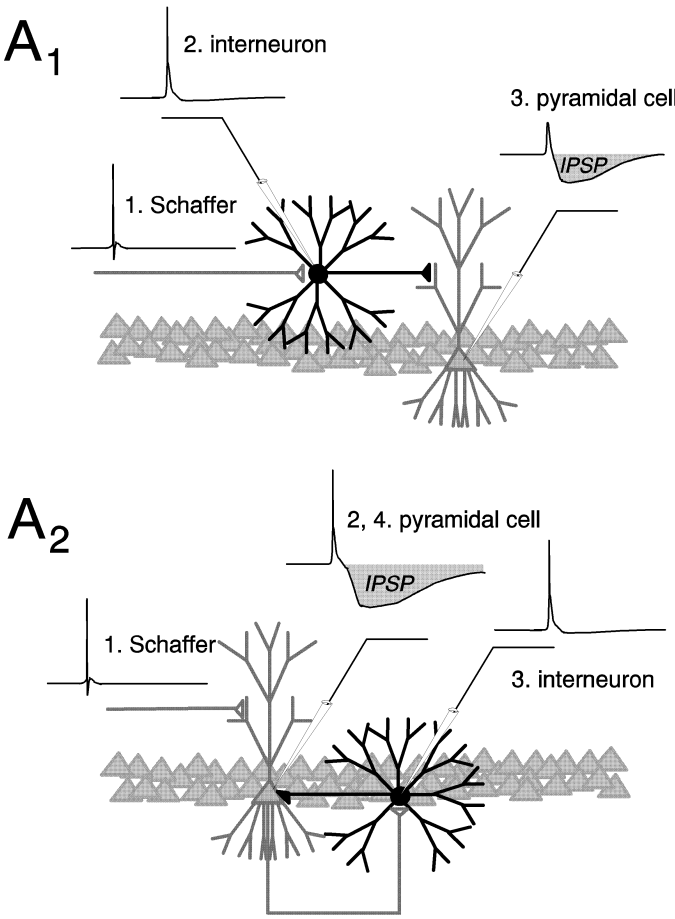



**Figure 2:** Two basic types of functional inhibition of principal cells in the hippocampal CA3-CA1 region. (*A1*) Feed-forward inhibition; action potential firing of a glutamatergic Schaffer collateral (1) that terminates on an interneuron (as well as on a pyramidal cell) elicits EPSP and action potential firing (2) in the target cell. Consequently, the activated GABAergic interneuron evokes IPSP (3) in the local pyramidal cell. (*A2*) Feed-back inhibition; the activated Schaffer collateral (1) excites its target (2) pyramidal cell. This, in turn, conveys excitation to the local GABAergic interneuron (3), and discharge of the interneuron feeds back IPSP to pyramidal cells of the area (4).

Kuffler and Edwards, 1958; Takeuchi and Takeuchi, 1966, 1967a, 1967b). Postsynaptic GABAergic inhibition results from activation of two different types of receptors: ligand-gated anion channels and metabotropic G-protein coupled receptors, the latter of which mediates slow potassium currents (Bormann, 1988). These two postsynaptic inhibitory mechanisms are referred to as $GABA_A$ and $GABA_B$, respectively. The novel, functionally and pharmacologically less well-characterized but controversial subclass of receptors, $GABA_C$, will not be dealt with here (see Barnard et al., 1998).

In the hippocampus, GABAergic neurons are mainly intrinsic cells, commonly referred to as interneurons. Electrophysiological properties of these neurons are typically different from those of pyramidal cells (for review see Freund and Buzsaki, 1996; Parra et al., 1998). For instance, most of them are able to fire action potentials at a very high frequency (up to several hundred Hz), having little adaptation of discharge during sustained depolarization. Interneurons are also far less numerous than principal neurons, comprising only 10-20% of the total number of hippocampal neurons. However, via extensive arborization, a single GABAergic nervous cell may contact 1000-3000 pyramidal cells (Li et al., 1992; Buhl et al., 1994). Anatomical variability of interneurons has been apparent since the days of Ramon y Cajal, but their electrophysiological properties are also diverse (Buhl et al., 1994; Miles et al., 1996; Parra et al., 1998; see Freund and Buzsaki, 1996). Parra et al. (1998) have described 16 different morphological types, three different firing models, and 25 different combinations of the most common neurotransmitter receptors, suggesting that at least 52 types of interneurons are contained within the CA1 region alone. In general, interneurons having their somas in the *stratum*

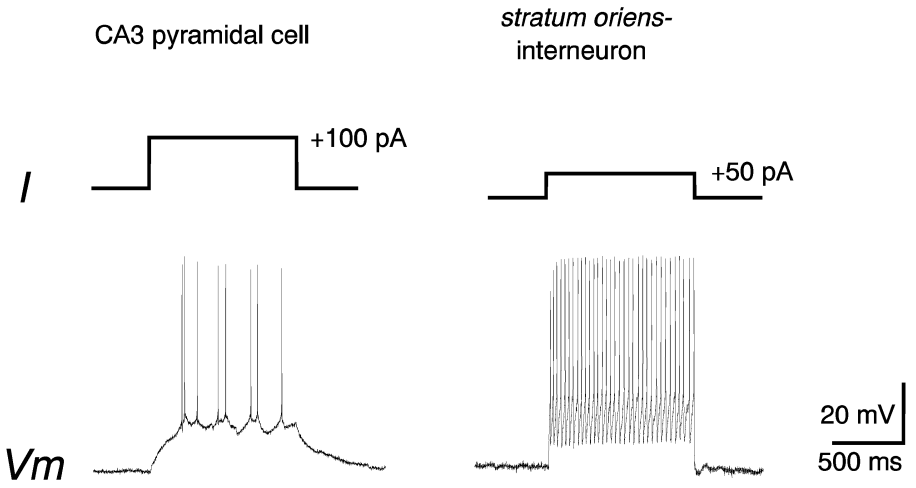



**Figure 3.** Electrophysiological properties of pyramidal cells and interneurons are typically different (recordings by K. Lämsä, unpublished). Membrane potential (*Vm*) responses of a CA3 pyramidal cell and a *stratum oriens*- interneuron to depolarizing current (*I*) pulses (100 pA and 50 pA, respectively). Interneurons often have a low firing threshold and weak attenuation of action potential frequency during sustaining depolarization. Note also the stronger response of the interneuron to the current pulse because of a higher input resistance (See Freund and Buzsaki, 1996).

*pyramidale* or *s. oriens* have efferents that ramify horizontally into the *pyramidal-oriens* layer, whereas interneurons in the *s. radiatum* may project their axons solely to the *radiatum* or through all three layers (Parra et al., 1998). The former group of interneurons consists mainly of *basket cells* and *axo-axonic* cells that heavily innervate the perisomatic region of pyramidal cells. Other interneurons terminate mostly on pyramidal cell dendrites (Miles et al., 1996). This suggests that perisomatic and dendritic inhibitory cells may also have functionally different roles in inhibition of the glutamatergic principal cells (Miles et al., 1996; Paulsen and Moser, 1998). Further, interneurons target not only pyramidal cells, but also other interneurons, forming an extensive GABAergic network (Gulyas et al., 1996). Moreover, interneurons that contact only other interneurons have been found (Acsady et al., 1996).

Functionally, the interneuron network provides two basic types of inhibition of pyramidal cells in the CA3-CA1 region. *1 )* Firing of CA3 pyramidal cell efferents directly excite the CA1 inhibitory neurons, which in turn "feed forward" the CA1 principal cells. By definition, this di-synaptic connection from the CA3 area is called *feed-forward inhibition* of pyramidal cells (Alger and Nicoll, 1982; Buzsaki, 1984). *2)* In the feed-back mechanism, excitation from the CA3 region first recruits CA1 pyramidal cells, whose excitatory output is "fed back" to the local inhibitory GABAergic neurons through axon collaterals (Andersen et al., 1964). Then, discharge of these interneurons recurrently inhibits the CA1 pyramidal cells, including those that had initially activated the interneurons. In general, direct stimulation of interneurons is more likely to induce feed-forward inhibition, since firing threshold of those interneurons is very low (Alger and Nicoll, 1982; Buzsaki, 1984). By contrast, more selective activation of the *feed-back (recurrent) inhibition* is achieved by antidromic stimulation of CA1 pyramidal cells and their axon collaterals from the *stratum oriens* (Alger and Nicoll, 1982). Most of the recurrent inhibition is probably mediated by perisomatic innervation of pyramidal cells via basket cells and axo-axonic interneurons that have their somata in the pyramidal layer and *s.oriens* (Parra et al., 1998). Figure 2 illus-

trates the inhibition of pyramidal cells in the CA3-CA1 area.

*3.1.3. Synaptic connectivity in newborn rat hippocampus*

While excitatory glutamatergic synapses made by CA3 pyramidal cells onto CA1 neurons are among the most extensively studied in the adult brain, only recently has their early development received focused attention. During the first postnatal days in the rat hippocampus, CA3 pyramidal cell dendrites and axons have simple morphology with very limited ramification and few branches. However, some distant projections do reach the CA1 region. At this age, the CA3-CA1 areas mostly lack the diagnostic morphological correlates of mature glutamatergic synapses, but multiple varicocities of the axons may represent functional synaptic contacts (Durand et al., 1996; Gomez-di Cesare et al., 1997; Hsia et al., 1998). During the first postnatal week, recurrent axon collaterals of CA3 pyramidal cells and their projections to the CA1 region undergo marked growth in length and branching (Bayer, 1980; Gomez-di Cesare et al., 1997). Studies in the cortex during early development have shown that these processes are largely driven by activity-independent mechanisms of growth, but neuronal activity is likely to take over as the predominant process that drives development of the initially coarse network into finely tuned neuronal circuits (for review Goodman and Shatz, 1993; see also Verhage et al., 2000).

At birth, most of the glutamatergic synapses in the hippocampal CA3-CA1 region are silent at resting membrane potential since they contain only functional NMDA receptors (Durand et al., 1996; Petralia et al., 1999). Single-pulse electrical stimulation of CA3-CA1 pathways elicits practically no AMPA-type response before the second postnatal day (P2) (Durand et al., 1996; Hsia et al., 1998; Tyzio et al., 1999; see Liao and Malinow, 1996). Paradoxically, pharmacological studies suggest an active role for AMPA-type transmission in hippocampal network functioning already at the perinatal stage (Bolea et al., 1999; Diabira et al., 1999; Khalilov et al., 1999a, 1999b). Despite the first postnatal week being
a period of intense development of excitatory glutamatergic circuitry in the hippocampus, mechanisms controlling pyramidal cell activity during this phase have received little attention.

In the hippocampus, as well as in various other areas of the brain, GABAergic interneurons appear to mature earlier than glutamatergic principal cells. In the hippocampal dentate gyrus, GABAergic neurons arise prenatally, whereas 80% of the glutamatergic neurons are generated after birth (Schlessinger et al., 1975; Soriano et al., 1989). Thus, most of the synaptic activity and functional connections in a newborn rat neocortex and hippocampus are presumably $GABA_AR$ mediated (Owens et al., 1999; Tyzio et al., 1999; see Hollrigel and Soltesz, 1998). While the threshold for electrically stimulated synaptic responses may be relatively high in the hippocampus during the first postnatal days (see Swann et al., 1989), the high spontaneous occurrence of action potential-dependent IPSPs in immature neurons indicates well-developed $GABA_A$-type synaptic circuitry. Many of the biochemical markers of GABAergic synaptic transmission are present only in low amounts in the brain at birth (Coyle and Enna, 1976; Skerritt and Johnston, 1982), but this is the case with other synaptic transmitter mechanisms as well (Wong and McGeer, 1981).

During the course of development, silent glutamatergic synapses are possibly transformed into functional ones through an LTP-like mechanism that involves induction of AMPA-type responses. Consistent with this hypothesis is the finding that the ratio of AMPA-R- to NMDAR-mediated synaptic currents increases remarkably during development (Hsia et al., 1998; but see Liao and Malinow, 1996). Indeed, Durand et al. (1996) demonstrated that repetitive stimulation of CA1 glutamatergic afferents combined with postsynaptic depolarization caused induction of AMPA-type responsiveness in connections initially displaying

only NMDA-type activity (see also Hanse et al., 1997).

### 3.2. Inhibition of hippocampal neurons via $GABA_A$ receptors

The dendrites, cell bodies, and the initial segment of axon of each principal cell in cortical structures are innervated by efferents of GABAergic inhibitory interneurons. Moreover, interneurons are heavily interconnected via GABAergic synapses. Fast GABAergic transmission is mediated by the ligand-gated ion channels, the $GABA_A$ receptors. Inhibition mediated by the GABA ionophores is examined here in two parts.

#### *3.2.1. Effect on input conductance*

The finding that $GABA_A$ receptor activation brings about vast anion conductances on the postsynaptic membrane while the potential may remain close to resting level has been central for understanding of the mechanism of GABAergic inhibition (Kuffler, 1958; Takeuchi and Takeuchi, 1967a, 1967b). $GABA_AR$ activation generates high conductance for chloride (and bicarbonate), which counteracts the action of excitatory currents to depolarize the cell (for review Kaila, 1994). The effect of any excitatory current on membrane potential ($V_m$) will be inversely related to the membrane input conductance according to Ohm`s law

$$\Delta Vm = \frac{I}{g_{input}} \qquad (3\text{-}1)$$

,where $\Delta V_m$ corresponds to the amplitude of postsynaptic potential, $g_{input}$ is cell input conductance, and $I$ is the synaptic net current. A $GABA_AR$-mediated increase in $g_{input}$ reduces $\Delta V_m$, generated by the excitatory current. Anion "leakage" of the membrane also decreases electrotonic propagation of the synaptic potentials. Passive spread of the EPSPs from dendrites (input site of most excitatory synapses) to the soma (action potential initiation site) is effectively attenuated when the spreading current is able to leak out through $GABA_AR$ channels. Thus, length constant of the membrane is decreased, which has the same result as an increase in the distance of the dendritic synapse from the soma (assuming a purely passive dendritic membrane). Inhibition mediated by increase in input conductance is called shunting. The inhibitory conductance is determined by permeability for the anions (i.e. number of $GABA_A$ receptors opened) and their extra- and intracellular concentrations. When the transmembrane ion concentrations are unequal, the conductance of a voltage-insensitive non-rectifying channel is larger when ions flow away from the more concentrated side (Goldman-Hodgkin-Katz rectification, see Hille, 1984).

Shunting is effective, even if $GABA_AR$-mediated potentials are depolarizing. For instance, in principal cells of dentate gyrus and neocortical pyramidal neurons, *in vitro* $GABA_AR$-mediated inhibitory responses are slightly depolarizing. Shunting of EPSP on the pyramidal cell soma (by basket cells) and close to the initial segment (by axo-axonic interneurons) can effectively control the temporal firing pattern of the neuron (Qian and Sejnowski, 1990; Miles et al., 1996). Since a single *stratum oriens* interneuron may terminate to hundreds of principal cells, inhibition via $GABA_A$ synapses is capable of a large-scale orchestration among glutamatergic neurons. Further, local shunting by dendritic $GABA_A$ synapses can strongly impede propagation of EPSPs from the dendritic branches to the soma. Since dendrites constitute the major input site to excitatory glutamatergic synapses, selective activation of $GABA_A$ synapses may produce compartmentalization of the dendritic tree into "effective and ineffective input pathways" for the somatic integration of EPSPs.

#### *3.2.2. Effect on membrane potential*

While shunting is the crucial aspect of $GABA_AR$-mediated inhibition decreasing ef-

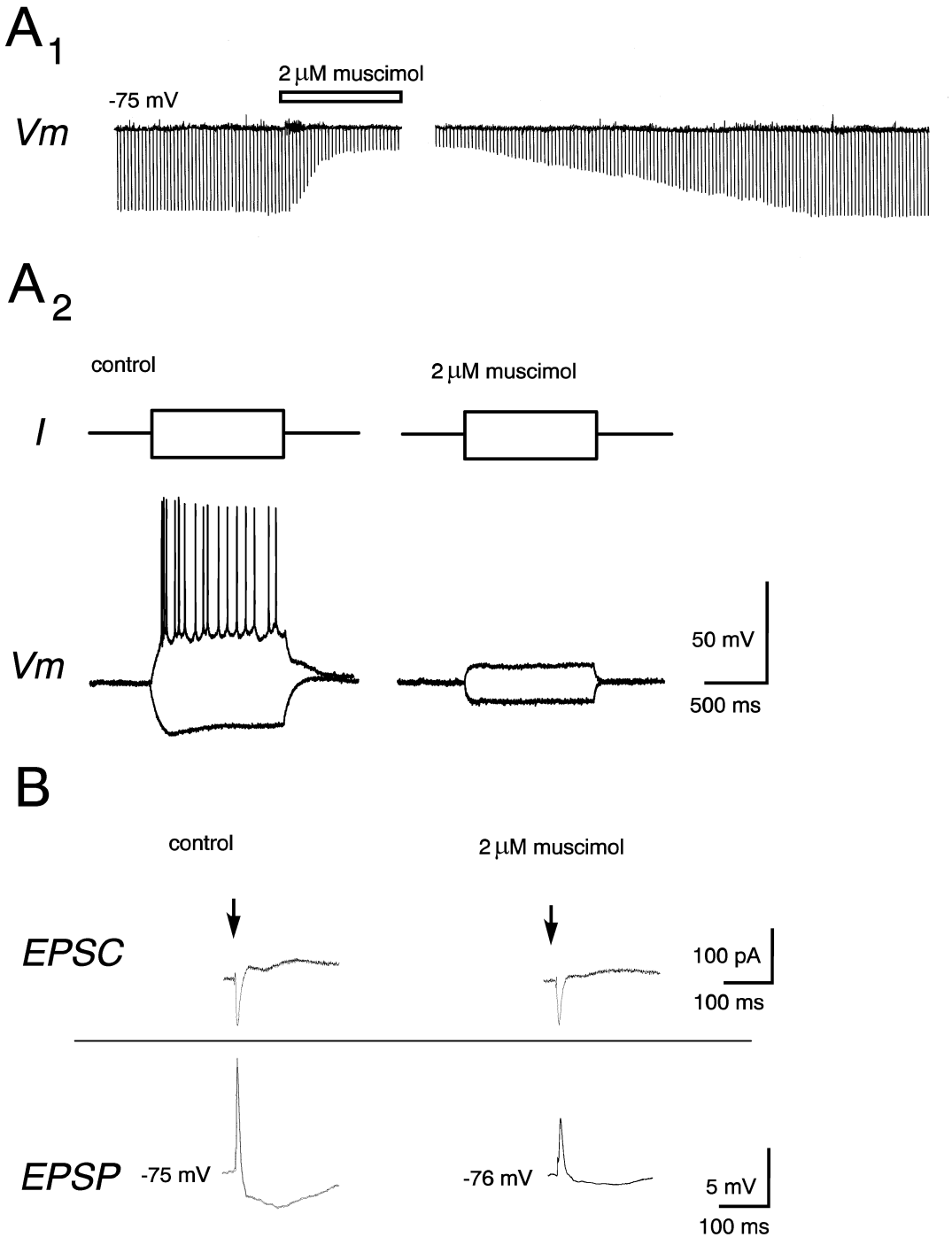



**Figure 4:** Input resistance and excitability of hippocampal neurons decrease during $GABA_A$ receptor activation while membrane potential remains unchanged (recordings by K. Lämsä, unpublished). **A)** ($A_1$) Decrease of input resistance in a CA3 neuron by exposure to $GABA_AR$ agonist muscimol is seen as diminished membrane potential (*Vm*) shift to a hyperpolarizing current step (-0.1 nA pulses with 5 s interval at $E_{GABAA}$). ($A_2$) Demonstration of the drop of excitability in the same neuron. Membrane potential (*Vm*) responses to ± 0.1 nA inputs (see Krjnevic, 1976). **B)** Shunting inhibition of synaptically activated glutamatergic EPSPs in a CA3 neuron. Responses to single-pulse electrical stimulus from *gyrus dentatus*. Inward EPSCs (upper traces) and EPSPs in control conditions and after exposure to $GABA_AR$ agonist muscimol (recordings close to $E_{GABAA}$) (Avoli and Perreault, 1987; Staley and Mody, 1992).

fectiveness of excitatory inputs, in most of the hippocampal neurons $GABA_AR$ activation has been shown to actually result in hyperpolarization. Then, the inhibitory action is due both to the effect of the GABAergic conductance and the negative shift of membrane potential. Polarity of the GABAergic inhibitory postsynaptic potentials (IPSPs) is determined by the difference between $GABA_A$ current reversal potential ($E_{GABAA}$) and membrane potential. When $E_{GABAA}$ is negative to $V_m$, net current through the $GABA_A$ receptor channel is outward, and IPSPs are hyperpolarizing.

Since the $GABA_AR$ channel is permeable for two physiological anions, namely $Cl^-$ and $HCO_3^-$ (Bormann et al., 1987; Kaila, 1994), reversal potential of the $GABA_A$ current is determined by

$$E_{GABAA} = -\frac{RT}{F} ln \frac{P_{Cl}[Cl^-]_o + P_{HCO3}[HCO_3^-]_o}{P_{Cl}[Cl^-]_i + P_{HCO3}[HCO_3^-]_i} \quad (3\text{-}2)$$

,where *P* represents relative permeability of the two ions (1 vs. 0.2-0.3, respectively).

Active extrusion of $Cl^-$ from the cytosol with a $K^+/Cl^-$ -cotransporter maintains a more negative chloride reversal potential ($E_{Cl}$) than resting membrane potential ($E_m$) in mature hippocampal pyramidal cells (Thompson and Gähwiler, 1989b; Rivera et al., 1999). Although bicarbonate reversal potential ($E_{HCO3}$) is more positive than $E_m$, high permeability of $GABA_AR$s to chloride vs. bicarbonate results in an outward, hyperpolarizing net current via $GABA_A$ receptors, except for cells with a strongly negative membrane potential (e.g. Kaila et al., 1993). However, during early postnatal development, $GABA_AR$-mediated responses are often depolarizing (Ben-Ari et al., 1989; Fiszman et al., 1990; Owens et al., 1996). This is due to inwardly directed net transport of chloride and thus elevated levels of intracellular $[Cl^-]$ in immature neurons (Rohrbough and Spitzer, 1996). In the rat hippocampus, hyperpolarizing $GABA_A$ responses appear during the second postnatal week (Ben-Ari et al., 1989). The ontogenic change in $GABA_AR$-mediated responses from depolarizing to hyperpolarizing is due to developmental induction of the expression of the neuronal $Cl^-$-extruding $K^+/Cl^-$ -cotransporter, KCC2 (Rivera et al., 1999). Via hyperpolarizing IPSPs, rhythmic discharge of single interneurons is able to impose a phase-locked membrane potential oscillation in its target cells. This kind of synchronous oscillation is known to entrain coherent action potential firing among CA3-CA1 glutamatergic principal cells (Cobb et al., 1995; Traub et al., 1998).

### 3.3. Generation of excitatory $GABA_A$ responses in mature hippocampal neurons

#### *3.3.1. Principal cells vs. interneurons*

Work on hippocampal slices has uncovered an increasing number of situations where initially hyperpolarizing postsynaptic $GABA_A$ responses shift to depolarizing during prolonged GABA release (Alger and Nicoll, 1982; Michelson and Wong, 1991; Xie and Smart, 1991; Avoli and Perreault, 1992; Davies and Collingridge, 1993; Grover et al., 1993; Staley et al., 1995; Kaila et al., 1997). In mature hippocampus, excitatory $GABA_A$ responses have been reported in CA3-CA1 pyramidal cells and, e.g., in interneurons in the hilar region (Alger and Nicoll, 1982; Michelson and Wong, 1991; Grover et al., 1993). However, important differences can exist between different cell types in this respect. Studies in hippocampal pyramidal cells have revealed that depolarizing $GABA_A$ responses are most likely generated in dendrites. Even strong activation of perisomatic GABAergic synapses is not very effective in eliciting $GABA_A$ depolarization in pyramidal cells (Alger and Nicoll, 1979, 1982; Wong and Watkings, 1982; Jackson et al., 1999a). In general, a positive shift in $GABA_A$ response is dependent on intensity of $GABA_AR$ activation: 1) high concentrations of the transmitter tend to produce more depolarizing responses, whereas low concentrations result in only hyperpolarizations (Alger and

Nicoll, 1979; Andersen et al., 1980). In line with this, 2) electrical stimulation of GABAergic synapses produces more depolarizing responses with increased stimulus intensity (Grover et al., 1993; Kaila et al., 1997; Cobb et al., 1999). The shift in $GABA_A$ response polarity is associated with a large $GABA_AR$-mediated conductance. A dissipation of the $Cl^-$ gradient and the $GABA_A$ current driving force in response to prolonged conductance might well explain the loss of hyperpolarizing responses (Thompson and Gähwiler, 1989a, 1989b). Nevertheless, such an explanation cannot account for the reversal in polarity of $GABA_AR$-mediated potential (Kaila et al., 1990).

The experiments of Michelson and Wong (1991, 1994) were the first to address mutual communication of interneurons during strong neuronal population bursts in the hippocampus. These studies showed that in *gyrus dentatus* $GABA_A$ depolarization was more likely generated in interneurons than in glutamatergic principal cells. During enhanced synaptic GABA release, excitatory GABAergic potentials were generated in interneurons. Simultaneously, in principal cells only prolonged, large-amplitude hyperpolarizing IPSPs were seen. In CA3-CA1 pyramidal cell dendrites, $GABA_A$ excitation can emerge during relatively short (few hundred millisecond) bursts of local interneuron population (Xie and Smart, 1991; Perreault and Avoli, 1992; Grover et al., 1993; Kaila et al., 1997; Cobb et al., 1999). To date no data exist on depolarizing $GABA_A$ responses in *cornu ammonis* interneurons.

### *3.3.2. Mechanisms*

Different hypotheses regarding the transformation of initially hyperpolarizing $GABA_A$ responses to depolarizations have been put forth. First, pyramidal cells were suggested to maintain a lower intracellular $[Cl^-]$ in soma than in their dendrites. This theory was based on the assumption that the chloride uptake mechanism is mainly localized in the dendrites, whereas outward transport occurs in the soma (Andersen et al., 1980; Misgeld et al., 1986; Hara et al., 1992). Yet, experimental data have indicated that differential $Cl^-$ distribution between soma and dendrites is improbable. In several studies, both hyperpolarizing and depolarizing responses have been evoked by activation of $GABA_ARs$ in pyramidal cell dendrites (Alger and Nicoll, 1982; Wong and Watkins, 1982; Lambert et al., 1991).

$E_{GABAA}$ is maintained in a "hyperpolarizing" mode in pyramidal cells by active outward transport of $Cl^-$. $E_{Cl}$ may become equal to membrane potential if there is sufficient chloride conductance through which $Cl^-$ can passively redistribute itself. This activity-induced reduction in the electrochemical gradient of the $Cl^-$ ion is due to intracellular accumulation of $Cl^-$ (Thompson et al., 1988; Thompson and Gähwiler, 1989b). Nevertheless, passive dissipation of $Cl^-$ gradient cannot drive $E_{GABAA}$ positive to membrane potential. One mechanism that can explain $GABA_AR$-mediated depolarization is the involvement of $HCO_3^-$ permeability of the $GABA_A$ receptor channel (Kaila and Voipio, 1987; Grover et al., 1993; Staley et al., 1995; Perkins and Wong, 1996; Kaila et al., 1997). In work on crayfish muscle fibers, direct measurements of $[Cl^-]$ and $[HCO_3^-]$, showed that prolonged activation of $GABA_ARs$ can lead to accumulation of intracellular $[Cl^-]$ and $[HCO_3^-]$ -dependent depolarization (Kaila and Voipio, 1987). Based on this work, Staley et al. (1995) have proposed that the depolarizing $GABA_A$ response is the result of an asymmetric, activity-dependent collapse of the opposing electrochemical gradients of $Cl^-$ and bicarbonate. In this model (Staley et al., 1995; Staley and Proctor, 1999), intense $GABA_AR$ conductance shifts $GABA_A$ reversal potential toward $E_{HCO3}$. At physiological internal pH (7.0-7.2), $E_{HCO3}$ is more positive than resting membrane potential (Roos and Boron, 1981; Kaila and Voipio, 1990; see Kaila, 1994). Net influx of $Cl^-$ and efflux of $HCO_3^-$ can lead to collapse of their chemical gradients. However, dissipation of

the bicarbonate gradient is opposed by intra- and extracellular pH buffering, as illustrated by the formula

$$\frac{[H^+]_o}{[H^+]_i} = \frac{[HCO_3^-]_i}{[HCO_3^-]_o} \qquad (3\text{-}3)$$

, where *o* and *i* indicate outer and inner sides of the cell membrane, respectively. This equation implies that the $HCO_3^-$ gradient is equal to the $H^+$ gradient. Because $[HCO_3^-]$ is determined by pH buffers acting in conjunction with the rapid diffusion of $CO_2$ across the cell membrane, catalyzed (intracellular) regeneration of $HCO_3^-$ by carbonic anhydrase (CA), according to the reaction *(3-4)*

$$H_2O + CO_2 \xleftrightarrow{CA} H_2CO_3 \longleftrightarrow H^+ + HCO_3^-$$

can maintain a relatively stable $[HCO_3^-]_i$. Then, the bicarbonate-carried inward current leads to a large depolarization-driven gain in postsynaptic $[Cl^-]$, producing a shift in $E_{GABAA}$ toward $E_{HCO3}$ (see equation *3-2*).

While later studies on activity-induced depolarizing $GABA_A$ responses support a key role for $HCO_3^-$ in hippocampal neurons, it has been shown in pyramidal cells (in which the mechanisms have been studied) that the postsynaptic $[HCO_3^-]$ alone cannot explain fast activity-induced positive shift of $E_{GABAA}$ (Kaila et al., 1997; Smirnov et al., 1999). For instance, CA activity in the postsynaptic pyramidal cell is not critical for GABAergic depolarizations (Kaila et al., 1997). Activity-induced $GABA_A$ depolarizations during strong interneuronal bursts are also significantly dependent on the shift of $E_{Cl}$ positive to $E_m$ in response to extracellular accumulation of $[K^+]$ (Kaila et al., 1997; Smirnov et al., 1999). Mechanisms of changes in $E_{GABAA}$ have not hitherto been studied in hippocampal interneurons.

## 3.4. $GABA_A$-type inhibition in hippocampal networks

### *3.4.1. GABAergic communication between interneurons*

The reversal potential of $GABA_A$R-mediated responses in CA3-CA1 interneurons is few millivolts negative to resting membrane potential, and hence, IPSPs are typically slightly hyperpolarizing (Misgeld and Frotscher, 1986; Lacaille and Schwartzkroin, 1988; Lacaille, 1991; Buhl et al., 1995). During tonic excitation of the CA3-CA1 interneuron network, interneurons can become synchronized via hyperpolarizing IPSPs (Whittington et al., 1995; Fisahn et al., 1998; for review Traub et al., 1998). In general, it is assumed that when GABAergic neurons are serially connected, increased activity of an interneuron will lead to decreased firing of the target interneuron. This process is referred to as disinhibition. As an example, the activity of CA3-CA1 interneurons that inhibit hippocampal principal cells is controlled by GABAergic afferents from the *septum* (Freund and Antal, 1988, Toth et al., 1997). Thus, suppression of *cornu ammonis* interneurons via extrahippocampal GABAergic connections results in enhancement of excitability of principal cells (Bilkey and Goddard, 1985).

Intriguingly, Michelson and Wong (1991, 1994) showed in the hilar region that IPSPs tend to become depolarizing in interneurons during a few hundred milliseconds of local bursting (see *3.3.1*). These authors reported that, via depolarizing shift of $GABA_A$ responses, a subpopulation of hilar interneurons can even excite each other. Excitatory $GABA_A$R-mediated coupling prolonged discharge of individual interneurons and resulted in a spread of interneuronal activity in the hippocampal network. This indicates that recruitment of interneurons does not solely depend upon glutamate-induced excitation from principal cells, but rather that the GABAergic neurons are capable of recruiting neighboring interneurons via depolarizing $GABA_A$ re-

sponses. The overall effect of excitatory coupling between inhibitory cells was an augmentation of inhibitory IPSPs in principal cells. Thus, excitatory action of $GABA_A$ receptors remained consistent with the role of GABA as an inhibitory transmitter in the hippocampal neuronal networks (Michelson and Wong, 1991).

Since synchronous bursts of interneurons are a common feature of hippocampal networks, GABAergic depolarization may represent a normal component of transmission in interneurons (see Grover et al., 1993). It has been suggested that the GABAergic excitatory mechanism may be generated between inhibitory interneurons throughout the hippocampus (Michelson and Wong, 1994). However, the significance of $E_{GABAA}$ in synchronization of the inhibitory circuitries has not been studied in the CA3-CA1 region.

### *3.4.2. Population activity of pyramidal cells*

Interneurons are involved in the induction and maintenance of hippocampal network oscillations at various frequencies (for review Freund and Buzsaki, 1996). We focus here on gamma (20-100 Hz) oscillation that is generated intrinsically in hippocampal formation (Jefferys et al., 1996). Importantly, hippocampal gamma rhythms are often seen simultaneously with other similar oscillations in the neocortex (see Gray, 1994). Synchronous principal cell gamma-frequency oscillations in the cortex probably have behavioural significance in that they occur during complex motor acts (Murthy and Fetz, 1992) as well as after specific sensory stimulation (Gray et al., 1989). Coherence of these oscillations has been suggested to represent a process for binding neuronal information of the oscillating cortical areas (see Buzsaki and Chrobak, 1995). Furthermore, it is known that when gamma oscillations are induced in one region, they can induce gamma oscillations in a connected region. This projection of gamma oscillation has been observed for CA1 to *subiculum* (Colling et al., 1998), for projections from entorhinal cortex to CA1 (Charpak et al., 1995), and for CA3 to CA1 (Fisahn et al., 1998).

Gamma-frequency oscillations have been shown to be generated in networks of CA3-CA1 interneurons after blockade of ionotropic glutamate receptors (Whittington et al., 1995; Fisahn et al., 1998). *Stratum pyramidale-oriens* interneurons that participate in the interneuron network gamma, entrain pyramidal cell firing by rhythmic inhibition (Cobb et al., 1995). Several observations indicate that synchronous gamma-frequency firing in the principal cell population is entrained by phase-locked $GABA_AR$-mediated hyperpolarizing IPSPs (Soltesz and Deschenes, 1993; Cobb et al., 1995). Moreover, ephaptic effects can significantly contribute to synchronization of principal cell outputs (Bracci et al., 1999). In general, gamma oscillation is thought to require hyperpolarizing $GABA_A$ responses in CA3-CA1 interneurons (for review Traub et al., 1998; Tamas et al., 1999).

Due to extensive arborization of axons and through their interconnectivity, hippocampal interneurons can produce coherent inhibition in a large number of anatomically distributed pyramidal cells and bring about sharply synchronized firing of the projective excitatory pathways. This is important since most single EPSPs, especially in pyramidal cells, are of insufficient amplitude to cause spike discharge of the neuron. If a target neuron is to fire, a number of temporally coincident EPSPs must be generated by synchronization of afferent glutamatergic neurons. During gamma oscillation, rhythmic GABAergic inhibition entrains firing in the principal cell population. Synchronization of the output of projective glutamatergic cells favors excitation of target neurons.

### *3.4.3. Induction of plastic changes in glutamatergic synapses*

Since $GABA_AR$-mediated activity controls excitability of principal cells, $GABA_A$-type

transmission is also critically involved in induction of long-term changes in efficacy of glutamatergic synapses. Plasticity (i.e. potentiation or depression in synaptic strength) of glutamatergic synapses have been extensively studied using patterned electrical stimulation of CA3-CA1 pathways. Long-term potentiation (LTP) can be induced in the hippocampal CA1 area by tetanic gamma-frequency (e.g. 100 Hz, 1s) stimulation of CA3 efferents (see Bliss and Collingridge, 1993). A key element in the induction of LTP is the accumulation of postsynaptic calcium, via glutamate NMDA receptors or voltage-gated calcium channels (VGCCs). The level of postsynaptic depolarization controls activation of these channels (Wigstrom and Gustafsson, 1983, 1985; Collingridge et al., 1987; Müller et al., 1988; Magee and Johnston, 1997). In tetanic LTP induction, the glutamatergic depolarization is augmented because repetitive stimulation reduces the amplitude and driving force of $GABA_A$ IPSPs (Thompson and Gähwiler, 1989a). Further, $GABA_B$ receptor-mediated autoinhibition suppresses GABA release (Davies et al., 1991), and pharmacological suppression of $GABA_A$-type inhibition has been shown to facilitate the induction of LTP (Wigström and Gustafsson, 1985; Bliss and Collingridge, 1993). However, high-frequency stimulation, similar to that used in LTP induction, has been shown often to make $GABA_A$ responses depolarizing in CA1 pyramidal cells (Grover et al., 1993; Kaila et al. 1997; Cobb et al., 1999). The possibility that an inhibitory postsynaptic GABA effect may become excitatory in this LTP induction model has received little attention. It appeared as interesting to know whether $GABA_A$ depolarization can contribute to post-tetanic excitation of CA1 pyramidal cells and neuronal uptake of calcium.

## 3.5. $GABA_A$ receptor-mediated transmission in newborn rat hippocampus

Biochemical markers of GABAergic transmission suggest that ontogenetically GABA-mediated signaling may develop quite early. Parameters, such as GABA concentration and immunoreactivity to $GABA_AR$ subunits or to glutamate decarboxylase (GAD), an enzyme responsible for the catalyzed production of the transmitter, can be found already in the embryonic cortex (Lauder et al., 1986; Van Eden et al., 1989). While these experiments give valuable information about maturation of the GABAergic system, they reveal no functional properties of the developing GABAergic transmission (but see Swann et al., 1989; Owens et al.,1996, 1999).

Perhaps the most striking feature in the functional development of the GABAergic system is that in early life $GABA_AR$ activation causes a depolarization in neurons of various regions of the CNS, whereas in adult neurons of the same areas, GABA is established as a predominantly hyperpolarizing transmitter (for review Ben-Ari et al., 1997). This difference in postsynaptic GABA response is based on the different regulation of intracellular $[Cl^-]$ in perinatal and adult nervous cells (Rohrbough and Spitzer, 1996; Rivera et al., 1999). Because of active uptake of chloride and hence higher intracellular $[Cl^-]$ in perinatal cells, activation of $GABA_A$ receptors leads to an inward current and depolarization of the cell at resting membrane potential. Therefore, mutual communication of CA3-CA1 interneurons and, e.g., mechanisms of synchronization of the interneuron network are assumed to be different from the adult brain (for review Holmes and Ben-Ari, 1998; Traub et al., 1998).

### *3.5.1. Spontaneous network activity*

Experiments on different parts of the nervous system over the last few decades have established that spontaneous activity is a characteristic feature of the maturating neuronal networks (Bekoff et al., 1975; Ben-Ari et al., 1989; Greer et al., 1992; Katz, 1993; Lippe, 1994; Fortin et al., 1995; Maeda et al., 1995). An important property of the developing networks appears to be that the neurons are often

active in periodic bursts, synchronous across the population of cells (for review O´Donovan, 1999). In structures with a highly organized laminar or columnar arrangement, such as the hippocampus, spinal cord or retina, the synchronous epochs have been found to propagate in a wave-like manner (Catscicas et al., 1998; Leinekugel et al., 1998; O`Donovan et al., 1998). In the neonate rat hippocampus, periodic bursting of immature networks can be observed until postnatal day 10-12 (Ben-Ari et al., 1989; Khazipov et al., 1997; Garaschuk et al., 1998).

The network-driven bursts seem to be initiated in the CA3 region (Khazipov et al., 1997; Garaschuk et al., 1998; Bolea et al., 1999), and thus, this area presumably has a particularly strong excitatory connectivity already at perinatal age (see O´Donovan et al., 1999). However, synaptic mechanisms behind the genesis and inhibition of the hippocampal bursts are not well understood. Although pharmacological studies have shown that the bursts are synaptic in origin, results are inconclusive since the synchronous epochs in slices are abolished by $GABA_A$R as well as AMPA-R antagonists, and moreover, they are inhibited by NMDA-R blockers (Ben-Ari et al., 1989; Hollrigel et al., 1998; Bolea et al., 1999). Further, they are blocked by antagonists for gap-junctions, suggesting that electrotonic coupling of immature neurons contributes significantly to synchronization of the spontaneous discharge (Strata et al., 1997; Draguhn et al., 1998).

Spontaneous $GABA_A$R-mediated bursts are seen in the newborn rat *gyrus dentatus* interneurons and principal cells (Strata et al., 1997; Hollrigel et al., 1998). In this region, glutamatergic principal cells do not burst during the population events (Hollrigel et al., 1998). While CA3 pyramidal cells are probably activated during the early hippocampal population bursts (Khazipov et al., 1997), their activity at early postnatal age has received little attention (for review see Ben-Ari et al., 1997; O`Donovan, 1999). No attempts have been made, for instance, to study output patterns of developing CA3 pyramidal cells during the early spontaneous events. Nor is there any data from patterning of the synchronous interneuron discharge during the spontaneous bursts. The newborn rat hippocampus has been assumed to lack the critical synaptic circuitry required for fast (e.g. gamma-range) network oscillations (Traub et al., 1998). The generation of gamma oscillations in the mature hippocampus relies on AMPA receptor-mediated excitation and $GABA_A$R-mediated inhibition (Cobb et al., 1995; Whittington et al., 1995; Fisahn et al., 1998).

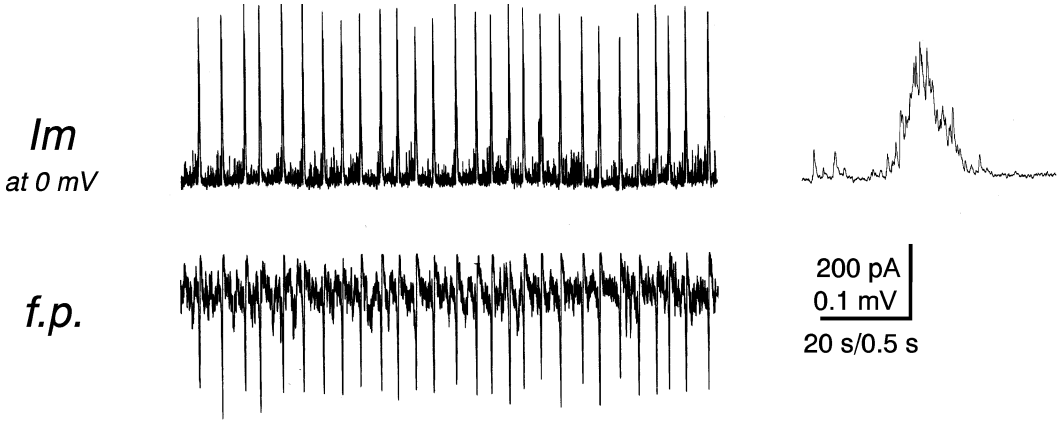
It has been shown in the visual cortex that it is critical for the developing neuronal populations to achieve an adequate pattern of output to get their postsynaptic targets established (Goodman and Shatz, 1993; Katz, 1993). Generally, for a given set of neurons to establish their connections, critical factors are their synchrony and recurrence of activation. While the spontaneous network activity has been proposed to drive maturation of the hippocampal circuitry, it remains unknown whether activation of principal cells displays any synchrony and patterning. At the age when arborization of pyramidal cell axons and establishment of their synaptic connections is most intense, mechanisms that control the aforementioned factors and the discharge of pyramidal cells are also likely to play a significant role in their development.

### *3.5.2. GABA as an excitatory transmitter*

Synaptically released GABA can exert a trophic effect on embryonic cortical neurons and can direct ramification of their growing axons. Whether these GABA effects are mediated non-synaptically or via activation of postsynaptic currents remains obscure. However, GABAergic depolarization is sufficient to trigger calcium influx to immature neurons via the activation of voltage-gated $Ca^{2+}$ channels as well as by removal of the $Mg^{2+}$ block of glutamate NMDA receptors (Leinekugel et al., 1995, 1997; Garaschuk et al., 1998). Intracel-

lular [$Ca^{2+}$] accumulation is known to act as an important signal in neuronal plasticity. Increased cytosolic calcium controls, e.g., gene expression and neurotrophin signaling in embryonic neurons (Lo Turco et al., 1995), suggesting that $GABA_A$R-mediated depolarization may play a role in activity-induced construction of the early neuronal networks.

**Figure 5:** Neonatal rat hippocampus displays endogenous population bursts (Recordings by K. Lämsä, unpublished). Simultaneous recording of extracellular potential (*f.p.*) and $GABA_A$R-mediated current (*Im*) in a CA3 neuron from a five-day-old rat hippocampal slice shows that the bursts accompany strong interneuronal discharge. *Right:* an expanded barrage of $GABA_A$ currents (see Ben-Ari et al., 1989; Khazipov et al., 1997).




A hypothesis originally put forward by Ben-Ari et al. (1989) presumes that excitation of CA3-CA1 pyramidal cells at birth occurs via a large GABAergic depolarization, initiated by spontaneous bursts of interneurons (Khazipov et al., 1997; see also Hanse et al., 1997). Thus, in this scenario, mutual GABAergic excitation of interneurons periodically gives rise to synchronous depolarizing events (also referred to as GABAergic depolarizing potentials, i.e. GDPs) sufficient to induce spiking in developing principal cells (Khazipov et al., 1997). Released glutamate, in turn, can produce cytosolic calcium accumulation via activation of NMDA receptors (Leinekugel et al., 1997). Accordingly, GABA acts as the major excitatory transmitter both in the immature CA3-CA1 pyramidal cell and interneurons. Further, the depolarizing (excitatory) effect of GABA has also been proposed to explain the high occurrence of epileptiform seizures in developing principal cell pathways in the neonatal brain (Holmes and Ben-Ari, 1998). Electrophysiological recordings using single-pulse stimulation of CA3-CA1 pathways speak for mainly NMDAR-based glutamatergic transmission in the hippocampus (Durand et al., 1996). Therefore, during the first postnatal days, glutamate AMPA-type transmission and glutamatergic recurrent excitation are assumed to play a functionally minor role in the hippocampal networks (Ben-Ari et al., 1997; Khazipov et al., 1997). However, it has also been shown that the periodic GABAergic depolarizing potentials (GDPs) are not solely GABAergic in origin and that their occurrence is effectively inhibited by glutamate AMPA-R antagonists (Bolea et al., 1999; Khalilov et al., 1999b).

### *3.5.3. Synaptic inhibition in neonatal rat hippocampus*

Although the synaptic inhibitory mechanisms in the adult hippocampus have been well elu-

cidated, little is known about their development, and even less about their operation during the early postnatal days. Because of the excitatory role of GABA reported in individual neurons, some open-minded hypotheses suggest that cortical networks at an early postnatal age might operate without transmitter-gated synaptic inhibition (for review Holmes and Ben-Ari, 1998). Yet, at this age hippocampal neurons form a very heterogeneous population of anatomically and physiologically developed neurons and neuroblasts (Durand et al., 1996; Gomez-Di Cesare, 1997; Hsia et al., 1998). Thus, it is also possible that transmitter-mediated responses are quantitatively or qualitatively different in cells of varying levels of maturity. This should be taken into account when predicting functioning of the neuronal networks.

Spontaneous bursting in the hippocampal networks has been proposed to be involved in the construction of neonatal neuronal circuits (Ben-Ari et al., 1997; Hanse et al., 1997). Conventional wisdom holds that in this kind of process physiologically relevant patterns of network activity must also be regulated by some inhibitory mechanisms (Hensch et al., 1998). In this context, it has been suggested that the critical inhibition of the synaptically driven population bursts occurs via control of GABA release by $GABA_B$-mediated autoinhibition or, simply, by run-down of the transmitter quantas (Ben-Ari et al., 1997; O`Donovan, 1999). Indeed, hippocampal network activity is regulated by presynaptic $GABA_B$ receptors, since application of the antagonist accentuates the spontaneous hippocampal bursts (McLean et al., 1996). Further, synchronous bursts are followed by a period of synaptic depression that, at least in part, regulates occurrence and duration of the synchronous population events (O`Donovan, 1999).

## 4. AIMS OF THE STUDY

$GABA_AR$-mediated inhibition is considered to be the major inhibitory transmitter mechanism in the brain. The purely inhibitory role of GABA has been challenged by recent findings showing that strong activation of $GABA_ARs$ can produce postsynaptic depolarization that promotes rather than attenuates neuronal activity in mature as well as in newborn rat hippocampus. However, physiological implications of the $GABA_AR$-mediated depolarization and consequent neuronal excitation are poorly understood. To elucidate the role of depolarizing $GABA_A$ responses in hippocampal networks, studies were done in the mature and the newborn rat CA3-CA1 region. The aims of this work were as follows:

1) To study whether $GABA_AR$-mediated depolarization can induce neuronal uptake of calcium in mature hippocampus. High-frequency proximal stimulation of GABAergic and glutamatergic afferents was used to elicit postsynaptic excitation and calcium uptake in the CA1 area **(I, II)**.

2) To compare the mechanisms and consequences of $GABA_A$ depolarization in interneurons and pyramidal cells. What is the role of depolarizing $GABA_A$ responses in synchronization of the interneuron network **(III, IV)**?

3) To compare the role of $GABA_AR$-mediated transmission in mature CA3-CA1 networks with that in the newborn rat hippocampus, where GABA is assumed to be the major excitatory transmitter both in interneurons and in pyramidal cells. This involved characterization of excitatory and inhibitory $GABA_AR$-mediated effects in the newborn hippocampal network to elucidate the possible physiological function of such dual role **(V, VI)**.

## 5. MATERIALS AND METHODS

### 5.1. Hippocampal preparations

The hippocampus is part of the cerebral cortical mantle. It is a cylindrical bilateral structure, which in a rat brain has a semicircular form. All studies have been performed in the hippocampus proper, at regions CA3-CA1 (see Fig. 1).

*5.1.1. Whole hippocampus preparations from neonatal rat brain and dissection of slices*

Neonatal rat pups (P0-P6) were anaesthetized by hypothermia prior to decapitation. The skull was opened and hippocampi were dissected from the brain submerged in oxygenated ice-cold standard solution (see *5.2.1.*). Preparation of whole hippocampal structures is described in detail in Khalilov et al. (1997). Since the whole hippocampus has intact internal circuitry, it was used, together with slices, in studies of hippocampal network functioning. In most cases, isolated hippocampi were cut into transverse slices (600 μm).

In the transverse hippocampal sections, the laminar organization of neurons is recognizable even under a low magnifying microscope. This is highly advantageous, since experiments require accurate placement of the electrodes in a particular layer or area within the hippocampus. Further, the intrinsic hippocampal circuitry is preserved best in slices taken transversely to its longitudinal (i.e. rostro-caudal) axis, although significant longitudinal pathways also exist. Because of the

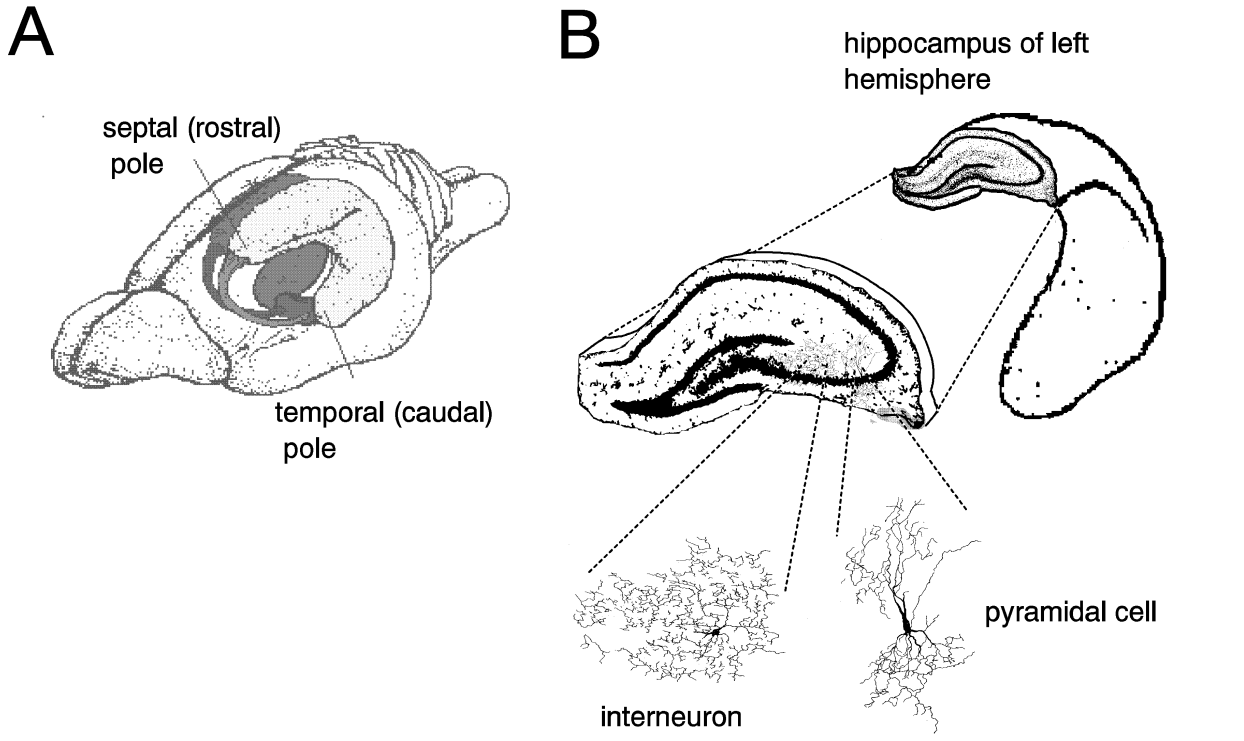



**Figure 6.** Location of hippocampus in the rat brain and illustration of slice preparation. **A)** Schematic representation of hippocampi in the whole rat brain, excluding the thalamus and overlying neocortex. **B)** Isolated hippocampus and transverse sectioning of slice preparations. A single CA3 interneuron and pyramidal cell from a five-day-old rat hippocampal slice (camera lucida drawings from biocytin-filled neurons. Karri Lämsä, Riitta Miettinen, and Tomi Taira, unpublished results).

small size and softness of neonate brain tissue, hippocampi were sliced using the tissue chopper (McIlwain). Following isolation or dissection procedures, preparations (slices as well as whole hippocampi) were allowed to recover in an oxygenated physiological solution (20-22°C) for at least 1 hour.

*5.1.2. Adult rat hippocampal slices*

Young (2-4 weeks) and adult rats (>4 weeks) were anaesthetized with an intraperitoneal injection of pentobarbital sodium (40-50 mg/kg). After decapitation, cerebral hemispheres were dissected from the brain in an ice-cold physiological solution. Transverse slices of a thickness of 400-500 μm were then cut from the whole hemispheres with a vibratome (Vibratome Series 1000, Technical Products Inc., USA). After the dissection procedure, slices were allowed to recover in an oxygenated physiological solution (20-22°C) for 1 hour.

## 5.2. Maintenance of preparations *in vitro*

Experimental milieu for the isolated brain tissues *in vitro* was maintained by accurate control of temperature, gas exchange, ionic environment, and rate of superfusion of the tissue.

*5.2.1. Solutions*

Standard physiological solution (simply termed standard solution) was used in the preparation process (ice cold bath) of the hippocampal tissue as well as for the recovery (at room temperature) and perfusion under experimental conditions (at 32°C). Composition of the standard solution was (in mM): 124-126 NaCl, 3.0-3.5 KCl, 2.0 $CaCl_2$, 25 $NaHCO_3$, 1.1-1.3 $NaH_2PO_4$, 1.3 $MgCl_2$ or 2.0 $MgSO_4$, and 10-11 glucose. The solution was gassed continuously and saturated with 95% $O_2$ + 5% $CO_2$ to yield pH 7.4 at 32°C.

*5.2.2. Interface-type chamber*

In the interface-type chamber, slice preparation occurs in the interface of the liquid and the gaseous phase. The upper part of the slice is covered by only a thin film of liquid, while the other surfaces are effectively flushed by the standard solution. This experimental set-up substantially enhances diffusion of $O_2$ and $CO_2$ between the tissue and the gaseous atmosphere (95% $O_2$ + 5% $CO_2$ in the chamber). Experiments with the adult rat (>4 weeks) hippocampus were performed in the interface-type chamber (studies I, II, IV). The slice in the recording chamber was superfused with the oxygenated standard solution at a rate of 1-1.5 ml/min (volume 0.6 ml).

*5.2.3. Submerged chamber*

In this type of recording chamber, preparations are fully submerged in the superfusing oxygenated standard solution. Softness of the neonatal brain tissue makes the hippocampal slices from newborn and young rats difficult to operate on in the interface-type chamber. Furthermore, whole hippocampus preparations survive only in submerged conditions (VI). Electrophysiological recordings (including measurements of extracellular pH) as well as anatomical studies have shown that intense superfusion with the oxygenated standard solution maintains physiological conditions within the submerged hippocampal tissue (500-600 μm thick slices 3-4 ml/min; whole hippocampus preparations 5-6 ml/min; volume of the recording chamber 0.35 ml) (see Khalilov et al., 1997). The fast perfused submerged chamber was used in studies III, V, and VI.

## 5.3. Extracellular electrophysiological recordings

### *5.3.1. Extracellular potential shifts*

Due to the laminar organization of the principal cell populations in the hippocampus, coherent activity of neuronal arrays easily elicits measurable deflections in extracellular potential. These responses offer a useful measure of population activity in hippocampal principal cell population. Field potential electrodes were pulled from borosilicate (GC150F, Clark Electromedical, UK) glass capillaries with the microelectrode puller (Scientific and Research Instruments Ltd, UK). The tip of the electrode was beveled to an approximate diameter of 5 μm. Electrodes were filled with 150 mM NaCl to give them a resistance between 5-20 MΩ. Recording of extracellular potential is also called here "field potential" measurement.

### *5.3.2. Ion-selective microelectrode recordings*

Ion-sensitive electrodes are devices with a Nernst-type voltage sensitivity to changes in activity of a particular ion in a measured solution. The recorded potential shift ($E_S$) in the ion-selective signal is:

$$E_S = E_C \log \frac{a2}{a1} \qquad (5\text{-}1)$$

, where $E_C$ is the slope (in millivolts) for a 10-fold change in ion concentration, and *a1* and *a2* are the baseline and the shifted activities of the ion, respectively. The general expression for the potential shift as function of the concentration of an ion is:

$$E_S = 2.3 \frac{RT}{Fz} ln \frac{a2}{a1} \qquad (5\text{-}2)$$

, where *R* is the gas constant, *T* is the absolute temperature (K), *F* is the Faraday constant, and *z* is the valency of the ion (see Table 1). Since $E_S$ reacts to activity shifts in the Nernstian manner, for a 10-fold change in monovalent ion activity, one would expect about a 60 mV shift in the recorded potential (at 32°C). For divalent ions, the ideal potential shift is close to 30 mV. For ion concentrations, activities should be divided by the specific activity coefficient. Free concentration of $Ca^{2+}$ in the normal bicarbonate-buffered physiological solution (stabilized with 5% $CO_2$) is only about 80% of the total concentration (Heinemann et al., 1977).

Microelectrodes for ion-selective recordings were pulled from double-barreled borosilicate glass (2GC150FS, Clark Electromedical, UK). The non-filamented barrel was silanized by exposure to vapor of TMSDMA (dimethyl-trimethyl-silylamine, Fluka) followed by baking in an oven at 200°C. After silanization, the tip diameter was beveled to 2-10 μm. The filamented barrel was used as a reference electrode, measuring field potential. The silanized barrel was back-filled with a solution which was specific to a measured ion. Then, a short column of the ion-selective sensor was taken into the tip using moderate suction. The same procedure was applied for $K^+$, $H^+$, $Ca^{2+}$, and tetramethylammonium ($TMA^+$) -selective electrodes. Measurements of the extracellular concentration of the bath-applied impermeable marker $TMA^+$ were used to study transient activity-induced changes in extracellular space volume. Detailed information about the electrodes is listed in Table 1 below. For the $TMA^+$ -sensitive sensor, see also Nicholson and Philips (1981).

## 5.4. Electrophysiological recordings of cellular parameters

### *5.4.1. Intracellular recordings with sharp electrodes*

Microelectrodes with an extremely sharp tip (diameter <0.5 μm) are widely used for recording intracellular potential in mammalian neurons. In this work, the sharp electrode recordings were obtained from pyramidal cells

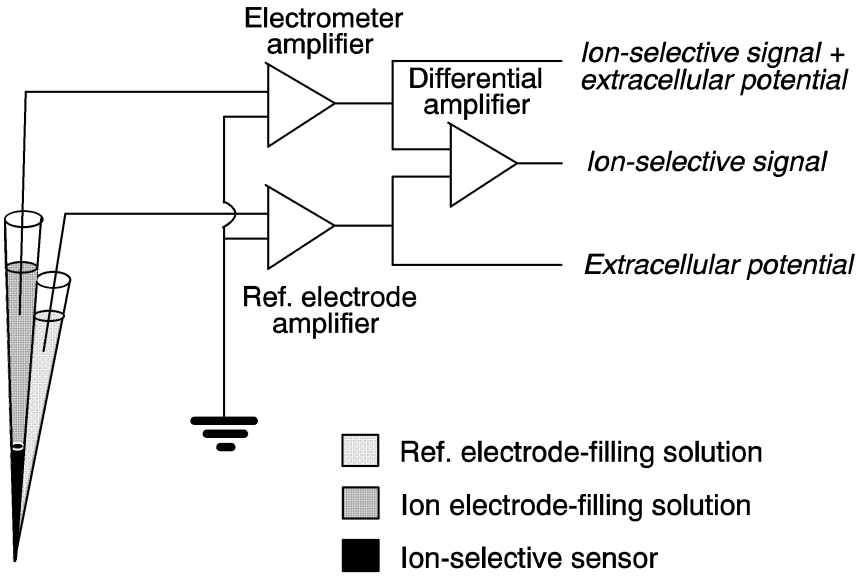



**Figure 7.** Recording of extracellular ion activity with a double-barreled microelectrode. Ion-selective change is a differential signal. Since the ion-sensitive barrel measures normal extracellular potential in addition to the activity of the specific ion, the reference electrode signal must be subtracted by the differential amplifier.

| Recorded ion | Sensor | Filling solution (in mM) | per decade change (mV) | Resistance |
|---|---|---|---|---|
| $K^+$ | Fluka 60398 | 150 NaCl, 3 KCl | 56-59 | 5-20 GΩ |
| $H^+$ | Fluka 95291 | 100 NaCl, 200 HEPES, 100 NaOH | 55-58 | 10-20 GΩ |
| $Ca^{2+}$ | Fluka 21048 | 100 NaCl, 1 $CaCl_2$, 1 HEPES | 28-30 | 15-20 GΩ |
| $TMA^+$ | Corning 477317 | 150 NaCl, 3 KCl, 0.5-5 TMACl | 55-59 | 0.5-1 GΩ |

**Table 1:** Data for ion-sensitive electrodes used.

of mature hippocampus (I, IV). The filling solution in the electrodes was *(a)* 0.5 M K-acetate, 5 mM KCl (pH 7.0 with $H_2SO_4$) for 120-200 MΩ resistance or *(b)* 1 M K-acetate, 1.5 M K-methyl sulphate, 6 mM KCl (pH adjusted to 7.0 with $H_2SO_4$) for 60-100 MΩ resistance. When passing current to the cell, the potential across the electrode resistance was compensated by a bridge balance (in NPI SECIL amplifier, NPI Electronic Gmbh, Germany). Cell input resistances in resting membrane potential (≤ -60 mV) were 20-100 MΩ.

*5.4.2. Whole-cell clamp recordings*

In whole-cell patch clamp recording, very low resistance electrodes are used. Intracellular contact is achieved by first sealing the tip of the pipette with the cell membrane and then rupturing the membrane from the patch by applying a slight suction (see Fig. 8). Low resistivity and effective capacitance compensation bring about a fast time constant for the electrode *RC* -circuit. Fast "reactivity" of the electrode makes this technique well-suited for single electrode voltage-clamp recordings. The voltage-clamp enables ion flow across the membrane to be measured as electric current,

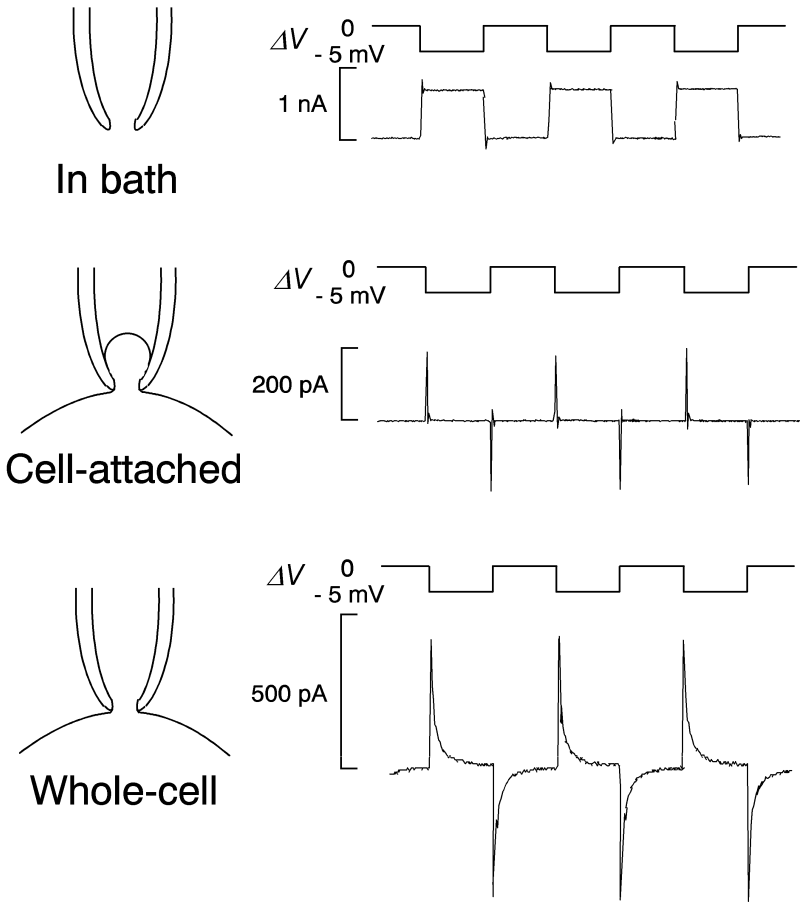



**Figure 8.** Voltage-clamp of the electrode in bath, in giga seal configuration before rupturing the patched membrane (cell-attached), and voltage-clamp of the pipette and "whole cell". Clamping currents for -5 mV (5 ms) steps illustrated.

whilst membrane voltage is held at a stable value with a feed-back amplifier. Perfusion of the cytoplasm with an electrode-filling solution through the wide tip has a strong influence on intracellular ion concentrations. This was of great value in separation of postsynaptic currents carried by different ions (III, V, VI). We also took advantage of this technique in cell-attached recordings, where action potentials of a single neuron can be measured without altering intracellular ionic content (III, V).

The pipettes were pulled from borosilicate glass capillaries (CG150TF, Clark Electromedical, UK) with a Narishige PP-83 micropipette puller. Electrodes had a resistance of 4-10 MΩ with the filling solutions used listed below (in mM).

**1)** 140 K-gluconate, 1.5-3 $CaCl_2$, 6 EGTA, 10 HEPES, 2 Mg-ATP (pH 7.0 with NaOH) (V).
**2)** 125-135 K-gluconate, 1-10 KCl, 2 $Ca(OH)_2$, 5 EGTA, 10 HEPES, 2 Mg-ATP (pH 7.0 with NaOH) (III, VI).

Cells were patched using the "blind method" of Blanton et al. (1989), from the CA3-CA1 area under a conventional binocular light microscope. Input resistance in the gigaseal configuration was 2-10GΩ. The access resistance in the whole-cell configuration was 5-20 MΩ.

Recordings were obtained using an Axoclamp 2B amplifier (Axon Instruments, Foster City, CA) in continuous voltage-clamp mode.

### *5.4.3. Gramicidin perforated-patch recordings*

In whole-cell patch-clamp recordings, intracellular ionic concentrations are effectively perturbed by dialysis of cytoplasmic contents with the electrode-filling solution. While this offers unique experimental approaches (see *5.4.2.*), the whole-cell clamp technique *per se* cannot be used for measurement of intact cellular potentials. Impalement of sharp microelectrodes into the very small and fragile neonatal cells as well as mature hippocampal interneurons may significantly lower their membrane potential. However, the method of perforated-patch recording can circumvent these problems. Among the commonly used antibiotic ionophores, gramicidin-formed pores are exclusively permeable to monovalent cations ($K^+$, $Na^+$ and $H^+$ in physiological solutions) and small uncharged molecules, but display negligible anion permeability, allowing for patch-clamp recordings, which leave intracellular chloride as well as second messenger systems undisturbed. Gramicidin perforated-patch recordings were used to avoid artifactual changes in membrane potential and $E_{GABAA}$ when effects of $GABA_AR$ activation (which opens chloride and bicarbonate conductance) in neonate hippocampal cells and mature interneurons were studied. Patch pipettes were made from the same glass capillary tubes as in whole-cell patch-clamp recordings. Detailed information about manufacturing gramicidin-perforated electrodes is described in Methods of the original publications (III, VI).

## 5.5. Pharmacological compounds

| Compound | Concentration (μM) | Mechanism of Action | Study |
|---|---|---|---|
| 4-aminopyridine (4-AP) | 50-100 | $K^+$ channel blocker, increases vesicle secretion | III, IV |
| Benzolamide (BA) | 10 | Poorly-permeant inhibitor of carbonic anhydrase | IV |
| Bicuculline methiodide | 10 | $GABA_A$ receptor antagonist | II, V, VI |
| 6-cyano-2,3-dihydroxy-7-nitroquinoxaline (CNQX) | 20-40 | glutamate AMPA/kainate receptor antagonist | III, VI |
| DL-2-amino-5-phosphonopentoate (AP5) | 40 | glutamate NMDA receptor antagonist | I, II, III, IV |
| Ethoxyzolamide (EZA) | 50 | membrane permeant inhibitor of carbonic anhydrase | I, II, IV |
| Ketamine | 50 | glutamate NMDA receptor antagonist | I, II |
| Muscimol | 0.01-5 | $GABA_A$ receptor agonist | VI |
| 6-nitro-7-sulfamoylbenzo[f]quinoxaline -2,3-dione (NBQX) | 10 | glutamate AMPA/kainate receptor antagonist | I, II, IV, VI |
| Pentobarbital (PB) | 100 | Modulator of the $GABA_A$ receptor channel | II, III, IV |
| Picrotoxin (PiTX) | 100 | $GABA_A$ receptor antagonist | I, II, IV |

**Table 2:** Summary of pharmacological substances used in the studies.

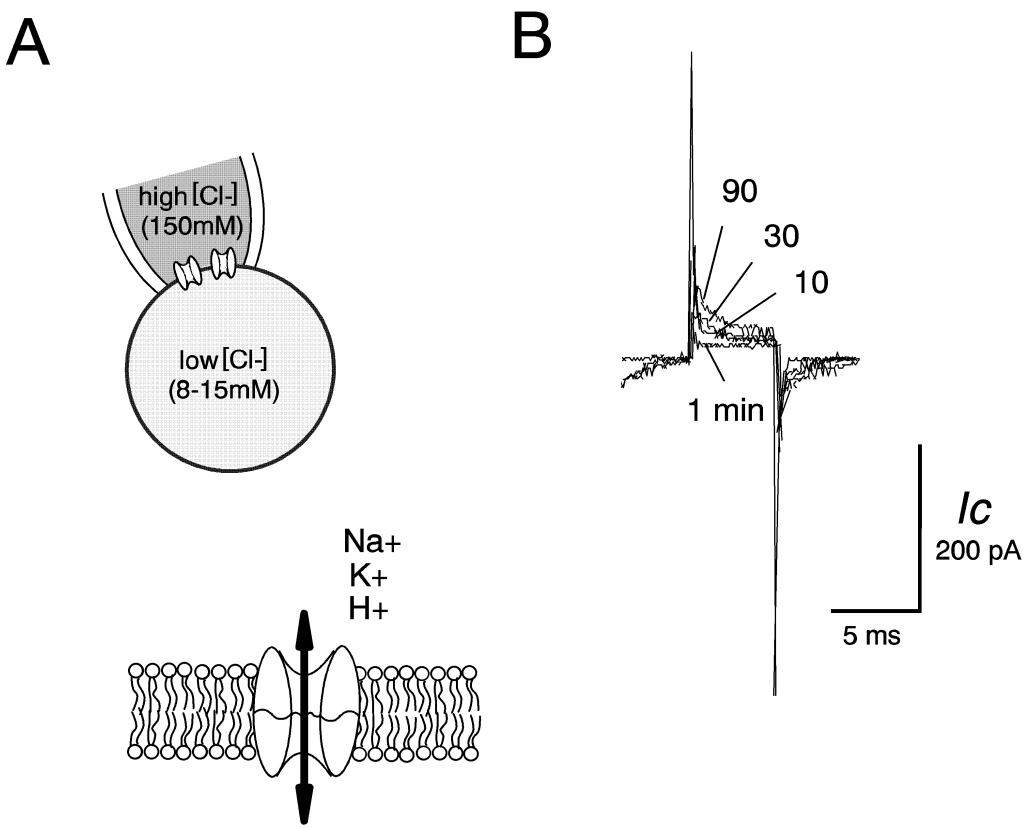



**Figure 9.** Gramicidin perforated-patch recording. **A)** Since gramicidin pores are impermeable to anions, intracellular recordings do not disturb the transmembrane $Cl^-$ gradient. The current across the perforated patch is carried by small monovalent cations. **B)** A gradual increase in a clamping current (*Ic*) indicates development (min) of gramicidin-perforated conductance through the patched cell membrane.

## 6. RESULTS

### 6.1. Contribution of GABAergic excitation to tetanically-induced network activity in rat hippocampal slices

Tetanic high-frequency (100-200Hz) stimulation of dendritic GABAergic synapses has proved to be an effective protocol to evoke depolarizations sufficient to elicit action potential discharges in pyramidal cells. However, information on this kind of excitatory effects of the interneuronal circuits relies heavily on experiments done in the presence of glutamate receptor antagonists. Here we studied roles of dendritic $GABA_A$ and glutamatergic connections in the network-driven excitation and neuronal uptake of calcium following high-frequency tetanic stimulus under intact synaptic transmission.

#### *6.1.1. Post-tetanic excitation of CA1 pyramidal cells via activation of dendritic $GABA_A$ receptors (I)*

Contribution of dendritic $GABA_A$R activity to the post-tetanic excitation of CA1 pyramidal cells was studied in adult rat (> 4 weeks) hippocampal slices using intra- and extracellular recording techniques. Synaptic responses for single-pulse and tetanic (100-200 Hz, 40-100 pulses) electrical stimuli were evoked in the *stratum radiatum* close (~0.5 mm) to the recording site. Tetanic stimulation resulted in a triphasic "fast depolarization/hyperpolarization/late depolarization" response in CA1 pyramidal cells.

Importantly, disappearance of the late depolarization at the early phase of picrotoxin (PiTX, 100 μM) application produced strong attenuation of post-tetanic excitation and action potential discharge. In extracellular recordings, a qualitatively similar reduction in population spikes was seen. The late depolarization (≤ 3 s) that gave rise to most of the spike firing was blocked completely by PiTX. However, the hyperpolarizing $GABA_A$ responses were not affected during the early stage of PiTX application, and thus, the initial glutamatergic component remained curtailed. Further, the monosynaptic EPSP/IPSP responses evoked by the single pulse were unchanged. On continuation of PiTX application, the $GABA_A$ -mediated IPSPs were gradually abolished, accentuating the glutamatergic excitation.

Application of ionotropic glutamate receptor antagonists NBQX (10 μM), AP-5 (80 μM), and ketamine (50 μM) blocked the initial fast depolarization and also suppressed the late, apparently $GABA_A$R-mediated response. Nevertheless, tetanus-induced firing was suppressed to a smaller degree than that seen at the early stage of PiTX application.

#### *6.1.2. $GABA_A$R -dependent neuronal $Ca^{2+}$ uptake (II)*

Uptake of extracellular calcium by tetanus-induced network activity was examined employing ion-selective microelectrode techniques in adult rat hippocampal slices. Microelectrode recordings of extracellular free $Ca^{2+}$ ($[Ca^{2+}]_o$) in the *stratum radiatum* of the CA1 area showed that high-frequency stimulation (100-200 Hz, 40-100 pulses) applied close to the recording site (~0.5 mm) was accompanied by a 0.1-0.3 mM transient fall in $[Ca^{2+}]_o$ from baseline activity (~1.6 mM). Recordings of tetramethyl ammonium ($TMA^+$) accumulation in the interstitial space (500 μM in standard solution) revealed a concomitant 30-40% shrinkage of extracellular volume, which was likely to partially compensate for the reduction of $[Ca^{2+}]_o$.

Exposure to $GABA_A$R antagonist picrotoxin (100 μM) first suppressed and only thereafter augmented the activity-induced $[Ca^{2+}]_o$ shifts. In contrast, exposure to ionotropic glutamate

receptor antagonists (NBQX, 10 μM; AP-5, 80 μM; ketamine, 50 μM) brought about a monotonic attenuation of the $Ca^{2+}$ transient to 60-70% of the original. $Ca^{2+}$ responses were further diminished by a subsequent application of $GABA_A$R antagonists (PiTX, 100 μM or bicuculline, 10 μM), but were strongly enhanced by $GABA_A$-receptor up-modulator pentobarbital sodium (100 μM). We observed suppression of $Ca^{2+}$ transients also in HEPES-buffered $HCO_3^-$ -free solution as well as by inhibition of intracellular carbonic anhydrase with ethoxozylamide (EZA, 50 μM) (see *6.2.4.*). These results suggest a possible contribution of post-tetanic $GABA_A$R-mediated depolarization to extracellular $Ca^{2+}$ shifts.

## 6.2. Pharmacologically induced GABAergic network activity in rat hippocampal slices

As shown by several previous studies, increased spontaneous activity of interneurons leads to their periodic synchronous bursting (Michelson and Wong, 1991, 1994; see also Whittington et al., 1995). However, it is an open question as to what physiological mechanisms control synchronous bursting of interneuronal ensembles. In this study, 4-aminopyridine (4-AP) was used to induce spontaneous population bursts in the CA3-CA1 interneuronal networks. 4-AP stimulates spontaneous transmitter release from presynaptic terminals by facilitating calcium-dependent secretion of transmitter quantas (Buckle and Haas, 1982; Tapia et al., 1985). In the following experiments, ion-selective ($H^+$ and $K^+$) microelectrode techniques, field potential measurements as well as recordings of the membrane potential and synaptic currents have been employed in hippocampal slices of two-to eight-week-old rats. In all experiments, ionotropic glutamate receptors were blocked by antagonists (NBQX, 10 μM / CNQX 20 μM and AP-5, 40 μM). In their presence, exposure to 4-AP (100 μM) led to periodic bursting in the interneuron network and occurrence of $GABA_A$R-mediated hyperpolarization/depolarization sequences in pyramidal cells. In CA3 interneurons, initial hyperpolarization was typically small and readily reversed to depolarization. These spontaneous GABAergic events (hereafter referred to as SGEs) were accompanied by extracellular signals; a negative shift of extracellular potential in the *stratum radiatum* and *stratum oriens*, transient $[K^+]$ accumulation (from 3.0 mM to ~4 mM), and an alkaline change of ~0.05 units in pH.

### *6.2.1. Role of $E_{GABAA}$ in generation of interneuronal population bursts (III, IV)*

In general, recordings from single cells revealed spontaneous firing of interneurons located in the CA3 *stratum pyramidale-oriens* (SP-O). In these SP-O interneurons, single GABAergic IPSPs were hyperpolarizing in the resting membrane potential (-64 ± 2 mV). However, during SGEs the neurons were depolarized by 5 ± 1 mV and they fired action potential bursts. These depolarizing events were carried by highly $[Cl^-]_i$ -sensitive currents, indicative of strong underlying $GABA_A$ receptor activity.

We next explored the role of mutual $GABA_A$ receptor-mediated communication of interneurons giving rise to the SGEs. First, slices were exposed to 100 μM pentobarbital sodium (PB), a positive allosteric modulator of the receptors (MacDonald et al., 1989). Application of PB resulted in an increase in the GABAergic depolarizations within the CA3 interneurons (from 4 ± 1 mV to 7 ± 1 mV). Similarly, the depolarizing phase of the GABAergic input in pyramidal cells was enhanced in the presence of PB. Synaptic input conductances during the SGEs in PB were stronger than in the control (73 ± 10 nS vs. 38 ± 5 nS, respectively). Concomitantly, amplitudes of the extracellular signals; field potential shifts, transients in $[K^+]$, and pH, were much higher (for the ionic transients 1.65 and 1.7-fold, respectively), reflecting the increased intensity of in-

dividual population bursts. Exposure to PB also increased the occurrence of the SGEs (~1.5 to 2 -fold).

We next manipulated $GABA_A$ reversal potential by replacement of 20-30 mM extracellular NaCl with Na-formate. Because conjugated forms of weak-acid anions are permeant across the cell membrane, distribution of the anions is governed by the transmembrane pH gradient, giving them a reversal potential of $H^+$ (Kaila and Voipio, 1990). These conditions caused a positive shift in $GABA_A$ responses, since the formate ion has a high permeability through $GABA_A$ receptor channels (Bormann et al., 1987; Mason et al., 1990). Membrane potential recordings of CA3 interneurons revealed that the positive shift of $E_{GABAA}$ was accompanied by an increased occurrence of SGEs. Since exposure to 20-30 mM formate may cause several secondary effects in neuronal functions, exposure to another weak-acid anion, propionate, was used as a control. Formate and propionate are very similar as weak-acid anions, except that propionate has practically no permeability across $GABA_A$ receptor channels. Equilibration of propionate across the cell membranes did not cause a positive shift in $E_{GABAA}$. However, with propionate, the occurrence of SGEs was slightly decreased.

### *6.2.2. Interneuronal gamma oscillations (III)*

Cell-attached and gramicidin perforated-patch recordings revealed that during the SGE SP-O interneurons delivered a burst of action potentials consisting of 14 ± 4 spikes. The predominant action potential interval in these bursts was 30-50 ms, yet intervals as short as 5 ms were seen. Whole-cell voltage-clamp recordings of the synaptic input in CA3 neurons revealed rhythmic occurrence of $GABA_AR$ – mediated current peaks during the interneuronal population bursts. Because of enormous underlying conductance, currents were likely to represent synchronous activity of numerous presynaptic GABAergic cells. Power spectra of the $GABA_A$ current bursts (500 ms) showed peak frequency at 20-40 Hz. Time-frequency analysis of averaged bursts uncovered that synchronous interneuronal oscillation typically started at gamma range (20-40 Hz) and slowed down to a < 20 Hz “tail activity”.

### *6.2.3. Role of gap junctions in interneuronal synchronization (III)*

Studies in hippocampal slices have demonstrated that interneurons can be electrotonically connected via gap junctions. To evaluate a possible contribution of the gap-junction-mediated electrical coupling to synchronous bursting of interneurons, two different gap-junction blockers, carbenoxolone (200 µM) and octanol (500 µM), were tested. In the presence of carbenoxolone as well as octanol, 4-AP-induced population bursts were strongly suppressed. Occurrence of the SGEs was decreased to 13 ± 10% and 24 ± 8% from the control, respectively. However, the interneuronal bursting could be restored (to ~85%) by replacing 30 mM extracellular NaCl with formate. Nevertheless, the power spectra taken from $GABA_A$ current bursts in the presence of carbenoxolone (n = 40, 5 slices) revealed that the rhythmic patterning was completely lost (n = 40, 5 slices).

### *6.2.4. $HCO_3^-$ -dependency of GABAergic network activity (IV)*

The role of $HCO_3^-$ in generation of spontaneous interneuronal population bursts was next examined. In the $HCO_3^-$ -free HEPES-buffered standard solution, the occurrence of SGEs was decreased to 20% of baseline activity. Further, depletion of bicarbonate was accompanied by a decrease in amplitude of the extracellular population signals, particularly in the alkaline transient of pH, which under present conditions is due to $GABA_AR$-mediated $HCO_3^-$ -outflux from neurons (Kaila et al., 1992). Recordings from CA1 pyramidal cells also revealed that the depolarizing phase of

$GABA_A$ response was selectively attenuated by $HCO_3^-$ depletion, while the fast hyperpolarization either remained unchanged or was slightly enhanced. These results suggest that transmembrane $HCO_3^-$ movements via $GABA_AR$s are closely linked to generation of SGEs.

Inhibition of carbonic anhydrase (CA) effectively slows down the equilibrium reaction of $CO_2/HCO_3^-$ buffer and hence inhibits maintenance of the transmembrane bicarbonate gradient during $GABA_AR$ activation (Kaila et al., 1992). Membrane-permeant CA inhibitors are also known to effectively suppress post-tetanic GABAergic depolarization (Kaila et al., 1997). In line with this, tetanus-induced excitation and extracellular $Ca^{2+}$ uptake in the CA1 area were attenuated by a membrane-permeant CA inhibitor, ethoxyzolamide (EZA, 50 μM) (see *6.1.2.*). Studies with two kinds of carbonic anhydrase inhibitors, a membrane-impermeant benzolamide (BA), and the permeant form, ethoxozylamide, were used to evaluate the role of $HCO_3^-$ availability in generation of SGEs. Selective inhibition of the interstitial CA by benzolamide (10 μM) had no effect either on the occurrence of the bursts or the amplitude of the extracellular signals, with the exception of the alkaline pH transients, which became acid shifts as reported earlier by Kaila et al. (1992). However, inhibition of extra- and intracellular CA by EZA (50 μM) resulted in a similar type of attenuation of SGEs to the $HCO_3^-$ -free medium. Occurrence of the SGEs in EZA was reduced to ~80%, extracellular potential shifts and $[K^+]$ transients were diminished to ~50% of the control in parallel with decline in the intracellular GABA depolarization.

To further explore the role of $GABA_AR$-permeable weak-acid anions in generation of SGEs, we tested the effects of formate and propionate in a $HCO_3^-$ -free medium. GABAergic population bursts, which were strongly suppressed by depletion of $CO_2/HCO_3^-$, were re-established in the formate- (20 mM) containing solution. In addition, extracellular recordings showed that amplitude and frequency of $[K^+]$ transients were comparable with those recorded in the $HCO_3^-$ -containing solution. Further, formate-induced SGEs were associated with alkaline transients. However, unlike those occurring by outflux of $HCO_3^-$, they were not affected by blockade of extracellular CA with benzolamide (10 μM). In the propionate- (20 mM) containing solution, SGEs were not restarted. These experiments suggest that regulation of the $GABA_AR$-permeant weak-acid anion, $HCO_3^-$, can significantly control mutual excitation of interneurons via the effect on $E_{GABAA}$.

To assess the role of bicarbonate-carried current in GABAergic interneuronal coupling and synchronization of the network, burst generation and rise of the depolarizing GABAergic component were studied applying weak electrical stimuli at a distance of ~1.5 mm from the recording site. A single-pulse stimulus elicited a propagating GABAergic population burst in the interneuronal network (see Perreault and Avoli, 1992). Generation of the SGEs, recorded from pyramidal cells, showed complete refractoriness to stimuli at intervals of ≤15 s. Peak amplitude of the depolarization was typically achieved in events evoked with a 30-35 s interval, and shortening of the interval selectively decreased the depolarizing phase. This indicates that the mechanisms generating the interneuronal bursts and accompanying depolarization have established periods of absolute as well as relative refractoriness. Upon application of PB (100 μM), the complete refractoriness was dramatically shortened (to ~5 s), indicating that thresholds to GABAergic depolarization and generation of SGEs were lowered concomitantly. By contrast, in the $HCO_3^-$ free medium, the SGEs could be evoked only at stimulus intervals of ~2-3 min. Moreover, the bursts were accompanied by a much reduced GABAergic depolarization. In the presence of EZA (50 μM), diminishment was mainly seen in the amplitude of the depolarizing response, not in the absolute frequency of the burst generation.

## 6.3. Contributions of GABAergic and glutamatergic transmission to spontaneous network activity in newborn rat hippocampus

To date, only excitatory $GABA_A$ responses have been reported in the newborn rat hippocampus. Until the end of second postnatal week, electrical activity in the rat hippocampus is characterized by synchronous bursts of neurons. The prominent interneuronal activity during these epochs as well as the depolarizing nature of $GABA_A$ responses have raised the possibility of GABA being the major excitatory transmitter early in life. In the following studies, the role of interneuronal circuits in network functioning of P0-P6 rat hippocampus was studied.

### *6.3.1. Pharmacological characterization of GABAergic inhibition and glutamatergic excitation (VI)*

Experiments with P0-P2 slices and whole hippocampus preparations disclosed that the spontaneous population bursts were strongly inhibited in the CA3 region by the glutamate AMPA-R antagonists CNQX (20 μM) and NBQX (10 μM). The interval between the spontaneous population bursts in control conditions was ~15-20 s. Because spontaneous as well as stimulus-evoked population events are also effectively inhibited by glutamate NMDA-R blockers (Bolea et al., 1999), the population bursts are likely to emerge from mutual action of fast glutamatergic and $GABA_A$R-mediated activity, much as in the adult hippocampus. Weak electrical single-pulse stimuli in the presence of CNQX (20 μM), however, gave rise to sustained discharge in the interneuronal network. This may speak for GABAergic excitation between interneurons in the neonatal rat hippocampus. While $GABA_A$R-mediated responses were depolarizing in all P0-P2 neurons studied, extra- and intracellular recordings showed that application of $GABA_A$R agonist muscimol (0.1 -5 μM) blocked the spontaneous population bursts, although the occurrence of unitary $GABA_A$ currents was simultaneously increased. Furthermore, in slices beginning at postnatal day 0, a $GABA_A$R antagonist bicuculline (10 μM) was able to augment stimulus-evoked network discharge in the CA3 area. In whole hippocampus preparations (P0-P2), bicuculline produced massive epileptiform glutamatergic bursts.

To further study the role of $GABA_A$R transmission in functioning of the CA3 pyramidal cell population, CA3-CA1 Schaffer collaterals were stimulated antidromically in P0-P1 rat slices. Single-pulse stimuli from the CA1 area elicited synchronous bursts in the CA3 region similar to the spontaneous epochs. Recordings of extracellular potential showed that blockade of $GABA_A$Rs by bicuculline (10 μM) accentuated stimulus-evoked population burst, bringing about more than a two-fold increase in the number of population spikes. This change was reversible and after wash-out of the antagonist, the number of population spikes was restored close to the control value. Gramicidin-perforated recordings of CA3 pyramidal cell membrane potential demonstrated that in 6 of 12 neurons both the network-driven depolarization as well as the number of action potentials following stimuli from CA1 were strongly increased after blockade of $GABA_A$Rs.

### *6.3.2. Gamma oscillation of the interneuron network (V)*

In order to gain insight into the operation of the GABAergic interneuron network in newborn rat hippocampus, $GABA_A$R-mediated currents during the spontaneous population bursts were first recorded from individual CA3 neurons simultaneously with extracellular measurements. Recordings from neurons voltage-clamped close to the reversal potential of glutamate AMPA-type currents (~0 mV) uncovered a barrage of $GABA_A$R-mediated outward PSCs during a deflection in extracellular potential, indicating synchronous bursting of hippocampal neurons (Ben-Ari et al., 1989).

Time-frequency (TF) representations of the barrages showed both gamma- (20-100 Hz) and high-frequency (>100 Hz) $GABA_A$ current peaks. Averaging of the TF-data smoothened the higher frequencies and emphasized the gamma-frequency band.

### *6.3.3. Temporally patterned firing of pyramidal neurons (V, VI)*

Function of the CA3 pyramidal cell population was first studied by extracellular recordings from the pyramidal layer of neonate rat (P0-P6) hippocampal slices. Recordings revealed bursts of fast (2.5 ± 0.5 ms at the base) negative field potential spikes (~0.1-0.2 mV) occurring during the spontaneous population bursts. In recordings from the *stratum radiatum*, the field potential spikes were reversed to positive deflections, indicating that they were most likely produced by action potential firing of pyramidal neurons. Strong cross-correlation of spikes between two simultaneous bursts separated by ~100 μm verified that they represented coherent population activity rather than random firing of single neurons in the pyramidal layer. Further analyses of the extracellular recordings in P3-P6 slices showed that the population spikes were temporally patterned in the bursts. While autocorrelation analyses from the spike bursts often uncovered several inter-spike frequencies, the predominant frequencies were found by averaging the autocorrelation data of all individual bursts in a single recording. In most of the slices (8 of 13), a prominent peak at 20-100 Hz (gamma-) inter-spike frequency remained after averaging the data. High frequencies (up to 350 Hz) were also present, but >>100 Hz frequency content was mainly produced by small clusters of spikes, whereas 20-100 Hz frequencies arose from the intervals of single spikes and of the high-frequency clusters.

Whole-cell voltage-clamp recordings from neurons at a distance of ~100-200 μm from the extracellular electrode showed that the field potential bursts in the CA3 region were accompanied by a barrage of AMPA-R mediated excitatory postsynaptic currents (EPSCs) in individual neurons. The number of EPSCs during the bursts varied from 7.6 ± 0.2 at P0-P2 to 11.0 ± 3.0 at P3-P6. To see whether the AMPA-R-mediated currents reflected synchronized afferent population spiking (see above), temporal patterning of the barrages was analyzed from P3-P6 neurons. Overall, time-frequency (TF) analyses of the barrages showed that the AMPA-R currents were patterned similarly to the extracellular bursts of spikes. In individual barrages, both gamma- and high-frequency intervals were identified, but again the high-frequency components were mostly formed by small clusters of EPSCs. In P0-P2 whole hippocampus, bicuculline (10 μM) augmented the spontaneous population bursts. The number and amplitude of population spikes as well as single AMPA-R PSCs were dramatically increased. A similar effect was seen in slice preparations at P3-P6.

### *6.3.4. Frequency dependency of synaptic transmission in glutamatergic CA3-CA1 synapses (VI, unpublished)*

Next, we studied activation of glutamatergic AMPA-type synapses in CA1 neurons during spontaneous bursts in the CA3 region. Combined field potential and whole-cell voltage-clamp recordings revealed that the synchronous bursts in the CA3 region were accompanied by a barrage of AMPA-R-mediated currents in several CA1 neurons already at P0. After surgical isolation of CA3 and CA1 areas, population bursts continued to occur at CA3, while barrages of AMPA currents disappeared in the CA1 region. Autocorrelation analyses of the AMPA-current bursts showed that single glutamatergic EPSCs were imposed onto CA1 neurons mostly with ~10-100 ms intervals. Amplitude spectrum computed from several correlograms demonstrated occurrence of the EPSCs at a 20-80 Hz frequency. Thus, the temporal patterning of CA1 glutamatergic input was similar to that in CA3 pyramidal cell population bursts (see *6.3.3.*).

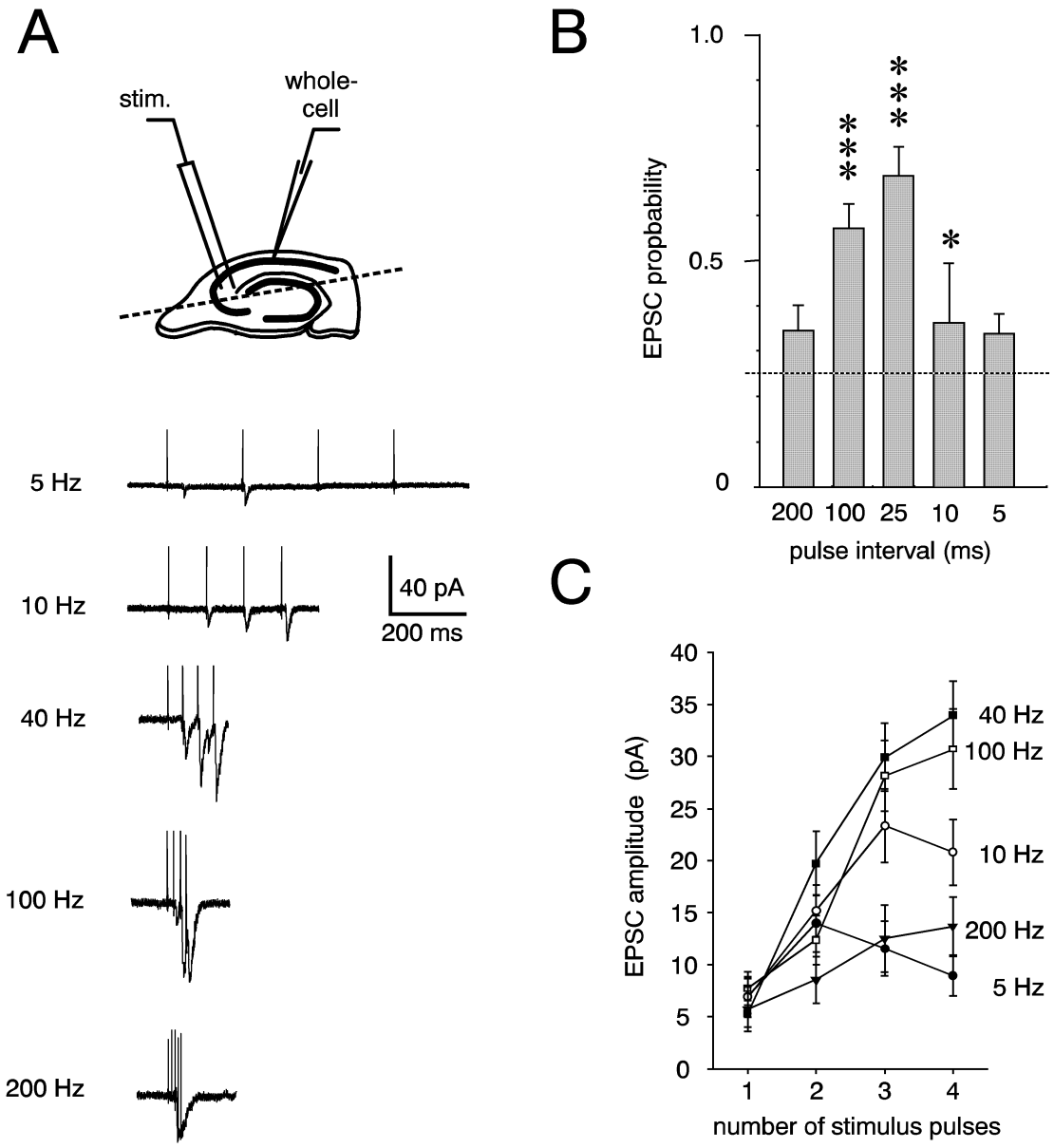



**Figure 10.** Generation of glutamate AMPA-R-mediated EPSCs in P0-P3 hippocampus requires activation of CA3-CA1 axons at gamma-frequency bursts (Lamsa K., Palva J.-M., Kaila K. and Taira T, unpublished results). **A)** After surgical removal of the CA3 area, a single-pulse stimulation of CA1 afferents regularly failed to evoke EPSCs. Whole-cell voltage-clamp recording from CA1 neuron at $E_{GABAA}$. While single-pulse responses were variabe, short stimulus trains at 10-100 Hz frequencies produced strong excitatory EPSCs. Locations of the recording and stimulation electrodes are depicted at the top of the figure. **B)** In slices where EPSCs were elicited by a single-pulse stimuli (5-10 V), the probability of postsynaptic response was very low. EPSCs were evoked as seldom as in 26% of all cases (n = 300 pulses in 5 slices). However, upon second stimulation with various delays, the probability of evoking EPSCs was increased to 1.3 (200 ms), 2.2 (100 ms), 2.6 (25 ms), 1.4 (10 ms), and 1.3 (5 ms) -fold as compared with that of the first stimulus. Maximal probability for AMPA-R-mediated EPSCs is achieved by 25 ms delay from the first stimulus. Mean ± SD for single-pulse stimulus is illustrated. Statistical significance, t-test * $P < 0.1$, *** $P < 0.01$. **C)** While 5 Hz and 200 Hz trains did not elicit AMPA-R-mediated excitation to CA1 neurons, 10 Hz, 40 Hz, and 100 Hz frequencies bring about prominent facilitation of consecutive currents. Data mean ± SD (for a single mean, n = 60 in 5 slices).

Finally, we examined the frequency dependency of glutamatergic inputs to CA1 neurons in P0-P3 rat hippocampal slices. First, the CA3 region was removed to abolish the spontaneous barrages of AMPA currents in CA1 neurons so that only single unitary EPSCs were seen. In the isolated CA1 area, a single-pulse electrical stimulation of Schaffer collaterals regularly failed to evoke AMPA EPSCs in CA1 neurons (n = 8 slices). However, upon a second pulse, the EPSC probability was increased at 25 ms and 100 ms intervals. In contrast, repeated stimuli at frequencies < 40 Hz and > 100 Hz were much less effective and did not significantly differ from single-pulse stimuli. Pulse train stimulation (4 pulses) using frequencies reflecting the synchronous bursting of CA3 pyramidal cells (10-100 Hz) induced strong responses also at 10 ms intervals, and prominent facilitation of the EPSC amplitude was noticed in 10-100 Hz trains. Both highest probability of AMPA EPSCs and strongest frequency facilitation were brought about by 25 ms intervals (40 Hz bursts). Thus, synaptic AMPA receptor-mediated responses were most efficiently evoked upon stimulation trains resembling the spontaneous activation of CA3-CA1 glutamatergic circuitry. Results are illustrated in Figure 10. These results have been published in abstract form (Lamsa et al., 1999).

## 7. DISCUSSION AND CONCLUSIONS

### 7.1. $GABA_AR$-mediated excitation generated in pyramidal cell dendrites and neuronal $Ca^{2+}$ uptake in mature hippocampus

Strong $GABA_A$ receptor-mediated input is known to result in effective shunting of the postsynaptic membrane, thus suppressing excitatory synaptic responses. Yet, it is becoming increasingly evident that $GABA_AR$-mediated input does not merely gate the activity of hippocampal principal cells, but upon strong activation of dendritic $GABA_A$ receptors, GABA can elicit postsynaptic firing (Grover et al., 1993; Staley et al., 1995; Kaila et al., 1997; Cobb et al., 1999). Because this novel idea of $GABA_AR$-mediated excitation in the mature hippocampus has been established only recently, the physiological consequences of this phenomenon are largely unknown. Thus, the first part of this work focused on whether $GABA_AR$-mediated depolarization, generated in pyramidal cell dendrites under physiological conditions, might accentuate tetanus-induced excitation and uptake of extracellular calcium.

Generation of excitatory $GABA_A$ responses in pyramidal cells requires targeted activation of synapses terminating on the dendritic tree (Jackson et al., 1999a). For instance, tetanic stimulation in the *alveus* that preferentially activates anatomically different population of interneurons, which contact close to the pyramidal cell soma, fails to trigger action potential firing in pyramidal cells (Alger and Nicoll, 1982; Jackson et al., 1999a). In our study, high-frequency pulse trains were delivered close to the recording site, so that dendritic GABAergic afferents of pyramidal neurons were directly stimulated (Davies et al., 1990; Taira et al., 1995; Kaila et al., 1997). This resulted in strong postsynaptic excitation and action potential firing. The major result was that GABAergic excitation can be even more pronounced than that produced by glutamatergic input.

The post-tetanic discharge was accompanied by uptake of extracellular $Ca^{2+}$, and both were attenuated by application of $GABA_A$ receptor antagonist PiTX. Previous studies in the presence of glutamate receptor antagonists have shown that depolarizing $GABA_A$ responses are dependent on the availability of bicarbonate (Grover et al., 1993; Staley et al., 1995; Kaila et al., 1997). Dependence of the excitation and accompanying calcium signal on bicarbonate was seen here under normal conditions upon application of a membrane-permeant inhibitor of carbonic anhydrase or by replacing the perfusion solution with nominally bicarbonate-free solution. Further, pentobarbital, which is known to enhance $GABA_A$ depolarization, augmented the activity-induced $Ca^{2+}$ transients (Alger and Nicoll, 1982). Thus, the results indicate that the post-tetanic $GABA_AR$-mediated excitation can induce neuronal uptake of calcium in the mature hippocampus.

The cellular elements in the nervous tissue taking up $Ca^{2+}$ upon tetanic stimulation cannot be inferred from the extracellular calcium recordings. It should be noted that activation of voltage-gated calcium channels in glial cells may also lead to uptake of $Ca^{2+}$. Yet, activation of glial $Ca^{2+}$ uptake requires a large elevation in extracellular $[K^+]$ (20-25 mM; Duffy and MacVigar, 1994) much higher than that seen in this experimental model (7-9 mM; Kaila et al., 1997). Furthermore, glial cells do not fire spikes, a property that underlies much of the calcium influx in neurons. Neuronal uptake of $Ca^{2+}$ can occur via direct activation of voltage-gated calcium channels as well as by increased conductance of glutamate NMDA-receptors. In this context, it is important to note that Staley et al. (1995) showed that dendritic $GABA_A$ depolarization is able to promote activation of NMDA-R-mediated currents in pyramidal cells. While it seems likely that interneuronal uptake of calcium occurred under the present conditions, the large membrane surface of pyramidal cell dendrites

in the *stratum radiatum* makes them likely to be the major sink for extracellular calcium.

It is important to note that the tetanic stimulation protocol used here (100-200 Hz, 0.4-1.0 s) is essentially the same as the one often used for induction of long-term potentiation (LTP) in adult Schaffer collateral synapses (see Bliss and Collingridge, 1993). A sustained postsynaptic depolarization *per se* can result in persistent modulation of EPSPs as well as IPSPs (Pitler and Alger, 1992; Stelzer et al., 1994; Wyllie et al., 1994). Since in the adult pyramidal cells GABAergic activity can induce marked excitation with accompanying $Ca^{2+}$ uptake (on which most of the plastic changes in postsynaptic neurons are dependent), it is highly plausible that GABAergic excitation, such as that described above, may also contribute to the processes underlying the plastic changes (see Stelzer and Shi, 1994). This raises the interesting possibility that at least part of the postsynaptic excitation and elevation of cytosolic calcium needed for the tetanus-induced forms of LTP results from the sustained bursting of interneurons (see Debray et al., 1997).

## 7.2. Excitatory $GABA_A$R-mediated coupling of interneurons in mature CA3-CA1 region

Work on hippocampal slices has uncovered a number of situations where postsynaptic GABA responses may shift to depolarizing mode during intense $GABA_A$R activation (Alger and Nicoll, 1982; Avoli and Perreault, 1987; Xie and Smart, 1991; Staley and Mody, 1992; Grover et al., 1993; Staley et al., 1995; Kaila et al., 1997). One of the most intriguing recent findings is the mutual excitatory coupling that is mediated by $GABA_A$ receptors among interneurons in the hilar region. In the *gyrus dentatus*, interneurons are capable of recruiting neighboring inhibitory interneurons via $GABA_A$ responses that become depolarizing during prolonged local bursting (Michelson and Wong, 1991; 1994; Forti and Michelson, 1998). It has been proposed that GABAergic excitatory communication between interneurons may also be generated in other regions of the hippocampus (Michelson and Wong, 1991). Our experiments provided direct evidence for the important role of GABAergic depolarization in synchronization of CA3-CA1 interneurons.

### *7.2.1. Synchronization*

Single inhibitory postsynaptic potentials (IPSPs) are hyperpolarizing in CA3 *stratum pyramidale-oriens* interneurons (Buhl et al., 1995). However, during strong postsynaptic $GABA_A$ conductance, IPSPs shifted positive to resting membrane potential. In addition, GABA depolarizations were more readily generated in interneurons than in glutamatergic principal cells (see also Michelson and Wong, 1991, 1994).

The positive shift of $GABA_A$ reversal potential appeared to be important for generation and propagation of interneuronal population bursts in the adult CA3-CA1 region. Pentobarbital, an effective up-modulator of $GABA_A$ receptor conductance, accentuated the depolarizing shift of $GABA_A$ responses in CA3 interneurons and lowered the threshold for population bursting in the GABAergic network. Moreover, direct manipulation of $E_{GABAA}$ with formate demonstrated that the occurrence of interneuronal population bursts increased concurrently with the depolarizing shift of the $GABA_A$ reversal potential in interneurons. This showed that the generation of the synchronous interneuronal bursts was based primarily on currents carried by $GABA_A$R-permeant anions, which under physiological conditions are $Cl^-$ and $HCO_3^-$ (Kaila, 1994).

Output of the local interneuron population was studied by analyzing $GABA_A$ receptor-mediated currents in CA3 neurons. Temporal structure of $GABA_A$ current peaks showed that in both the adult and the neonatal rat the hippocampal CA3 interneurons tend to dis-

charge synchronously at low-gamma frequency (20-40 Hz) during activation of large populations of GABAergic cells. Synaptic conductances in CA3 neurons during the GABAergic population bursts were often 40-50 nS, while conductances of single IPSCs were usually below 5 nS. Such high conductances during the bursts are strong evidence for synchronous activation of numerous GABAergic afferents, since estimated conductance of a single GABAergic synapse is around 1 nS (Buhl et al., 1995). Single CA3 interneurons terminating on the perisomatic area usually have 2-6 synapses per pyramidal cell (Miles et al., 1996). Therefore, the $GABA_A$ current peaks were likely produced by synchronous activity of several interneurons.

It is widely assumed that in interneuronal gamma-frequency network oscillations, individual GABAergic neurons entrain each other via hyperpolarizing IPSPs (Traub et al., 1996, 1998; Tamas et al., 2000). Yet, recordings here revealed only depolarizing $GABA_A$ responses in the CA3 interneurons during their synchronous bursting. Therefore, it is likely that the network gamma oscillations during these bursts were generated by some mechanism other than hyperpolarizing IPSPs. Since dendrites of interneurons in the CA3-CA1 region are known to be interconnected via gap junctions, electrotonic coupling is a highly probable candidate underlying synchronization of the neuronal network (Gulyas et al., 1996; Katsumaru et al., 1988). Accordingly, the gap-junction blockers octanol and carbenoxolone were used to study this question. Gap-junction blockers were found to strongly suppress interneuronal population bursting, a result also reported in the neonate hippocampus (Strata et al., 1997). More important, however, was the finding that in the presence of gap-junction blockers gamma rhytmicity disappeared in the interneuronal population events, even though the population bursts could be restored. This indicates that the synchronous fast oscillations can be produced in the CA3 interneuron network via electrotonic coupling, although $GABA_A$ responses are depolarizing in individual interneurons (see Tamas et al., 2000).

### *7.2.2. Mechanisms*

Previous studies in principal cells have demonstrated the dependence of post-tetanic $GABA_A$ depolarization on bicarbonate availability (Grover et al., 1993; Staley et al., 1995; Kaila et al., 1997). Experiments here provided direct evidence for the important modulatory role of bicarbonate in interneuronal population behavior; generation of interneuronal population bursts was strongly inhibited by bicarbonate depletion as well as by blockade of cellular carbonic anhydrase. Moreover, restoration of the population bursts by loading neurons with $GABA_A$R-permeant weak-acid anion formate indicates that availability of a weak-acid anion such as $HCO_3^-$ can control mutual GABAergic excitation of interneurons. Dependence on bicarbonate is of particular importance since bicarbonate content is regulated by the $CO_2/HCO_3^-$ equilibrium reaction acting as a physiological buffer system (e.g. Kaila and Voipio, 1987). For instance, processes decreasing cellular $CO_2$/ $HCO_3^-$ are likely to increase the threshold of the depolarizing shift in $E_{GABAA}$ and thereby control the propensity for synchronous interneuronal bursting. This kind of situation could theoretically be produced, e.g., by hyperventilation (Huttunen et al., 1999). In addition, activity-induced intracellular acidification e.g. during epileptic bursts is likely to suppress the bicarbonate-carried depolarizing current (see Kaila and Voipio, 1990; Kaila et al., 1993).

Since alterations in interneuronal population behavior are also reflected in the function of pyramidal cells, it would be interesting to see how manipulations that change the depolarizing shift of $GABA_A$ responses affect epileptiform activity. It is noteworthy that the GABA uptake inhibitors nipecotic acid and tiagabine, which increase depolarizing $GABA_A$ responses, have been reported to inhibit epileptic bursting of principal cells (Avoli et al.,

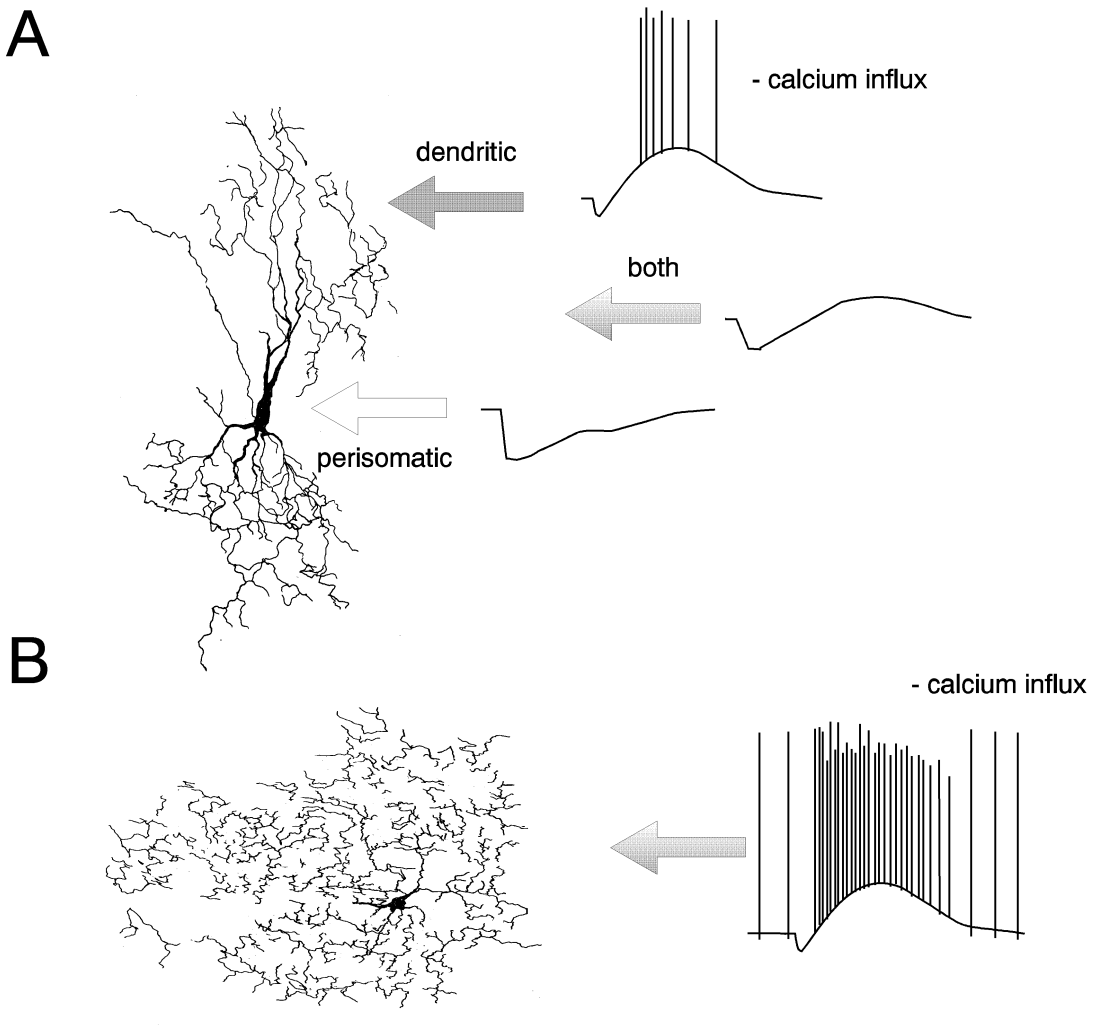



**Figure 11.** Postsynaptic responses to prolonged $GABA_A$ receptor activity in CA3-CA1 pyramidal cells (*A*) and interneurons (*B*). **A)** Initially hyperpolarizing $GABA_A$ responses can become depolarizing and even excitatory in dendrites. Yet, activity-induced depolarizing shift in perisomatic $GABA_A$ response is fairly small (Alger and Nicoll, 1982; Jackson et al., 1999a, 1999b). Therefore, during simultaneous activation of dendritic and perisomatic $GABA_A$ conductances, responses are only moderately depolarizing and are much below firing threshold. In the dendritic branches, however, depolarizing $GABA_A$ response is likely to promote calcium uptake. **B)** In interneurons, $GABA_A$ reversal potential is also hyperpolarizing. Prolonged synaptic activation of $GABA_A$ receptors (including "dendritic" and "somatic") results in prompt depolarizing shift of $GABA_A$ response. Since firing threshold of interneurons is often very close to the resting membrane potential, $GABA_A$R-mediated depolarization is usually sufficient to increase action potential discharge. It is possible that also in interneurons $GABA_A$ depolarization enhances calcium influx.

1993; Jackson et al., 1999a; 1999b). This is consistent with the idea that $GABA_A$R-mediated depolarization primarily promotes firing of GABAergic interneurons and that the overall effect of excitatory coupling between the GABAergic cells in the CA3 region is an enhanced inhibition of the glutamatergic network (Michelson and Wong, 1991).

### 7.3. Comparison of $GABA_A$ excitation in mature CA3-CA1 pyramidal cells and interneurons

The data presented here show that GABAergic excitation in the CA3-CA1 area occurs more readily in interneurons than in pyramidal

cells. Presumably due to dense perisomatic GABAergic innervation of pyramidal cells, even strong $GABA_A$ depolarization in the dendritic area is rather ineffective in depolarizing the soma (Jackson et al., 1999a). This was seen during spontaneous interneuronal population bursts; while synaptic conductance was similar to the post-tetanic activity (see Kaila et al., 1997), $GABA_A$ responses in pyramidal cells were mostly hyperpolarizing. However, concurrently in CA3 interneurons, the initially hyperpolarizing GABAergic IPSPs were transformed into excitatory responses. Since distal dendritic electrogenesis can practically remain unaffected by the postsynaptic currents generated at the soma and vice versa (Traub et al., 1994; Buzsaki and Chrobak, 1995), interneurons terminating on the pyramidal cell dendrites may promote local excitation and calcium influx, while having negligible effect at the soma and at the action potential initiation segment (Traub et al., 1994; Miles et al., 1996).

The basic mechanisms of the depolarizing shift in $GABA_A$ response were similar in CA3-CA1 interneurons and pyramidal cells, being dependent on $GABA_AR$ conductance and availability of bicarbonate. Since the average number of GABAergic synapses in interneurons is not markedly different from that in pyramidal cells (Parra et al., 1998), it is likely that the difference in $GABA_A$ response lability is mainly due to the cellular architectures and different termination sites of GABAergic synapses. In line with this, the depolarizing shift is stronger in dendrites, where membrane surface area is high as compared with cytoplasmic volume (Grover et al., 1993; Staley et al., 1995). This property is expected to be more pronounced in interneurons. On the basis of the above results, the activity-induced GABAergic excitation in interneurons is suggested be an important mechanism in promoting inhibition of CA3-CA1 principal cells during hippocampal population bursts. Therefore, the activity-induced depolarizing shift of $E_{GABAA}$ in interneurons may provide a crucial antiepileptic mechanism in the hippocampus.

## 7.4. Interneuron network activity in the newborn rat CA3-CA1 region

The depolarizing nature of GABA in the neonate hippocampus is well established (Ben-Ari et al., 1989; Fizsman et al., 1990; Hollrigel et al., 1998; Rivera et al., 1999), and strong activation of $GABA_A$ receptors may evoke action potential firing in immature CA3-CA1 neurons (Khazipov et al., 1997; Leinekugel et al., 1997; Khalilov et al., 1999a). GABA has been suggested to be the major excitatory transmitter in principal cells and interneurons at an early postnatal age (for review Ben-Ari et al., 1997; Hanse et al., 1997).

### *7.4.1. Excitatory and inhibitory $GABA_A$ effects*

The role of GABAergic transmission in the neonate hippocampus has mainly been studied by recording single-cell responses to pharmacological or stimulus-evoked activation of $GABA_A$ receptors. Under these conditions, action potential firing as well as accumulation of cytosolic calcium can be seen in individual CA3 neurons (Leinekugel et al., 1995; 1997; Khazipov et al., 1997; Garaschuk et al., 1998). It should be emphasized that at an early postnatal age the hippocampal CA3-CA1 network consists of a very heterogeneous population of variable mature nervous cells as well as neuroblasts (Gomez-Di Cesare et al., 1997). Hence, it is very likely that postsynaptic transmitter responses may also vary between individual cells (see Fiszman et al., 1990) and observation of only single-cell reactions may give a biased view of the role of GABA in the function of the local networks. At the network level, the excitatory role of GABA was seen in interneurons; the application of $GABA_A$ receptor agonist muscimol increased the occurrence of unitary IPSCs, suggesting increased activity of the GABAergic cells. Khalilov et al. (1999a) recently revealed that low concentrations of $GABA_A$ receptor agonists or upmodulators may promote synchronous interneuronal bursting in the newborn rat hippo-

campus. This finding is in perfect agreement with the data presented here.

However, studies on spontaneous bursts revealed that although depolarizing by nature, $GABA_AR$ activation is in essence inhibitory by action in the newborn hippocampal CA3 pyramidal cells. This result is clearly in contrast to interpretations in the existing literature (Leinekugel et al., 1995; 1997; Ben-Ari et al., 1997; Hanse et al., 1997; Garaschuk et al., 1998; Holmes and Ben-Ari, 1998). Nonetheless, it should be stressed that the depolarizing effects do not necessarily imply an excitatory role for GABA (Staley and Mody, 1992; Hollrigel et al., 1998). It is worth recalling that excitatory $GABA_AR$-mediated responses and calcium uptake can be evoked during intense interneuron network activity and consequent GABA release in adult hippocampal neurons as well, yet the principal inhibitory role of GABA in the mature hippocampus has not been questioned. $GABA_AR$-mediated inhibition was found to control the size of the activated principal cell population, since EPSC amplitude of spontaneous as well as stimulus-evoked responses increased in the presence of the $GABA_AR$ antagonist bicuculline. Moreover, stimulus-induced depolarizations that were driven by the CA3 network were augmented after blockade of $GABA_A$ receptors. Further, CA3 pyramidal cell population bursts were abolished upon tonic activation of $GABA_A$ receptors by muscimol. Thus, as described in the adult hippocampus (Staley and Mody, 1992; see Jackson et al., 1999b), $GABA_AR$-mediated shunting also exerts inhibition in the newborn hippocampal principal cells.

In light of the existing literature, the barrages of fast glutamatergic (AMPA-R-mediated) currents in CA3-CA1 neurons occurring during the first postnatal days were rather surprising (see Durand et al., 1996; Ben-Ari et al., 1997; Hanse et al., 1997; but see Tyzio et al., 2000). In the *gyrus dentatus*, for instance, glutamatergic bursts do not occur at this age (Hollrigel et al., 1998). The EPSCs were rather modest in amplitude and their occurrence was temporally restricted to the network-driven bursts. Since the EPSC amplitude is determined by temporal integration of synaptic inputs and therefore represents a good measure of synchronously activated afferents, it seems likely that only a small population of the maturating CA3 principal cells drove this glutamatergic bursting. This may also explain why functional glutamatergic transmission has rarely been detected in neonate hippocampal neurons (Ben-Ari et al., 1989; Durand et al., 1996; Khazipov et al., 1997; Hollrigel et al., 1998; Hsia et al., 1998).

### *7.4.2. Physiological implications*

It is generally accepted that endogenous activity plays a central role in the formation of developing neuronal networks (see Goodman and Shatz, 1993). In this context, the spontaneous hippocampal activity at perinatal age has received much attention (Hanse et al., 1997; Traub et al., 1998). The finding that spontaneous hippocampal activity is accompanied by patterned bursting of CA3 glutamatergic cells, which is inhibited by activation of $GABA_A$ receptors, is interesting.

Spontaneous discharge of CA3-CA1 pathways occurred in transient gamma-frequency bursts. Closer study revealed that their activation at gamma frequency has particular importance in the early hippocampal transmission. AMPA-type postsynaptic responses may be difficult to evoke upon single-pulse electrical stimulation during the first postnatal days (Durand et al., 1996; Hsia et al., 1998). To recruit immature CA1 AMPA-type synapses, activation of CA3 efferents should occur as a burst. Intriguingly, activation of CA1 afferents by stimulation that mimicked the natural pattern of discharge seen in CA1 neurons (4 stimuli at 40 Hz frequency) was most effective in eliciting AMPA EPSCs. This speaks for coding in the CA3-CA1 transmission, in which the short gamma burst represents the

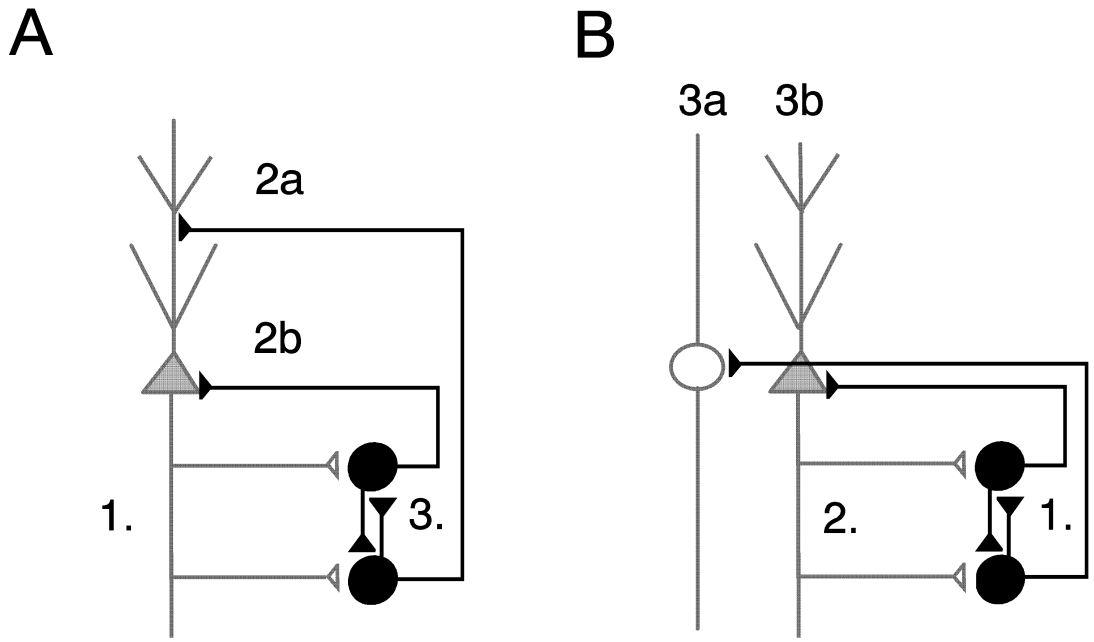



**Figure 12.** $GABA_A$ receptor-mediated excitation in the mature (*A*) and neonate (*B*) rat hippocampal CA3-CA1 network. **A)** Glutamatergic pyramidal cells (*depicted as gray*) employ (1) GABAergic interneurons (*black*), which in turn inhibit the pyramidal cells via dendritic (2a) and somatic (2b) synapses. However, strong glutamatergic excitation and the consequent discharge in GABAergic circuits can result in a positive shift of $E_{GABAA}$ in interneurons, giving rise to $GABA_AR$-mediated excitation in the interneuron network (3). In perisomatic pyramidal cell synapses (which comprise most of the GABAergic input to pyramidal cells), the shift in $E_{GABAA}$ is weak and, consequently, inhibitory input is augmented (2b). However, depolarizing $GABA_A$ responses may be generated locally in distal dendrites (2a). If only dendritic $GABA_A$ synapses are activated, $GABA_A$ depolarization may promote pyramidal cell action potential firing. **B)** Under resting conditions, $E_{GABAA}$ is positive to membrane potential and interneurons are excitatorily connected via $GABA_AR$-mediated synapses (1). Yet, interneuron population bursts are normally triggered by activity of a maturated, functionally connected population of glutamatergic principal cells (2). Local interneuronal bursting may excite developing neuroblasts in the pyramidal layer (3a) (Fiszman et al., 1990; Leinekugel et al., 1995, 1997; Owens et al., 1996), but it inhibits the functionally connected CA3-CA1 glutamatergic pyramidal cells (3b).

optimal pattern to employ glutamatergic synapses (Lisman, 1997). Therefore, it is tempting to speculate that the gamma-frequency bursts of the immature CA3-CA1 connections described here are also used for shaping of the developing synaptic contacts.

In mature neuronal systems, bursts appear to have a special role in synaptic plasticity and information processing (for review Lisman, 1997). Spontaneous activity of the newborn neuronal networks may function as a force guiding local synaptic circuit formation (Goodman and Shatz, 1993). The early pyramidal cell activity and its inhibitory GABAergic control described here may play an important role in this process.

### 7.5. Comparison of $GABA_A$ excitation in the newborn and mature CA3-CA1 networks

Here, experimental approaches revealed that despite being depolarizing by nature, $GABA_AR$ activation is inhibitory by action in the newborn as well as in the mature rat hippocampal pyramidal cells. Recent data also suggest that a subset of CA3 pyramidal cells may be the ultimate drivers of the large depo-

larizing bursts seen throughout the CA3-CA1 regions and the *gyrus dentatus* area of the newborn hippocampus (Khazipov et al., 1997; Hollrigel et al., 1998; Bolea et al., 1999). This periodic glutamatergic drive is likely to arise from synchronization of the CA3 recurrent excitatory loop. As in synchronous CA3 bursts in the mature hippocampus (induced, e.g., by 4-AP; Traub et al., 1996), AMPA-type excitation appears to be responsible for the excitation of pyramidal cells, whereas $GABA_A$-type transmission is clearly inhibitory.

Since also in the newborn rat CA3-CA1 region $GABA_A$ responses are more likely to be excitatory in interneurons than in glutamatergic principal cells, via GABAergic interactions, interneurons can promote local interneuronal bursting as well as propagation of inhibitory activity in the hippocampal network. As was shown here, this is an important mechanism for promoting inhibition of CA3 principal cells in the mature hippocampus.

In the adult CA3-CA1 region, gamma oscillations may arise in networks of interneurons that entrain the firing of pyramidal cells by inhibitory $GABA_AR$-mediated input (Cobb et al., 1995; Fisahn et al., 1998). The GABAergic current peaks during the neonatal population burst occurred predominantly at gamma frequencies, similar to what was seen in adult hippocampal interneuronal bursts. $GABA_A$ currents were shown to be out-of-phase with synchronous firing of CA3 pyramidal cells, and $GABA_AR$ activity restrained both the activity of individual pyramidal cells as well as the pyramidal cell population. It is put forward here that the newborn rat CA3 pyramidal neurons, synchronized by gap junctions (Draguhn et al., 1998), form the 300-400 Hz frequency oscillation that is temporally and spatially modulated to synchronous gamma-frequency bursts by rhythmic $GABA_AR$-mediated inhibition. This mechanism is very similar to that seen in the generation of gamma- and high-frequency oscillations in the mature hippocampus in that it relies on AMPA-type excitation as well as rhythmic $GABA_AR$-mediated inhibition (Cobb et al., 1995; Whittington et al., 1995; Ylinen et al., 1995; Fisahn et al., 1998).

### 7.6. Conclusions

In the mature hippocampus, GABAergic and glutamatergic synaptic mechanisms can act in concert to enhance neuronal excitation. Strong postsynaptic $GABA_A$ receptor activity can shift initially hyperpolarizing IPSPs to depolarizations and under certain conditions (e.g. proximal high-frequency stimulation of CA1 afferents) provide strong excitatory drive to interneurons as well as the dendritic tree of pyramidal cells. $GABA_AR$-mediated excitation is also accompanied by neuronal calcium uptake. It is suggested that excitatory $GABA_A$ responses may contribute to synaptic plasticity and epileptogenesis **(I, II)**.

Some basic properties of the activity-induced $GABA_A$ depolarization are similar in interneurons and pyramidal cell dendrites (i.e. dependence of the $E_{GABAA}$ shift on the postsynaptic $GABA_AR$ conductance as well as on the availability of bicarbonate). GABAergic excitation between interneurons synchronizes discharge in the interneuron network, generating prolonged inhibitory interneuronal input to pyramidal cells. The excitatorily connected GABAergic interneuron network can generate self-sustained synchronous 20-40 Hz (gamma) oscillation in the CA3 region **(I, III, IV)**.

Previously, only the excitatory role of depolarizing $GABA_A$ responses has been demonstrated in the newborn rat hippocampus. However, here it was shown that already from birth $GABA_AR$-mediated activity has an important inhibitory effect. Although $GABA_A$ responses can be strongly depolarizing at this age, they act as inhibitory to a functionally maturated population of CA3-CA1 glutamatergic neurons. Interneurons are excitatorily connected via $GABA_AR$-mediated synapses. It is concluded that synaptic mechanisms of synchronous CA3 principal cell bursts are basi-

cally similar to those seen in the adult, since they are driven by fast (AMPA/kainate-type) glutamatergic excitation and restricted by network-driven $GABA_A$R-mediated inhibition. The dual role of $GABA_A$R-mediated transmission is involved in synchronous gamma-frequency bursting of the CA3 network; mutual excitation of interneurons generates rhythmic oscillation of the interneuron network (e.g. at gamma frequency), which provides inhibition of principal cells **(V, VI)**.

A picture emerges where the $GABA_A$R-mediated transmission may simultaneously enhance local excitation and synaptic plasticity in the dendritic area of pyramidal cells, and entrain or damp general excitability by input in the perisomatic area. The strength of both actions is adaptively regulated by the autoexcitation of the interneuron network, developing when the interneuronal activity rises to a certain level. In all these respects, differences between mature and neonate hippocampus appear as quantitative rather than qualitative.

## 8. REFERENCES


Acsady I., Gorcs T. J., Freund T. F. (1996). Different populations of VIP-immunoreactive interneurons are specialized to control pyramidal cells or interneurons in the hippocampus. *Neuroscience* 73:317-334.

Alger B. E., Nicoll R. (1979). GABA-mediated biphasic inhibitory responses in hippocampus. *Nature* 281:315-317.

Alger B. E., Nicoll R. (1982). Feed-forward dendritic inhibition in rat hippocampal pyramidal cell studied in vitro. *J Physiology (Lond)* 328:105-123.

Andersen P., Dingledine R., Gjerstad L., Langmoen I. A., Mosfeldt-Laursen A. (1980). Two different responses of hippocampal pyramidal cells to application of gamma-aminobutyric acid. *J Physiology (Lond)* 305:279-296.

Andersen P., Eccles J. C., Loyning Y. (1964). Pathway of postsynaptic inhibition in the hippocampus. *J Neurophysiology* 27:608-619.

Avoli M., Perreault P. (1987). A GABAergic depolarizing potential in the hippocampus disclosed by the convulsant 4-aminopyridine. *Brain Research* 400:191-195.

Avoli M., Psarropoulou C., Tancredi V., Fueta Y. (1993). On the synchronous activity induced by 4-aminopyridine in the CA3 subfield of juvenile rat hippocampus. *J Neurophysiology* 70(3): 1018-1029.

Barnard E., Skolnick P., Olsen R. W., Mohler H., Sieghart W., Biggio G., Braestrup C., Bateson A., Langer S. Z. (1998). International union of pharmacology: XV. Subtypes of γ-aminobutyric $acid_A$ receptors: classification on the basis of subunit structure and receptor function. *Pharmacological reviews* 50(2):291-313.

Bazemore A. W., Elliott K. A. C., Florey E. (1957). Isolation of factor I. *J Neurochemistry* 1:334-339.

Bekoff A., Stein P. S. G., Hamburger V. (1975). Coordinated motor output in the hindlimb of the 7-day-old chick embryo. *Proc Natl Acad Sciences* 72:1245-1248.

Ben-Ari Y., Cherubini E., Corradetti R., Gaiarsa J.-L. (1989). Giant synaptic potentials in immature rat CA3 hippocampal neurones. *J Physiology (Lond)* 416:303-325.

Ben-Ari Y., Khazipov R., Leinekugel X., Caillard O., Gaiarsa J.-L. (1997). $GABA_A$, NMDA and AMPA receptors: a developmentally regulated menage a trois. *Trends Neurosci* 20(11):523-529.

Bilkey D., Goddard G. (1985). Medial septal facilitation of hippocampal granule activity is mediated by inhibition of inhibitory interneurons. *Brain Research* 361: 99-106.

Blanton M. G., Lo Turco J., Kriegstein A. (1989). Whole cell recording from neurons in slices of reptilian and mammalian cerebral cortex. *J Neurosci Methods* 30:203-210.

Bliss T. V. P., Collingridge G. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361:31-39.

Bolea S., Avignone E., Berretta N., Sanchez-Andres J., Cherubini E. (1999). Glutamate controls the induction of GABA-mediated giant depolarizing potentials through AMPA receptors in neonatal rat hippocampal slices. *J Neurophysiology* 81:2095-2102.

Bormann J. (1988). Electrophysiology of $GABA_A$ and $GABA_B$ receptor subtypes. *Trends Neurosci* 11(3):11-116.

Bormann J., Hamill O. P., Sakmann B. (1987). Mechanism of anion permeation through channels gated by glycine and γ-aminobutyric acid in mouse cultured spinal neurones. *J Physiology (Lond)* 385:243-286.

Bracci E., Vreugdenhil M., Hack S. P., Jefferys J. G. R. (1999). On the synchronizing mechanisms of tetanically induced hippocampal oscillations. *J Neuroscience* 19(18): 8104-8113.

Buckle P. J., Haas H. L. (1982). Enhancement of synaptic transmission by 4-aminopyridine in hippocampal slices. *J Physiology (Lond)* 326:109-122.

Buhl E. H., Cobb S., Halasy K., Somogyi P. (1995). Properties of unitary IPSPs evoked by anatomically identified basket cells in the rat hippocampus. *Eur J Neuroscience* 7:1989-2004.

Buhl E. H., Han Z. S., Lorinczi Z., Stezhka V. V., Somogyi P. (1994). Physiological properties of ana-

tomically identified axo-axonic cells in the rat hippocampus. *J Neurophysiology* 71:1289-1307.

Buzsaki G. (1984). Feed-forward inhibition in the hippocampal formation. *Prog Neurobiology* 22:131-153.

Buzsaki G., Chrobak J. (1995). Temporal structure in spatially organized neuronal ensembles: a role for interneuronal networks. *Curr Opin Neurobiology* 5:504-510.

Cobb S., Buhl E. H., Halasy K., Paulsen O., Somogyi P. (1995). Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. *Nature* 378:75-78.

Cobb S., Manuel N., Morton R., Gill C., Collingridge G., Davies C. H. (1999). Regulation of depolarizing $GABA_A$ receptor-mediated synaptic potentials by synaptic activation of $GABA_B$ autoreceptors in the rat hippocampus. *Neuropharmacology* 38:1723-1732.

Charpak S., Pare D., Llinas R. (1995). The entorhinal cortex entrains fast CA1 hippocampal oscillations in the anaesthetized guinea-pig: role of the monosynaptic component of the perforant path. *Eur J Neuroscience* 7(7): 1548-1557.

Colling S. B., Stanford I. M., Traub R. D., Jefferys J. G. (1998). Limbic gamma rhythms. I. Phase-locked oscillations in hippocampal CA1 and subiculum. *J Neurophysiology* 80: 155-161.

Collingridge G. L., Herron C. E., Lester R. A. (1987). Frequency-dependent N-methyl-D-aspartate receptor-mediated synaptic transmission in rat hippocampus. *J Physiology (Lond)* 399:301-312.

Coyle J. T., Enna S. (1976). Neurochemical aspects of the ontogenesis of GABAergic neurons in the brain. *Brain Research* 111(1):119-133.

Dailey M., Smith S. (1994). Spontaneous $Ca^{2+}$ transients in developing hippocampal pyramidal cells. *J Neurobiology* 25(3):243-251.

Daval J.-L., Sarfati A. (1987). Effects of bicuculline-induced seizures on benzodiazepine and adenosine receptors in developing rat brain. *Life Science* 41:1685-1693.

Davies C. H., Collingridge G. L. (1993). The physiological regulation of synaptic inhibition by $GABA_B$ autoreceptors in rat hippocampus. *J Physiology (Lond)* 472:245-265.

Davies C. H., Davies S. N., Collingridge G. L. (1990). Paired-pulse depression of monosynaptic GABA-mediated inhibitory postsynaptic responses in rat hippocampus. *J Physiology (Lond)* 424: 513-531.

Davies C., H., Starkey S., J., Pozza M. F., Collingridge G. (1991). $GABA_B$ autoreceptors regulate the induction of LTP. *Nature* 349:609-611.

Debray C., Diabira D., Gaiarsa J.-L., Ben-Ari Y., Gozlan H. (1997). Contributions of AMPA and GABA(A) receptors to the induction of NMDAR-dependent LTP in CA1. *Neurosci Letters* 238(3): 119-122.

Diabira D., Hennou S., Chevassus-au-Louis N., Ben-Ari Y., Gozlan H. (1999). Late embryonic expression of AMPA receptor function in the CA1 region of the intact hippocampus in vitro. *Eur J Neuroscience* 11:4015-4023.

Draguhn A., Traub R. D., Schmitz D., Jefferys J. G. R. (1998). Electrical coupling underlies high-frequency oscillations in the hippocampus in vitro. *Nature* 349:189-192.

Duffy S., MacVigar B. (1994). Potassium-dependent calcium influx in acutely isolated hippocampal astrocytes. *Neuroscience* 61:51-61.

Durand G. M., Kovalchuk Y., Konnerth A. (1996). A long-term potentiation and functional synapse induction in developing hippocampus. *Nature* 381:71-75.

Fisahn A., Pike F. G., Buhl E. H., Paulsen O. (1998). Cholinergic induction of network oscillations at 40 Hz in the hippocampus in vitro. *Nature* 394: 186-189.

Fiszman M., Novotny E., Lange G. D., Barker J. L. (1990). Embryonic and early postnatal hippocampal cells respond to nanomolar concentrations of muscimol. *Dev Brain Research* 53:186-193.

Forti M., Michelson H. (1998). Synaptic connectivity of distinct hilar interneuron subpopulation. *J Neurophysiology* 79:3229-3237.

Fortin G., Kato F., Lumsden A., Champagnat J. (1995). Rhythm generation in the segmented hindbrain of chick embryos. *J Physiology (Lond)* 486:735-744.

Freund T., Antal M. (1988). GABA-containing neurons in the septum control inhibitory interneurons in the hippocampus. *Nature* 336:170-173.

Freund T., Buzsaki G. (1996). Interneurons in the hippocampus. *Hippocampus* 6:347-470.

Gaiarsa J.-L., Corradetti R., Cherubini E., Ben-Ari Y. (1991). Modulation of GABA-mediated synaptic potentials by glutamatergic agonists in neonatal CA3 rat hippocampal neurons. *Eur J Neuroscience* 3:301-309.

Garaschuk O., Hanse E., Konnerth A. (1998). Developmental profile and synaptic origin of early network oscillations in the CA1 region of rat neonatal hippocampus. *J Physiology (Lond)* 507:219-236.

Gomez-Di Cesare C., Smith K., Rice F., Swann J. (1997). Axonal remodelling during postnatal maturation of CA3 hippocampal pyramidal neurons. *J Comp Neurology* 384:165-180.

Goodman C., Shatz C. (1993). Developmental mechanisms that generate precise patterns of neuronal connectivity. *Cell/Neuron* 72/10 (Suppl.): 77-98.

Gray C. M. (1994). Synchronous oscillations in neuronal systems: mechanisms and functions. *J Comput Neuroscience* 1:11-38.

Gray C. M., König P., Engel A., Singer W. (1989). Oscillatory responses in cat visual cortex exhibit intercolumnar synchronization which reflects global stimulus properties. *Nature* 338: 334-337.

Greer J. J., Smith J. C., Feldman J. L. (1992). Respiratory and locomotor patterns generated in the fetal rat brain stem-spinal cord in vitro. *J Neurophysiology* 67:996-999.

Grover L., Lambert N. A., Schwartzkroin P., Teyler T. J. (1993). Role of $HCO_3$-ion in depolarizing $GABA_A$ receptor-mediated responses in pyramidal cells of rat hippocampus. *J Neurophysiology* 69:1541-1555.

Gulyas A. L., Hajos N., Freund T. (1996) Interneurons containing calretinin are specialized to control other interneurons in the rat hippocampus. *J Neuroscience* 16:3397-3411.

Hanse E., Durand G. M., Garaschuk O., Konnerth A. (1997). Activity-dependent wiring of the developing hippocampal neuronal circuit. *Sem Cell Dev Biology* 8:35-42.

Hara M., Inoue M., Yasukura T., Ohnishi S., Mikami Y., Inagaki C. (1992). Uneven distribution of intracellular $Cl^-$ in rat hippocampal neurons. *Neurosci Letters* 143:135-138.

Hayashi T. (1959). The inhibitory action of β-hydroxy-γ-aminobutyric acid upon the seizure following stimulation of the motor cortex of the dog. *J Physiology (Lond)* 145:570-578.

Heinemann U., Lux H. D., Gutnick M. J. (1977). Extracellular free calcium and potassium during paroxysmal activity in the cerebral cortex of the rat. *Exp Brain Resear*ch 27:237-243.

Hensch T., Fagiolini M., Mataga N., Stryker M., Baekkeskov S., Kash S. F. (1998). Local GABA circuit control of experience-dependent plasticity in developing visual cortex. *Science* 282:1504-1507.

Hille B. (1984). Ionic channels of excitable membranes. Sinauer Associates, Sunderland, MA.

Hollmann M., Hartley M., Heinemann S. (1991). $Ca^{2+}$ permeability of KA-AMPA-gated glutamate receptor channels depends on subunit composition. *Science* 252:851-853.

Hollrigel G., Ross S., Soltesz I. (1998). Temporal patterns and depolarizing actions of the spontaneous $GABA_A$ receptor activation in the granule cells of the early postnatal dentate gyrus. *J Neurophysiology* 80:2340-2351.

Hollrigel G., Soltesz I. (1998). Slow kinetics of miniature IPSCs during early postnatal development in granule cells of the dentate gyrus. *J Neuroscience* 17(13): 5119-5128.

Holmes G., Ben-Ari Y. (1998). Seizures in the developing brain: Perhaps not so benign after all. Neuron 21:1231-1234.

Hsia A., Malenka R., Nicoll R. (1998). Development of excitatory circuitry in the hippocampus. *J Neurophysiology* 79:2013-2024.

Huttunen J., Tolvanen H., Heinonen E., Voipio J., Wikstrom H., Ilmoniemi R. J., Hari R., Kaila K. (1999). Effects of voluntary hyperventilation on cortical sensory responses. Electroencephalographic and magnetoencephalographic studies. *Exp Brain Research* 125(3):248-254.

Isaac J. T. R., Crair M. C., Nicoll R., Malenka R. (1997). Silent synapses during development of thalamocortical inputs. *Neuron* 18:269-280.

Jackson M. F., Esplin B., Capek R. (1999a). Activity-dependent enhancement of hyperpolarizing and depolarizing γ-aminobutyric acid (GABA) synaptic responses following inhibition of GABA uptake by tiagabine. *Epilepsy Research* 37:25-36.

Jackson M. F., Esplin B., Capek R. (1999b). Inhibitory nature of tiagabine-augmented $GABA_A$ receptor-mediated depolarizing responses in hippocampal pyramidal cells. *J Neurophysiology* 81:1192-1198.

Jefferys J. G. R., Traub R. D., Whittington M. (1996). Neuronal networks for induced “40 Hz” rhythms. *Trends Neurosci* 19:202-208.

Kaila K. (1994). Ionic basis of $GABA_A$ receptor channel function in the nervous system. *Prog Neurobiology* 42:489-537.

Kaila K., Lamsa K., Smirnov S., Taira T., Voipio J. (1997). Long-lasting GABA-mediated depolarization evoked by high-frequency stimulation in pyramidal neurons of rat hippocampal slice is attributable to a network, bicarbonate-dependent $K^+$ transient. *J Neuroscience* 17(20):7662-7672.

Kaila K., Paalasmaa P., Taira T., Voipio J. (1992). pH transients due to monosynaptic activation of $GABA_A$ receptors I rat hippocampal slices. *Neuroreport* 3:105-108.

Kaila K., Voipio J. (1987). Postsynaptic fall in intracellular pH induced by GABA-activated bicarbonate conductance. *Nature* 330:163-165.

Kaila K., Voipio J. (1990). GABA-activated bicarbonate conductance. In F. Alvarez-Leefmans, J. Russell (eds.): “Influence on $E_{GABA}$ and on postsynaptic pH regulation. In: “Chloride channels and carriers in nerve, muscle and glial cells.” Pp. 331-353. Plenum-New York.

Kaila K., Voipio J., Paalasmaa P., Pasternack M., Deisz R. A. (1993). The role of bicarbonate in GABAA receptor-mediated IPSPs of rat neocortical neurones. *J Physiology (Lond)* 464:273-89.

Katsumaru H., Kosaka T., Heizmann C. W., Hama K. (1988). Gap junctions on GABAergic neurons containing the calcium-binding protein parvalbumin in the rat hippocampus (CA1 region). *Exp Brain Research* 72:363-370.

Katz L. C. (1993). Coordinate activity in retinal cord and cortical development. *Curr Opin Neurobiology* 3:93-99.

Katz L. C., Shatz C. J. (1996). Synaptic activity and the construction of cortical circuits. *Science* 274:1133-1138.

Khalilov I., Dzhala V., Ben-Ari Y., Khazipov R.(1999a). Dual role of GABA in the neonatal rat hippocampus. *Dev Neuroscience* 21:310-319.

Khalilov I., Dzhala V., Medina I., Leinekugel X., Melyan Z., Lamsa K., Khazipov R., Ben-Ari Y. (1999b). Maturation of kainate-induced epileptiform activities activities in interconnected intact neonatal limbic structures in vitro. *Eur J Neuroscience* 11:3468-3480.

Khalilov, I., Esclapez M., Medina I., Aggoun D., Lamsa K., Leinekugel X., Khazipov R., Ben-Ari Y. (1997). A novel in vitro preparation: the intact hippocampal formation. *Neuron* 19:743-749.

Khazipov R., Leinekugel X., Khalilov I., Gaiarsa J.-L., Ben-Ari Y. (1997). Synchronization of GABAergic interneuronal network in CA3 subfield of neonatal rat hippocampal slices. *J Physiology (Lond)* 498:763-772.
Kravitz E. A., Kuffler S. W., Potter D. (1963). Gamma amino butyric acid and other blocking compounds in crustacea. III. Their relative concentrations in separated motor and inhibitory axons. *J Neurophysiology* 26:739-751.

Krnjevic K. (1976). Inhibitory action of GABA and GABA-mimetics on vertebrate neurons. In Roberts E., Chase T., Tower D. (eds): “GABA in nervous system function.” New York: Raven Press, pp. 269-281.

Kuffler S. W. (1958). Synaptic inhibitory mechanisms. Properties of dendrites and problems of excitation in isolated sensory nerve cells. *Exp Cell Res Suppl* 5:493-519.

Kuffler S. W., Edwards C. (1958). Mechanism of gamma aminobutyric acid (GABA) action and its relation to synaptic inhibition. *J Neurophysiology* 21:589-610.

Lacaille J.-C. (1991). Postsynaptic potentials mediated by excitatory and inhibitory amino acids in interneurons of stratum pyramidale of the CA1 region of rat hippocampus slices in vitro. *J Neurophysiology* 66:1441-1454.

Lacaille J.-C., Schwartzkroin P. (1988). Stratum lacunosum-moleculare interneurons of hippocampal CA1 region. I. Intracellular response characteristics, synaptic responses, and morphology. *J Neuroscience* 8:1400-1410.

Lambert N. A., Borroni A., Grover L. M., Teyler T. J. (1991). Hyperpolarizing and depolarizing $GABA_A$ receptor-mediated dendritic inhibition in area CA1 of the rat hippocampus. *J Neurophysiology* 66:1538-1548.

Lamsa K., Palva J.-M., Kaila K., Taira T. (1999). AMPA-R-mediated EPSCs in CA1 neurons evoked by synchronous gamma-frequency bursts of CA3 region in the P0-P2 rat hippocampus. *Soc Neurosci Abstract* 29:399.11.

Lauder J., Han V., Henderson P., Verdoorn T., Towle A. (1986). Prenatal ontogeny of the GABAergic system in the rat brain: an immunocytochemical study. *Neuroscience* 19:465-493.

Leinekugel X., Khalilov I., Ben-Ari Y., Khazipov R. (1998). Giant depolarizing potentials: the septal pole of the hippocampus paces the activity of the developing intact septohippocampal complex in vitro. *J Neuroscience* 18:6349-6357.

Leinekugel X., Medina I., Khalilov I., Ben-Ari Y., Khazipov R. (1997). $Ca^{2+}$ oscillations mediated by the synergistic excitatory actions of $GABA_A$ and NMDA receptors in the neonatal hippocampus. *Neuro*n 18:243-255.

Leinekugel X., Tseeb V., Ben-Ari Y., Bregestovski P. (1995). Synaptic $GABA_A$ activation induces $Ca^{2+}$ rise in pyramidal cells and interneurons from rat neonatal hippocampal slices. *J Physiology (Lond)* 487: 319-329.

Li X.-G., Somogyi P., Tepper J.M., Buzsaki G. (1992). Axonal and dendritic arborization of an intracellularly labeled chandelier cell in the CA1 region of rat hippocampus. *Exp Brain Res* 90:519-525.

Liao D., Malinow R. (1996). Deficiency in induction but not expression of LTP in hippocampal slices from young rats. *Learning and memory* 3(2-3):138-149.

Lippe W. R. (1994). Rhythmic spontaneous activity in the developing avian auditory system. *J Neuroscience* 14:1486-1495.

Lisman J. E. (1997). Bursts as a unit f neural information: making unreliable synapses reliable. *Trends Neurosci* 20(1):38-43.

Lo Turco J., Owens D., Health M., Davis M. B. E., Kriegstein A. (1995). GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. *Neuron* 15:1287-1298.

MacDonald R. L., Rogers C. J., Twyman R. (1989). Barbiturate regulation of kinetics properties of the $GABA_A$ receptor channel of mouse spinal neurones in culture. *J Physiology (Lond)* 417:483-500.

Maeda E., Robinson H. P. C., Kawana A. (1995). The mechanisms of generation and propagation of synchronized bursting in developing networks of cortical neurons. *J Neuroscience* 15:6834-6845.

Magee J. C., Johnston D. (1995). Synaptic activation of voltage-gated channels in the dendrites of hippocampal pyramidal neurons. *Science* 268:301-304.

Mason M. J., Pasternack M., Rydqvist B., Kaila K. (1990). Effect of γ-aminobutyric acid (GABA) on intracellular pH in the crayfish stretch receptor neurone. *Neuroscience* 34:359-368.

Mayer M. L., Westbrook G. L. (1987) Permeation and block of N-methyl-D-aspartic acid receptor channels by divalent cations in mouse cultured central neurones. *J Physiology (Lond)* 394:501-527.

McLean H. A., Caillard O., Khazipov R., Ben-Ari Y., Gaiarsa J.-L. (1996). Spontaneous release of GABA activates $GABA_B$ receptors and controls network activity in the neonatal rat hippocampus. *J Neurophysiology* 76:1036-1046.

Merlin L. R., Wong R. K. S. (1993). Synaptic modifications accompanying epileptogenesis in vitro:long-term depression of GABA-mediated inhibition. *Brain Research* 627:330-340.

Michelson H., Wong R. K. S. (1991). Excitatory synaptic responses mediated by $GABA_A$ receptors in the hippocampus. *Science* 253:1420-1423.

Michelson H., Wong R. K. S. (1994). Synchronization of inhibitory neurones in the guinea-pig hippocampus in vitro. *J Physiology (Lond)* 477.1:35-45.

Miles R., Toth K., Gulyas A., I., Hajos N., Freund T. F. (1996). Differences between somatic and dendritic inhibition in the hippocampus. *Neuron* 16:815-823.

Misgeld U., Deisz R., Dodt H. U., Lux H. D. (1986). The role of chloride transport in postsynaptic inhibition of hippocampal neurons. *Science* 232:1413-1415.

Misgeld U., Frotscher M. (1986). Postsynaptic GABAergic inhibition of non-pyramidal neurons in the guinea-pig hippocampus. *Neuroscience* 19:193-206.

Müller D. Joly M., Lynch G. (1988). Contributions of quisqulate and NMDA receptors to the induction and expression of LTP. *Science* 242:1694-1697.

Murthy V. N., Fetz E. E. (1992). Coherent 25- to 35-Hz oscillations in the sensorimotor cortex of awake behaving monkeys. *Proc Natl Acad Sciences* 89: 5670-5674.

Nicholson C., Phillips J. M. (1981). Ion diffusion modified by tortuosity and volume fraction in the extracellular microenvironment of the rat cerebellum. *J Physiology (Lond)* 321:225-257.

Nowak L., Bregestovski P., Ascher P., Herbet A., Prochiantz A. (1984). Magnesium gates glutamate-

activated channels in mouse central neurones. *Nature* 307:462-465.

O`Donovan M. (1999). The origin of spontaneous activity in developing networks of the vertebrate nervous system. *Curr Op Neurobiology* 9:94-104.

O`Donovan M., Chub N., Wenner P. (1998). Mechanisms of spontaneous activity in developing spinal networks. *J Neurobiology* 37:131-145.

Owens D., Boyce L., Davis M. B. E., Kriegstein A. (1996). Excitatory GABA responses in embryonic and neonatal cortical slices demonstrated by gramicidin perforated patch recordings and calcium imaging. *J Neuroscience* 16:6414-6423.

Owens D., Xiaolin L, Kriegstein A. (1999). Changing properties of $GABA_A$ receptor-mediated signaling during early neocortical development. *J Neurophysiology* 82:570-583.

Parra P., Gulyas A., Miles R. (1998). How many subtypes of inhibitory cells in the hippocampus? *Neuron* 20:983-993.

Paulsen O., Moser E. I. (1998). A model of hippocampal memory encoding and retrieval: GABAergic control of synaptic plasticity. *Trends Neurosci* 21:273-278.

Perkins K. (1999). $Cl^-$ accumulation does not account for the depolarizing phase of the synaptic GABA response in hippocampal pyramidal cells. *J Neurophysiology* 82:768-777.

Perkins K., Wong R. K. S. (1996). Ionic basis of the postsynaptic depolarizing GABA response in hippocampal pyramidal cells. *J Neurophysiology* 76:3886-3893.

Perrault P., Avoli M. (1992). 4-aminopyridine-induced epileptiform activity and a GABA-mediated long-lasting depolarization in the rat hippocampus. *J Neuroscience* 12(1):104-115.

Petralia R., Esteban J. A, Wang Y.-X., Partridge J., Zhao H.-M., Wenthold R., Malinow R. (1999). Selective acquisition of AMPA receptors over postnatal development suggests a molecular basis for silent synapses. *Nature Neurosci* 2(1):31-36.

Pitler T. A., Alger B. E. (1992). Postsynaptic spike firing reduces synaptic $GABA_A$ responses in hippocampal pyramidal cells. *J Neuroscience* 12:4122-4132.

Psarropoulou C., Avoli M. (1999). Differential bicuculline-induced epileptogenesis in rat neonatal, juvenile and adult CA3 pyramidal neurons in vitro. *Dev Brain Research* 117:117-120.

Qian N., Sejnowski T. J., (1990). When is an inhibitory synapse effective? *Proc Natl Acad Sciences* 87:8145-8149.

Ramon y Cajal S. (1893). Estructura del asta de ammon y fascia dentata. *Ann Soc Esp Hist Nat* 22.

Rivera C., Voipio J., Payne J., Ruusuvuori E., Lahtinen H., Lamsa K., Pirvola U., Saarma M., Kaila K. (1999). The $K^+/Cl^-$ co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature* 397:251-255.

Rohrbough J., Spitzer N. (1996). Regulation of intracellular $Cl^-$ levels by $Na^+$ -dependent $Cl^-$ cotransport distinguishes depolarizing from hyperpolarizing $GABA_A$ receptor-mediated responses in spinal neurons. *J Neuroscience* 16(1):82-91.

Roos A., Boron W. (1981). Intracellular pH. *Physiol review* 61:296-433.

Sarantis M., Ballerini, L., Miller B., Silver R. A., Edwards M., Attwell, D. (1993). Glutamate uptake from the synaptic cleft does not shape the decay of the non-NMDA component of the synaptic current. *Neuron* 11:541-549.

Schlessinger A., Cowan W. M., Gottlieb D. (1975). An autoradiographic study of the time of origin and the pattern of granule cell migration in the dentate gyrus of the rat. *J Comp Neurology* 159:149175.

Skerritt J., Johnston G. A. (1982). Postnatal development of GABA binding sites and their endogenous inhibitors in rat brain. *Devel Neuroscience* 5(2-3):189-197.

Smirnov S., Paalasmaa P., Uusisaari M., Voipio J., Kaila K. (1999). Pharmacological isolation of the synaptic and nonsynaptic components of the GABA-mediated biphasic response in rat CA1 hippocampal pyramidal cells. *J Neuroscience* 19(21):9252-9260.

Soltesz I., Deschenes M (1993). Low- and high-frequency membrane potential oscillation during theta activity in CA1 and CA3 pyramidal neurons of the rat hippocampus under ketamine-xylazine anesthesia. *J Neurophysiology* 70:97-116.

Soriano E., Cobas A., Fairen A. (1989). Neurogenesis of glutamic acid decarboxylase immunoreactive cells in the hippocampus of the mouse. II. Area dentata. *J Comp Neurology* 281:603-611.

Staley K., Mody I. (1992). Shunting of excitatory input to dentate gyrus granule cells by depolarising $GABA_A$ receptor-mediated postsynaptic conductance. *J Neurophysiology* 68: 197-212.

Staley K. Proctor W. (1999). Modulation of mammalian dendritic $GABA_A$ receptor function by the kinetics of $Cl^-$ and $HCO_3^-$ transport. *J Physiology (Lond)* 519.3:693-712.

Staley K., Soldo B., Proctor W. (1995). Ionic mechanisms of neuronal excitation by inhibitory $GABA_A$ receptors. *Science* 269:977-981.

Stelzer A., Shi H. (1994). Impairment of $GABA_A$ receptor function by N-methyl-D-aspartate-mediated calcium influx in isolated CA1 pyramidal cells. *Neuroscience* 62(3): 813-828.

Stelzer A., Simon G., Kovacs G., Rai R. (1994). Synaptic disinhibition during maintenance of long-term potentiation in the CA1 hippocampal subfield. *Proc Natl Sciences* 91(8):3058-3062.

Strata F., Atzori M., Molnar M., Ugolini G., Tempia F., Cherubini E. (1997). A pacemaker current in dye-coupled hilar interneurons contributes to the generation of giant GABAergic potentials in developing hippocampus. *J Neuroscience* 17(4):1435-1446.

Swann J. W., Brady R., Martin D. (1989). Postnatal development of GABA-mediated synaptic inhibition in rat hippocampus. *Neuroscience* 28(3):551-561.

Taira T., Voipio J., Paalasmaa P., Kaila K. (1995). Relative contributions of excitatory and inhibitory neuronal activity to alkaline transients evoked by stimulation of Schaffer collaterals in the rat hippocampal slice. *J Neurophysiology* 74(2): 643-649.

Takeuchi A., Takeuchi N. (1966). A study of the inhibitory action of γ-aminobutyric acid on neuromuscular transmission in the crayfish. *J Physiology (Lond)* 183:418-432.

Takeuchi A., Takeuchi N. (1967a). Anion permeability of the inhibitory postsynaptic membrane of the crayfish neuromuscular junction. *J Physiology (Lond)* 191:575-590.

Takeuchi A., Takeuchi N. (1967b). Electrophysiological studies of the action of GABA on the synaptic membrane. *Fed Proc* 26:1633-1638.

Tamas G., Buhl E., Lörincz A., Somogyi P. (2000). Proximally targeted GABAergic synapses and gap junctions synchronize cortical interneurons. *Nature Neurosci* 3(4): 366-371.

Tapia R., Sitges M., Morales E. (1985). Mechanism of the calcium-dependent stimulation of transmitter release by 4-aminopyridine in synaptosomes. *Brain Research* 361:373-382.

Thompson S. M., Deisz R., Prince D. A. (1988). Relative contributions of passive equilibrium and active transport to the distribution of chloride in mammalian cortical neurons. *J Neurophysiology* 60:105-120.

Thompson S. M., Gähwiler B., H. (1989a). Activity-dependent disinhibition. I. Repetitive stimulation reduces IPSP driving force and conductance in the hippocampus in vitro. *J Neurophysiology* 61: 501-511.

Thompson S. M., Gähwiler B., H. (1989b). Activity-dependent disinhibition. II. Effects of extracellular potassium, furosemide, and membrane potential on $E_{Cl^-}$ in hippocampal neurons. *J Neurophysiology* 61: 512-523.

Toth K., Freund T., Miles R. (1997). Disinhibition of rat hippocampal pyramidal cells by GABAergic afferents from the septum. *J Physiology (Lond)* 500.2:463-474.

Traub R. D., Jefferys J. G. R., Miles R., Whittington M., Toth K. (1994). A branching dendritic model of a rodent CA3 pyramidal neurone. *J Physiology (Lond)* 481:79-95.

Traub R. D., Spruston N., Soltesz I., Konnerth A., Whittington M., Jefferys J. G. R. (1998). Gamma-frequency oscillations: a neuronal population phenomenon regulated by synaptic and intrinsic cellular processes. *Prog Neurobiology* 55:1-13.

Traub R. D., Whittington M., Colling S., Buzsaki G., Jefferys J. G. R. (1996). Analysis of gamma rhythms in the rat hippocampus in vitro and in vivo. *J Physiology (Lond)* 493.2:471-484.

Tyzio R., Represa A., Jorquera I., Ben-Ari Y., Gozlan H., Aniksztejn L. (1999). The establishment of GABAergic and glutamatergic synapses on CA1 pyramidal neurons is sequential and correlates with the development of the apical dendrite. *J Neuroscience* 19(23): 10372-10382.

Van Eden C., Mrzljak L., Voorn P., Uyling H. (1989). Prenatal development of GABAergic neurons in the neocortex of the rat. *J Comp Neurology* 6:213-227.

Verhage M., Maia A., Plomp J., Brussaard A., Heeroma J., Vermeer H., Toonen R., Hammer R., Van den Berg T., Missler M., Geuze H., Südhof T. (2000). Synaptic assembly of the brain in the absence of neurotransmitter secretion. *Science* 287:864-869.

Whittington M., Traub R. D., Jefferys J. G. R. (1995). Synchronized oscillations in interneuron networks driven by metabotropic glutamate receptor activation. *Nature* 373:612-615.

Wigström H., Gustafsson B. (1983). Facilitated induction of hippocampal long-lasting potentiation during blockade of inhibition. *Nature* 301:603-604.

Wigström H., Gustafsson B. (1985). Facilitation of hippocampal long-lasting potentiation by GABA antagonists. *Acta Physiol Scandinavica* 125:159-172.

Wong P.T., McGeer E. G. (1981). Postnatal changes of GABAergic and glutamatergic parameters. *Brain Research* 227(4):519-529.

Wong R. K. S., Watkins D. J. (1982). Cellular factors influencing $GABA_A$ response in hippocampal pyramidal cells. *J Neurophysiology* 48:938-954.

Wyllie D. J. A., Manabe T., Nicoll R. A. (1994). A rise in postsynaptic $Ca^{2+}$ potentiates miniature excitatory postsynaptic currents and AMPA responses in hippocampal neurons. *Neuron* 12:127-138.

Xie X., Smart T. G. (1991). A physiological role for endogenous zinc in rat hippocampal synaptic transmission. *Nature* 349:521-524.

Ylinen A., Bragin A., Nadasdy Z., Jando G., Szabo I., Sik A., Buzsaki G. (1995). Sharp wave-associated high frequency oscillation (200 Hz) in the intact hippocampus: network and intracellular mechansims. *J Neuroscience* 15:30-46.