Tonically Active Kainate Receptors (tKARs): A Novel Mechanism Regulating Neuronal Function in the Brain

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My beloved Grandma passed away 1998 and I want to dedicate this thesis to her. When I was a kid she was my best friend. I will always remember the times when I sat on a branch of a pine tree and called myself tohtori pöllö (Dr. Owl). My grandmother took the role of sick animals in the forest and I always had the remedy even in the most demanding cases. Back then I called myself a doctor and now I´m finally getting the degree.Thank you dear Mummi for inspiring me to become what I am!

Table of contents

1 Original publications

This thesis is based on the following publications, referred to by Roman numerals (I-III) and on unpublished results

I: Lauri SE, Segerstrale M, Vesikansa A, Maingret F, Mulle C, Collingridge GL, Isaac JTR, Taira T (2005) Endogenous activation of kainate receptors regulate glutamate release and network activity in the developing hippocampus. J Neurosci 25:4473–4484.

II: Segerstrale M, Juuri J, Lanore F, Piepponen P, Lauri SE, Mulle C, Taira T (2010) High firing rate of neonatal hippocampal interneurons is caused by attenuation of afterhyperpolarizing potassium currents by tonically active kainate receptors. J Neurosci 19:6507-6514.

III: Lauri SE, Vesikansa A, Segerstrale M, Collingridge GL, Isaac JTR, Taira T (2006) Functional maturation of CA1 synapses involves activity- dependent loss of tonic kainate receptor-mediated inhibition of glutamate release. Neuron 50:415–429.

Author's contribution to the studies incuded in the thesis:
I The author participated in the electrophysiological work (studying the effects of ATPA, LY, TBOA and glutamate scavenger on spontaneous activity in CA3), the data-analysis and writing of the manuscript.
II The author designed most of the experiments, did all the electrophysiological experiments on GluK1 knock-out mice in the lab of prof. Christophe Mulle (Université Bordeaux 2), participated in most of the pharmacological studies, analyzed the data and participated in writing the manuscript.
III The author participated in the electrophysiological work (studying the specificity of the GluK1 antagonist LY382884 and elucidating the effects of ATPA, LY382885, TBOA and glutamate scavenger on spontaneous activity in CA1), contributed to the data-analysis and writing of the manuscript.

2 Abbreviations

AC	adenylyl cyclase
AHP	afterhyperpolarizing potential
AMPA	2-amino-3-(5-methyl-3-oxo-1,2- oxazol-4-yl)propanoic acid
CA1	cornu ammonis 1
CA3	cornu ammonis 3
cAMP	cyclic AMP
EPSC	excitatory postsynaptic current
GABA	gamma amino butyric acid
GDP	Giant depolarizing potential
GluA1-4	glutamate AMPA receptor subunits 1-4
GluK1-5	glutamate kainate receptor subunits 1-5
Im_{AHP}	medium afterhyperpolarizing current
IPSC	inhibitory postsynaptic current
Is_{AHP}	slow afterhyperpolarizing current
KAR	kainate receptor
LTD	long term depression
LTP	long term potentiation
mEPSC	miniature excitatory postsynaptic current
mIPSC	miniature inhibitory postsynaptic current
NMDA	*N*-Methyl-D-aspartatic acid
PKA	protein kinase A
PKC	protein kinase C
PLC	phospholipase C
Pr	release probability
tKAR	tonically activated kainate receptors
trkB	BDNF-neurotrophic tyrosine kinase receptor type 2

3 Abstract

Fast excitatory transmission between neurons in the central nervous system is mainly mediated by L-glutamate acting on ligand gated (ionotropic) receptors. These are further categorized according to their pharmacological properties to AMPA (2-amino-3-(5-methyl-3-oxo-1,2- oxazol-4-yl)propanoic acid), NMDA (*N*-Methyl-D-aspartic acid) and kainate (KAR) subclasses. In the rat and the mouse hippocampus, development of glutamatergic transmission is most dynamic during the first postnatal weeks. This coincides with the declining developmental expression of the GluK1 subunit-containing KARs. However, the function of KARs during early development of the brain is poorly understood. The present study reveals novel types of tonically active KARs (hereafter referred to as tKARs) which play a central role in functional development of the hippocampal CA3-CA1 network. The study shows for the first time how concomitant pre- and postsynaptic KAR function contributes to development of CA3-CA1 circuitry by regulating transmitter release and interneuron excitability. Moreover, the tKAR-dependent regulation of transmitter release provides a novel mechanism for silencing and unsilencing early synapses and thus shaping the early synaptic connectivity.

The role of GluK1-containing KARs was studied in area CA3 of the neonatal hippocampus. The data demonstrate that presynaptic KARs in excitatory synapses to both pyramidal cells and interneurons are tonically activated by ambient glutamate and that they regulate glutamate release differentially, depending on target cell type. At synapses to pyramidal cells these tKARs inhibit glutamate release in a G-protein dependent manner but in contrast, at synapses to interneurons, tKARs facilitate glutamate release. On the network level these mechanisms act together upregulating activity of GABAergic microcircuits and promoting endogenous hippocampal network oscillations. By virtue of this, tKARs are likely to have an instrumental role in the functional development of the hippocampal circuitry.

The next step was to investigate the role of GluK1 -containing receptors in the regulation of interneuron excitability. The spontaneous firing of interneurons in the CA3 stratum lucidum is markedly decreased during development. The shift involves tKARs that inhibit medium-duration afterhyperpolarization (mAHP) in these neurons during the first postnatal week. This promotes burst spiking of interneurons and thereby increases GABAergic activity in the network synergistically with the tKAR-mediated facilitation of their excitatory drive. During development the amplitude of evoked medium afterhyperpolarizing current (Im_{AHP}) is dramatically increased due to decoupling tKAR activation and Im_{AHP} modulation. These changes take place at the same time when the endogeneous network oscillations disappear.

These tKAR-driven mechanisms in the CA3 area regulate both GABAergic and glutamatergic transmission and thus gate the feedforward excitatory drive to the area CA1. Here presynaptic tKARs to CA1 pyramidal cells suppress glutamate release and enable strong facilitation in response to high-frequency input. Therefore, CA1 synapses are finely tuned to high-frequency transmission; an activity pattern that is common in neonatal CA3-CA1 circuitry both *in vivo* and *in vitro*. The tKAR-regulated release probability acts as a novel presynaptic silencing mechanism that can be unsilenced in response to Hebbian activity.

The present results shed new light on the mechanisms modulating the early network activity that paves the way for oscillations lying behind cognitive tasks such as learning and memory. Kainate receptor antagonists are already being developed for therapeutic use for instance against pain and migraine. Because of these modulatory actions, tKARs also represent an attractive candidate for therapeutic treatment of developmentally related complications such as learning disabilities.

4 Introduction

L-glutamate is the major transmitter mediating fast excitatory transmission in the mammalian central nervous system. Glutamate binds to three subtypes of ionotropic glutamate receptors (iGluRs) namely AMPA, NMDA and KARs (Jahr & Stevens, 1987; Ozawa et al, 1998). In addition to iGluRs glutamate is a ligand to three sub-groups of metabotropic, seven transmembrane region, G-protein coupled receptors, mGluRs (Conn & Pinn, 1997). The iGluRs are homo- or heteromeric tetrameric receptor channels composed of multiple, often alternatively spliced and edited subunits; GluA1-4 for AMPA receptors, NR1,2A-D for NMDA receptors and GluK1-5 for kainate receptors (Lerma 2003; Lerma 2006). The ionotropic action of AMPA and NMDA receptors is well understood (Ben-Ari et al., 1997; Wisden & Seaburg, 1993) whereas the function of kainate receptors is slowly emerging from obscurity (Contractor et al., 2011; Jane et al., 2009; Kullmann 2001).

Glutamate elicits its actions predominantly through phasic synaptic transmission but recent results have shown, however, that this is not the whole picture. Many studies have confirmed the finding that ambient levels of glutamate activate presynaptic NMDARs (Sah et al, 1989) and mGluRs (Losonczy et al., 2003) and subsequently regulate transmitter release. Glutamatergic transmission in the hippocampus (fig. 1) is under dynamic development during the first postnatal weeks in rats (Fiala et al., 1998; Hsia et al., 1998; Tyzio et al., 1999), and ambient glutamate levels are high. At the same time KARs are heavily expressed (Bahn et al., 1994; Ritter et al., 2002).

Figure 1: Major glutamatergic connections in the hippocampal formation

There are three main types of excitatory glutamatergic principal cells in the hippocampal formation. Dentate gyrus (DG) cells recieve input from entorhinal cortex via perforant path (pp) and project their axons to CA3 pyramidal cells through the mossy fibers (mf). In turn the CA3 pyramidal cells project their axons to pyramidal cells in CA1. Together these connections form the trisynaptic pathway, a term coined by Per Andersen in 1979. In addition to the mossy fibers, CA3 pyramidal cells receive input from septum and the contralateral CA3 through the associational commissural (A/C) pathway and from the entorhinal cortex through the perforant path (PP). Entorhinal cortex also innervates the CA1 area directly (Schematic of the rat brain adapted from Andersen et al., 2007)

4.1 KAR-mediated transmission in mature hippocampus

In the adult brain postsynaptic KARs mediate slow, small amplitude excitatory postsynaptic currents (Castillo et al., 1997; Vignes & Collinridge 1997) while their presynaptic function is to act as auto- or heteroreceptors regulating transmitter release at both glutamatergic and GABAergic synapses in many areas of the nervous system (Kullmann, 2001; Lerma, 2003; Isaac et al., 2004; Lauri et al., 2001a&b; Pinheiro & Mulle 2008; Jane et al., 2009). The roles of KARs in the neonatal brain are not well understood.

Kainate receptors are structurally similar to AMPARs since they also are tetramers, but built from 5 subunits; GluK1-5. The basic subunits GluK1-3 (formerly known as GluR5-7) can form functional homomers when expressed in recombinant expression systems (Barberis et al., 2008; Perrais et al., 2009) whereas the high affinity subunits GluK4-5 (formerly known as KA1-2) cannot. GluK4-5 can co-assemble with GluK1-GluK3 altering biophysical properties of the receptors (Pinheiro & Mulle, 2006; Contractor et al., 2011). This gives a rise to diverse properties and function of KARs and variability of the KAR subunit composition at tissue- (Wisden and Seeburg, 1993) and single cell level (Bureau et al., 2000; Ruano et al., 1995). GluK1 and GluK2 also coassemble forming recombinant and native receptors with novel functional properties (Cui & Mayer, 1999; Mulle et al., 2000; Paternain et al., 2000). Complexity is further increased by RNA editing and by the existence of splice variants for GluK1, GluK2, and GluK3 receptor subunits (Bettler and Mulle, 1995; Dingledine et al., 1999; Jaskolski et al., 2004; Lerma et al., 2001)

4.2 KARs control GABAergic input to hippocampal pyramidal cells

Synaptically-released L-glutamate acting on KARs can regulate the activity of interneurons by providing postsynaptically-mediated excitatory drive (Cossart et al., 1998; Frerking et al., 1998; Frerking & Ohliger-Frerking, 2002; Goldin et al., 2007) or directly depolarizing axons (Semyanov & Kullmann, 2001). Activation of somatodendritic KARs presumably underlies interneuron depolarization and, in concert with direct axonal excitation, increases spontaneous action potential-dependent release of GABA from interneuron synapses to pyramidal cells. This result is reproduced with exogenous application of KAR agonists (Cossart et al, 1998; Frerkinget al., 1998). Both pharmacological evidence (Cossart et al., 1998) and studies using GluK1 deficient mice (Bureau et al., 1999) support a role for GluK1-containing subtypes in this effect, probably in heteromeric combination with GluK2 (Mulle et al., 2000).

In addition, pharmacological studies suggest that KARs can directly depress GABAergic synapses (Clarke et al., 1997; Rodríguez-Moreno et al., 1997). Whether this represents a distinct effect of KARs, located on the presynaptic terminals of interneurons themselves, or results from an indirect consequence of interneuronal depolarization through the activation of somatodendritic KARs is somewhat controversial. It was proposed that the resultant increase in GABA as a result of spontaneous interneurone activity lead to a direct reduction of evoked GABA release following the activation of presynaptic $GABA_B$ receptors and a passive shunting of the postsynaptic GABAergic response via the activation of postsynaptic $GABA_A$ receptors (Frerking et al., 1999). However, such an explanation appears insufficient to account for the observed effects of kainate receptor activation on GABAergic transmission in area CA1. In particular, most studies agree that $GABA_B$ antagonists have no effect on the depressant effect of KARs on evoked GABA release (Clarke et al., 1997; Frerking et al., 1998; Min et al., 1999; Rodríguez–Moreno et al., 1997; 2000). Furthermore, the two effects, namely interneuron depolarisation and depression of evoked GABAergic transmission, can be dissociated pharmacologically (Cossart et al., 1998; Rodríguez-Moreno et al., 2000) and appear to couple to separate signalling systems (Rodríguez-Moreno & Lerma, 1998; Rodríguez-Moreno et al., 2000).

The observation that KARs at the presynaptic terminals directly depresses release via a distinct metabotropic mechanism is consistent with the observation that KAR-mediated depression of GABA release from synaptosomes is dependent on G-proteins (Cunha et al., 1999) and phospholipase C (Cunha et al., 2000). Thus, presynaptic activation of KARs activates G-proteins which in turn stimulate phospholipase C and produce diacylglycerol (DAG). DAG consequently activates protein kinase C which phosphorylates a currently unknown target, and decreases GABA release (fig. 2A). In addition, Min et al., 1999 were able to show that synaptically-released L-glutamate released from Schaffer collaterals could attenuate evoked IPSCs in CA1 pyramidal cells in an apparent heterosynaptic manner. This disinhibitory mechanism that depresses GABA release, may also involve the cannabinoid type 1 (CB1) receptor. A recent study showed that synaptic activation of GluK1 containing KARs depresses GABAergic input to CA1 pyramidal cells by facilitating presynaptic CB1 receptor-mediated signaling. In synapses lacking CB1 but containing GluK1 short term facilitation was seen, suggesting a role for KARs in gating CB1 signaling at GABAergic synapses and eventually controlling the direction of short-term heterosynaptic plasticity (Lourenço et al., 2010).

In addition KAR-mediated facilitation of GABA release has been demonstrated. In paired recordings from synapses between unknown interneuron types and CA1 pyramidal cells, low concentrations of kainate leads to facilitation of release in cell pairs which initially show low release probability (*Pr*) (Jiang et al., 2001). Interestingly the same study showed that cell pairs with high *Pr* are depressed by KAR antagonism. Future studies will probably show if these two interneuron populations are anatomically or neurochemically different (Klausberger and Somogyi, 2008). The facilitatory effect could be mediated via tonically active somatodendritic KARs on interneurons. These can be activated by spillower glutamate or glutamate released by adjacent astrocytes (Liu et al., 2004). This mechanism could protect principal cells from overexcitation during heavy activity. KAR-mediated facilitation of GABA release has also

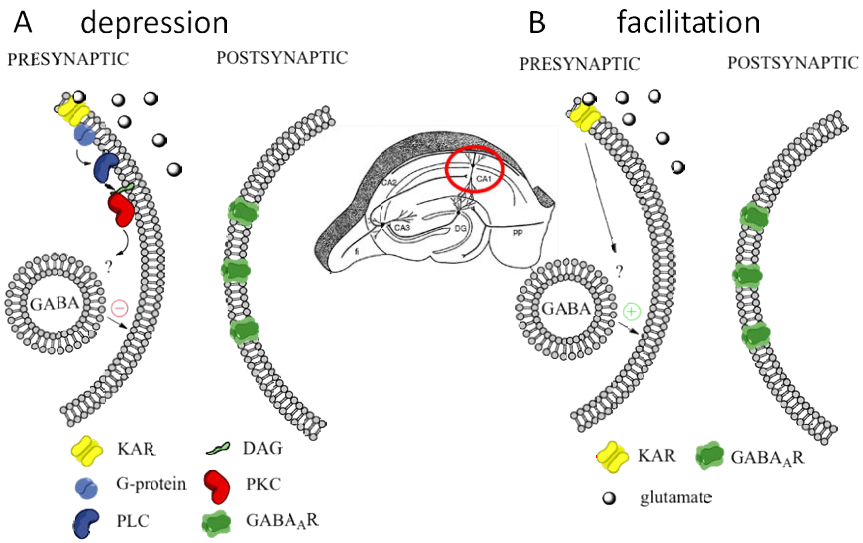

Figure 2: KAR-mediated regulation of GABA release in the CA1 area

Both depression and facilitation of GABA release has been demonstrated in hippocampal area CA1: A) presynaptic activation of KARs activate G-proteins which stimulate phospholipase C to produce diacylglycerol (DAG). DAG then activates protein kinase C, which phosphorylates an unknown target, to decrease GABA release (Rodriguez-Moreno & Sihra 2007). On the postsynaptic side GABA binds to GABAA receptors increasing the membrane conductance for Cl^- and HCO_3^- ions (Kaila & Voipio 1987). B) Presynaptic KARs react to elevated levels of ambient glutamate and facilitate GABA release to both pyramidal cells (Jiang et al., 2001) and interneurons (Cossart et al., 2001) in a PKC/PKA independent manner.

been shown in CA1 interneuron pairs (Cossart et al., 2001). In both cases above the facilitatory action of KARs is PKC- and PKA-independent (fig 2B).

Thus, in neonatal CA1 pyramidal neurons exogenous KA application leads to a large increase in sIPSC frequency in pyramidal cells (Cossart et al., 1998; Frerking et al., 1998; Frerking & Ohliger-Frerking, 2002; Goldin et al., 2007) but a depression in evoked IPSCs (Clarke et al., 1997; Rodríguez-Moreno et al., 1997). Interestingly, the GluK1-selective agonist ATPA decreases frequency of mIPSCs without affecting their kinetics suggesting a depression in *Pr* (Maingret et al., 2005). The KAR-mediated effect is also age-dependent; KAR activation after the second postnatal week does not decrease the frequency of mIPSCs as efficiently (Cossart et al. 1998; Rodríguez-Moreno et al. 1997; Rodríguez-Moreno & Lerma, 1998) suggesting developmental regulation. Depression of GABA release by diffused glutamate can be spatially restricted to defined circuits only (Maingret et al., 2005), and could represent a physiologically relevant regulatory role of activity by KARs during development. In apparent agreement, KARs tonically depress GABA release in neonatal mossy fiber-CA3 synapses in a developmentally regulated and G-protein-mediated manner (Caiati et al., 2010).

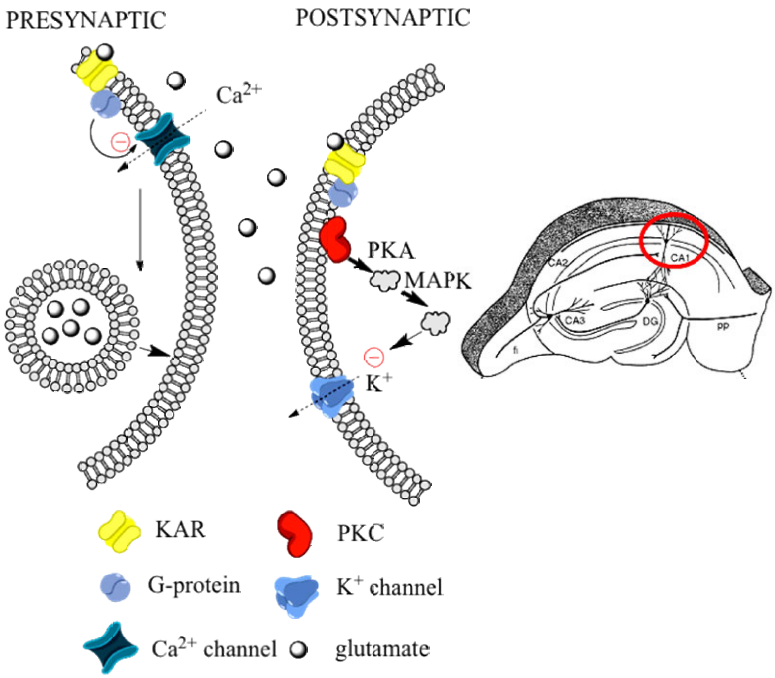

Figure 3: KARs involved in pre- and postsynaptic mechanisms in glutamatergic synapses to pyramidal cells in CA1

Presynaptic KARs in CA1 depress calcium entry and consequently glutamate release via a pertussis toxin sensitive G-protein mediated mechanism. Postsynaptically KARs cause a long-lasting suppression of a Ca^{2+}-activated K^+ current (I_{AHP}). THis involves G protein-mediated activation of phospholipase C (PLC) and PKC downstream (Melyan et al., 2004). Further studies have shown that in addition to PKC, also PKA and and mitogen-activated protein kinases contribute to the regulation of the Is_{AHP} (Grabauskas et al. 2007).

In summary, roles of KARs in GABAergic signaling in the CA1 region are complex. By providing significant excitatory postsynaptic drive, ionotropic somatodendritic KARs promote GABAergic release onto principal CA1 neurons. However, activation of KARs located on the presynaptic terminals of interneurons may be limited to periods of intense afferent firing (Min et al., 1999) and may represent a role to facilitate information transfer under such conditions.

4.3 KARs control glutamate release in the hippocampus

The first presynaptic KARs regulating transmitter release were discovered at CA1 synapses in the hippocampus where they downregulated glutamate release to pyramidal cells upon pharmacological activation (Chittajallu et al., 1996; Clarke & Collinridge, 2002; Frerking et al., 2001; Kamiya & Ozawa, 1998; Vignes et al., 1998). These KARs have pharmacologically been shown to contain at least GluK1 subunits (Clarke et al., 1997; Vignes et al., 1998). Activation of KARs could theoretically decrease glutamate release via ionotropic action inactivating voltage dependent Ca^{2+} channels (Chittajallu et al., 1996; Kamiya and Ozawa, 1998). However, experiments have shown that the regulation is sensitive to G-protein inhibitors such as pertussis toxin. It is suggested that rather than acting ionotropically, presynaptic KARs in CA1 depress calcium entry and consequently glutamate release through a pertussis toxin sensitive G-protein mediated mechanism. Schematic for the mechaisms is shown in fig. 3 (Frerking et al., 2001). Paradoxically no physiological function had been ascribed to KARs depressing glutamate release in CA1 before the studies presented in this thesis. Presynaptic KARs in CA1 also regulate glutamate release in synapses onto a subpopulation of unidentified GABAergic interneurons by depressing glutamate release (Sun & Dobrunz, 2006).

Interestingly, in mossy fiber synapses in area CA3 KARs show a biphasic response to kainate. Concentrations below 100 nmol induce facilitation- whereas concentrations above 100 nmol depress glutamate release (Schmitz et al., 2001). Both cases are dependent on the second messenger cyclic adenosine monophosphate (cAMP) and activation of protein kinase A (PKA). However, only the depression depends on G protein-signalling mechanism. The facilitation is regulated by a Ca^{2+}-dependent activation of the adenylate cyclace (AD)/cAMP/PKA pathway (fig. 4), as shown in regulation of mossy fiber-CA3 long term

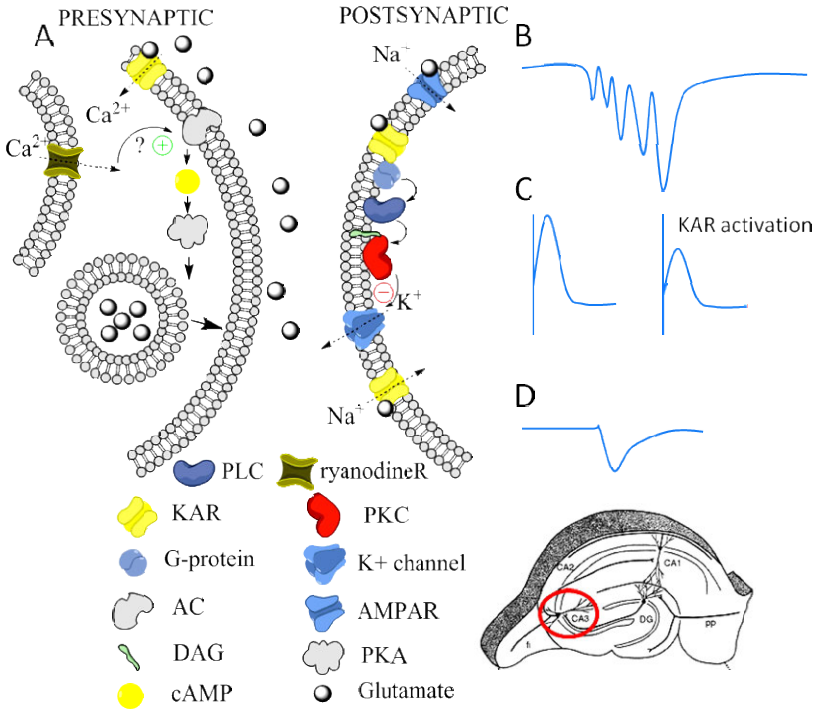

Figure 4: Facilitatory KARs involved in the mossy fiber synapse

A: In synapses to CA3 pyramidal cells presynaptic KARs facilitate glutamate release in a G-protein-AC-cAMP-PKA mediated manner. An increase in glutamate release is G-protein, adenylyl cyclase (AC) and protein kinase A (PKA) dependent (Rodriguez-Moreno & Sihra 2004). B: Postsynaptically the presynaptic facilitation of release is seen as a frequency facilitation in response to a 5 pulse train at 50Hz (Schmitz et al., 2001). C: Postsynaptic kainate receptors depress the G-prot-PLC-PKC dependent K^+ current (Is_{AHP}) increasing the excitability of CA3 pyramidal cells (Ruiz et al., 2005). D: Postssynaptically localized ionotropic KARs produce an excitatory post-synaptic current with slow kinetics (Vignes & Collinridge 1997). The traces in parts B-D are line drawings depicting currents measured intracellularly in voltage clamp mode.

potentiation (LTP) (Weisskopf et al., 1994). The Ca^{2+} release from intracellular stores can be triggered by Ca^{2+}-permeable presynaptic KARs (Lauri et al., 2003a). Presynaptic KARs in these synapses contain GluK2, GluK5 (Bureau et al., 1999; Wisden & Seeburg 1993) and possibly GluK4 and GluK3 subunits (Pinheiro et al., 2007). Although GluK1 subunits were not detected in the *in situ* hybridization experiments (Bureau et al., 1999; Wisden & Seeburg 1993), pharmacological evidence speaks for their existence (Lauri et al., 2001b) and involvement in mossy fiber LTP (Contractor et al., 2001).

An inherent property of mossy fiber synapses (fig 4B) is their prominent KAR dependent frequency facilitation which facilitates transmission during rapid afferent stimulation (Lauri et al., 2001a, 2001b; Schmitz et al., 2001; Contractor et al., 2001). Furthermore KARs can rapidly regulate glutamate release (within 10 ms) and thus can efficiently control short-term dynamics of synaptic transmission (Lauri et al., 2001a, Lauri et al., 2001b, Schmitz et al., 2001, Contractor et al., 2001, Kidd et al., 2002, Delaney and Jahr, 2002).

In addition to mossy fibers, CA3 pyramidal cells receive input from septum and the contralateral CA3 through the associational commissural (A/C) pathway and from the entorhinal cortex through the perforant path (PP). The A/C terminals express GluK1 and GluK2-containing KARs that depress glutamate release. On the contrary PP terminals express GluK1 and GluK2 containing KARs that facilitate glutamate release (Contractor et al., 2000) emphasizing circuit-specific modulation by KARs.

KARs have been shown to play important role in maturation of synaptic connectivity during development. Activation of KARs at early postnatal stage in hippocampus depresses glutamate release and decreases synaptic network activity. Prolonged activation of KARs in organotypic slices however results in massive formation of new glutamatergic synapses (Vesikansa et al., 2007; see also Lauri et al., 2003b). Furthermore, KARs have important roles in presynaptic regulation of neonatal long term depression (LTD), LTP and short term plasticity (STP) (Sallert et al., 2007, Lauri et al 2007).

4.4 KARs control cellular excitability

In some CA1 interneurons KARs mediate excitatory synaptic currents with remarkably slow kinetics (Cossart et al., 1998; Frerking et al., 1998). Similar somatodendritic KAR-mediated currents have also been reported in CA3 pyramidal cells (Vignes & Collinridge, 1997; Castillo et al,. 1997) (fig. 4D). In these synapses, sustained kinetics may be an adaptation to integrate excitatory inputs over a larger time window (Lerma, 2003). In addition, CA1 pyramidal cells have GluK2 and GluK5 -containing KARs (Bureau et al., 1999) which can be activated synaptically to elicit sustained suppression of a Ca^{2+}-activated K^+ current (I_{AHP}). This signaling cascade requires G-protein-mediated activation of phospholipase C (PLC) and subsequently PKC (Melyan et al.,2002;2004). Further studies have shown that in addition to PKC, both PKA and and mitogen-activated protein kinases are involved in the regulation of the Is_{AHP} (Grabauskas et al. 2007).

The afterhyperpolarizing current (Is_{AHP}) is a calcium-activated hyperpolarizing current which is mediated via activation of K^+ channels. The current has a slow rising phase and duration of seconds (Lancaster & Adams, 1986; Sah, 1996). In CA1, Is_{AHP} is activated by increased action potential firing and consequent calcium influx, and it provides an effective negative

feedback mechanism to protect the network from overexitability (Madison & Nicoll, 1984; Traub et al., 1993). Hyperpolarising Is_{AHP} supresses action potential firing and is responsible for spike frequency adaptation during repetitive discharge. Inhibition of the slow afterhyperpolarizing current KARs can thereby upregulate cellular excitability (fig 4C).

A study by Fisahn et al. (2005) showed that the Is_{AHP} in CA3 pyramidal cells is intact in GluK1 but absent in GluK2-/- mice suggesting a role for the GluK2 containing receptors in regulation of the current. Similar to these results Ruiz et al. (2005) concluded that both GluK2-/- and GluK5-/- mice have attenuated Is_{AHP} in CA3 pyramidal cells. Interestingly Fernandez et al. (2009) introduced somewhat contradictory results showing that ablation of subunits GluK4&5 completely removes the ionotropic response in CA3 pyramidal cells, whereas the KAR-regulated Is_{AHP} is unaffected. In the CA3 pyramidal cells the Is_{AHP} regulation depends on G-proteins, PLC and PKC (fig 4C) (Ruiz et al., 2005). An afterhyperpolarizing current is also seen in CA3 interneurons but with faster kinetics than in pyramidal cells. This medium afterhyperpolarizing current (Im_{AHP}) is mediated by a Ca^{2+} -dependent apamin sensitive K^+ conductance (Aoki et al., 2000; Savic et al., 2001). No KAR dependence for this current had been reported.

4.5 KARs in synchronous network oscillations

Kainate injections into the brain have been used as a model for mesial temporal lobe epilepsy because the symptoms are reminiscent of those reported in humans having this disease (Ben-Ari, 1985; Nadler et al., 1978). Early studies suggested that kainate causes its neurotoxic effects by acting on excitatory amino acid receptors (Herndon et al., 1977) but the identity of the receptors has remained a mystery until development of specific pharmacological tools (reviewed in Lodge, 2009). Since the division of excitatory amino acid receptors into AMPARs, NMDARs and KARs, the subunits comprising these receptors have been well described and the subunit specific drugs as well as genetically modified animals have been developed (reviewed in Contractor et al., 2011). This has revealed that animals lacking GluK2 subunit are less susceptible to kainate injections than wildtypes suggesting a contribution of this subtype in generation of pathological synchrony (Mulle et al., 1998). Further GluK1 antagonism protects from pilocarpine-induced seizures (Smolders et al., 2002) while injection of GluK1 agonist causes seizures apparently by an action in the amygdala (Rogawski et al., 2003; Kaminski et al., 2004).

In vitro kainate-induced gamma (20-80 Hz) oscillations are reduced in CA3 of GluK2-/- mice while GluK1-/- mice have an increased susceptibility to kainate (Fisahn et al., 2004) suggesting opposite roles for the two subunits. Further, GluK1 antagonism has been shown to reduce the frequency of hippocampal theta oscillations in vivo (Huxter et al., 2007). Other studies, however, have shown that GluK1 agonism does not induce gamma band oscillatory activity but antagonism reduces the power of these kainate induced oscillations (Brown et al., 2006). The opposite results could be explained by functional compensation and alterations in trafficking and expression of KAR subunits in the knockout mice (Christensen et al., 2004, Jaskolski et al, 2004).

Spontaneous synchronous activity characterizes immature neuronal networks and it is thought to play an important role in controlling the development of synaptic circuitry (for review, see Zhang and Poo, 2001). In the neonatal hippocampus, spontaneous network

bursts are seen both *in vitro* (Ben-Ari et al., 1989; Garaschuk et al., 1998; Palva et al., 2000) and *in vivo* (Lahtinen et al., 2001; Leinekugel et al., 2002). This activity consists of glutamate receptor-driven synchronous firing of neurons, which are rhythmically paced by $GABA_A$ receptor-mediated conductances (Khazipov et al., 1997; Bolea et al., 1999; Lamsa et al., 2000, Lamsa & Taira, 2003). The bursts appear to be critical for normal hippocampal development (Groc et al., 2002; Lauri et al., 2003; Huupponen et al., 2007), and are developmentally down-regulated such that in a mouse or rat brain they are no longer seen by the end of the second postnatal week (Ben-Ari et al., 1989; Garaschuk et al., 1998; Khazipov et al., 2004).

Interestingly, transient upregulation of GluK1 expression in the hippocampus coincides with the timeline for appearance of spontaneous network bursts (Bahn et al., 1994; Ritter et al., 2002; Ben-Ari, 1989). The strong developmental correlation between KAR expression profile and hippocampal spontaneous activity hints that KAR might play a role in the regulation of this activity during this crucial phase of development. The idea is supported by the roles KARs play in the modulation of glutamatergic and GABAergic transmission in hippocampus.

In addition to the work presented in my thesis, relatively few studies have addressed the role of high affinity KARs in the neonatal hippocampal CA3 area (Caiati et al, 2010 and Juuri et al., 2010). Caiati and colleagues show that presynaptic KARs on immature mossy fiber terminals in the neonatal CA3 tonically depress GABA release. Juuri et al. (2010) demonstrate that KARs can initiate network bursts by inducing ectopic spikes in CA3 pyramidal cells. Allene et al. (2008) have further shown that a certain form of early network oscillations (cENOs) is dependent on KARs and endogenous glutamate.

5 Aims of the study

The aim of this thesis was to elucidate the role of KARs in the modulation of excitatory transmission and hippocampal network activity in the hippocampus during early postnatal development. The physiological function of GluK1 subunit (formerly known as GluR5) containing KARs was of particular interest since expression of the subunit is strictly developmentally regulated and it coincides temporally with the dramatic changes in the synaptic functions in the hippocampus. Hitherto, very little has been shown about the KARs in the hippocampal synaptic transmission early in development.

The specific aims were to:
1: Study whether KARs are endogenously activated during early postnatal development (original publications I, II, III)
2: Study what possible physiological functions KARs might have in the hippocampus at early developmental stage, in particular in modulation of glutamatergic transmission. I decided to look into

a) pyramidal-pyramidal cell synapses (I, III) and
b) pyramidal cell-interneuron synapses (I)

3: Study whether KARs play a role in regulation of interneuron's excitability and the early synchronous activity controlled by these cells (I, II).
4: Elucidate the mechanisms by which KARs tune the glutamate release and synaptic dynamics during early postnatal development (III).

6 Materials and methods

The detailed descriptions of the experimental protocols can be found in the original publications. The following is a brief explanation of what was performed and why.

300-400 µm thick transversal slices from rat and mouse hippocampi were cut in ice cold ringer's solution using a vibratome. The slices were kept in rest in room temperated (20-24 °C) ringer's solution for at least 1h before the recordings. Cell somata were found under visual guidance using infrared illumination with differential contrast imaging technique or Dodt gradient optics. In addition, in publication II biocytin was routinely added to the pipette filling solution and the cells were visualized by Alexa 568-avidin immunostaining and fluorescence microscopy.

The recordings were done at 32^0C, with exception of the evoked EPSCs in publication III, in a flowthrough submerged chamber. To answer the questions stated in the aims of the study, different electrophysiological and molecular methods stated in table 1 were used. In order to isolate events, different drugs were administered in the flowthrough system (table 2). Likewise to focus on certain mechanisms in the neuronal membrane, different filling solutions in the measuring pipette were used (table 3). pH of the filling solutions was adjusted to 7.2 and osmolarity to 285. The resistance of the pipettes was 3-5 mOhm except for pipettes used in perforated patch recordings that were 12-14 mOhm. Voltage clamp recordings where access resistance exceeded 25 Mohms or where the range exceeded 15% were discarded.

Data were collected using PClamp-, WinLTP-program-, Win-EDR- and pulse softwares. For statistical analysis, ANOVA following Tukey's honestly significant difference (HSD comparison), Student's two-tailed t test or Pearson's χ^2 was used. $P < 0.05$ was considered as statistically significant.

All experiments were done in accordance with the guidelines given by the ethics committee for animal research at the University of Helsinki.

Table 1: Methods used

Method	Use	Publication
patch clamp	voltage clamp	I-III
patch clamp	current clamp	II
perforated patch	voltage clamp	III
cell-attached recording	monitor cell firing	II
immunostaining, infrared optics	visualize interneurons	II
HPLC	verify the effect of the glutamate scavenger	II
genetically modified animals	GluK1 -/- mice were used to I: check the specificity of the GluK1 agonist and antagonist used II: study the responses in hippocampal cells lacking the GluK1 subunit	I-III

Table 2: Drugs used

Drug	Concentration	Action	Publication
ATPA	1µM	GluK1 agonist	I – III
LY382884	10µM	GluK1 antagonist	I – III
Tetrodotoxin	1µM	Blocker of voltage gated Na^+ channels	I – III
Picrotoxin	100µM	$GABA_AR$ antagonist	I – III
CGP55845A	1µM	$GABA_BR$ antagonist	I – III
GYKI53655	50µM	AMPAR antagonist	I – III
NBQX	20µM	AMPA and KAR antagonist	I – III
LY341495	100µM	mGluR1-8 antagonist	I – III
TBOA	50µM	glutamate uptake inhibitor	I – III
GPT + pyruvate	–	glutamate scavenger	I – III
GDPβS	0.3mM	blocker of G-proteins (intracellular)	II – III
PTX	5µM/ml	blocker of G-proteins (incubation)	II – III
KAINATE	50nM	KAR agonist	III
CPA	1µM	adenosine receptor agonist	III
D-AP5	50µM	NMDA antagonist	I – III
propanolol	1µM	blocker of noradrenergic receptors	II
yohimbine	5µM	blocker of noradrenergic receptors	II
coryanthine	5µM	blocker of noradrenergic receptors	II
SB-269970	0.1µM	blocker of serotonergic receptors	II
ketanserin	10µM	blocker of serotonergic receptors	II
WAY-100635	1µM	blocker of serotonergic receptors	II
SCH-23390	10µM	blocker of dopaminergic receptors	II
sulpiride	20µM	blocker of dopaminergic receptors	II
atropine sulfate	1µM	blocker of cholinergig muscarinic receptors	II
SR-141716	5µM	blocker of CB1 cannabinoid receptors	II
BIS	1µM	PKC inhibitor	II-III
APAMIN	100nM	SK2 channel blocker	II

Table 3: Filling solutions used

Filling solution	Use	Publication
Cesium based	mPSC recordings	I-III
K-gluconate based	current clamp	II
K-gluconate based low EGTA	ImAHP recordings	III
filling solution + 10mM BAPTA	recording of NMDA responses	III
filling solution + GDP betas	block G-protein mediated transmission intracellularly	II-III
filling solution + BIS	block PKC mediated transmission intracellularly	II-III
K-gluconate with gramicidin or amphoterricin	perforated patch recordings	III

7 Results

7.1 tKARs regulate glutamate release in the hippocampal CA3 area at early postnatal age (I)

7.1.1 GluK1-containing tKARs differentially regulate glutamatergic input to CA3 pyramidal cells and interneurons

In order to study the patterns and regulation of spontaneous network activity in area CA3 of the neonatal (P3-P6) hippocampus, whole cell patch clamp recordings were made from pyramidal cells and interneurons. A low chloride (2mM) potassium gluconate filling solution was used to discriminate excitatory postsynaptic currents (EPSCs) from inhibitory postsynaptic currents (IPSCs). When clamped to -60 mV, glutamatergic conductances generated inward- and GABAergic conductances outward currents.

To find out if GluK1 subunit containing kainate receptors modulate the spontaneous activity the selective agonist ATPA (1μM) was applied in the perfusion bath (Clarke et al., 1997). In pyramidal cells the pharmacological activation of tKARs increased spontaneous IPSC (sIPSC) frequency, decreased the spontaneous EPSC (sEPSC) frequency and decreased the occurrence of the synchronous spontaneous bursts characteristic to the neonatal hippocampal network (Ben-Ari 2001; Palva et al., 2000). Hereafter, these events will be referred to as GDPs (giant depolarizing potentials) as they were first introduced (Ben-Ari et al., 1989). In interneurons ATPA had stereotypically similar effects on sIPSC- and GDP frequency but opposite effect on the occurrence of sEPSCs compared to pyramidal cells.

To understand the physiological role of these tKARs the GluK1-selective antagonist LY382884 (Bortolotto et al., 1999; Lauri et al., 2001a) was used. Bath application of the antagonist reduced the occurrence of GDPs recorded in CA3 pyramidal cells and interneurons but had no effect on the occurrence of sIPSCs. The frequency of sEPSCs, however, was increased in pyramidal cells and decreased in interneurons. In order to further understand the role of GluK1 containing tKARs the mice lacking GluK1 subunit were used. In GluK1 -/- interneurons, the occurrence of sEPSCs was lower than in the wildtypes. There were no differences in the occurence of sIPSCs between the wildtype and the GluK1 -/- interneurons. In GluK1 -/- pyramidal cells the frequency of sEPSCs was higher than in wild type mice. In contrast, the frequency of sIPSCs in GluK1 -/- pyramidal cells was lower than in the wild types (table 4, unpublished data). The results thus supporting the view that GluK1 containing tKARs depress glutamate release to CA3 pyramidal cells and facilitate glutamate release to CA3 interneurons.

To confirm the specifity of the drugs the pharmacological experiments were repeated with wildtype- and GluK1 -/- mice. In pyramidal cells LY382884 did not affect sEPSC frequency in GluK1 -/- mice but increased sEPSC frequency in wildtype mice similarily as in rats confirming that the effects of LY382884 are specifically mediated by tKARs containing the subunit GluK1. In GluK1-/- interneurons ATPA had similar effects as in GluK1 -/- pyramidal cells. ATPA did not affect the sEPSC frequency but elevated the frequency of sIPSCs and network bursts. In GluK1 -/- pyramidal cells ATPA did not affect the frequency of sEPSCs but

elevated the frequency of sIPSCs and network bursts thus calling for further investigations on the pharmacological selectivity of ATPA (table 5, unpublished data).

7.1.2 GluK1-containing tKARs modulate presynaptic glutamate release in age-dependent manner

The amplitude and kinetics of the sEPSCs were unaltered by ATPA or LY382884 which suggests a presynaptic mechanism of regulation. One way to determine whether the mechanism regulating synaptic transmission is pre- or postsynaptic is to measure miniature postsynaptic currents (mPSPs) in the presence of TTX and to isolate the tKAR mediated transmission, by blocking NMDA-, $GABA_A$-, $GABA_B$ - and metabotropic glutamate receptors. In neonatal pyramidal cells LY382884 increases and ATPA decreases the frequency of mEPSCs. On the contrary in neonatal interneurons LY382884 decreases- and ATPA increases the mEPSC frequency.

A comparison between GluK1-/- and wildtype mice further support presynaptic regulation of transmission. In pyramidal cells, the mEPSC frequency was higher in the GluK1-/- compared to the wildtype mice. In interneurons the mEPSC frequency was lower in the GluK1 -/- than in the wildtype mice (table 5, unpbublished results). Thus tKARs inhibit glutamate release to pyramidal cells and facilitate release at glutamatergic terminals onto interneurons in an action potential independent manner. Experiments on older animals showed that the mechanism is developmentally regulated and no longer seen in P14-P16 animals.

7.1.3 tKARs do not modulate presynaptic GABA release in neonatal rat CA3 area

KARs have been shown to regulate action potential-independent GABA release in adult CA1 (Cossart et al., 2001; Mulle et al., 2000; Rodríguez-Moreno and Lerma, 1998) (but see Frerking et al., 1999; Jiang et al., 2001; Semyanov and Kullmann, 2001). To study whether tKARs influence GABA release in the neonatal hippocampus by a direct action on GABAergic terminals, miniature IPSCs (mIPSCs) were recorded from CA3 pyramidal neurons and interneurons. Neither ATPA nor LY382884 had significant effect on mIPSCs in interneurons or pyramidal cells suggesting that GluK1 is not clearly regulating presynaptic GABA release in neonatal CA3 area. In interneurons, the mIPSCs did not differ significantly between GluK1 -/- and wildtype mice. In pyramidal cells, however, the mIPSC frequency was lower in GluK1-/- compared to wildtypes (table 5, unpublished data), a finding which is somewhat contrast to what has been previously reported (Maingret et al., 2005; Caiati et al., 2010).

7.1.4 Suppressing but not facilitatory effects of tKARs on glutamate release dependend on pertussis toxin sensitive G-proteins and PKC

The depression of excitatory synaptic transmission in area CA1 by pharmacological activation of KARs (Chittajallu et al., 1996; Frerking et al., 2001; Clarke and Collingridge, 2002) has been suggested to involve a G-protein-mediated mechanism (Frerking et al., 2001), similar to that involved in the regulation of GABA release in CA1 (Rodríguez-Moreno and Lerma, 1998). In order to investigate the G-protein dependency, slices were treated overnight with pertussis toxin (PTX). Pharmacological activation or blockade of GluK1 containing tKARs affects the frequency of mEPSCs in both pyramidal cells and interneurons

in acute slices. In PTX treated slices, however, neither ATPA nor LY382884 had any effect on mEPSC frequency in pyramidal cells suggesting a G-protein dependent regulation of glutamate release. In interneurons, PTX treatment did not block the original effect in mEPSC frequency by ATPA or LY382884 indicating that G-protein dependent mechanism is not involved.

The KARs acting via a G-protein-mediated signaling mechanism have been shown to couple to activation of PKC (Rodríguez-Moreno and Lerma, 1998). To investigate if the tKARs regulating glutamate release in CA3 are PKC dependent, slices were preincubated in 1 µMbisindolylmaleimide VIII acetate for 30 min (Toullec et al., 1991) before the effects of pharmacological activation or blockade of tKARs on mEPSC frequency was tested. Inhibition of PKC blocked the regulatory actions of tKARs on mEPSC frequency in pyramidal cells but not in interneurons, having an effect similar to that of inhibition of G-proteins. Thus the inhibitory but not facilitatory effects of tKARs are coupled to intracellular mechanisms involving G-proteins and PKC.

7.1.5 Presynaptic tKARs are regulated by ambient levels of L-glutamate

One possible explanation for the tonic activation of presynaptic KARs in neonatal, but not in 2-week-old animal CA3 is a difference in the ambient glutamate concentration and/or diffusion of glutamate. These parameters are attributable to developmental changes because both the glutamate transport mechanisms and tortuosity of the extracellular space are in a dynamic range during that period (Khalilov et al., 1997; Rusakov and Kullmann, 1998; Sykova et al., 2000; Danbolt, 2001).

To test whether manipulation of the extracellular glutamate concentration affects the activation of presynaptic tKARs and consequently change mEPSC frequency, an enzymatic "glutamate scavenger" was used (Overstreet et al., 1997; Min et al., 1998). In line with the hypothesis removal of extracellular glutamate increased the frequency of mEPSCs. In addition the facilitatory effect of LY382884 on mEPSC frequency was blocked in these conditions suggesting that in control conditions high ambient levels of glutamate tonically depress glutamate release through tKARs. In contrast raising glutamate levels by blocking the glutamate uptake inhibitor with TBOA decreased the mEPSC frequency. Neither the scavenger nor TBOA had significant effects in P14-P16 rats. Endogenous glutamate thus provides a tonic but submaximal activation of presynaptic tKARs at CA3 terminals in the neonatal hippocampus.

7.2 tKARs increase spike firing in neonatal CA3 interneurons by attenuating afterhyperpolarizing K+ current (II)

7.2.1 GluK1 subunit-containing tKARs regulate spontaneous interneuron firing at P3–P5 but not at P14–P16

The former results demonstrated that glutamatergic input to interneurons in CA3 is facilitated by GluK1 containing receptors. To take a closer look at the tKAR function in interneurons in neonatal CA3, on-cell recordings were made from wildtype and GluK1-/- mice from both neonates (P3-P5) and juveniles (P14-P16). First, we found that the frequency of spontaneous firing in wildtype interneurons is strongly downregulated during the first weeks of life, a finding that had not been reported previously. Interestingly, the firing of interneurons in GluK1 -/- mice was found to be significantly lower compared to wildtypes. Indeed, the frequency was very similar to that seen in wildtypes at P14-P16. In the GluK1 -/- mice no differences in firing frequency was seen between the two age groups.

Application of the GluK1 antagonist LY382884 attenuates interneuronal firing reversibly in wildtype- but not in GluK1 -/- or juvenile (P14-P16) mice. At least partially the altered firing rate in GluK1 -/- interneurons could be explained by the developmentally regulated tKAR mediated presynaptic facilitation of glutamate release

7.2.2 tKARs increase interneuron firing by depressing an afterhyperpolarizing current at P3–P5

Another explanation for the tKAR mediated effect of interneuronal firing would be a postsynaptic somato-dendritic depolarization. Accordingly, pharmacological activation of tKARs with the GluK1 agonist ATPA depolarized the interneurons. LY382884, however did not alter the resting membrane potential, suggesting that no tKARs are present postsynaptically, consistent with the earlier findings that LY382884 has no effect on sIPSCs in either pyramidal cells nor interneurons.

It has been shown that in CA1 and CA3 pyramidal cells the slow afterhyperpolarizing current (Is_{AHP}) is regulated by KARs (Melyan et al., 2002; Melyan et al., 2004; Ruiz et al., 2005). In order to see if a similar mechanism exists in neonatal CA3 stratum lucidum interneurons intrasomatic depolarizing pulses were applied in voltage clamp mode. The experiments revealed a current with the characteristics of a medium afterhyperpolarizing current (Aoki & Baraban, 2000). To see if this current is modulated by tKARs, GluK1 activation and blockade was tested. Indeed, ATPA decreased the current whereas LY382884 increased it. The current was partially inhibited by apamin suggesting it to be mediated via Ca^{2+} sensitive K^+ channels previously described in adult CA3 inteneurons (Savic et al 2001, Aoki & Baraban 2000). However, the modulatory actions of tKAR antagonists and agonists on the medium afterhyperpolarizing current (Im_{AHP}) were not seen in juvenile mice indicating developmental regulation.

7.2.3 tKAR-coupled depression of ImAHP is mediated via a G-protein- dependent mechanism and activated by ambient glutamate

The afterhyperpolarizing currents can be modulated by noradrenaline-, serotonin-, dopamine-, aceylcholine-, mGlu- and cannabinoid receptors (Sah & Faber, 2002; Vogalis et al., 2001). To see if tKARs depresses the Im_{AHP} indirectly through some of these mechanisms, a cocktail containing antagonists for all of the above receptors was used. In these conditions ATPA still depressed the Im_{AHP}. Since ambient glutamate tonically regulates synaptic connections to CA3 neurons (Lauri et al., 2005) we went on and studied the effect of glutamate scavenger on the Im_{AHP}. Interestingly the scavenger increased the Im_{AHP} amplitude and masked the effect of LY382884 suggesting a tKAR mediated regulation of Im_{AHP} and thus interneuronal excitability. This tonic modulation of Im_{AHP} was not seen in P14-16 mice.

Intracellular application of GDPbetaS increased the mAHP and masked the effect of ATPA, suggesting a G-protein dependent mechanism. Blocking PKC mediated transmission with BIS did not affect the mAHP or alter the actions of ATPA. The downstream mechanism is thus G-protein but not PKC dependent.

7.2.5 tKAR-coupled AHP regulates endogenous activity patterns in the immature hippocampus

At the level of hippocampal network, selective inhibition of the Im_{AHP} by apamin increased the frequency of spontaneous network bursts in P3-P5 neurons. This was accompanied by an increased firing of interneurons. The apamin-induced increase in firing was more prominent in the GluK1 -/- mice than in wildtype littermates which is consistent with the idea that tKAR mediate tonic depression of the Im_{AHP} in the wildtype mice.

7.3 Functional maturation of CA1 Synapses Involves Activity-Dependent Loss of tKAR-mediated Inhibition of Glutamate Release (III)

7.3.1 Presynaptic tKARs inhibit glutamate release in developing pyramidal cell synapses in the area CA1

In the adult hippocampus, pharmacological activation of KARs in area CA1 leads to depression of glutamatergic transmission through a $Ca2^{+}$- (Chittajallu et al., 1996; Kamiya & Ozawa 1998) and G-protein (Frerking et al., 2001) mediated mechanism. These KARs have also been shown to contain the GluK1 subunit (Vignes et al., 1998; Clarke & Collingridge 2002).

The expression of the GluK1 subunit in CA1 is high during the first postnatal week (Bahn et al., 1994; Bettler et al., 1990; Ritter et al., 2002). To study whether these receptors are endogenously activated in P3-P5 rats, the GluK1-selective KAR antagonist LY382884 was used. LY382884 reversibly increased evoked EPSC (eEPSC) amplitude and decreased the failure rate. Additionally LY382884 increased the mEPSC frequency in the presence of the $GABA_B$ antagonist CGP. Interestingly the above effects of LY382884 were absent in P14-16 rats although experiments with ATPA proved that KARs still depress glutamate release at

this developmental stage. The above results suggest that presynaptic tKARs tonically decrease the probability of glutamate release at immature CA1 synapses and that the tonic control is present only early in development. The results in neonates could also be explained by insertion of AMPA receptors at silent synapses (Malinow & Malenka, 2002) or indirect activation of KARs by a modulatory substance such as acetylcholine (Maggi et al., 2004), ATP/adenosine (Zhang et al., 2003, Safiulina et al., 2005) or glutamate binding to metabotropic glutamate receptors (Scanziani et al., 1997). Antagonism of these receptors, however, had no effects on the actions of tKARs in neonatal CA1 (see publication III for details).

7.3.2 Ambient glutamate reduces release probability via presynaptic tKARs and modifies synaptic facilitation

Given that tKARs excert a tonic inhibitory action on glutamate release in the neonatal CA1, their role in frequency dependent synaptic transmission was investigated. Immature synapses in CA1 are heterogeneous in terms of their short-term dynamics. Although some of the excitatory synapses have a high probability of release and are characterized by synaptic depression, others have a low probability of release and facilitate release during repetitive activity (Hanse and Gustafsson, 2001; Dobrunz & Stevens, 1997; Palmer et al., 2004). Similar facilitation is a characteristic feature of the mossy fiber synapse in CA3, in which presynaptic KARs play an important role in regulating glutamate release (Lauri et al., 2001a, 2001b; Schmitz et al., 2001).

To investigate the short term dynamics of synapses in neonatal CA1, EPSCs were evoked with train of 5 stimuli at 50 Hz. Based on the responses to these trains the synapses were categorized into facilitatory and non facilitatory. Application of LY382884 turned facilitatory synapses into non-facilitatory while having no effect on non-facilitatory synapses. At an age of P14 no facilitatory synapses were seen anymore and LY382884 did not have any effect on the EPSCs. These findings suggest that presynaptic tKARs are endogenously activated only in the facilitating neonatal inputs and that they tonically keep *Pr* low, allowing for the large facilitation during repetitive activity, such as high frequency bursts spontaneously generated in the newborn hippocampus (Lamsa et al., 2000; Palva et al., 2000).

As we demonstrated (publications I, II), ambient glutamate activates tKARs in the neonatal hippocampus. As expected removing ambient glutamate with the glutamate scavenger mimicked the pharmacological effect of LY382884 turning facilitatory synapses into non-facilitatory and having no effect on non-facilitatory synapses. The scavenger also completely occluded the effects of LY382884. The results strongly suggest that the tKARs regulating *Pr* in neonatal facilitatory CA1 synapses are tonically activated by ambient glutamate. Removing glutamate in older animals (P14) did not have any significant effect. In order to test if the lack of effect was due to low glutamate levels the glutamate concentration was experimentally increased with the glutamate uptake inhibitor TBOA. Increasing glutamate concentration did not have any effect suggesting a developmentally regulated change in the functional properties of presynaptic tKARs.

7.3.3 Presynaptic tKARs maintain low release probability in facilitatory synapses via G-proteins

G-proteins have been shown to be involved in the inhibition of synaptic transmission between CA3 and CA1 neurons induced by pharmacological activation of KARs (Frerking et al., 2001). In line with these results, incubating neonatal hippocampal slices in pertussis toxin masked the effects of LY382884, glutamate scavenger and ATPA on mEPSC frequency suggesting a G-potein mediated mechanism. To isolate the mechanism to the presynaptic side, GDPbetaS was applied through the measuring pipette. This manipulation did not prevent the facilitation of mEPSC frequency in response to LY382884. The depression of glutamate release in neonatal CA1 synapses induced by presynaptic tKARs is thus G-protein mediated, but differing from adult neurons the mechanism is tonically activated by ambient glutamate.

7.3.4 tKARs explain heterogeneity in excitatory synapses' facilitation properties in developing CA1

In the studies of short term dynamics of the CA1 synapses the GluK1 antagonist LY382884 had an effect on facilitatory synapses only. One explanation for this is that the affinity of tKARs at facilitatory synapses is higher compared to non-facilitatory inputs. In order to clarify this, experiments were done with 50 nM kainate which selectively activates high- but no low-affinity KARs (Lauri et al., 2001a; Schmitz et al., 2001).

50nM kainate did not have any detectable effect on eEPSCs in control conditions but when glutamate was removed with the glutamate scavenger, 50nM kainate depressed eEPSC amplitude at facilitatory synapses having no effect on non-facilitatory inputs. Accordingly the depression of eEPSC was also associated with an increased facilitation in response to 5-pulse stimuli at 50Hz. Further, the differences in facilitatory and non facilitatory synapses persisted after altering extracellular divalent concentrations. The above results strongly suggest that high-affinity tKARs are selectively expressed at facilitatory neonatal CA1 synapses.

7.3.5 Induction of LTP at facilitating neonatal synapses rapidly alters tKAR activation and short term dynamics

The dynamic properties of glutamatergic CA1 synapses are closely correlated with the tonic regulation of the KARs suggesting a developmental mechanism rendering immature, facilitatory synapses to mature none-facilitatory ones. Because LTP-like processes are thought to be important in synaptic maturation (Abbot & Nelson, 2000) we next tested whether LTP-induction affects the tKAR-dependent synaptic dynamics. In two pathway experiments the synapses were first categorized as facilitatory or non facilitatory. Pathway specific LTP (pairing protocol) was more robust in facilitatory synapses compared to non facilitatory ones. Application of LY382884 increased EPSC amplitude in facilitatory synapses in the non LTP pathway but not in the LTP pathway regardless if the input was initially facilitatory or non facilitatory. LTP also masked the previously shown effects of the glutamate scavenger. Further, induction of LTP caused a decrease of facilitation in initially facilitating synapses.

7.3.6 LTP induces a change in affinity of tKARs regulating glutamate release

LTP caused a rapid loss in presynaptic tKAR activity but whether this was due to internalization of receptors or due to changes in the properties of tKARs was still unknown. To investigate if functional tKARs remain in presynaptic terminals after LTP induction, the KAR agonist ATPA was applied. ATPA caused a depression in EPSC amplitude suggesting that the tKARs still are present and that the loss of activity after LTP rather depends on a change in the functional properties of tKARs.

The tonic activation of tKARs apparently requires high affinity receptors. The loss in functional tKAR activity after LTP could thus be due to a decrease in affinity. To elucidate this, 50nM kainate was applied to the initially facilitatory pathways before and after removal of glutamate. In the pathways where LTP was induced, 50nM kainate failed to have any detectable effect both before and after application of the glutamate scavenger. In the control pathway, however, kainate still depressed the eEPSC.

The results suggest that LTP causes a rapid decrease in the affinity of presynaptic tKARs at facilitating inputs.

Table 4. Effect of ATPA and LY382884 on spontaneous activity in CA3 pyramidal cells and interneurons in wildtype and GluK1-/- mice compared. NA = not available, no eff = no effect, - = suppressing effect on frequency, + = enhancing effect on frequency.

Event	pyramidal cells				interneurons			
	ATPA wt	ATPA GluK1-/-	LY382884 wt	LY382884 GluK1 -/-	ATPA wt	ATPA GluK1-/-	LY382884 wt	LY382884 GluK1 -/-
sEPSC	-	no eff	+	no eff		NA	-	NA
sIPSC	++	+	no eff	NA	++	+	no eff	NA
mEPSC	-	NA	+	NA	+	NA	-	NA
mIPSC	no eff	NA	no eff	NA	no eff	NA	no eff	NA
GDPs	-	+	-	no eff	-	+	-	no eff

Table 5. Summary data showing the occurrence of spontaneous activity in CA3 in wildtype and GluK1-/- mice. Diff = difference, the symbol states the difference between the genotypes.

Event	events / minute in pyramidal cells			events / minute in interneurons		
	wildtype	diff	GluK1-/-	wildtype	diff	GluK1-/-
sEPSC	5.9 ± 1.1, n=7	<	17.8 ± 4.9,n=7	36.6 ± 8, n=7	>	18.09 ± 6.2 n=15
sIPSC	83.9 ± 20.2, n= 3	>	13.1 ± 4.3, n=7	30.4 ± 7.3, n=6	=	32.5 ± 6.9, n=9
mEPSC	2.54 ± 0.2, n=8	<	3.92 ± 0.7, n=19	32.9 ± 8.1 n=7	>	13.1 ± 3.7 n=14
mIPSC	33,98 ± 7,2 n=5	>	12 ± 3.4, n=6	36.3 ± 10.8 n=4	>	9.8 ± 3.4, n=6
GDP	0.7 ± 0.09, n=7	<	2.7 ± 0.4, n=7	0.9 ± 0.11, n=7	<	1.86 ± 0.2, n=15

8 Discussion

During the course of this study, several novel features of KAR-mediated synaptic signaling have been discovered. The major finding is that a population of GluK1 subunit-containing KARs is tonically activated by ambient glutamate (the receptors referred to as tKARs) during a restricted developmental period. tKARs are involved in several neuronal functions in the hippocampus, and are likely to be important for the development of hippocampal circuitries. The physiological implications of these tKAR-associated functions are widespread, ranging from synapse formation to emergent cognitive-range oscillations in the hippocampus. These will be discussed below.

8.1 tKARs in the neonatal hippocampus are activated by ambient glutamate in a developmentally restricted manner

By manipulating the levels of extracellular glutamate it was possible to modulate tKAR activation and its effects (on spontaneous activity, eEPSCs and Im_{AHP}) bidirectionally. Interestingly, the tKAR activation-linked effects were lost by the end of the second postnatal week. There are two plausible but not mutually exclusive explanations for this. First, it is known that the expression of glutamate transporters is upregulated during development thus resulting in more powerful glutamate clearance from the extracellular volume (Danbolt 2001). Also, the tortuosity of the extracellular space increases during development restricting the glutamate diffusion (Sykova et al., 2000). Consequently, by maturation there would be less ambient glutamate available to activate tKARs which explains the developmental decline in the tKAR-linked functions. Experiments where glutamate concentration was elevated in the P14-P16 tissue, however, showed that the tKAR function cannot be restored solely by increasing the ambient glutamate concentration. The GluK1 KARs could still be activated pharmacologically by the selective agonist ATPA indicating persistent presence of the receptor. The most parsimonious explanation for these results is that such developmental alterations in the function depend on changes in the receptor affinity (because of lack of effect of LY382884 in the presence of TBOA) or in signaling mechanisms downstream to the receptor activation.

8.2 Downstream signalling and developmental changes in tKARs

The affinity of kainate receptors depend on the composition of subunits. The high affinity subunits GluK4 and GluK5 cannot form homomers but act to increase the affinity of GluK1-3 containing receptors (Werner et al., 1991; Herb 1992). The developmentally regulated change in affinity of the tKARs could thus reflect a changed composition of the receptors. In addition, the mRNA editing and possibly the alternate splicing of KAR subunits change during development (Bernard et al., 1999; Boutz et al., 2007). The presynaptic tKARs in the neonatal CA1, however, shifted from a high affinity- to a low affinity mode rapidly in response to induction of LTP. At least in these synapses, the functional regulation seems to be because of fast alterations of the affinity of the receptor *per se*. This event appears too fast to be explained by changes in subunit composition. The initial high affinity state of the tKAR suggests the presence of either GluK4 or GluK5 subunits. What the mechanism decreasing the affinity so swiftly is remains to be elucidated, but a direct modification of the receptor, such as phosphorylation, is a strong candidate.

A recent study provides novel information about the molecular switch responsible for this transition in affinity. It is established that BDNF increases the release probability at CA1 synapses to pyramidal cells (Tyler et al., 2001 & 2006; Mohajerani et al., 2007). Sallert et al. (2009) showed that blocking BDNF-neurotrophic tyrosine kinase receptor type 2 (TrkB) prevented the transition in tKAR-mediated signaling in response to LTP in neonatal CA1 pyramidal cell synapses. In addition, the BDNF-mediated enhancement of glutamate release is age dependent (Gottschalk et al., 1998), similar to the developmental downregulation of the function of tKARs which is likely to depend on a developmentally regulated increase in release probability similar to that shown by Wasling et al. (2004).

KARs mediate both ionotropic and metabotropic actions in target neurons (Rodríguez-Moreno and Lerma, 1998; Melyan et al., 2002; Rozas et al., 2003). The tonically active KARs that depress glutamate release at terminals to CA3 and CA1 pyramidal cells are G-protein coupled. The effects of both GluK1 agonist and antagonist were blocked by pertussis toxin. In addition, the depression of glutamate release in CA3 was PKC dependent, similar to that shown for regulation of GABA release in CA1 (Rodríguez-Moreno et al., 1998). These findings demonstrate that tKARs depress glutamate release in a metabotropic manner similar to KARs in mature CA1 area (Frerking et al., 2001). Yet, the mechanism is tonically regulated by tKARs binding ambient glutamate. Interestingly, the tKAR-dependent facilitation of glutamate release to interneurons was insensitive to inhibitors of G-proteins and PKC suggesting other signalling cascades. In the immature brain, GluK1 and GluK2 are mostly unedited at the Q/R site and thus Ca^{2+} permeable (Bernard et al., 1999; Lee et al., 2001). tKAR-mediated Ca^{2+} influx could facilitate glutamate release to interneurons in an analogous fashion to the facilitation of glutamate release at mature mossy fiber terminals (Lauri et al., 2003a).

A metabotropic G-protein mediated action of KARs have been shown to regulate AHPs and consequently action potential firing in CA3 and CA1 pyramidal cells (Melyan et al., 2002, 2004; Ruiz et al., 2005). In adult CA3 pyramidal cells, Is_{AHP}s and Im_{AHP}s can be suppressed by Gluk2 but not GluK1-containing KARs leading to increased firing frequency (Fisahn et al., 2005). It has also been reported that Is_{AHP}s in CA3 pyramidal cells can be inhibited by endogenously activated G-protein coupled KARs and that these KARs require the coexpression of the high affinity subunit GluK5 (Ruiz et al., 2005). In the present study, a GluK1 antagonist revealed endogenously active KARs that depress an apamin sensitive Im_{AHP} in a G-protein but not PKC dependent manner. Similar to the KARs in pyramidal cells, the activation of tKARs depresses the Im_{AHP} and thus increases interneuronal firing. The study by Ruiz et al, 2005 suggested that activation of KARs regulating Is_{AHP} requires the coexpression of high affinity subunit GluK5. In the present study experiments done with GluK5-/- mice still showed the regulation of interneuronal excitability by tKARs indicating that GluK5 is not required for tKAR-mediated regulation of Im_{AHP} in interneurons.

8.3 Presynaptic tKARs in neonatal CA3 facilitate glutamate release to interneurons but depress release to pyramidal cells

The tKARs in area CA3 regulate glutamate release differentially depending on the target cell type. In synapses to pyramidal cells they depress glutamate release while they facilitate glutamate release to interneurons (fig. 5). This functional discrepancy of tKARs at

glutamatergic terminals in CA3 is probably attributable to their diverse synaptic origin. In adult tissue, CA3 interneurons in straum lucidum receive 10 times more mossy fiber (mf) synapses than pyramidal cells (Acsady et al., 1998). The mf synapse to pyramidal cells develops predominantly during the second postnatal week in rats (Stirling and Bliss, 1978; Amaral and Dent, 1981; Marchal and Mulle, 2004). The glutamatergic input to CA3 area pyramidal cells in P3-P6 rats originates from septum and the contralateral hippocampal CA3 area via the associational commissural (A/C) pathway. In addition the area is innervatedfrom the entorhinal cortex through the perforant path (PP). This data suggests that the predominant glutamatergic input to neonatal CA3 pyramidal cells is not coming from mf terminals. Studies using GluK1 and GluK2 -/- mice suggest that A/C and mf terminals express GluK1 and GluK2 containing KARs that depress glutamate release. On the contrary the PP terminals express GluK1 and GluK2 containing KARs that facilitate glutamate release (Contractor et al., 2000).

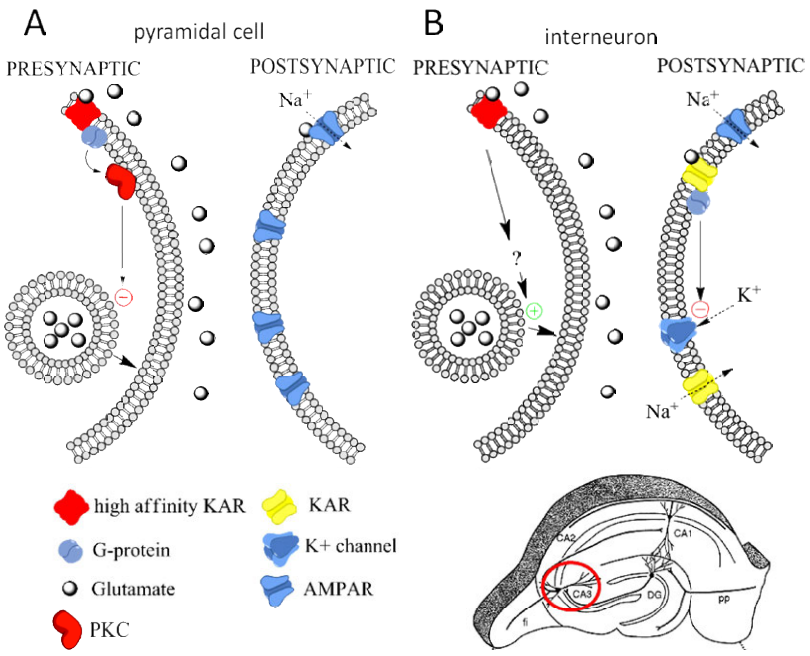

Figure 5: KARs depress glutamate release to pyramidal cells but facilitate release to interneurons in CA3

A: High affinity KARs at terminals to CA3 pyramidal cells depress glutamate release by a G-protein-PKC mediated mechanism. B: At neonatal CA3 terminals to interneurons the KARs facilitate release enhancing the synaptic response. Both mechanisms are developmentally regulated and the effect is not seen after the second postnatal week. (Lauri et al., 2005).

By depressing excitatory glutamatergic transmission and enhancing GABAergic input to pyramidal cells the tKARs could be instrumental in regulating the balance between excitation and inhibition in the network. The finding that both activation and blockade of tKARs attenuate the frequency of GDPs strongly supports this view.

8.4 The role of KARs controlling GABA release in neonatal CA3

In neonatal CA3, ATPA directly depolarizes interneurons revealing the presence of GluK1 containing KARs and giving rise to elevated sIPSC frequency in pyramidal cells. However, experiments with LY382884 showed that inhibition of GluK1-containing receptors did not affect the holding current, implying that these receptors are not tonically active. The present study also suggests that no tonically active KARs directly regulate GABA release to interneurons or pyramidal cells in CA3, because neither ATPA nor LY382884 had any effect on the mIPSC frequency in either cell type. This suggests that the regulation of sIPSCs in

neonatal CA3 is mediated via somatodendritic and/or axonal KARs on GABAergic interneurons.

Recordings from neonatal GluK1 -/- mice showed a depression in both sIPSCs and mIPSCs in CA3 pyramidal cells (Table 5, unpublished results) suggesting a KAR mediated facilitation of GABA release. These results are seemingly in contrast with the results from rats where LY382884 failed to affect sIPSC frequency in pyramidal cells and interneurons. A recent study did indeed show that GluK1 containing tKARs are present at mossy fiber terminals to neonatal CA3 pyramidal cells (Caiati et al., 2010). These KARs, however, depressed GABA release. The discrepancy in these results could be attributable to differences in animal strains or compensatory mechanisms in GluK1 deficient mice (Christensen et al., 2004; Jaskolski et al., 2004)

8.5 Postsynapic tKARs regulate interneuron excitability in neonatal CA3

When studying spontaneous activity in CA3 interneurons in GluK1-/- mice it was apparent that the spontaneous firing in neonatal CA3 interneurons was lower in the mutants than in the wildtypes. In fact, the frequency of neonatal GluK1 -/- firing was similar to that seen in P14-P16 wildtype interneurons (see fig. 6B,C). The simplest explanation is that the GluK1 subunit containing KARs are responsible for the high firing rate and that changes in ambient glutamate or in the receptor itself are explain the shift to lower firing rates in P14-P16 mice. The change in firing rate could be explained by reduced sEPSC frequency (decreased glutamate release) onto interneurons in the absence of GluK1 function as shown in I. However, in addition, a much bigger afterhyperpolarizing potential (AHP) in response to a depolarizing current step was observed in CA3 interneurons in GluK1-/- than in wildtype mice under current clamp

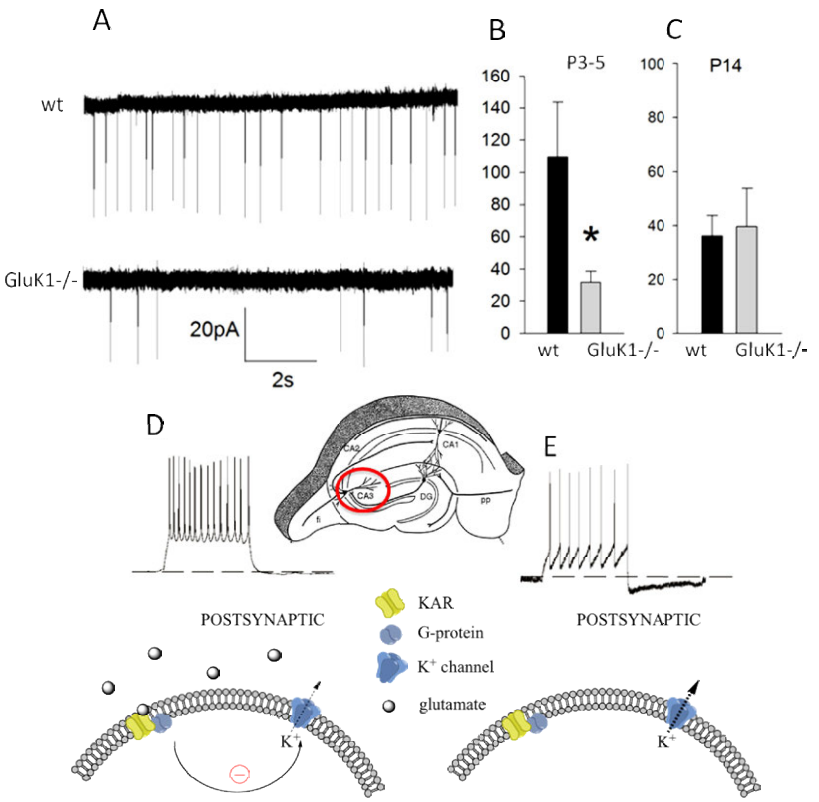

Figure 6: Regulation of interneuronal firing by tKARs in CA3

A: Representative traces from on-cell recordings from interneurons in CA3 radiatum/lucidum. B: In GluK1-/- mice the interneuronal firing is attenuated. C: In P14 there is no longer a difference in firing frequency indicating a developmental downreguation of AP firing. D: The mechanism enhancing the firing in neonatal interneurons is a tonic KAR mediated depression of an apamin dependent Im_{AHP}. Pharmacological blockade or genetic ablation (E) of the GluK1 subunit abolishes the inhibition of the K+ current, increasing the ImAHP and thus decreasing firing. The same is seen in older (P14-P16) animals. (Segerstrale et al., 2010).

experiments (see sample traces in fig 6D,E). Further, in wildtype mice, a more robust AHP was seen upon application of the GluK1 antagonist LY382884. Thus, at least two separate, tKAR-linked mechanisms regulate interneuronal firing in the newborn hippocampus. It appeared that the tKARs directly regulate interneuronal firing by depressing an apamin sensitive K^+ current, Im_{AHP} (fig 6). This represents a novel physiological role for tKARs: the depression of the Im_{AHP} promotes repetitive firing of interneurons during the first postnatal week, a time when characteristic high frequency network bursting is seen in hippocampal networks both *in vivo* and *in vitro* (Lahtinen et al. 2002; Palva et al., 2000). The tonic control diminishes during the development increasing the Im_{AHP} and consequently, decreasing the interneuron's firing rate. Thus, the findings reveal a critical endogenous mechanism that controls GABAergic transmission during development that can be of vital importance in the development of functional synaptic circuits in the hippocampus.

According to the statistics the tKAR dependent Im_{AHP} is a generic property of the most of the interneurons at this developmental stage. Interestingly Bonifazi et al recently revealed the existence of HUB interneurons that orchestrate synchrony in the developing hippocampal networks. Given the strong role in controlling the overall activity, it is possible that most of the recorded interneurons in this thesis were of the HUB-interneuron type (Bonifazi et al., 2009).

8.6 tKARs operate as gatekeepers at glutamatergic synapses to CA1 pyramidal cells

During the first postnatal days CA1 hippocampal glutamatergic transmission is based almost entirely on NMDA receptors and AMPAR-deficient silent synapses (fig. 7A) are converted to synapses with both AMPA and NMDA responses by associative pairing of pre- and postsynaptic activity (Durand et al., 1996; Liao et al., 1995). Silent glutamatergic synapses thus are developmentally expressed so that virtually all CA3-CA1 synapses to pyramidal cells are silent at birth and by the end of the second postnatal week around half of them are activated. This can be seen as an age dependent increase in the AMPA/NMDA ratio measured by evoked EPSCs (Hsia et al., 1998).

In terminals to CA1 pyramidal cells where high affinity tKARs are present, the release probability is low due to tonic inhibition of glutamate release. This mechanism depresses transmission between pyramidal cells, filtering out background activity and only allowing high frequency activity to pass through (fig. 8). In this way high affinity tKARs act as presynaptic silencers (fig. 7B) of CA1 synapses, enabling

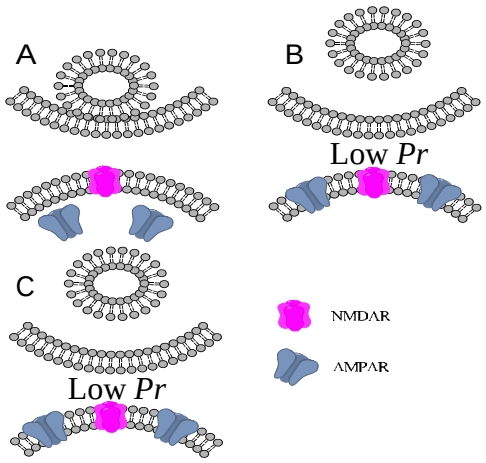

Figure 7. Silent synapses

Three alternative mechanisms for developmental silencing of glutamatergic synapses: A) postsynaptic silencing where the presynaptic terminal is working normally but the postsynaptic side has only NMDA receptors for the time being (Montgomery et al., 2001). B) presynaptic silencing where the release probability of transmitter is low or zero (Gasparini et al., 2000; Maggi et al., 2003) and C) synapses where glutamate release is impared because of a slow fusion pore (Choi et al., 2001) or because of a non-functional presynaptic terminal and diffusion of glutamate from nearby synapses (Kullmann et al., 1996; Rusakov & Kullmann, 1998). For a review on silent synapses see Kerchner & Nicoll (2008).

pathways recieving high-frequency input to enhance *Pr* and stabilize the synapse. This is associated with transformation of high-affinity KARs to low-affinity receptors. Remarkably the expression of this mechanism is restricted to the first postnatal weeks coinciding with the decrease in the number of silent synapses. This finding suggests a novel presynaptic developmentally regulated mode of silencing in the neonatal hippocampus, where expression of high affinity tKARs acts as the silencer that can be unsilenced by specific activity patterns such as high-frequency input or a GDP.

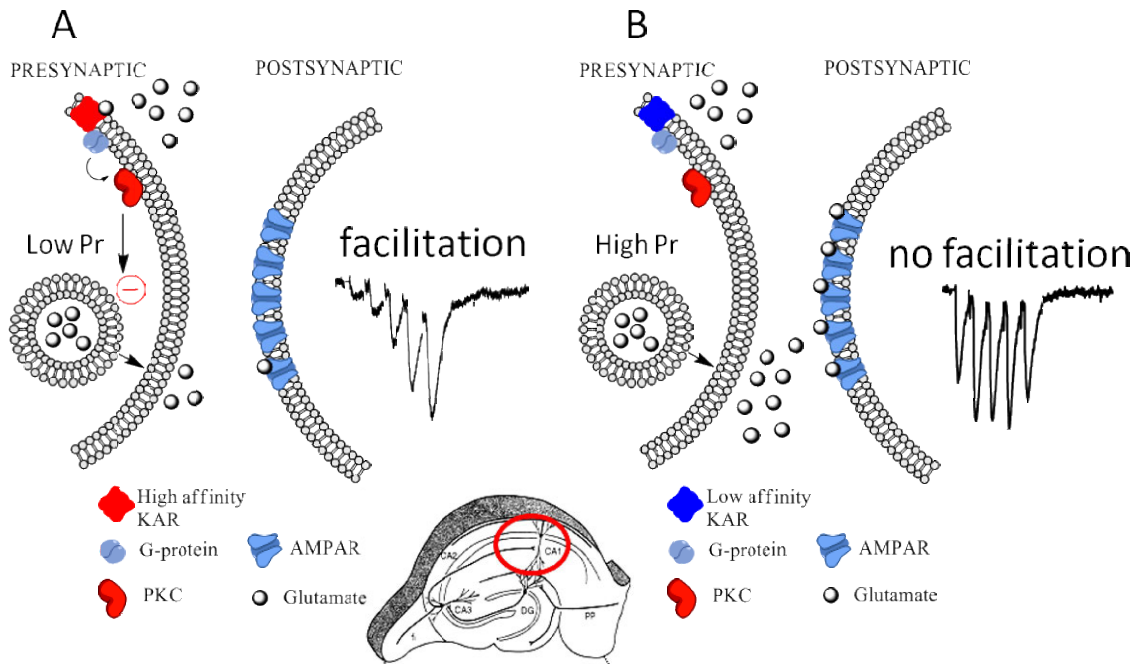

Figure 8. High affinity KARs enable frequency facilitation at terminals to neonatal CA1 pyramidal cells

A: Presynaptic high affinity KARs lower the release probability by a G-protein – PKC mediated mechanism and which can seen as a frequency facilitation in response to a 5 pulse train at 50Hz. B: After LTP induction the affinity of the KARs suddenly change increasing release probabilty (Pr) und thus unsilencing the synapse and converting it to ”mature” form. As a result, the facilitation is much less prominent after the second postnatal week. (Lauri et al., 2006).

It has been shown that in hippocampal neurons, repeated synapse activation leads to AMPAR silencing that can be reversed by Hebbian induction in a developmentally regulated manner (Montgomery and Madison, 2004; Xiao et al., 2004; Abrahamsson et al., 2005, 2007, 2008). The silencing could depend on endocytosis of AMPARs from the postsynaptic membrane (Daw et al., 2000; Man et al., 2000). Likewise, the unsilencing could depend on recruitment and insertion of AMPARs from intracellular pools (Lledo et al., 1998; Shi et al., 1999). The discovery of tKARs, however, offers an alternative or concomitant mechanism of action. The lability of neonatal synapses seems thus to depend on both presynaptic and postsynaptic mechanisms (Hanse et al., 2009). It is thought that Hebbian type of activity can stabilize these synapses and the silent ones could thus represent a pool of nascent synapses waiting for activation. A prolonged silence leads to synapse elimination and conversely activation of the synapse to its stabilization.

Glutamate receptors have been shown to participate in the activity dependent shaping of synaptic circuitry (McKinney et al. 1999; Fischer et al. 2000; Chang & De Camilli, 2001; Luthi

et al., 2001; Tashiro et al., 2003; Richards et al., 2005). It was recently shown that prolonged activation of tKARs in CA1 in slice cultures leads to an increase in the number of functional synapses seen in frequency of mEPSCs (Vesikansa et al., 2007). These data together with the findings in this thesis suggest that GluK1-containing tKARs in CA1 probably have an important role in formation- and stabilization of synapses in the immature hippocampus.

8.7 The role of tKARs in early synchronized network activity

Oscillatory activity is found in many areas of the brain such as the olfactory bulb, hippocampus, thalamus and the neocortex (Buzsaki & Draguhn, 2004). Oscillations in the frequency bands theta (4-12 Hz) and gamma (30-90 Hz) have received most of the attention because they are thought to be involved in higher brain functions such as temporal encoding, sensory binding of information and storage and recollection of information (Lisman 1995, 1999). Conversely, disruption of gamma oscillations could underlie cognitive symptoms in psychiatric disorders such as schizophrenia (Spencer et al., 2003, Lewis et al., 2005). Inhibitory interneurons have a key role in regulating time and space in the oscillatory activity, balancing excitation and controlling pyramidal cell spike timing (Mann & Paulsen 2007; Klausberger & Somogyi, 2008). Although the importance of interneurons is well established it has recently become evident that KARs affect interneuronal function and thus have an impact on network behavior. Activation of somatodendritic KARs depolarize interneurons in both CA1 an CA3 which leads to strong increase in tonic GABAergic input to principal cells in the above areas (Cossart et al., 1998; Fisahn et al., 2004; Frerking et al., 2002). In addition, KARs may also directly depolarize some interneuron's axons in CA1 (Semyanov & Kullmann, 2001). *In vitro* kainate-induced oscillations are reduced in CA3 of GluK2-/- mice while GluK1-/- mice have a higher susceptibility to kainate (Fisahn et al., 2004) suggesting opposite roles for the two subunits. Other studies, however, have shown that GluK1 agonism does not induce epileptiform activity but antagonism reduces the power of kainate induced oscillations (Brown et al., 2006).

In the neonatal brain, spontaneous oscillatory activity is thought to play an important role in controlling the development of synaptic circuitry (for review, see Zhang and Poo, 2001). The high-frequency bursts appear to be critical for normal hippocampal development (Groc et al. 2002; Lauri et al. 2003, Huupponen et al., 2007), and they are developmentally down-regulated such that they are no longer seen by the end of the second postnatal week (Ben-Ari et al. 1989; Garaschuk et al. 1998; Khazipov et al. 2004). In addition to interneuronal activity, glutamatergic transmission is involved in generating and regulating the activity and excitability of the immature hippocampal network (Bolea et al., 1999; Garashuk et al., 1999, Khalilov et al., 1999, Lamsa et al., 2000). Interestingly, the disappearance of the bursts coincides with the developmental downregulation of tKARs.

Blockade of GluK1-containing tKARs decreases the occurrence of the high frequency bursts. One explanation is that inhibition of the tKARs reduces interneuron activity and synchronization and therefore reduces the frequency of network bursts. Activation of tKARs by the agonist ATPA completely blocks the occurrence of neonatal network bursts accompanied by an increase in sEPSCs and a tenfold increase in sIPSCs. The depression of bursts is likely because of increased shunting of the network by increased asynchronous GABAergic transmission and a shift in the balance between excitation and inhibition (Lamsa et al., 2000). Interestingly application of kainate at concentrations 25-50nM, selective for

high affinity kainate receptors increases the frequency of network bursts (Juuri et al., 2010). This effect is attributable to KAR-mediated ectopic spiking of CA3 pyramidal cells. An increase in burst frequency can also be induced by increasing interneuronal excitability in CA3 by blocking the apamin sensitive, tKAR mediated mAHP. Interestingly both ATPA and high concentrations of kainate initially increases the burst frequency before attenuating it, suggesting that initial activation of high affinity tKARs increase the synchrony of the network, but when the concentration of kainate overrides the threshold for selective activation of high affinity receptors, the synchrony gets disrupted and the bursting disappears.

In mice lacking the GluK1 subunit the frequency of network bursts is higher compared to wildtypes (table 4). This result is in contrast with the results from recordings of spontaneous activity when blocking GluK1 with LY382884. However, the frequency of mIPSCs and IPSCs to CA3 pyramidal cells was lower in GluK1 -/- mice compared to wildtype littermates. Maybe a lower inhibition of pyramidal cells enables them to fire more and thus increase the burst frequency, in a similar way that of low concentrations of kainate that induce ectopic spiking and increased bursting. The high bursting could be a reflection of compensatory mechanisms in GluK1 -/- that rescues the function of the network. Interestingly the bursts in GluK1 -/- mice disappeared at an age of P12 compared to P10 in wildtypes, suggesting an impaired speed of maturation of the hippocampal network (unpublished results). Results using genetically modified mice must be interpreted with caution, however, since functional compensation can occur (Christensen et al., 2004).

Gamma band oscillations in the newborn rat brain starts to emerge around P5, seen both *in vivo* (Lahtinen et al., 2002) and *in vitro* (Palva et al., 2000). The first detectable frequencies are in the gamma band and, around P8, *in vivo* theta band frequencies are also seen. Given the regulatory role of tKARs, these receptors are likely to participate in the developmental emergence of oscillations relevant to cognition. It would be interesting to investigate putative roles and pharmacological potential of KARs in cognitive impairments such as learning disabilities and concentration difficulties. Remarkably recent studies have shown that there is a link between the gene coding for GluK2 (GRIK2) and autism (Jamain et al., 2002; Shuang et al., 2004 but see Dutta et al., 2007).

8.8 Conclusions

Our understanding of the physiological roles of KARs in the brain has only rececently started to emerge. The results obtained in the course of this study on KAR functions are of pioneering nature, highlighted by the discovery of tonically activated kainate receptors or tKARs. These receptors provide a novel, developmentally restricted mechanism involved in several pre- and postsynaptic functions concerned with neuronal maturation (fig. 9). First, a completely novel mode of action of KARs was discovered. Namely, it was found that early in postnatal development certain GluK1-containing KARs are tonically activated by ambient glutamate, and that this type of activation recedes by the end of the second postnatal week. tKARs regulate glutamate release and dynamics of synaptic strength to pyramidal cells and inhibitory interneurons. The loss of this modulation coincides with a switch from high- to low affinity KARs. Second, another tKAR-linked regulatory mechanism was found in hippocampal CA3-CA1 circuitry; tKARs postsynaptically modulate afterhyperpolarizing K^+ current and interneuron spiking.

Third, tKARs have an instrumental role in regulating hippocampal network activity leading to several physiological implications from synapse formation to emergent cognitive-range oscillations in the hippocampus.

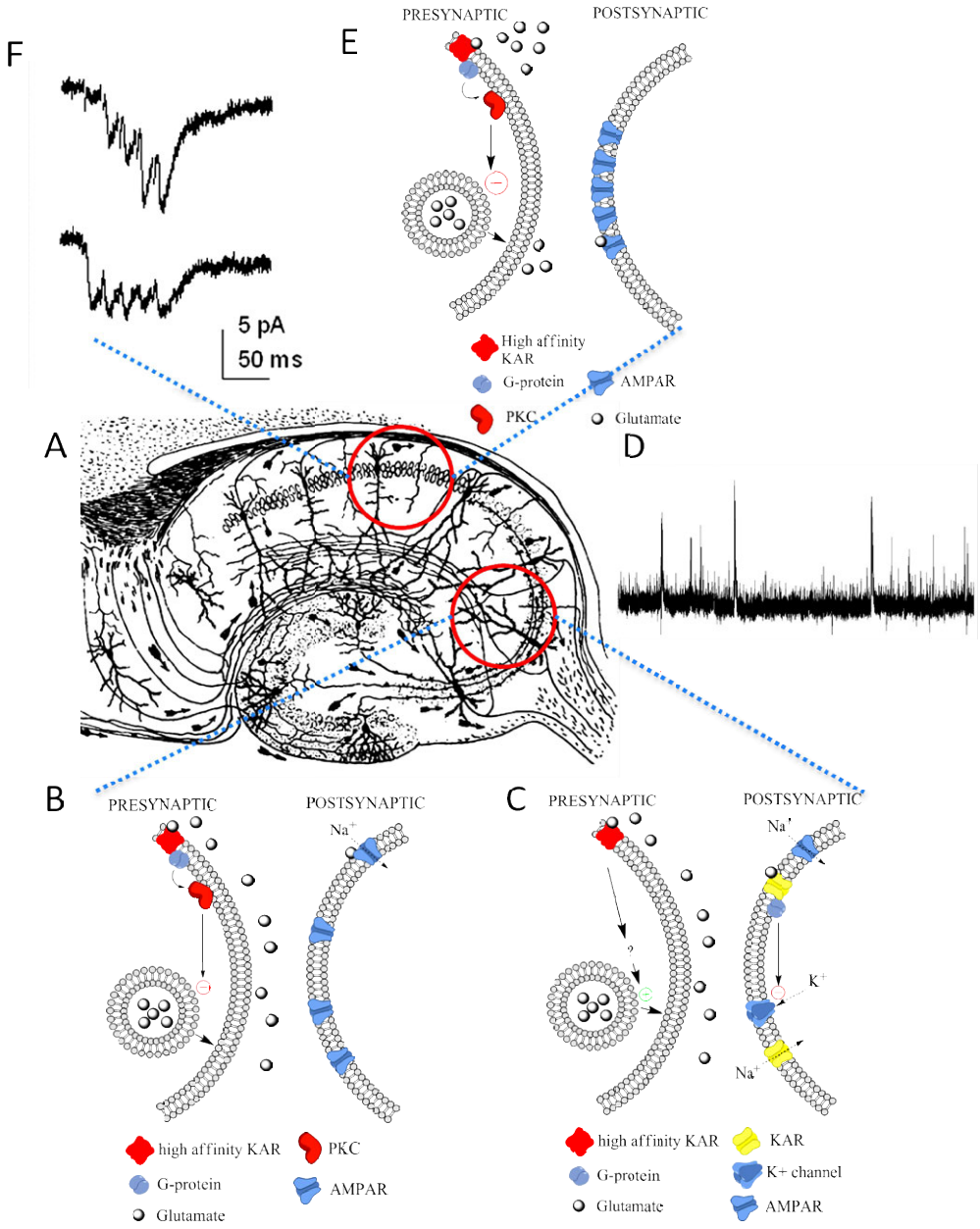

Figure 9. Function of tKARs in the neonatal hippocampus

A: A copy of the schematic of the hippocampus by Santiago Ramón y Cajal 1911. Since the first characterisations of the connections in the hippocampus, neuroscience has gone a long way. The development of new methods has enabled scientists to look deeply into the physiology of the hippocampus. The research in this thesis shows a novel type of modulatory role for KARs early in development. Ambient levels of glutamate in the newborn hippocampus are high enough to tonically activate high affinity KARs. B: At synapses to CA3 pyramidal cells tKARs depress glutamate release and at synapses to CA3 interneurons C: they facilitate release. In addition CA3 interneurons hold tKARs that upregulate interneuronal firing during the first postnatal weeks. By upregulating GABAergic transmission these mechanisms are likely to be involved in controlling the spontaneous bursts of activity D: typical for the developing hippocampus. The high frequency activity is eventually conveyed to CA1 where tKARs tune the filtering properties of the inputs E: by depressing glutamate release. The synapses receiving high frequency input are preferred because the high frequency input turn the F: facilitatory synapses to non facilitatory enhancing synaptic transmission and thus unsilencing the idle connections.

9. Acknowledgements

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