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DISSERTATION

Cell-Type-Specific Modulation of a Hippocampal Microcircuit by Serotonin

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von

Jochen Winterer

aus St. Georgen im Schwarzwald

Gutachter/in: 1. Prof. Dr. D. Schmitz

2. Prof. Dr. I. Vida

3. Prof. Dr. med. H. Beck

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Synopsis

The serotonergic system is a subcortical neuromodulatory center that controls cortical information processing in a state-dependent manner. Dysregulations of serotonergic neurotransmission and/or neuromodulation are thought to play an important role in many neuropsychiatric diseases such as depression and schizophrenia. In the hippocampus, ascending serotonergic fibers originating from the median raphe nuclei project mainly onto GABAergic interneurons. This highly diverse subgroup of hippocampal neurons effectively controls the input and the output of principal neurons, which represent by far the most abundant group of neurons in the hippocampus. We addressed the question of how serotonin affects excitatory glutamatergic transmission onto these interneurons, in particular onto basket cells, which provide the major source of perisomatic inhibition. This form of inhibition is ideally suited to control the spiking and therefore the output of pyramidal neurons. Basket cells are divided into two subgroups, one expressing the calcium-binding protein parvalbumin, the other expressing the neuropeptide cholecystokinin. We found that serotonin selectively reduces synaptic excitation of cholecystokinin-expressing interneurons via presynaptic serotonin 1B (5-HT_{1B}) heteroreceptors that are highly expressed in CA1 pyramidal neurons. We were able to show that this reduction is input-specific and that, as a result, serotonin selectively decreases feedback inhibition in hippocampal area CA1 via activation of 5-HT_{1B} heteroreceptors. This reduction allows CA1 pyramidal neurons to integrate inputs for spike-generation during a broader time window. Furthermore, we found that on the network level, activation of 5-HT_{1B} receptors increases the power of gamma oscillatory activity in hippocampal area CA1 both *in vitro* and *in vivo*. To conclude, in this work we provide evidence for a specific serotonergic modulation of a defined microcircuit in area CA1 of the hippocampus.

Zusammenfassung

Das serotonerge System ist ein subkorticales, neuromodulatorisches Zentrum, welches die Informationsverarbeitung im Kortex abhängig vom Zustand des Organismus kontrolliert. Es wird angenommen, dass Störungen der serotonergen Neurotransmission und/oder Neuromodulation eine bedeutende Rolle für viele neuropsychiatrische Erkrankungen spielen, zum Beispiel bei rezidivierenden depressiven Störungen oder bei Erkrankungen aus dem schizophrenen Formenkreis. Serotonerge Fasern des Nucleus raphe medianus projizieren im Hippocampus vornehmlich auf GABAerge Interneurone. Diese äußerst heterogene Zellgruppe kontrolliert die afferenten wie efferenten Signale von Pyramidenzellen, welche die quantitativ größte Zellgruppe im Hippocampus darstellen. Die vorliegende Arbeit befasst sich mit der Frage, ob und auf welche Weise die erregende, glutamaterge Neurotransmission, die auf diese Interneurone projiziert, von Serotonin beeinflusst wird. Dabei interessierten wir uns vor allem für eine Untergruppe dieser Interneurone, sogenannte Korbzellen, die den Hauptanteil der perisomatischen Hemmung ausmachen. Diese spezielle Form der Hemmung ist besonders geeignet, das Auftreten von Aktionspotentialen und daher die efferenten Signale von Pyramidenzellen zu kontrollieren. Korbzellen können wiederum in zwei Untergruppen unterteilt werden, nämlich in jene, die das kalzium-bindende Protein Parvalbumin und in jene, die das Neuropeptid Cholezystokinin (CCK) exprimieren. Ein Ergebnis dieser Arbeit ist nun, dass Serotonin, vermittelt durch präsynaptische 5-HT_{1B} Rezeptoren, selektiv die erregende, glutamaterge Neurotransmission auf CCK-positive Interneurone reduziert. Diese sogenannten Heterorezeptoren werden besonders ausgeprägt in CA1 Pyramidenzellen des Hippocampus exprimiert. Daran anschließend konnten wir aufzeigen, dass die Reduzierung der glutamatergen synaptischen Übertragung vom Ursprung der afferenten Fasern abhängt und auf Fasern die von CA1 Pyramidenzellen herrühren beschränkt ist. Als Folge dieser Reduktion durch 5-HT_{1B} Rezeptoren zeigte sich eine selektive Verringerung der rückgekoppelten Hemmung von CA1 Pyramidenzellen durch CCK-positive Interneurone und konsequenterweise wurde es den Pyramidenzellen damit ermöglicht, unterschiedliche Afferenzen in einem erweiterten Zeitfenster für die Initiierung von Aktionspotentialen zu integrieren. Nicht zuletzt konnten wir schließlich zeigen, dass die Aktivierung von 5-HT_{1B}

Heterorezeptoren auf glutamatergen Fasern Gamma-Oszillationen in ihrer Ausprägung sowohl *in vivo* als auch *in vitro* verstärkt. Zusammenfassend konnte in dieser Arbeit eine spezifische neuromodulatorische Funktion von Serotonin in einem reduzierten und definierten neuronalen Schaltkreis aufgezeigt werden.

1 Introduction

1.1 The hippocampal formation: structure and function

The hippocampus proper with its three subdivisions area CA3, area CA2 and area CA1 is part of the hippocampal formation, a functional system consisting of several related brain regions embedded in the medial temporal lobe of the mammalian brain. The hippocampal formation comprises the entorhinal cortex, the dentate gyrus, the hippocampus proper, the subiculum and the para- and presubiculum (for review see Amaral and Lavenex in Andersen et al., 2006). Contrasting the six-layered architecture of the neocortex with its reciprocal excitatory connections, the hippocampal formation impresses with a unique set of predominantly unidirectional excitatory pathways and in its allocortical parts consisting of the hippocampus proper, the dentate gyrus and the subiculum with a single layer of principal neurons. This arrangement entails a three-layered structure with fiber-rich layers above and below the principal cell layer. Together with afferent fiber projections from distant sources terminating in distinct layers in a non-overlapping fashion, this assembly gives rise to the so-called lamination of the hippocampus (for review see Förster et al., 2006): fibers from the entorhinal cortex project onto dendrites of dentate gyrus granule cells in the outer molecular layer. The inner molecular layer of the dentate gyrus is the termination zone for afferents from mossy cell projections from the ipsilateral and contralateral sides: the associational/ commissural pathway. Dentate gyrus granule cells project via mossy fibers onto CA3 pyramidal neurons, mainly impinging on dendrites in stratum lucidum. CA3 and CA2 pyramidal neurons in turn project onto themselves on the ipsilateral (associational) and contralateral (commissural) sides in stratum radiatum of area CA3 and give rise to the Schaffer collateral pathway. This fiber tract is comprised of axons terminating on CA1 pyramidal dendrites in stratum radiatum and stratum oriens of area CA1. Stratum lacunosum moleculare is the termination zone for fibers originating in the enthorinal cortex. Pyramidal cells of area CA1 again project onto two intrahippocampal projections, first to the adjacent subiculum, second to the enthorinal cortex. The subiculum is the major output structure of the hippocampus with efferents projecting to

the pre-and parasubiculum, to the enthorinal cortex, to the neocortex and the amygdala as well as subcortical structures.

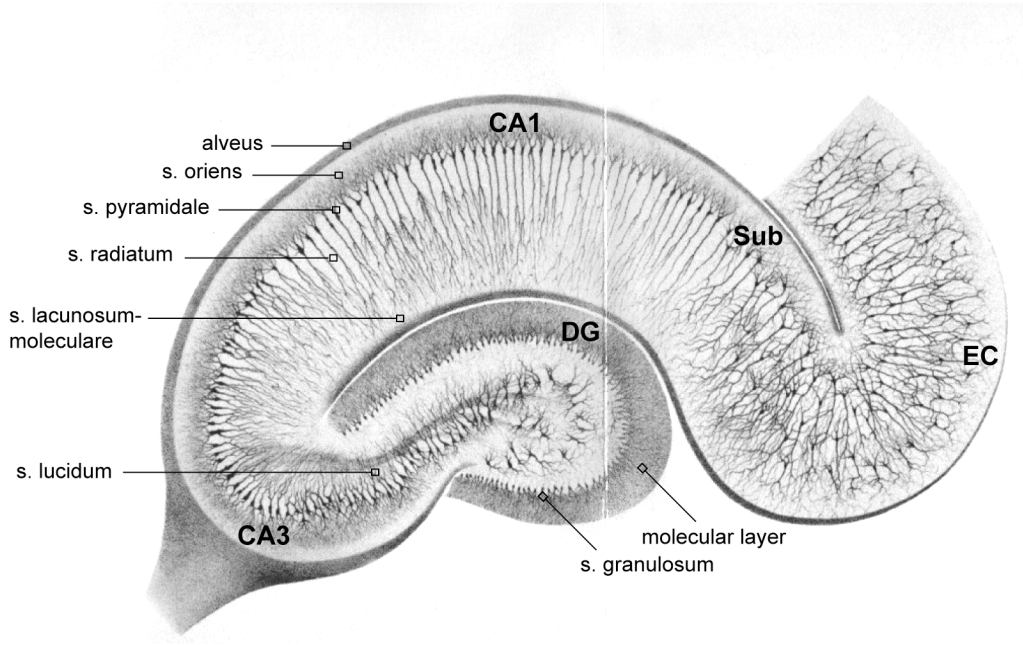

Fig.1.1: Principal neurons of the hippocampal formation and their connectivity.

A classical Golgi stain drawing of a sagittal hippocampal slice of a newborn kitten. DG: dentate gyrus, CA: cornu ammonis, Sub: subiculum, EC: entorhinal cortex, s.: stratum. Adapted from Camillo Golgi, first published in 1883, republished in Golgi, 2001.

The hippocampal formation is essential for spatial navigation and episodic memory. But how is this accomplished and, more generally, how is information encoded in the central nervous system (CNS)? Being far too much for a question to be answered in this work, it is nevertheless helpful to consider the different concepts that have been related to this question. One has been the idea that neurons act as individual computing elements, transmitting information to each other in discrete bundles via their axonal projections. A different view is that ensembles of cells, acting in concert by synchronous firing, provide representing reference signals for information. For both point of views there are fascinating examples to be found in the hippocampal formation: On the one hand there has been the identification of synaptic plasticity, where a single cell responds to a specific pattern of activation with long-lasting increases or decreases of synaptic efficacy, termed long-term potentiation (LTP) and long-term depression (LTD) respectively and it has been suggested that this form of plasticity is the synaptic correlate for memory formation (for review see Martin et al., 2000; Bliss et al.

in Andersen et al., 2006). Furthermore there are multiple examples of a single hippocampal pyramidal neuron increasing it's firing rate at specific locations in space, thereby representing this specific location of the moving animal (O'Keefe, 1979; Moser et al., 2008). On the other hand, it has long been shown that a specific rhythmic activity of multiple neurons, i.e. theta rhythm, occurs during movement of an animal in space and research on hippocampal network activity has shed light on beautiful examples of how different types of oscillatory activity can be related to different behavioral states (Soltesz and Deschênes, 1993; Buzsáki, 2002; Lee and Wilson, 2002). Furthermore, neuromodulatory inputs originating in brainstem nuclei or neuronal ensembles in the septum provide further regulatory mechanisms for both computational levels, thereby adding additional complexity to the computational patterns expressed by hippocampal neurons and neuronal ensembles (Bliss et al. in Andersen et al., 2006; Kocsis et al., 2006).

This being said, it is clear that most probably both computational strategies are used by the hippocampus and it seems likely that the two functional levels interact. In this work, we tried to approach both the synaptic and the network level using acute hippocampal slices as well as *in vivo* recordings in the hippocampus of the freely moving animal.

1.2 The serotonergic system

1.2.1 Serotonin: an evolutionary perspective

Serotonin is a monoaminergic neurotransmitter/neuromodulator that is synthesized from tryptophan via the action of two enzymes, the oxygen-dependent tryptophan hydroxylase and the pyridoxal phosphate-dependent general L-amino acid decarboxylase. Tryptophan is an aromatic amino acid that cannot be synthesized by animals, in contrast to primitive unicellular organisms and plants. Albeit animals lack the genes for tryptophan synthesis and are therefore dependent on external supplies of tryptophan for serotonin synthesis, there is an amazing consistency of the function of serotonin throughout evolution, reflected in the close relationship of serotonin to sunlight (for review see Azmitia in Muller and Jacobs, 2009).

The serotonin precursor tryptophan contains an indole backbone that absorbs light (Lin and Sakmar, 1996). In cyanobacteria, algae and plants this feature of tryptophan is used for photosynthesis, where CO_2 and water are converted into oxygen and glucose (Azmitia, 2007).

A problem that occurs with the production of oxygen in cells is the generation of free radicals, namely singlet oxygen and superoxide that are harmful to protein function and membrane integrity. Therefore, in simple cell organisms and fungi, many of the derivatives of tryptophan (like serotonin, melatonin and others) evolved antioxidant features that support these primitive organisms to maintain homeostasis, regulate differentiation and promote cell division. In plants, tryptophan derivates like serotonin evolved into modulators of growth and limited movements such as the turning of leaves towards the sunlight. Additionally, serotonin and melatonin synthesis have diurnal and seasonal rhythms (Azmitia in Muller and Jacobs, 2009). In animals, as mentioned above, the capability to synthesize tryptophan is lost. Nevertheless, highly branched networks of serotonergic cells in the brain and in the gut are capable of taking up tryptophan and are endowed with enzymes required for serotonin synthesis. In addition, the serotonergic system is equipped with a vast and diverse set of specialized receptors to maximize serotonergic actions (for review see Barnes and Sharp, 1999). It is worth noting that the impact of serotonin on various types of behavior is still related to light as can be seen by the effects of sunlight on serotonin levels in the blood, on mood, sleep and even suicide ideation in humans (Mann, 1999; Lambert et al., 2002).

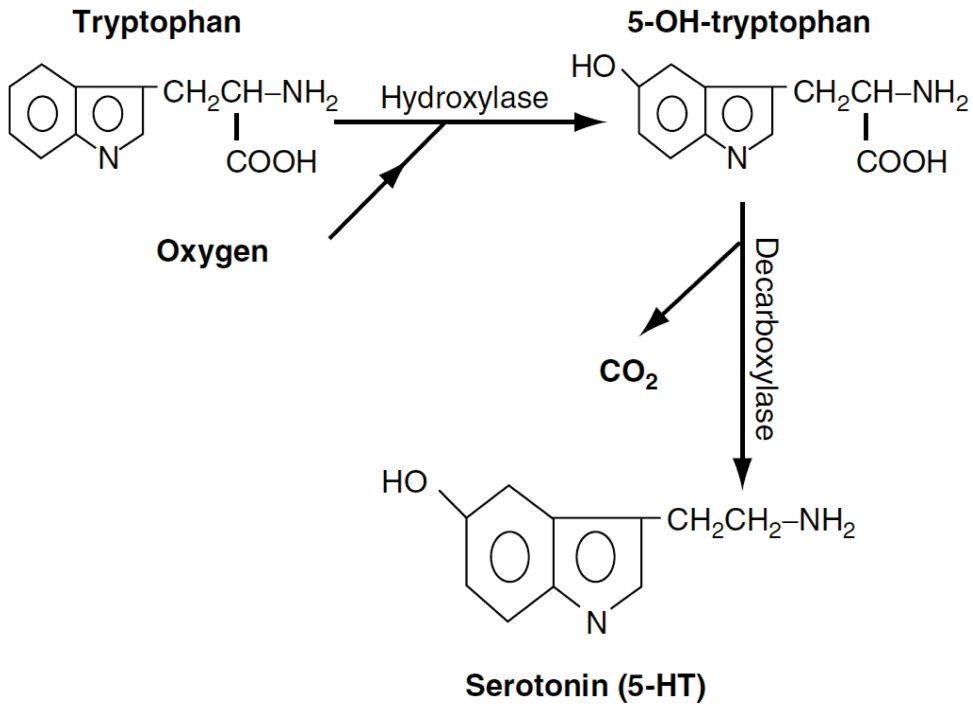

Fig.1.2.1. The metabolic pathway for serotonin synthesis

The synthesis of serotonin from tryptophan involves two enzymes, tryptophan hydroxylase and aromatic amino acid decarboxylase. Adapted from Azmitia, 2007.

1.2.2 Neuroanatomy of the serotonergic system

In the CNS, neurons using serotonin as a neurotransmitter are restricted to the brainstem. Initially described by Dahlström and Fuxe in 1964, these neurons are arranged in cell clusters, named B1 to B9, that are mainly centered on the midline raphe nuclei. These clusters can be further subdivided into two groups, a rostral group (B4-B9) in the midbrain and the rostral pons and a caudal group (B1-B3) residing in the caudal pons and in the medulla. Also, each cluster comprises a different proportion of serotonergic neurons: roughly speaking, the relative amount of serotonergic neurons decreases from rostral to caudal. Besides serotonergic neurons, the cell clusters contain peptidergic neurons expressing substance P, cholecystokinin, dynorphin, enkephalin and neurotensin as well as nitric oxide neurons and neurons immunopositive for tyrosin hydroxylase (for review see Hornung in Muller and Jacobs, 2009).

The rostral group contains the caudal linear nucleus, the most rostral population of serotonergic neurons, representing the rostral population of the B8 group. At its dorsal end, this nucleus merges with the most rostral part of the dorsal raphe nucleus, which represents the second nucleus of the rostral group and the largest collection of serotonergic neurons in the brain. The dorsal raphe represents the B7 and B6 group and is located dorsal to the medial longitudinal fasciculus, while all other raphe nuclei are positioned ventrally to this fiber tract. The third cluster of serotonergic cells can be further subdivided in three groups, the median raphe nucleus, corresponding to B8 and B5 and the oral pontine nucleus and the supralemniscal nucleus in the pontomesencephalic reticular formation, corresponding to B9. Together these clusters represent the second largest group of serotonergic neurons. The caudal group comprises three raphe nuclei, the raphe magnus nucleus (B3 group), the raphe obscurus nucleus (B2 group) and the raphe pallidus nucleus (B1 group), and a population of serotonergic neurons in the lateral medullary reticular formation.

Serotonergic neurons of the raphe nuclei send projections to all parts of the adult brain. The main projections of the raphe nuclei, with focus on the rostral group, are mentioned in the following section. The caudal group sends projections mainly to the lower brainstem and the spinal cord, whereas the rostral group has only a minor projection to the brainstem in addition to a major projection to the forebrain. From the rostral group a dorsal and a ventral pathway ascend to their target regions in parallel. The anterior division of the dorsal raphe nuclei

projects to the cerebral cortex, the neostriatum, the amygdala and the substantia nigra. The caudal parts send projections to the hippocampal formation and the locus coeruleus. There are also reports that show projections to the thalamus and the cerebellum. The median raphe projects mainly to the basal forebrain, the septal regions, the ventral tegmental area, the hypothalamus, the thalamic nuclei and the hippocampus. There are also projections to the cerebral cortex, but to a lesser extent than the projections from the dorsal raphe nuclei. Together with the dorsal raphe nuclei, the median raphe nuclei contribute to the cerebellar innervation (for review see Hornung in Muller and Jacobs, 2009).

Serotonergic neurons from the raphe nuclei display a different morphological pattern of their axonal varicosities. They segregate into axons with large and axons with small varicosities, the latter being devoid of associated postsynaptic specializations, whereas the large ones form classical chemical synapses. In the hippocampus these fibers have been shown to impinge exclusively on cholecystokinin (CCK)-positive and/or calbindin-positive interneurons. Furthermore, these different fibers have distinct sources; axons with large varicosities originate from serotonergic neurons of the median raphe nuclei, while their counterparts originate in the dorsal raphe (Freund et al., 1990; Vertes, 1991; Vertes et al., 1999).

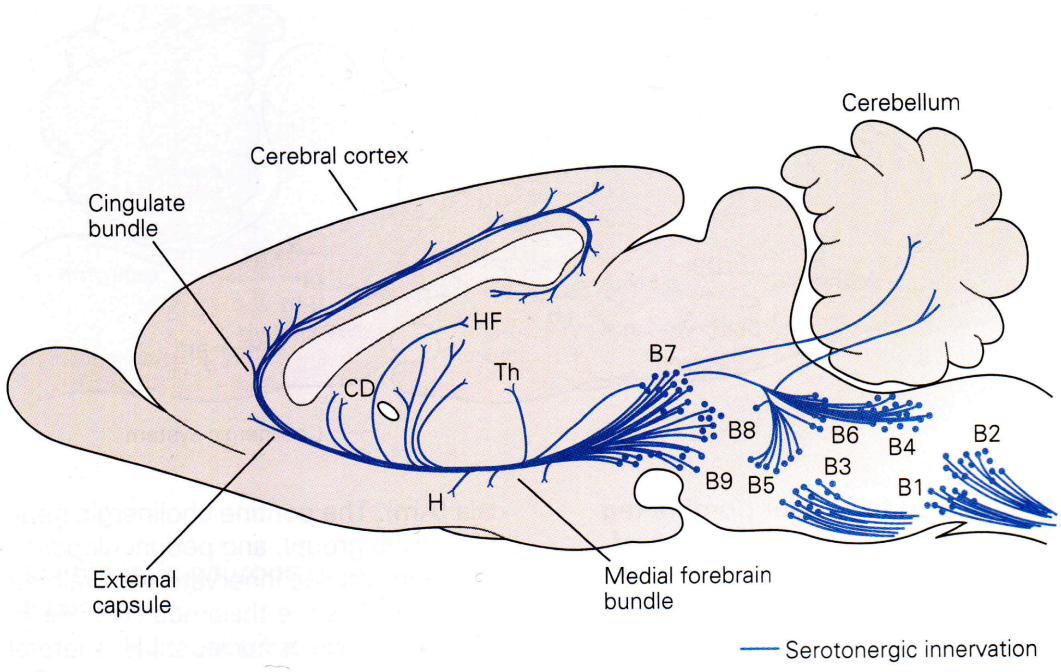

Fig.1.2.2. Serotonin cell clusters of the brainstem

Adapted from Kandel, 2008. CD = caudate nucleus; HF = hippocampal formation; H = hypothalamus; Th = thalamus. B1-B9 = serotonergic cell clusters of the brainstem.

1.2.3 Classification and signaling of 5-HT receptors

To date serotonin receptors comprise seven classes with a total of 14 receptors encoded by 17 genes (for review see Bockaert et al. in Muller and Jacobs, 2009; Barnes and Sharp, 1999). Historically, two classification periods can be distinguished: a first, pharmacological period that described four receptor classes (5-HT_{1-4}) and a molecular cloning period that revealed another three classes (5-HT_{5-7}) and also added additional subclasses. All 5-HT receptors are metabotropic G-protein-coupled receptors (GPCRs) with the exception of 5-HT_3 receptors, which are ionotropic ligand-gated ion channels. The signaling of metabotropic 5-HT receptors is not restricted to a single transduction mechanism. In recent years it has been shown that there are signaling pathways that are non-G-protein dependent, implicating additional interactions of GPCRs with other proteins (Bockaert et al., 2004). Some GPCRs display a constitutive activity, where the activation of the receptor is independent of the agonist. Another phenomenon of metabotropic 5-HT receptors is the so called agonist-directed signaling, where the receptor is stabilized in different active states, depending on the nature of the agonist, also described as ligand-directed signaling (Kenakin, 2007). Different agonists at a given 5-HT receptor show different efficacies to activate different subunits of the GPCR. Last but not least, there is an increasing body of literature describing interacting proteins that form multiprotein complexes with metabotropic 5-HT receptors (Bockaert et al., 2006). The functional implications of these interactions range from modulations of the receptors trafficking, the desensitization properties of the receptor to signaling efficacy of the receptor. In the following, the different classes of 5-HT receptors with their different subclassifications and main signaling characteristics are outlined.

The 5-HT_1 receptor class:

Like other 5-HT_1 receptors, 5-HT_{1A} receptors are coded by intronless genes. They are coupled to Gi/Go, thereby inhibiting adenylyl cyclases. Via their Gβγ subunit they open G-protein-gated inwardly rectifying K^+ channels (GIRK) (Colino and Halliwell, 1987; Andrade and Nicoll, 1987) and inhibit Ca^{2+} channels.

5-HT_{1B} receptors are negatively coupled to adenylyl cyclase via their Gi/Go subunit. They also inhibit Ca^{2+} channels and open K^+ channels. 5-HT_{1B} receptors interact with P11, a member of the S100 Ca^{2+}-binding protein superfamily. This interaction promotes the

expression of the receptor at the plasma membrane and enhances the signal transduction efficacy (Svenningsson et al., 2006).

5-$HT_{1D/E/F}$ receptors are also negatively coupled to adenylyl cyclase via their Gi/Go subunit.

The 5-HT_2 receptor class:

5-HT_{2A} receptors activate Phospholipase C (PLC) via their Gq/G11 subunit. These receptors also show ligand-directed signaling. Both hallucinogenics (LSD, DOI) and non-hallucinogenics (lisuride) utilize the Gq/PLC pathway leading to induction of c-fos, a transcription factor of the immediate early gene family. However, only hallucinogenics activate an additional Gi/Go- Src (a non-receptor tyrosine kinase) pathway leading to the induction of the expression of the transcription factor early growth response protein-2 (egr-2) (González-Maeso et al., 2007). Recently, a direct interaction of the 5-HT_{2A} receptor with the metabotropic glutamate receptor $mGluR_2$ has been shown. Activation of $mGluR_2$ leads to the formation of heterodimers with the 5-HT_{2A} receptor, which in turn inhibits the induction of egr-2 expression, without affecting the Gq/PLC pathway. As a consequence, the activation of $mGluR_2$ attenuates the behavioral effects of hallucinogenics (González-Maeso et al., 2008).

5-HT_{2B} receptors are coupled to Gq/G11 and activate PLC as well.

5-HT_{2C} receptors share the same main signal transduction pathway with the other members of the 5-HT_2 receptor class. They activate PLC via Gq/g11. In addition, 5-HT_{2C} receptors are subject to mRNA editing. The non-edited form activates PLC more potently and shows a higher constitutive activity in contrast to the edited receptor. To date, 5-HT_{2C} receptors are the only 5-HT receptors, for which mRNA editing has been shown to exist (Fitzgerald et al., 1999). These receptors also display non-G-protein dependent signaling. For example, the activation of extracellular-signal-regulated kinases (ERK) mediated by 5-HT_{2C} receptors is independent of the G-protein subunits of the receptor (Bockaert et al. in Muller and Jacobs, 2009).

The 5-HT_3 receptor class:

5-HT_3 receptors are ligand-gated ion channels. The cationic channel is permeable for Na^+, Ca^{2+} and K^+ and upon activation, will lead to a depolarization of the cell expressing the receptor. There are five genes that encode for the different subunits of the pentameric 5-HT_3

ion channel (5-HT_{3A-E}), possibly leading to homo- and hetero-pentameric forms of the receptor (Bockaert et al. in Muller and Jacobs, 2009).

The 5-HT_4 receptor class:

5-HT_4 receptors couple positively to adenylyl cyclase via a Gs subunit. The activation of Protein kinase A (PKA) via cyclic adenosine monophosphate (cAMP) has downstream effects on K^+ currents (inhibiting), on the afterhyperpolarization (AHP) of action potentials (inhibiting) and on current flowing through hyperpolarization-activated cyclic nucleotide-gated (HCN) channels (I_H-activating). Furthermore, like 5-HT_{2C} receptors, 5-HT_4 receptors display non-G-protein dependent signaling. 5-HT_4 receptor mediated ERK activation has been shown to be mostly independent of Gs, but dependent on Src tyrosine kinase (Bockaert et al. in Muller and Jacobs, 2009).

The 5-HT_5 receptor class:

The signaling pathways of the 5-HT_5 receptors have not been elucidated as yet (Bockaert et al. in Muller and Jacobs, 2009).

The 5-HT_6 receptor class:

5-HT_6 receptors are coupled to Gs and activate, like 5-HT_4 and 5-HT_7 receptors, adenylyl cyclase. They can also activate the ERK pathway (Sebben et al., 1994).

The 5-HT_7 receptor class:

5-HT_7 receptors, as mentioned above, are positively coupled to adenylyl cyclase via their Gs subunit. They have also been shown to activate the ERK1/2 pathway via the activation of Epac (exchange proteins directly activated by cAMP) (Lin et al., 2003).

1.2.4 Cellular effects of serotonin in the hippocampus proper

In hippocampal area CA1 pyramidal cells and interneurons show different expression of 5-HT receptor subtypes, resulting in a differential modulation of intrinsic and synaptic properties (for review see Andrade, 1998). One of the first descriptions of how serotonin affects the intrinsic properties of it's target cell was the serotonin-induced hyperpolarization of CA1 pyramidal cells (Segal, 1980). This effect could later on be attributed to a 5-HT_{1A} receptor-

mediated opening of a GIRK channel, (Colino and Halliwell, 1987; Andrade and Nicoll, 1987). Similarly, a 5-HT_{1A} receptor mediated hyperpolarization is apparent in granule cells of the dentate gyrus and CA3 pyramidal neurons. Consistent with this inhibitory effect of 5-HT, in the dentate gyrus a decrease in unit firing *in vivo* could be observed (Richter-Levin and Segal, 1990). Another receptor that is expressed in pyramidal neurons of hippocampal areas CA1 and CA3 is the 5-HT_4 receptor (in CA3, pyramidal cells additionally express the 5-HT_7 receptor). 5-HT_4 and 5-HT_7 signaling exerts an increased excitability on the respective cells via a combination of (1) a reduction of the Ca^+-activated AHP and (2) a depolarization of the cell's membrane. The reduction in AHP was shown to depend on cAMP and PKA (Torres et al., 1995; 1996). A recent study attributed the membrane depolarization to cAMP activation and the subsequent opening of a proposed, not yet identified cyclic nucleotide gated channel (Chapin et al., 2002, but see Gasparini and DiFrancesco, 1999). Last but not least CA1, but not CA3 pyramidal cells express 5-HT_{1B} receptors (Voigt et al., 1991). As there is not just one direction for serotonin to modulate the intrinsic properties of pyramidal neurons, the same holds true for interneurons. In an ambitious attempt to classify hippocampal CA1 interneurons, Parra et al. found hyperpolarizing as well as depolarizing effects of serotonin (Parra et al., 1998). The latter effect has been in part attributed to the 5-HT_3 receptor subtype mediating a transient increase of fast inhibitory postsynaptic potentials (IPSPs) (Ropert and Guy, 1991). Interestingly, there is a differential expression of the 5-HT_3 receptor in interneurons. Whereas CCK-positive basket cells do express this ionotropic receptor mediating a membrane depolarization of the neuron, parvalbumin-expressing basket cells do not.

The modulation of synaptic transmission by serotonin is as diverse as the modulation of the intrinsic neuronal properties. Measuring excitatory postsynaptic potentials (EPSPs), Schmitz et al. reported a reduction of the EPSPs only at very high concentrations of 5-HT (>100 μM) while at lower concentrations occasionally an increased EPSP amplitude was observed (Segal, 1980; Schmitz et al., 1995). These findings contrast the possibility of serotonin to reduce EPSP amplitudes substantially at lower doses in subicular pyramidal neurons via a presynaptic 5-HT_{1B} receptor subtype mediated suppression of glutamate release (Boeijinga and Boddeke, 1993; 1996). Synaptic inhibition in the hippocampal area CA1 is modulated by serotonin via a reduction in the polysynaptic fast and slow IPSPs (Segal, 1980; Oleskevich and Lacaille, 1992; Schmitz et al., 1995). These reports indicate that 5-HT effects fast and

slow synaptic inhibition of principal cells by a presynaptic mechanism involving the inhibition of inhibitory interneurons by the activation of the 5-HT_{1A} receptor subtype (Schmitz et al., 1995). Additionally, a facilitatory effect of 5-HT_2 receptor signaling on GABAergic neurotransmission onto pyramidal neurons was observed (Shen and Andrade, 1998) that is most likely mediated by serotonin-induced depolarization of different 5-HT_2 receptor-expressing interneurons.

1.2.5 The role of serotonin in neuropsychiatric disorders

The extensive serotonergic projections to virtually all parts of the adult brain together with the diversity of serotonergic receptors differentially expressed in distinct neuronal populations and target regions, as well as their different transduction pathways, help to explain how, by a single neurotransmitter/neuromodulator, it is possible to exert a multitude of effects that in turn relate to such a large number of behaviors: serotonin is linked to neuronal development, to basal sensory-motor control, to appetite and ingestion, to sexual behavior, mood and emotion, impulsivity and compulsivity, to learning and memory functions, to social behavior and pain (for review see Muller and Jacobs, 2009). As all of these behaviors have the possibility of being dysfunctional, it is not surprising that serotonin has been implicated in a corresponding number of diseases, in their etiology as well as a possible therapeutical target. In the following, some of the concepts and recent advances in understanding the role of serotonin in different diseases will be outlined with a focus on the role of serotonin in depression and schizophrenia.

1.2.5.1 The role of serotonin in depression

Two main findings motivated the assumption that serotonin is implicated in the pathophysiology of major depression. Firstly, in the early 1950s, the antidepressant iproniazid was identified as a monoaminooxidase inhibitor (MAOI) and secondly, decreased levels of 5-HT metabolites in the cerebrospinal fluid of patience suffering from major depression and suicidal behavior were reported. Since then, there have been substantial advances in understanding the role of serotonin in major depression, but still a congruent picture has not yet emerged. Especially the concept of a simple monoamine deficiency, as expressed by the "monoamine hypothesis" of depression, has been challenged by several findings, most prominently by the fact that serotonin reuptake inhibitors (SSRIs) and MAOIs, still being at the frontline of antidepressive therapy, induce an immediate increase in monoaminergic

transmission, whereas the symptoms of patients suffering from major depression take weeks to be relieved. Extensions to the "simple" monoamine hypothesis concentrate on secondary neuroplastic changes that arise from the acute enhancement of monoamine transmission by antidepressants like SSRIs and MAOIs (Krishnan and Nestler, 2008). Recently, it has been demonstrated that the dynamic modulation of 5-HT_{1B} receptor function is an important adaptation mechanism downstream of 5-HT reuptake inhibition by antidepressants (Svenningsson et al., 2006). Chronic treatment with a tricyclic antidepressant evoked an upregulation of the calcium-binding protein p11 in the cerebral cortex of mice. In cell culture systems, p11 induced an increased localization of 5-HT_{1B} receptors on the cell surface. Subsequent experiments could demonstrate a depression-like behavior of p11 knockout mice, accompanied by parallel changes in 5-HT_{1B} receptor expressions and, vice versa, that the up-regulation of p11, which increases 5-HT_{1B} receptor function, reduced depression-like symptoms. Other research has focused on associations between genes that regulate serotonergic transmission or 5-HT receptors and major depression. To date, there is good evidence for a link to major depression for genes that regulate the 5-HT_{1A}, 5-HT_{2A} receptors, the serotonin transporter and the monoamino oxidase (Holsboer, 2008).

1.2.5.2 The role of serotonin in schizophrenia

Historically, the idea that serotonin is linked to the pathophysiology of schizophrenia has been probably proposed the first time by Kurt Beringer in the 1920s, although at that time serotonin was not yet discovered. Beringer suggested the use of mescaline, a hallucinogen later discovered to be a 5-HT_{2A} receptor agonist, as an experimental model of psychosis (Quednow et al. in Muller and Jacobs, 2009). About twenty years later, again before the first description of serotonin, Albert Hofmann discovered the psychotomimetic potency of LSD, a 5-HT_{2A} receptor agonist as well. Subsequent experiments with schizophrenic patients and healthy volunteers compelled the psychiatrist Walther Stoll and colleagues at the University Hospital of Psychiatry in Zürich to propose that a toxic substance similar to LSD may cause schizophrenic psychosis. After the discovery of serotonin, Hoffer, Osmond and Smythies proposed that schizophrenia could result from an „aberrant endogenous biosynthesis of methylated indolamine hallucinogens such as LSD" (Hoffer et al., 1954). The finding that the neuroleptic drugs chlorpromazine and haloperidol signal via postsynaptic dopamine receptors eclipsed the idea of a serotonin hypothesis of schizophrenia. At the end of the 1950s, the dopamine hypothesis became the most influential concept on the etiopathogenesis of

schizophrenia for the following 30 years. The advent of ‚atypical' neuroleptic drugs, with a higher affinity for 5-HT_{2A} receptors than dopamine D_2 receptors and a nevertheless high antipsychotic potency, caused a resurgence of the idea that serotonin is involved in the pathophysiology of schizophrenia. Recent advances in understanding the role of serotonin in the pathophysiology of schizophrenia emphasize the role of 5-HT_{1A} and 5-HT_{2A} receptors. Post-mortem and PET studies implicate an increase in 5-HT_{1A} receptors and a decrease in 5-HT_{2A} receptors in the prefrontal cortex of patients suffering from schizophrenia (Bantick et al., 2001; Quednow et al. in Muller and Jacobs, 2009). Hallucinogenic 5-HT_{2A} receptor agonists resemble parts of the symptoms of schizophrenic patients (Geyer and Vollenweider, 2008). Association studies link especially the A-1438G polymorphism of the 5-HT_{2A} receptor to schizophrenia (SchizophreniaGene database). Nevertheless it has also become evident that the serotonin system, or parts of it, is not the only neurotransmitter system that is affected in this multifactorial disease. An intriguing example of how different neurotransmitter systems might interact has been recently proposed by González-Maeso and colleagues (González-Maeso et al., 2008). They provide evidence that activation of $mGluR_2$, and the subsequent formation of functional complexes of $mGluR_2$ and 5-HT_{2A} receptors, precludes the hallucinogenic effects of 5-HT_{2A} receptor agonists. Interestingly, in the light of this possible interaction, a prodrug for an $mGluR_{2/3}$ selective agonist has been shown to display significant antipsychotic efficacy.

1.3 Inhibitory interneurons of the hippocampus

Neurons of the hippocampus can be divided into two major classes: principal cells, projecting predominantly from one hippocampal subfield to another and interneurons, mainly restricted to hippocampal subfields and therefore thought to be local-circuit neurons. Interneurons make up only about 10% of the total number of neurons in the hippocampus. Although less in number, interneurons show an overwhelming diversity in respect to their morphology and their functional properties (for review see Freund and Buzsáki, 1996; Soltesz, 2005). One unifying property of interneurons is the use of GABA as neurotransmitter, rendering their function inhibitory in the adult brain. A consequence of the morphology of interneurons (local-circuit) and their use of GABA (with mostly inhibitory effects) is the build-up of local circuits of neurons with distinct excitatory and inhibitory input and output patterns. In the hippocampus, principal neurons predominantly give rise to an unidirectional excitatory

pathway. In the target zone of this pathway, the afferent excitatory signal will be concurred by a delayed feedforward inhibitory signal, generated by local inhibitory interneurons, receiving the same excitatory input. „Feedforward inhibition serves to impose a temporal framework on a target area on the basis of inputs received“ (Buhl and Whittington in Andersen et al., 2006), as further excitatory drive onto the target region will be diminished by the feedforward loop of the local circuit. Correspondingly, the excitatory output pattern of the local circuit will be shaped by local inhibitory interneurons receiving strong recurrent excitation and in turn target “back“ onto local principal cells. The excitatory signal onto efferent target regions will be followed by a marked local feedback inhibition. These local circuits are further characterized by a huge divergence of the axons of numerous inhibitory interneurons present; i.e. a given inhibitory interneuron has many target cells, and vice versa a given principal cell receives a large number of convergent inhibitory inputs from different interneurons.

The input and output patterns of inhibitory interneurons and subsequently the pattern of the local circuits depend on the afferents interneurons receive, on their intrinsic properties and on their efferent target regions. Given the huge diversity of hippocampal interneurons described and taking into account, that “it has, as yet, proved impossible to find a set of physiological parameters that allow a meaningful classification scheme of the hippocampal interneurons to be established“ (Buhl and Whittington in Andersen et al., 2006), the subsequent description of hippocampal interneurons will follow with respect to their relative dendritic and axonal distribution as compared with the different laminae of the hippocampus and is based on the description of Freund and Buzsáki from 1996. This implies a segregation of interneurons in perisomatic vs. dendritic inhibitory interneurons. A third subgroup of interneurons that innervate primarily or exclusively other interneurons will be mentioned briefly. The focus will be on inhibitory interneurons in hippocampal area CA1.

1.3.1 Perisomatic inhibitory interneurons

Chandelier cells or axo-axonic cells, first described by Szentágothai and Arbib in 1974, are inhibitory interneurons with their somata lying in or close by the pyramidal cell layer of area CA1. The dendritic arborization spans over all layers, endowing them with inputs from all the major excitatory pathways. Their axons form a dense arbor in the pyramidal cell layer and at the border of stratum pyramidale and stratum oriens, corresponding to the very specialized termination zone of the axon: the axon initial segment of CA1 pyramidal cells. Axo-axonic

cells express the Ca^{2+} binding protein parvalbumin (PV). There has been a controversy regarding the effect of GABA released from axo-axonic terminals onto pyramidal cells, where depolarizing as well as hyperpolarizing events have been described (Szabadics et al., 2006; Glickfeld et al., 2009). The other, more abundant perisomatic inhibitory cells are basket cells. The somata of basket cells are found in stratum pyramidale and in the adjacent regions of stratum oriens and stratum radiatum. Like axo-axonic cells, the dendrites of basket cells span all layers of area CA1. They receive input from the Schaffer-collaterals, from enthorinal and commissural afferents as well as from recurrent collaterals of local pyramidal cells and therefore are activated in a feedforward and feedback manner. The axonal arborization is predominantly if not exclusively restricted to the pyramidal cell layer, where the axons impinge on the soma and the perisomatic region (region approx. ≤50μm from the soma) of CA1 pyramidal cells. Basket cells can be further subdivided in cells that express PV, have a low input resistance and a characteristic fast spiking, non-adapting spike pattern upon membrane depolarization and in cells that express the neuropeptide CCK, have a higher input resistance and a regular, adapting spike pattern. Despite their similar morphology, PV-positive and CCK-positive show marked differences not only in their intrinsic properties, but also in a different endowment of extrinsic afferents and different expression pattern of various receptors. Whereas it has been shown that CCK-positive basket cells receive serotonergic input from the median raphe nucleus via classical chemical synapses and additionally are equipped with ionotropic 5-HT_3 receptor, PV-positive basket cells are devoid of this specific serotonergic neuromodulatory input (Freund, 2003). Furthermore, the axon terminals of both basket cell types show differences in the coupling of the Ca^{2+} sensor and the release machinery resulting in a different timing of action potential triggered release. The PV-positive basket cell - pyramidal synapse shows synchronous release characteristics, whereas the CCK-positive basket cell - pyramidal synapse displays asynchronous release (Hefft and Jonas, 2005; Daw et al., 2009). Even the postsynaptic pyramidal neurons show a segregation of the GABA-A receptor subunit composition. The axon terminals of PV-positive basket cells match with GABA-A receptors, containing the $\alpha 1$ subunit, the axon terminals of CCK-positive basket cells match with $\alpha 2$ containing GABA-A receptors (Freund, 2003). Recently, it has been suggested that perisomatic inhibitory cells display a functional dichotomy: while PV-positive basket cells provide a highly reliable clockwork that generates network oscillations, CCK-positive basket cells might serve as modulators that adapt network activity to behavioral states (Freund and Katona, 2007).

1.3.2 Dendritic inhibitory interneurons

Oriens - lacunosum moleculare (O-LM) interneurons are interneurons that have their axonal termination zone in conjunction with the inputs from layer III of the enthorinal cortex in stratum lacunosum moleculare. The fusiform somata of O-LM interneurons lie in stratum oriens of hippocampal area CA1 and give rise to dendrites that travel parallel to the alveus, where they receive excitatory input predominantly from recurrent CA1 pyramidal cell axons. O-LM interneurons can therefore be considered as feedback inhibitory interneurons (Blasco-Ibáñez and Freund, 1995). The axon is ascending directly from the soma or a dendrite to stratum lacunosum moleculare, where it arborizes and gives rise to a dense cloud of collaterals. O-LM cells can, depending on intensity and frequency of the activation stimulus, generate action potentials in the dendrite or the axon (Martina et al., 2000). Experimental and theoretical work has demonstrated the importance of O-LM cells in synchronizing activity by means of their large longitudinal arborization and their preferred firing at hippocampal theta frequency (Gloveli et al., 2005; Tort et al., 2007). Other features of O-LM neurons are the expression of somatostatin and a prominent hyperpolarization-activated conductance, I_h, that is important for their oscillatory behavior (Griguoli et al., 2010).

Bistratified and trilaminar cells are interneurons innervating the dendrites of pyramidal cells in stratum oriens and in stratum radiatum (for review see Freund and Buzsáki, 1996). The somata of both cell types lie within or adjacent to the pyramidal cell layer and at the border of stratum oriens and alveus. Both cell types have predominantly radially oriented dendrites, and the vertical dendrites of bistratified cells avoid stratum lacunosum moleculare. The dendrites of the horizontal trilaminar cell are confined to stratum oriens. The axons of trilaminar cells can also be found in stratum pyramidale, whereas the termination zone of bistratified cells is restricted to the dendrites of pyramidal cells in strata oriens and radiatum, in this respect being located complementary to basket cells. Bistratified cells express the calcium-binding proteins parvalbumin, somatostatin and neuropeptide Y (NPY). Trilaminar cells show an intense immunoreactivity of the muscarinic receptor M_2. The laminar distribution of bistratified cells is in conjunction with the Schaffer-collaterals and with the associational-commissural input. As recurrent collaterals of CA1 pyramidal cells are known to innervate local interneurons, it can be assumed that bistratified cells are driven in a feedforward and feedback manner, whereas trilaminar cells are most likely driven in a feedback manner.

Schaffer-collateral associated interneurons are interneurons with axon and dendrites restricted to stratum radiatum and to a minor extend to stratum oriens (Vida et al., 1998). They have their somata in distal stratum radiatum and at the border to stratum lacunosum moleculare. Their axons impinge on the proximal and distal apical dendrites of CA1 pyramidal neurons and to a lesser extend on basal dendrites in stratum oriens. The dendrites of Schaffer – collateral associated interneurons are restricted to strata radiatum and oriens as well. These cells receive input from Schaffer-collaterals, from associational-commissural fibers and from recurrent CA1 pyramidal cell axons. Thus they are driven in a feedforward and in a feedback manner. Immunohistochemical examinations revealed that Schaffer-collateral associated interneurons are immunopositive for CCK, yet some for Calbindin as well.

Neurogliaform cells, that express NPY, have a characteristic dense axonal cloud impinging on dendrites and spines of CA1 pyramidal cells. The dendritic fields of these cells are relatively small and their somata can be found in stratum lacunosum moleculare, in conjunction with afferent projections from the enthorinal cortex. Neurogliaform cells evoke slow $GABA_A$ and $GABA_B$ receptor mediated IPSPs in their target cell (Tamás et al., 2003; Capogna and Pearce, 2011).

Lacunosum-moleculare (-radiatum) perforant path-associated cells. These interneurons are immunopositive for CCK and Calbindin. Their somata lie in distal radiatum near stratum lacunosum moleculare. The axons of lacunosum-moleculare-radiatum perforant path-associated cells are mainly associated with the perforant path input from the enthorinal cortex, but have, unlike the lacunosum-moleculare perforant path-associated cells, a significant spillover to stratum radiatum and even to the dentate gyrus. Dendrites of the lacunosum-moleculare-radiatum perforant path-associated cells are spanning several layers, from stratum lacunosum moleculare to stratum oriens (for review see Soltesz, 2005).

There are very few examples of back-projection cells (Sik et al., 1994; 1995). These interneurons have their somata in stratum oriens, the dendrites reside in the same layer and the axon innervates area CA1 of the hippocampus and also projects back to CA3 and the hilus.

1.3.3 Interneurons specialized to innervate other interneurons

There are four groups of interneurons innervating other interneurons. Firstly, the hippocampo-septal cells, predominantly projecting to interneurons in the septum. Secondly the group of interneuron-specific interneurons, type I-III. They express calretinin (type I), vasoactive intestinal polypeptide (VIP; type II) or both of the immunocytochemical markers (type III). Their axons innervate other calretinin positive interneurons (type I), VIP/ CCK-positive basket cells (type II) and O-LM cells (type III). The dendrites of these cells mostly span all layers, except those of type II, that are mainly restricted to stratum lacunosum moleculare (for review see Soltesz, 2005).

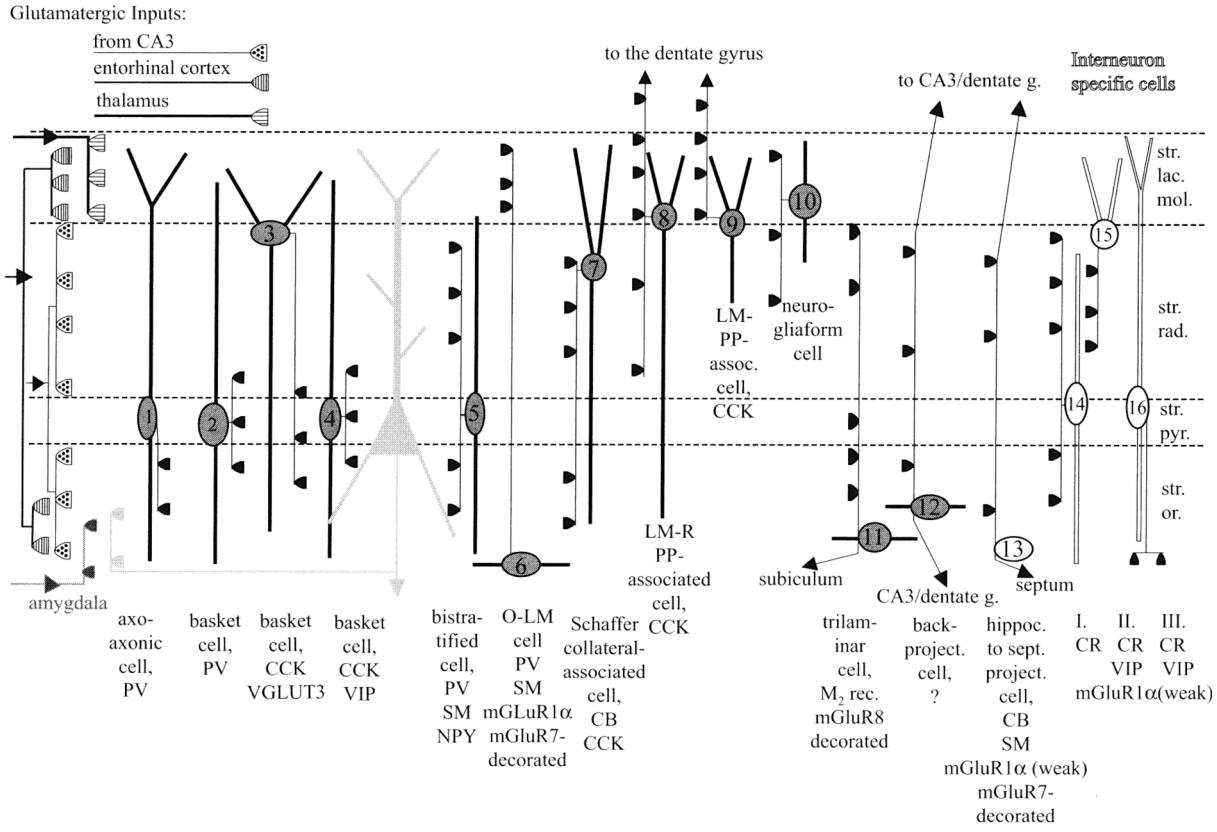

Fig. 1.3. Innervation of pyramidal cells by different GABAergic interneurons and interneurons by different interneuron-specific cells in area CA1 of the hippocampus.

Adapted from Soltesz, 2005. CB: calbindin, CR: calretinin, CCK: cholecystokinin, LM-PP: lacunosum-moleculare perforant path, LM-R-PP: lacunosum-moleculare radiatum perforant path, mGluR1α/7/8: metabotropic glutamate receptor 1α/7/8, M_2: muscarinic receptor type 2, NPY: neuropeptide Y, PV: parvalbumin, SM: somatostatin, VGLUT3: vesicular transporter 3.

1.3.4 Perisomatic vs. dendritic inhibition

The conjunction of distinct axonal projections from different interneurons and distinct membrane compartments of pyramidal cells innervated by these interneurons suggests different functional roles for perisomatic and dendritic inhibitory interneurons. An important step to show differences between these two forms of inhibition was the study by Miles and colleagues from 1996 (Miles et al., 1996). They found that IPSPs initiated by perisomatic inhibitory cells were able to suppress the repetitive generation of Na^+-dependent action potentials and therefore control the output and synchronize the action potential firing of their target cells. In contrast, IPSPs initiated by dendritic inhibitory cells were more effective than somatic IPSPs in suppressing dendritically generated Ca^{2+}spikes.

CA1 pyramidal cells have a narrow time window for spike generation, i.e. two inputs have to be closely timed (≤2ms) to reach the threshold for spike generation. It has been shown that this precise coincidence detection in CA1 pyramidal cells results from the delayed disynaptic activation of inhibitory interneurons, as the integration time window increases dramatically if inhibition is blocked. Furthermore, simultaneous recordings of somata and dendrites revealed that the inhibition, responsible for the narrow time window for spike generation is most likely provided by perisomatically targeting interneurons, whereas dendritic inhibition allows the integration of inputs over broader time windows (Pouille and Scanziani, 2001).

1.3.5 Interneurons and their role in synchronization

Neuronal oscillatory activity is a key feature of cortical networks and occurs in multiple brain regions, including the olfactory bulb, the thalamus, the hippocampus and the neocortex. These oscillations span a broad range of different frequency bands from slow oscillations in the delta (0.5-3 Hz) and theta (3-8 Hz) range to fast oscillations in the gamma (30-90 Hz) and ultrafast (90-200 Hz) range. In the rodent hippocampus, different oscillations have been correlated to different behavioral states: oscillations in the theta range, typically coexisting with gamma oscillations, occur during exploratory behavior and REM sleep, while ultrafast oscillations, so called sharp waves ripples, occur during wakeful rest and non-REM sleep. Gamma oscillations have been associated with higher brain functions, for example with attentional selection (Fries et al., 2001), working memory (Howard et al., 2003) as well as binding of features of an object in the sensorium into a coherent aspect (Gray and Singer, 1989). In the following, the focus will lie on gamma oscillatory activity in the hippocampus. Gamma

oscillations in the hippocampus have first been described as „fast activity“ or Small-amplitude irregular activity (SIA), in contrast to Large-amplitude irregular activity (LIA), where sharp waves and ripples do occur (Stumpf, 1965; Vanderwolf, 1969; Buzsáki et al., 1983). Hippocampal gamma oscillatory activity has been proposed to be associated with the storage and recall of information (Lisman and Idiart, 1995; Lisman, 1999) and has attracted research not least because of the relatively high power of extracellularly recorded gamma, which is due to the simple, three-layered structure of the hippocampus proper and the dentate gyrus. A further argument for studying gamma oscillations in the hippocampus rises from the possibility to induce synchronized gamma oscillations in acute hippocampal slices *in vitro*, either through electrical stimuli or application of chemical agonists. As a consequence, in the past decade research in this particular field has yielded a deeper insight into the synaptic mechanisms underlying synchronized gamma oscillations and the different cell types participating in this network activity (Bartos et al., 2007; Whittington et al., 2011).

Although the properties of the different *in vitro* gamma models differ considerably, they all rely on inhibition (for review see Whittington et al., 2011). The marked divergence of the axonal projections of GABAergic interneurons thereby provides the framework for the entrainment of synchronized inhibitory postsynaptic potentials in a large number of pyramidal neurons, no matter if the interneurons are activated directly (e.g. by mGluR activation) or indirectly (by phasic excitation provided by pyramidal cells). Early on it has been shown that even in the most simple *in vitro* gamma model, the interneuron network gamma, perisomatic targeting, fast spiking, PV-positive basket cells play a pivotal role in the generation of gamma rhythms (Whittington et al., 1995; Traub et al., 1996). In many respects, the properties of this specific cell type argue for this prominent position. Fast spiking, PV-positive basket cells are highly active during gamma and fire phase locked to the gamma cycle (Tukker et al., 2007). They are mutually interconnected and provide a highly divergent yet coherent synaptic output to pyramidal cells. Additionally, it has been shown that optogenetic activation of PV-positive interneurons in the neocortex increases cortical gamma activity (Cardin et al., 2009). In summary, it is now commonly accepted that inhibition provided by this type of interneuron is essential for the generation of gamma rhythmic activity in multiple brain regions, *in vivo* and *in vitro* (Bartos et al., 2007; Whittington et al., 2011). Nevertheless, the possible contribution of other interneuron subtypes to the generation or modulation of gamma rhythmic activity is a matter of ongoing research and there are examples where other types of interneurons also

participate in gamma activity (Gloveli et al., 2005). Notably, for CCK-positive basket cells a clear picture of their contribution to gamma rhythmic activity has not yet emerged (Hájos et al., 2000; Tukker et al., 2007; Holderith et al., 2011).

Aims of this work

Our goal was to characterize the role of serotonin in shaping the strong excitatory innervation of interneurons in hippocampal area CA1. As will be presented in the results section, we began our investigation by comparing the modulation of evoked glutamatergic transmission by serotonin in CA1 pyramidal neurons with different types of interneurons. We found evidence that serotonin does indeed affect the excitatory innervation of interneurons, although the observed reduction of glutamatergic transmission seemed to be restricted to an interneuron subpopulation. To test this hypothesis we established a set of experiments to further characterize those cells that were sensitive to serotonin application on the basis of their intrinsic properties, their anatomy and immunohistochemical markers.

We further wanted to know by which receptor subtype this effect of serotonin was mediated and whether this receptor mediates the reduction of excitation on the presynaptic or the postsynaptic side of the glutamatergic synapse. To address these questions we used multiple receptor subtype specific agonists and antagonists, as well as several different experimental protocols and evaluation techniques to characterize the effects of serotonin on short-term plasticity, failure rate, on the coefficient of variation and on photolytically activated glutamate.

After characterizing the cell type that was affected and after determining the location of the responsible serotonin receptor subtype we analyzed the functional consequences of this surprisingly specific neuromodulation. We asked whether the modulation of this defined type of local inhibitory interneuron is able to affect the output element and thereby the output of the microcircuit under investigation. Thus we characterized the effect of serotonin on disynaptic inhibition in CA1 pyramidal neurons by determining the consequences of serotonin on the integration time for spike generation of CA1 pyramidal neurons. To address the question whether this serotonergic neuromodulation may affect network activity as well, in a last set of experiments we studied gamma oscillatory activity *in vitro* and *in vivo*.

2 Materials and experimental methods

All experiments including animals were performed according to the regulations of Berlin animal experiment authorities and the animal welfare committee of the Charité Berlin (File reference: T 0100/03, T 0073/04 and T 0124/05).

2.1 Electrophysiology

2.1.1 Hippocampal slice preparation

Hippocampal slices were prepared from Wistar rats and C57/Bl6 mice (P16-24; both sexes). The animals were anesthetized with isoflurane and decapitated. To remove the brains, first the scalp was cut form the neck to the nose. Afterwards, by cutting along the median line of the suttura sagittalis, the skullcap was opened. After careful removal of the dura mater with a forceps, the two pieces of the divided skullcap were bent aside to achieve access to the brains. They were then removed with a small spoon and stored for 5 minutes in a beaker filled with ice-cold artificial cerebrospinal fluid, ACSF, containing (in mM): NaCl, 87; sucrose, 75; $NaHCO_3$, 26; KCl, 2.5; NaH_2PO_4, 1.25; $CaCl_2$, 0.5; $MgCl_2$, 7; glucose, 25, saturated with 95 % O_2, 5% CO_2, pH 7.4. Tissue blocks containing the subicular area and hippocampus were cut and mounted on a Vibratome (Leica VT1200) in a chamber filled with the same ice-cold ACSF. Transverse slices were cut at 300-350µm thickness and were kept at 35^{o}C for 30 minutes. Slices were then cooled to room temperature and transferred to ACSF containing (in mM): NaCl, 119; $NaHCO_3$, 26; glucose, 10; KCl 2.5, $CaCl_2$, 2.5; $MgCl_2$ 1.3; NaH_2PO_4, 1. All ACSF was equilibrated with 95% O_2 and 5% CO_2. The slices were stored in a submerged chamber, where they were kept for 1-7 hours before being transferred to the recording chamber. In the recording chamber slices were perfused with ACSF at a rate of 3-4ml/min at room temperature.

2.1.2 Whole cell patch clamp experiments in hippocampal slices

Whole cell recording electrodes were filled with (in mM): K-gluconate 135, Hepes 10, Mg-ATP 2, KCl 20, EGTA 0.2, Phosphocreatine 5, or for EPSP-IPSP sequences: K-gluconate 150, Hepes 5, $MgCl_2$ 0.5, EGTA 1.1, Phosphocreatine 10; pH was adjusted to 7.3 with KOH. For staining and reconstruction of the recorded neurons, 0.2% biocytin was added to the intracellular solution. Excitatory postsynaptic responses were evoked by electrical stimulation (100μs at 0.1Hz) in stratum radiatum of area CA1 via a broken patch-pipette (~15 μm) filled with ACSF, in some experiments by alveus stimulation. Minimal stimulation was defined as follows: after establishing a synaptic input, the stimulus intensity was decreased until no synaptic response could be detected anymore, then the stimulus intensity was increased again slowly, until EPSCs of stable amplitudes and synaptic failures in an alternating manner appeared. For the minimal stimulation experiments the average stimulation strength was 0.012 ± 0.002mA. Experiments were done in the presence of the $GABA_A$ receptor-antagonist gabazine (1μM) and 100nM NBQX to prevent epileptiform activity and to minimize polysynaptic activity except where EPSP-IPSP sequences and monosynaptic IPSCs were measured. EPSP-IPSP sequences were performed in the presence of 25μM D-AP5 and 10μM SCH 50911, monosynaptic IPSCs were performed in the presence of 25μM D-AP5 and 10μM NBQX. Access resistances ranged between 9 and 28MΩ for interneurons and between 6 and 20MΩ for pyramidal cells. They were continuously monitored during the recording and were not allowed to vary more than 25% during the course of the experiment. No series resistance compensation was used. Electrode resistances ranged from 2 to 5MΩ.

The intrinsic properties of cells were measured in whole-cell current-clamp mode. The resting membrane potential was estimated during the absence of a holding current, as recorded cells did not fire action potentials at rest. The input resistance was recorded in whole-cell voltage-clamp mode. Only those recordings were included in the statistical analysis for input resistance measurements where the input resistance did not vary more than 10% during the baseline recording before drug application.

Serotonin-sensitivity of interneurons was determined by the reduction of the EPSC amplitude after application of 10μM 5-HT. If the reduction of the EPSC amplitude ≥ 30% of the baseline response and if this reduction was reversible, interneurons were considered to be 5-

HT sensitive. At lower concentrations (0.1µM and 0.3µM) a successive application of higher concentrations or, after washout of 5-HT, application of the 5-HT_{1B} agonist defined the 5-HT sensitivity.

2.1.3 Identification of interneurons

The identification of the CCK-positive interneurons and the PV-positive basket cells was based on morphology and immunohistochemistry (see below: *Immunohistochemistry and neuroanatomy of interneurons*). However, it is possible to selectively record from these distinct populations with a sufficient degree of confidence even before post-hoc identification based on the position and shape of the cell body in stratum radiatum and pyramidale, the morphology of the dendrites visible in the IR-DIC and the electrophysiological characteristics in firing properties and input resistances (Neu et al., 2006; Földy et al., 2007). To characterize the discharge behavior of the cells, depolarizing current steps of 1000ms duration were applied. Fast spiking interneurons displayed firing frequencies of 50Hz - 120.5Hz at room temperature (mean firing frequency: 75.4 ± 5.0Hz), while CCK-positive interneurons had a mean firing frequency of 11.1 ± 1.0Hz. Yet the maximum firing frequency also depends on temperature and on the amount of current injected. The mean input resistance of fast spiking interneurons was 165.2 ± 11.3MΩ, the mean input resistance of CCK-positive interneurons was 302.8 ± 17.6MΩ. CCK-positive interneurons were selected mainly in stratum radiatum and at the border of stratum radiatum/stratum pyramidale. PV-positive interneurons were mainly selected at the border of stratum radiatum/stratum pyramidale and in stratum pyramidale.

2.1.4 Local field potentials

2.1.4.1 *In vivo* experiments

Mice were stereotaxically implanted under general anesthesia (350mg/kg chloralhydrate i.p.) with monopolar stainless steel wire electrodes soldered on a male connector (Plastics One, Roanoke, VA). Field potential recordings were done in the stratum radiatum of the CA1 region of the dorsal hippocampus (AP, -2.0mm; ML, -1.8mm; DV, -1.5mm). EEG recordings were collected in freely moving mice during spontaneous exploration in the home cage. Recordings were low-pass filtered at 1kHz with a custom-made Bessel filter, digitized at 2kHz by using an ITC-16 A/D board (Instrutech, Mineola, NY), and analyzed using WinTida

software (Heka, Lambrecht, Germany). The selective 5-HT_{1B} receptor agonist CP94253 (10mg/kg) and saline were i.p. injected and the corresponding EEG was recorded 4h after injection. The power spectra were calculated by Fourier transformation.

2.1.4.2 *In vitro* experiments

Transverse slices (400µm thickness) were obtained from the hippocampus of C57/Bl6 adult mice. The slices were transferred into an interface chamber, where they were continuously superfused with pre-warmed (34±1°C) ACSF containing (in mM): NaCl, 129; KCl, 3; NaH_2PO_4, 1.25; $CaCl_2$, 1.6; $MgSO_4$, 1.8; $NaHCO_3$, 21; glucose, 10, and saturated with 95% O_2 and 5% CO_2. Extracellular recordings were obtained from stratum radiatum of areas CA1 (intact or mini slices) and CA3 (mini slices) with ACSF-filled glass pipettes. Kainate (200nM intact slices and 400nM mini slices) was bath-applied to obtain network oscillations. Field potentials were low pass filtered at 1kHz with a custom-made Bessel filter, digitized using a Digidata 1322A and recorded using pClamp software (Axon Instruments, USA).

2.1.5 Glutamate uncaging

20ml of 200µM MNI-caged-L-glutamate (Tocris, Bristol, UK) were reperfused at 2.5 – 3.0ml/min. Uncaging was done using a UV pulsed laser (Rapp Optoelektronik, Wedel, Germany) attached with a 200µm optical fiber coupled into the epifluorescence port of the microscope with an OSI-BX adapter (Rapp Optoelektronik, Wedel, Germany) and focused on the specimen by the objective lens. This yielded an illuminated circle of 20-50µm. The duration of the laser flash was 5ms. The laser power under the objective corresponding to the stimulus intensity levels used was monitored using a photo diode array-based photodetector (PDA-K-60, Rapp Optoelectronics, Wedel, Germany) and did not change over time. Glutamate was uncaged over the cell soma in the presence of the $GABA_A$ receptor-antagonist gabazine (1µM) and 100nM NBQX. For the experiments in which both somatic and synaptic currents were recorded, EPSCs were evoked with a stimulation electrode in stratum radiatum, thus evoking a synaptic response and, after 500ms, a laser pulse was flashed to uncage MNI-glutamate, evoking a somatic current.

For the determination of the input specificity of the 5-HT effect, a grid of stimulation points with a raster size of 30µm was defined over areas CA3 and area CA1 of the hippocampus. After finding an input, repetitive uncaging was done at that specific location (for further

details of the procedure see Bendels et al., 2008). EPSCs elicited by photostimulation of single presynaptic neurons were Gaussian-filtered and aligned to the peak of the response for subsequent averaging and quantification of the responses before and after application of CP93129.

2.2 Immunohistochemistry and neuroanatomy of interneurons

After recording, slices were transferred into a fixative solution containing 4% paraformaldehyde and 0.2% saturated picric acid in 0.1M phosphate buffer. Slices were resectioned into 70µm thin sections. Immunoreactivity for CCK was revealed with a mouse monoclonal antibody (mAB 9303, generously provided by the CURE Digestive Disease research Center, Antibody RIA Core, Los Angeles (NIH grant #DK41301); diluted 1:1000 in Tris-buffered saline containing 2% normal goat serum, TBSS; immunoreactivity for PV was tested with a rabbit polyclonal antibody (PV-28; Swant, Bellinzona, Switzerland; diluted 1:1000 in TBSS). The reactions were visualized with a goat anti-rabbit IgG conjugated to Alexa 488 (diluted 1:500 in TBSS; Molecular Probes) and a goat anti-mouse IgG conjugated to Alexa 594 (diluted 1:500 in TBSS), streptavidin conjugated to Alexa-350 for biocytin (diluted 1:500 in TBSS). The sections were then mounted in Vectashield (Vector Laboratories) and analyzed with a fluorescence microscope. In order to reveal the presynaptic axonal arborization and dendritic arbors in detail, the biocytin-filled cells were subsequently visualized with 3,3'-diaminobenzidine tetrahydrochloride (0.015%) using standard ABC kit (Vector) and reconstructed with the aid of a Neurolucida 3D reconstruction system (MicroBrightField, Inc., Williston, VT, USA).

2.3 Material

Vibratome	VT 1200 Leica, Wetzlar, Germany
Amplifier and digitizer	Multiclamp 700 A or Multiclamp 700B, Molecular Devices, Toronto, Canada
Digitizer	BNC 2090, National Instruments, Austin, Texas, USA
Extracellular stimulation unit	Iso Flex, A.M.P.I, Jerusalem, Israel
Stimulus generator	Master 8, A.M.P.I, Jerusalem, Israel

Oscilloscope	HG-1507-3, HAMEG Instruments, Mainhausen, Germany
Glass electrode puller	DMZ universal puller, Zeitz-Instrumente, Munich, Germany
Glass pipettes	Science products, Hofheim, Germany
Ag/AgCl electrode	Science products, Hofheim, Germany
Bath electrode	Science products, Hofheim, Germany
Upright microscopes	Olympus BX-51WI, equipped with Differential Interference Contrast (DIC) optics and video microscopy
	Olympus LumPlan FI 60x 0.9NA water immersion
	Olympus UPlanFL N 4X×0.13 PhP
	Olympus BX61 TRF, configured for NeuroLucida transmitted light brightfield/ fluorescence
	Mirror unit U-MWU2 with excitation filter 330-350
	Mirror unit U-MWIB2 with excitation filter 460-490
	Mirror unit U-MWIG2 with excitation filter 520-550
Micromanipulators	Mini 25, 3 axes, Luigs & Neuman, Ratingen, Germany
488 nm solid state laser	Sapphire 488 nm 50 mW Laser; Coherent, Utrecht, Netherlands
Plastic syringes	B. Braun, Melsungen, Germany
Perfusion tubing	Carl Roth, Karlsruhe, Germany
Software:	
IGOR Pro 4.0	WaveMetrics Inc., OR, USA
pClamp 9.1	Molecular Devices, Toronto, Canada
Morgentau M1	Morgentau Solutions GmbH, München, Germany

2.3.1 Solutions for slice preparations and electrophysiological recordings

All standard salts were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Carl Roth (Karlsruhe, Germany).

Slicing solution

87mM NaCl
26mM $NaHCO_3$
75mM Sucrose
25mM Glucose
2.5mM KCl
1.25mM NaH_2PO_4
0.5mM $CaCl_2$
7mM $MgCl_2$
saturated with 95% O_2–5% CO_2
350mOsm

Extracellular ("Ringer") solution for slice recordings

119mM NaCl
26mM $NaHCO_3$
10mM Glucose
2.5mM KCl
1mM NaH_2PO_4
2.5mM $CaCl_2$
1.3mM $MgCl_2$
saturated with 95% O_2–5% CO_2
300mOsm

Intracellular solution I

135mM K-gluconate
20mM KCl
2mM MgATP
10mM Hepes
0.2mM EGTA
5mM phosphocreatine
pH adjusted to 7.3 with KOH
300mOsm

Intracellular solution II

150mM K-gluconate
0.5mM KCl
5mM Hepes
1.2mM EGTA
10mM phosphocreatine
pH adjusted to 7.3 with KOH
300mOsm

Table 1: Drugs used for pharmacological manipulations

Drug, Company	Chemical name	Biological activity	Stock, solvent	Max. conc.
5-HT, Sigma	5-hydroxytryptamine creatine sulfate complex	Monoamine neurotransmitter	10mM, H_2O	30µM
8-OH-DPAT, Tocris	(±)-7-hydroxy-2-dipropylaminotetralin hydrobromide	Selective 5-$HT_{1A/7}$ receptor agonist	10mM, H_2O	1µM
CP93129, Tocris	1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5H pyrrol [3,2-b] pyridin-5-one dihydrochloride	Selective 5-HT_{1B} receptor agonist	10mM, H_2O	1µM

CP94253, Tocris	5-propoxy-3-(1,2,3,6-tetrahydro-4-p yridinyl)-1*H*-pyrrolo[3,2-*b*]pyridine hydrochloride	Selective 5-HT_{1B} receptor agonist	10mM, H_2O	10mg/kg
D-AP-5, Tocris	D-(-)-2-amino-5-phosphonopentanoic acid	Competitive NMDA receptor antagonist	50mM, H_2O	25µM
Fenfluramine, Sigma	(+)-N-ethyl-α-methyl-*m*-[trifluoromethyl]phenethyl amine hydrochloride	Serotonin releaser	200mM, H_2O	200µM
Gabazine (SR 95531 hydrobromide), Tocris	6-imino-3-(4-methoxy-phenyl)-1(6H)-pyri-dazinebutanoic acid hydrobromide	Competitive $GABA_AR$ antagonist	20mM, H_2O	1µM
GR127935, Tocris	*N*-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2-methyl-4-(5-methyl-1,2,4-oxadiazol-3-yl)-1–1-biphenyl-4-carboxamide	Selective 5-HT_{1B} receptor antagonist	10mM, H_2O	10µM
Kainate,	(2*S*,3*S*,4*S*)-carboxy-4-(1-methylethenyl)-3-pyrrolidineacetic acid	Selective agonist at kainate receptors	1mM, H_2O	400nM
(MNI)-caged-L-glutamate, Tocris	(*S*)-α-amino-2,3-dihydro-4-methoxy-7-nitro-δ-oxo-1*H*-indole-1-pentanoic acid	Photolytically activated AMPA receptor agonist	50mM, H_2O	200µM
NBQX, Tocris	2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxa line -7-sulfonamide disodium salt	Competitive AMPA receptor antagonist	25mM, H_2O	10µM
SCH50911, Tocris	[(2S)-(+)-5,5-dimethyl-2-morpholineacetic acid	Competitive $GABA_BR$ antagonist	10mM, H_2O	10µM

2.4 Data analysis and statistics

Average values in the text and figures are expressed as means ± s.e.m.. The Student's *t*-test was used for statistical comparisons, differences were considered statistically significant if $p<0.05$. All example traces in the figures are averages of 10-30 sweeps unless otherwise stated. All points in the time course plots are individual EPSCs from individual recordings.

3 Results

3.1 Serotonin differentially modulates excitatory transmission

First, we established whole-cell voltage-clamp recordings from pyramidal cells (n=7) within area CA1 of the hippocampus and studied the role of serotonin (5-hydroxytryptamine, 5-HT) on glutamatergic transmission onto these cells (**Fig.3.1A**).

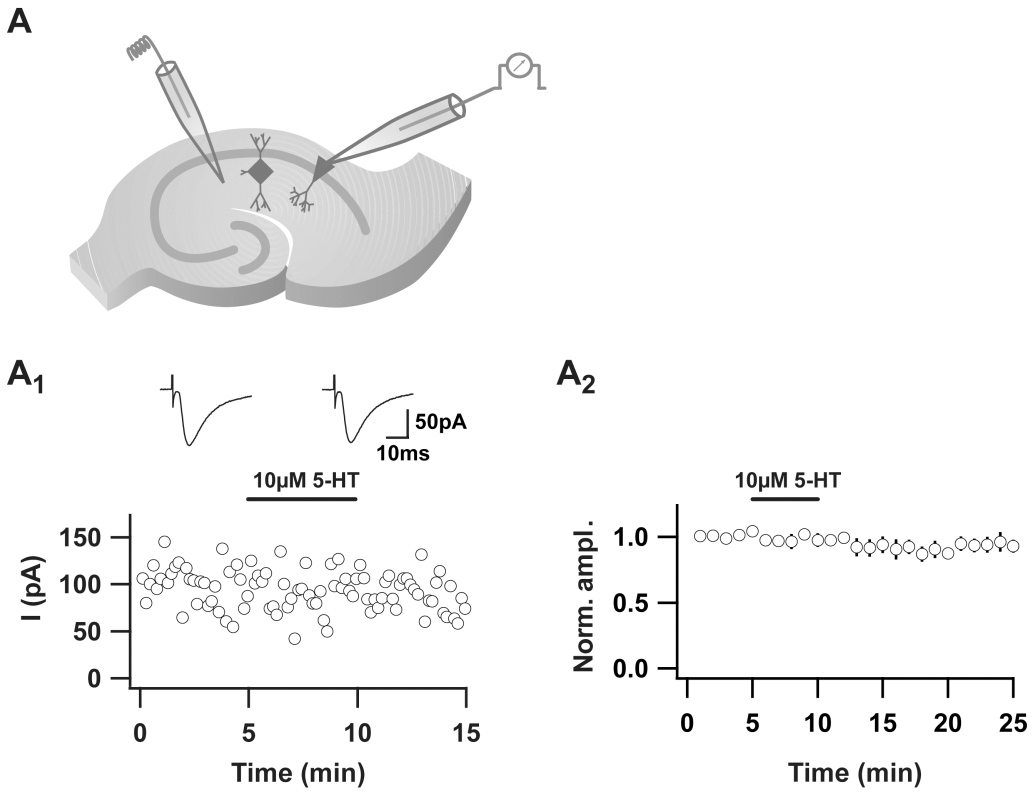

Fig.3.1A. Modulation of glutamatergic transmission onto pyramidal cells by 5-HT. ($V_{holding}$=-60 mV). (A) Schematic of the recording configuration. (A1) Top, example traces of EPSCs in a CA1 pyramidal cell evoked by stimulation in stratum radiatum before and in 10µM 5-HT. Bottom, time course of the measured EPSCs in the same cell (Scale bar: 50pA; 10ms). (A2) Summary of the time course of normalized and binned (1min) EPSC amplitudes for pyramidal cell recordings (n=7). Black bar indicates the application of 10µM 5-HT.

The results presented in this Chapter are based on the manuscript *Cell-type-specific modulation of feedback inhibition by serotonin in the hippocampus* by Winterer J, Stempel AV, Dugladze T, Földy C, Maziashvili N, Zivkovic AR, Priller J, Soltesz I, Gloveli T, Schmitz D (2011) J. Neurosci. 31: 8464–8475.

We found that stimulus-induced excitatory postsynaptic currents (EPSCs) elicited by electrical stimulation in stratum radiatum remained unaltered upon bath application of 10µM 5-HT when recorded from CA1 pyramidal neurons (**Fig. 3.1A$_1$; 3.1A$_2$**). Likewise we obtained whole-cell voltage-clamp recordings from different types of interneurons (n=86) located in stratum radiatum and stratum pyramidale of hippocampal area CA1. In a subset of interneurons (34.9%) located in both strata, stimulus-induced EPSCs elicited by electrical stimulation in stratum radiatum were not affected by application of serotonin (see below). In sharp contrast, 5-HT effectively and reversibly inhibited glutamatergic transmission in a second subset of interneurons (65.1%) within the same hippocampal area (**Fig. 3.1B$_1$; 3.1B$_2$**).

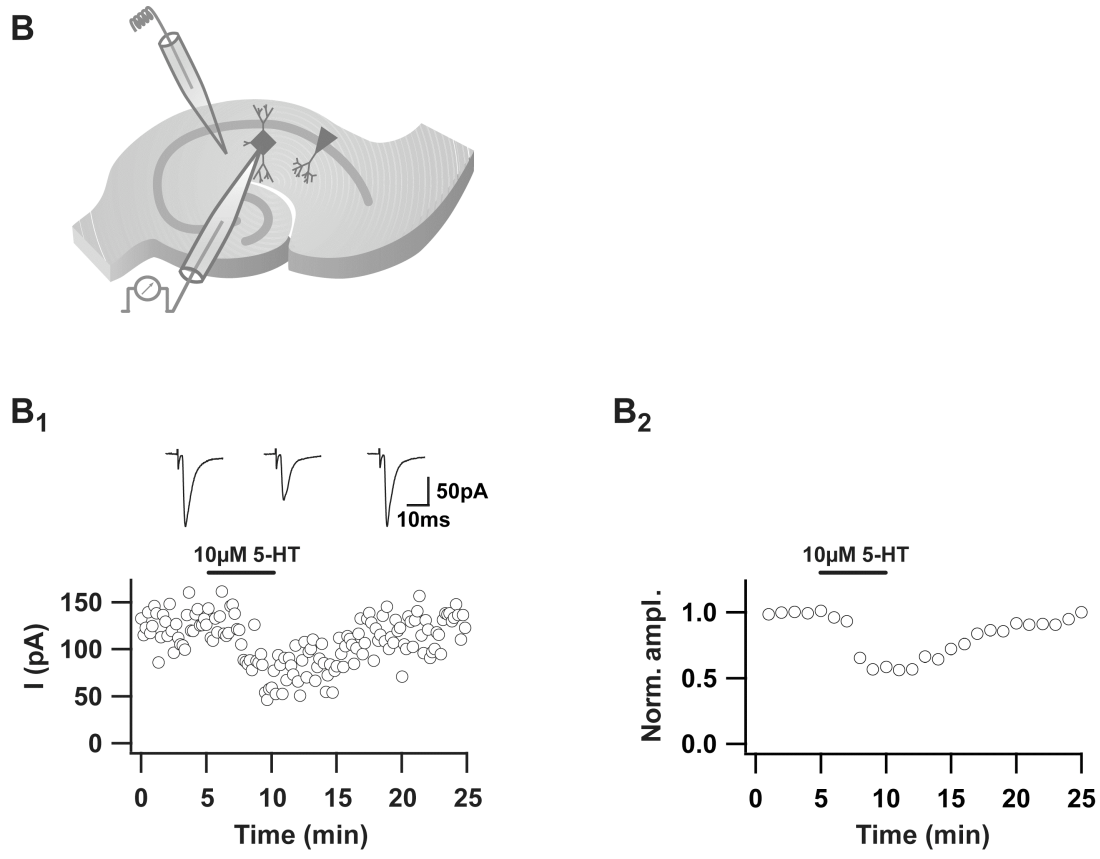

Fig.3.1B. Modulation of glutamatergic transmission onto interneurons by 5-HT. ($V_{holding}$=-60 mV). (B) Schematic of the recording configuration. (B_1) Top, example traces of EPSCs in an interneuron evoked by stimulation in stratum radiatum before, in 10µM 5-HT and after washout. Bottom, time course of the measured EPSCs in the same cell (Scale bar: 50pA; 10ms). (B_2) Summary of the time course of normalized and binned (1min) EPSC amplitudes for 5-HT-sensitive interneurons (open circles; n=18, selected from all 5-HT-sensitive interneurons (65.1%) of n=86 recorded interneurons). Black bar indicates the application of 10µM 5-HT.

Interneurons were included as serotonin-sensitive if the reduction of the EPSC amplitude after application of 10µM 5-HT ≥ 30% of the baseline response and if this reduction was

reversible. These data indicate that 5-HT is able to modulate excitatory inputs onto interneurons and that this modulation is specific to a fraction of interneurons.

Very low concentrations of 5-HT already had significant effects on excitatory inputs to these interneurons (**Fig. 3.1C$_1$**). A detailed assessment of the concentration dependence is shown in **Fig. 3.1C$_2$**. The concentration range we tested was 0.1, 0.3, 1, 3, 10 and 30µM serotonin.

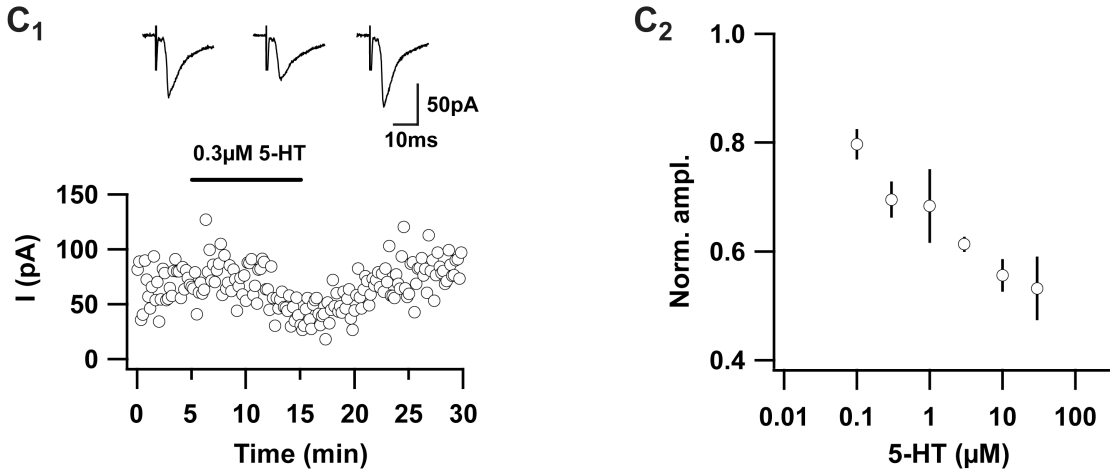

Fig.3.1C. Concentration dependency of 5-HT effect on interneurons. (C_1) Top, example traces of EPSCs in an interneuron evoked by stimulation in stratum radiatum before, in 0.3µM 5-HT and after washout. Bottom, time course of the measured EPSCs in the same cell. Black bar indicates application of 0.3µM 5-HT (Scale bar: 50pA; 10ms). (C_2) Dose response curve for 5-HT concentrations. Normalized EPSC amplitudes are plotted against logarithmic scaled 5-HT concentrations (0.1µM (n=3); 0.3µM (n=8); 1µM (n=9); 3µM (n=2); 10µM (n= 31) and 30µM (n=3)).

3.2 Serotonin affects intrinsic properties in a dose dependent manner

In a fraction of interneurons, bath application of 5-HT was paralleled by a decrease of the input resistance of the membrane of the recorded cells (**Fig.3.2A$_1$**). Unlike the effects on excitatory inputs to interneurons, the decrease in input resistance could be observed reliably only at higher concentrations of 5-HT. We found a significant and reversible decrease in the input resistance at 10µM 5-HT (% of control in 10µM 5-HT: 77.3 ± 5.6; n=12; $p<0.05$ paired *t*-test; % of control after wash: 100.9 ± 4.6; n=12), whereas at concentrations of 0.3µM 5-HT no significant decrease could be detected (**Fig.3.2A$_2$**; p=0.18 paired *t*-test; n=4).

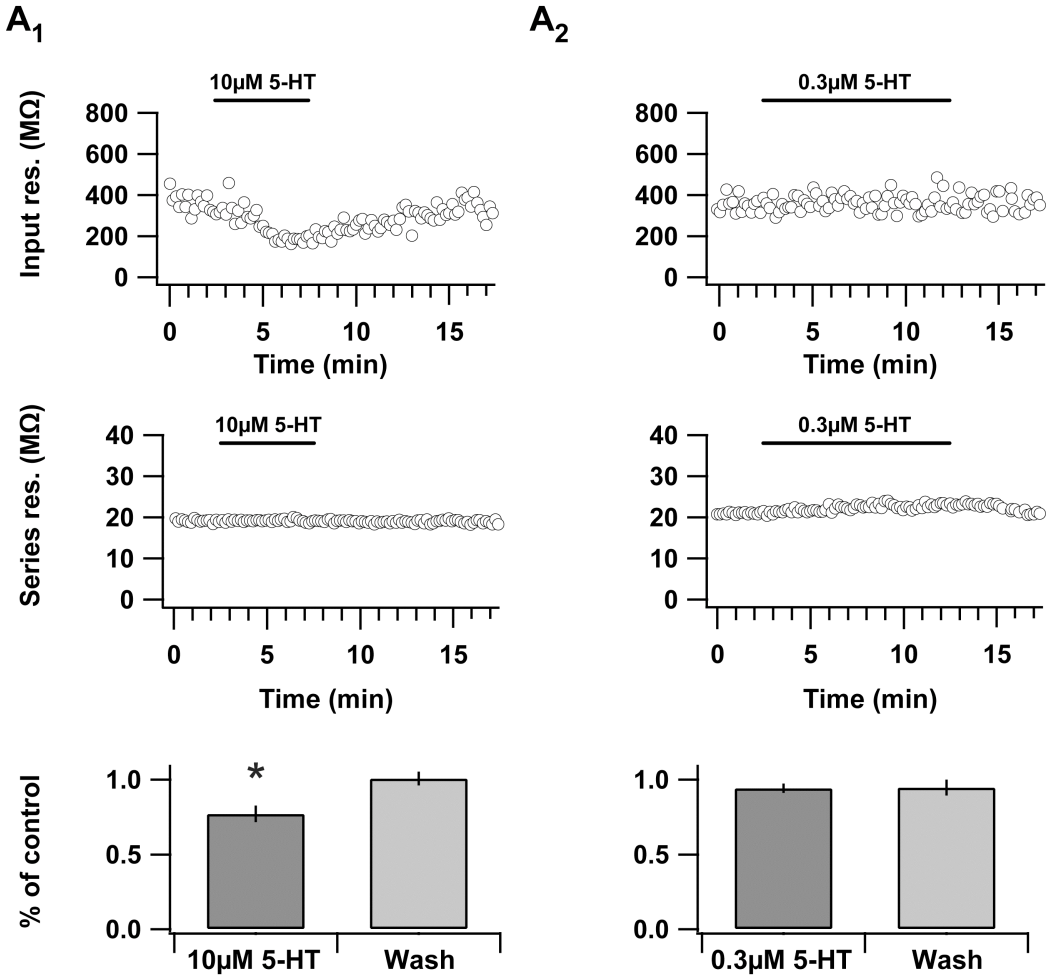

Fig.3.2A. The effect of 5-HT on the input resistance is dose dependent. Top, time course of input resistance in example cells for 10µM 5-HT (A_1) and 0.3µM 5-HT (A_2). Black bars indicate the application of 10µM 5-HT and 0.3µM 5-HT, respectively. Middle, time course of series resistance in the same cells for 10µM 5-HT (A_1) and 0.3µM 5-HT (A_2). Bottom, summary graph of the normalized input resistance in 10µM 5-HT and in 0.3µM 5-HT, respectively and after washout (n=12; $p<0.05$ for 10µM 5-HT and n=4; $p=0.18$ for 0.3µM 5-HT; paired *t*-test).

3.3 Differential modulation of basket cell types by serotonin

The observation that only a subset of interneurons responded to 5-HT application suggested a cell-type-specific modulation among different classes of interneurons. In the following set of experiments we therefore further differentiated interneurons on the basis of their electrical properties, such as input resistance and firing properties, followed by immunohistochemical characterization and morphological reconstruction (**Tab. 1,** n=41).

Tab. 1

	5-HT sensitive: 11			5-HT insensitive: 30				
spiking	regular spiking: 11			regular spiking: 21			fast spiking: 9	
immuno	CCK(+): 11			CCK(+): 5		CCK(-): 16	PV(+): 4	
morphology	basket: 8	Schaff.: 2	failure: 1	basket: 4	failure: 1	not further specified	basket: 7	failure: 2

Tab.1. Characterization of interneuron subclasses. immuno: immunohistochemistry; CCK: Cholecystokinin; PV: Parvalbumin; Schaff. : Schaffer-collateral associated interneuron; failure: morphological reconstruction failed.

We found that 5-HT exclusively reduced the EPSC amplitude of CCK-positive interneurons, which had a high input resistance and slow firing frequency upon depolarizing current injection (**Fig. 3.3A$_3$**; n=11). The mean input resistance of CCK-positive interneurons was 349.7 ± 38.7MΩ, the mean firing frequency was 15.5 ± 3.6Hz. Among these serotonin-sensitive cells (26.8%), CCK-positive basket cells represented the biggest fraction (**Fig. 3.3A$_{1-3}$**; n=8; 62.8 ± 5.9% of control; $p<0.05$). Another subclass of neurons that were sensitive to 5-HT could be identified as CCK-positive Schaffer-collateral associated interneurons (**Fig. 3.3B$_{1-3}$**; n=2; normalized EPSC amplitude: 42.9 ± 2.2% of control). For one CCK-positive and 5-HT-sensitive interneuron, the morphological reconstruction failed. We also found CCK-positive cells that were not sensitive to 5-HT application (n=5; see discussion), of which 4 could be identified as basket cells and one could not be classified. In contrast to CCK-positive interneurons, CCK-negative interneurons with regular spike patterns (n=16; normalized EPSC amplitude: 108.2 ± 7.8% of control; p=0.31; data not shown) were never sensitive to 5-HT. Furthermore, interneurons with a lower input resistance and fast firing properties (**Fig. 3.3C$_{1-3}$**; n=9; 93.1 ± 4.0% of control; p=0.15) were never responsive to bath application of serotonin as well. We further classified these particular interneurons on the basis of their neuroanatomical and immunohistochemical features. Morphological reconstruction revealed that seven out of nine fast-spiking interneurons could be classified as basket cells. Four were fully reconstructed and characterized as fast-spiking PV-positive basket cells. Unlike 5-HT sensitive interneurons, the input resistance of fast-spiking interneurons was not affected by 5-HT application (% of control in 10µM 5-HT: 90.7 ± 6.3; n=5; p=0.22; data not shown). In summary, all interneurons that were sensitive to 5-HT application were CCK-positive (see also discussion), whereas fast-spiking PV-positive interneurons were never responsive.

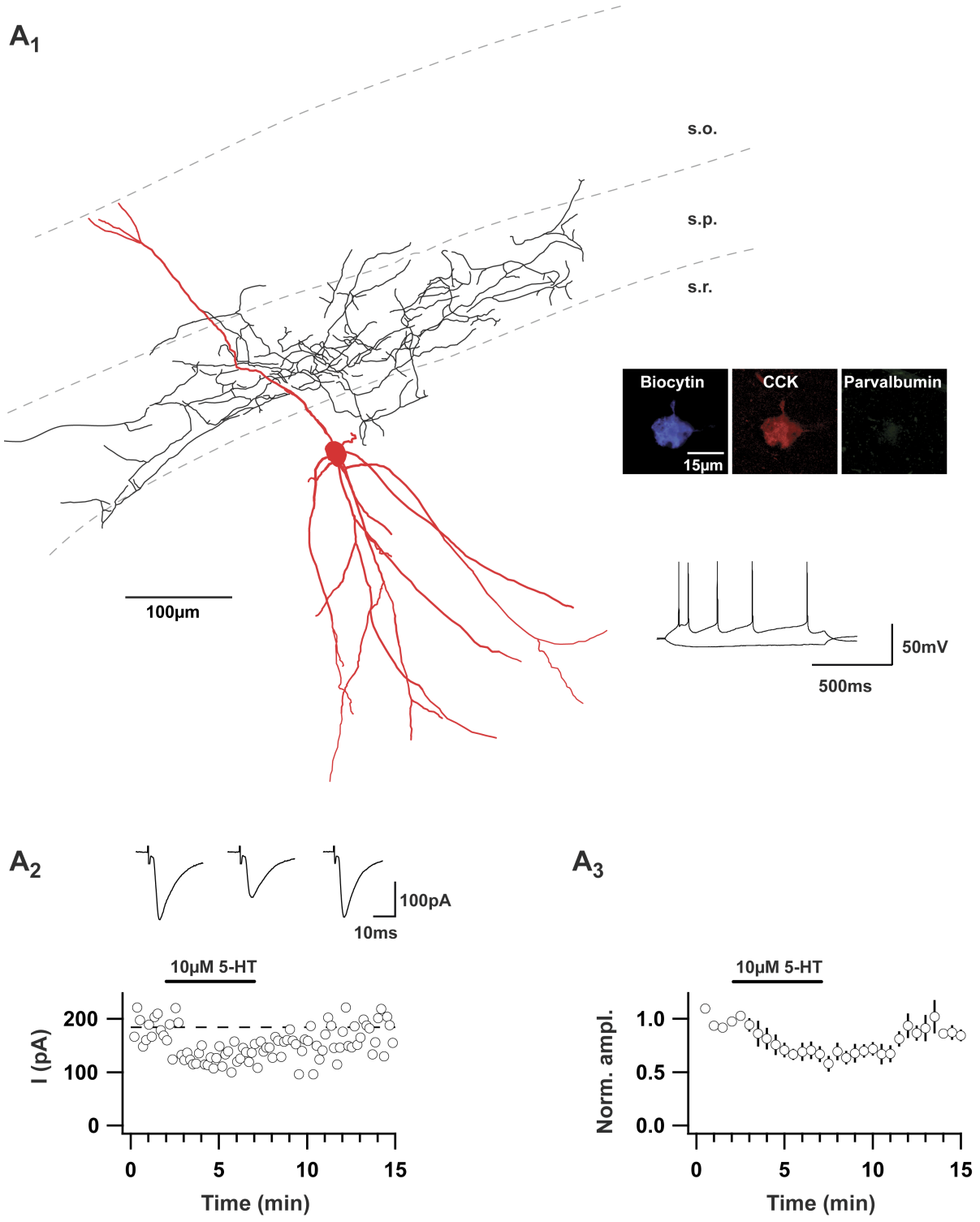

Fig.3.3A. Characterization of morphological, immunohistochemical and firing properties of serotonin-sensitive basket cells. Left, reconstruction of a CCK-positive basket cell (A_1). Scale bar: 100µm; dendrites in red, axons in black; s.o.: stratum oriens, s.p.: stratum pyramidale, s.r.: stratum radiatum. Right, immunohistochemistry (top) of biocytin filled CCK-positive cell body and spike pattern (bottom) of the interneuron. (A_2) Top, example traces of EPSCs of the characterized cells before, in 10µM 5-HT and after washout. Bottom, time courses of EPSCs in the same cells. (A_3) Summary of the time course of normalized and binned (1/2min) EPSC amplitudes (n=8 for CCK-positive basket cells). Black bars indicate the application of 10µM 5-HT.

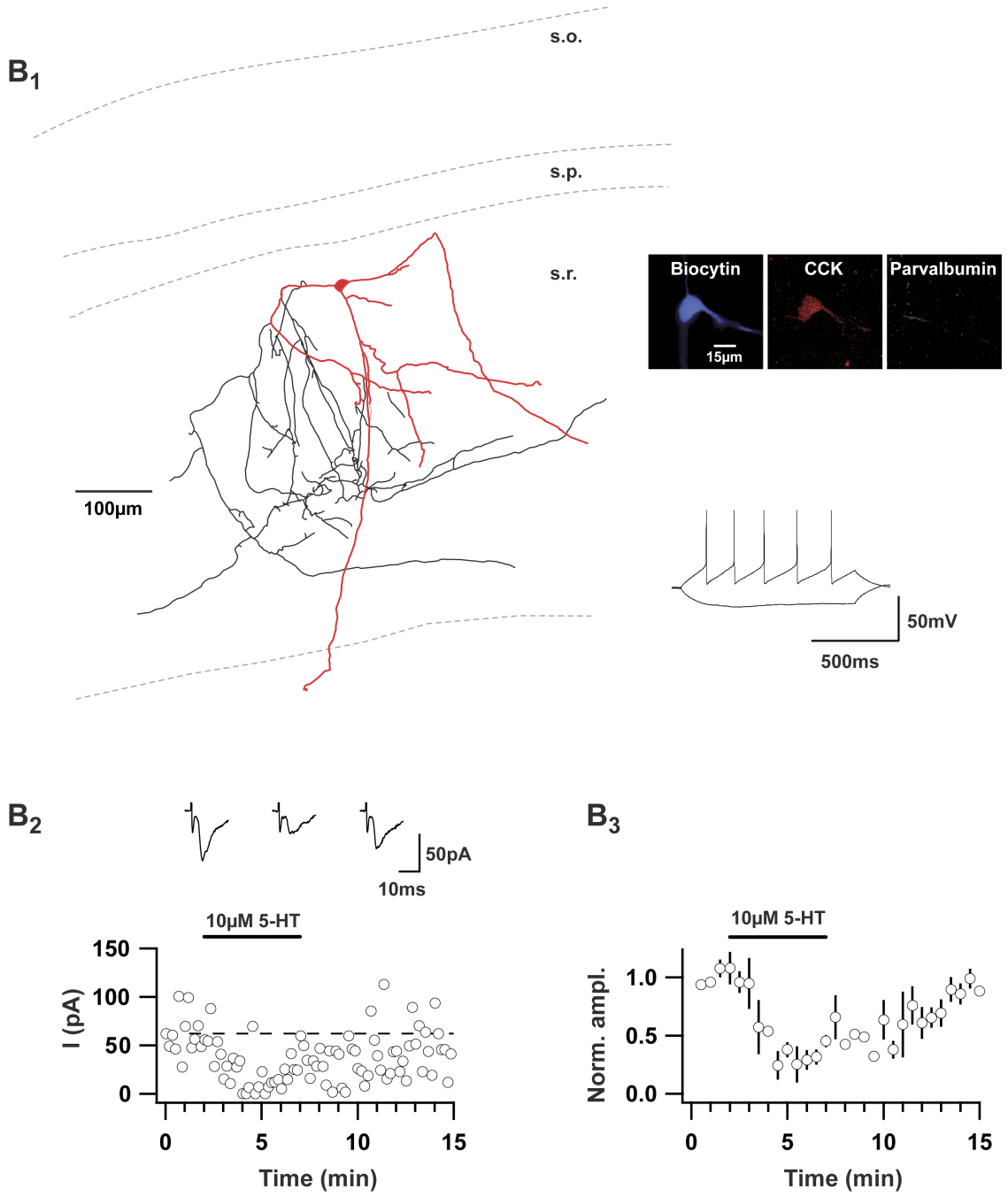

Fig.3.3B. Characterization of morphological, immunohistochemical and firing properties of serotonin-sensitive Schaffer-collateral associated interneurons. Left, reconstruction of a CCK-positive Schaffer-collateral associated interneuron (B_1). Scale bar: 100µm; dendrites in red, axons in black; s.o.: stratum oriens, s.p.: stratum pyramidale, s.r.: stratum radiatum. Right, immunohistochemistry (top) of biocytin filled CCK-positive cell body and spike pattern (bottom) of the reconstructed interneuron. (B_2) Top, example traces of EPSCs of the characterized cells before, in 10µM 5-HT and after washout. Bottom, time courses of EPSCs in the same cells. (B_3) Summary of the time course of normalized and binned (1/2min) EPSC amplitudes (n=2 for CCK-positive Schaffer-collateral associated interneuron). Black bars indicate the application of 10µM 5-HT.

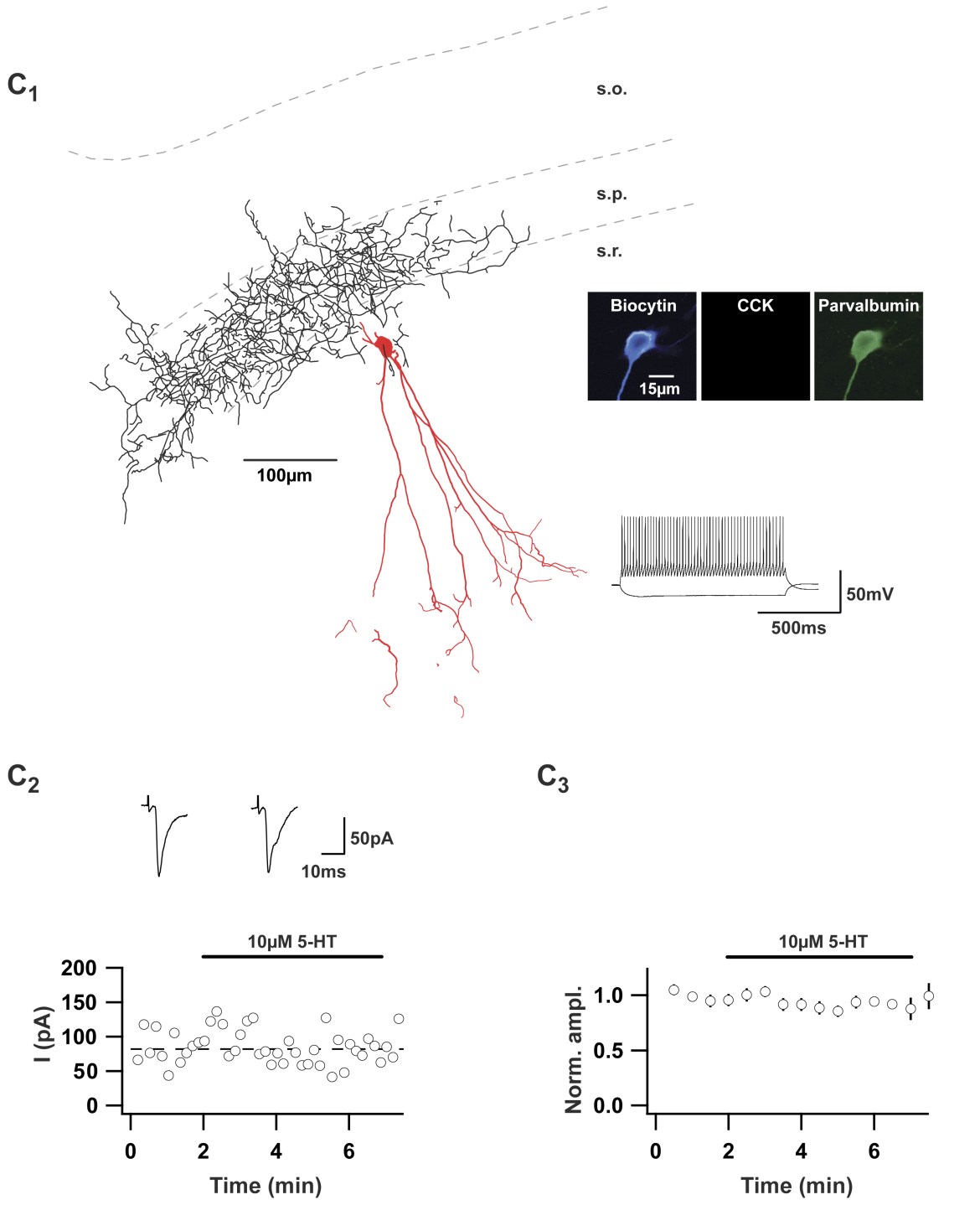

Fig.3.3C. Characterization of morphological, immunohistochemical and firing properties of fast spiking interneurons. Left, reconstruction of a PV-positive basket cell (C_1). Scale bar: 100µm; dendrites in red, axons in black; s.o.: stratum oriens, s.p.: stratum pyramidale, s.r.: stratum radiatum. Right, immunohistochemistry (top) of biocytin filled PV-positive cell body and spike pattern (bottom) of the reconstructed interneuron. (C_2) Top, example traces of EPSCs of the characterized cells before, in 10µM 5-HT and after washout. Bottom, time courses of EPSCs in the same cells. (C_3) Summary of the time course of normalized and binned (1/2min) EPSC amplitudes (n=9 for fast spiking interneurons, including 7 basket cells, of which 4 were PV-positive; 1 fast-spiking interneuron

showed PV-positive staining, but the axonal arborization was lost; 1 fast-spiking interneuron could not be reconstructed). Black bars indicate the application of 10μM 5-HT

In order to exclude species differences we confirmed in an additional set of experiments that the differential modulation of basket cells by serotonin is evident in mice as well (**Fig. 3.3D$_{1-3}$**).

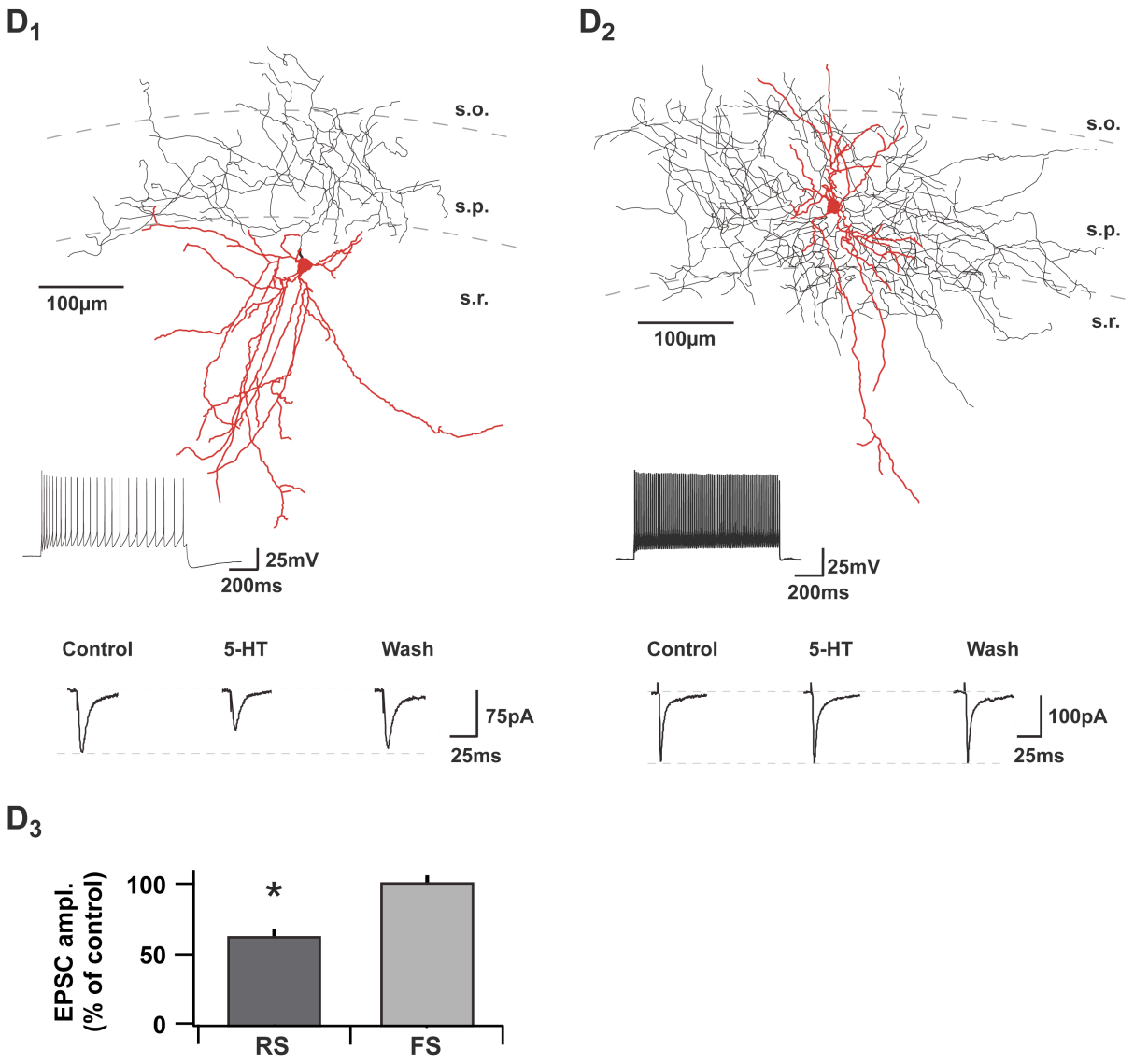

Fig.3.3D. Cell-type-specific reduction of glutamate transmission by 5-HT in mice. (D1) Top, reconstruction and spike pattern of a regular-spiking basket cell. Bottom, example traces of EPSCs of the characterized cells before, in 10μM 5-HT and after washout. (D2) Top, reconstruction and spike pattern of a fast-spiking basket cell. Bottom, example traces of EPSCs of the characterized cells before, in 10μM 5-HT and after washout. (D3) Summary graph of the normalized, residual EPSC amplitudes in 10μM 5HT for regular-spiking basket cells (n=10) and fast-spiking basket cells (n=5).

Tamar Dugladze performed the recordings and the morphological reconstructions of interneurons in mice.

3.4 Serotonin mediates reduction of excitation via a presynaptic mechanism

The observed reduction of EPSC amplitudes by 5-HT may be caused by a presynaptic reduction of transmitter release, a postsynaptic effect, or both. To discriminate between these different scenarios, we performed the following sets of experiments on 5-HT responsive cells. A presynaptic decrease in transmitter release probability will result in a reduced postsynaptic response, but is also predicted to increase short-term facilitation (Zucker and Regehr, 2002). Typically, short-term plasticity is tested by synaptic stimulation with two closely timed pulses evoking paired-pulse facilitation or depression. Application of 5-HT led to an increase in the ratio of the 2nd to 1st response from 1.58 ± 0.19 to 2.17 ± 0.29 (**Fig. 3.4A**; n=16; p<0.05), suggesting a presynaptic locus of serotonin action. This increase in paired pulse ratio was reversible upon washout of 5-HT.

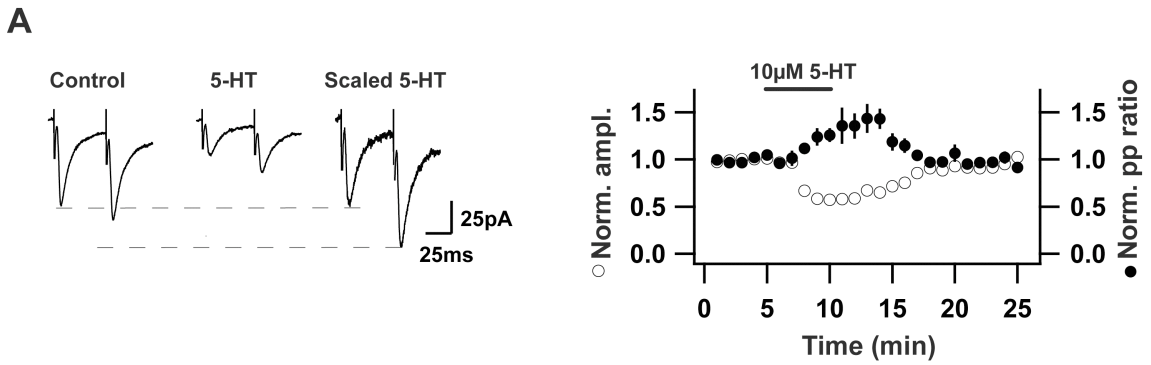

Fig.3.4A. Increased paired pulse ratio after application of 5-HT. (A) Left, example traces show paired-pulse facilitation (50ms inter-stimulus interval) in control conditions, in 10µM 5-HT and in 10µM 5-HT, scaled to the peak of 1st EPSC amplitude under control conditions (Scale bar: 25pA; 25ms). Note the relative increase in the second EPSC amplitude in 10µM 5-HT. Right, summary of the time course of the normalized and binned (1min) 1st EPSC (open circles) and of the normalized and binned (1min) paired-pulse ratio (filled circles; n=16). Black bar indicates application of 10µM 5-HT.

A reduction in presynaptic transmitter release is additionally expected to result in a higher incidence of synaptic failures, i.e. presynaptic stimulation without successful synaptic transmission. Indeed, the probability of failures increased from 39.5 ± 8.1% under control conditions to 63.3 ± 7.1% in the presence of 5-HT (**Fig. 3.4B**; n=6; p<0.05).

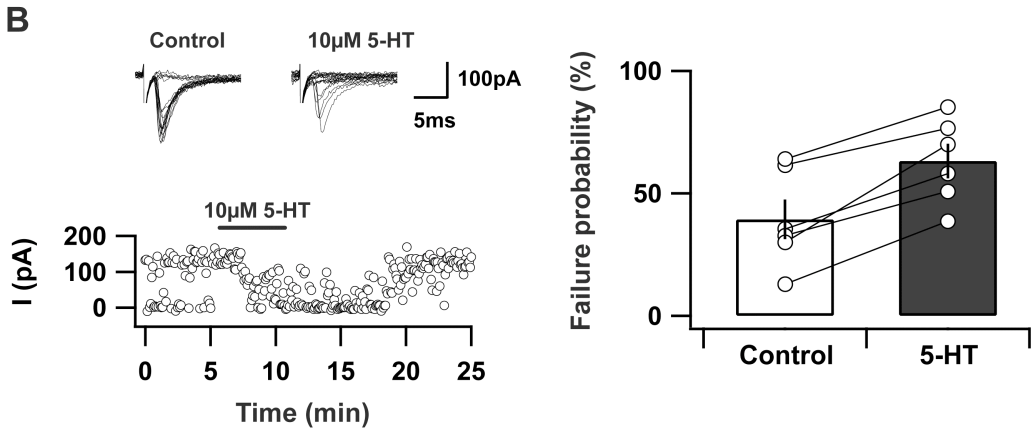

Fig.3.4B. Increased failure probability after application of 5-HT. Left, example traces (top, single sweeps) showing synaptic failures in control and in 10µM 5-HT (Scale bar: 100pA; 5ms). Bottom, time course of the same experiment. Black bar indicates the application of 10µM 5-HT. Right, summary graph of the probability of synaptic failures in control and in 10µM 5-HT (n=6; $p<0.05$ paired *t*-test).

Based on the analysis of the coefficient of variation (CV), a presynaptic locus of action is most likely as well, as the ratio $CV^2_{control}/CV^2_{serotonin}$ scaled linearly with the change of the mean response upon application of 5-HT (n=26, see **Fig. 3.4C**). Even though the analysis of the coefficient of variation is commonly used to differentiate between pre- and postsynaptic mechanisms, this method is limited when using multi-afferent stimulation (Faber and Korn, 1991). We therefore performed an additional set of experiments, in which we made use of the minimal stimulation technique and gained similar results (see filled circles in **Fig. 3.4C**).

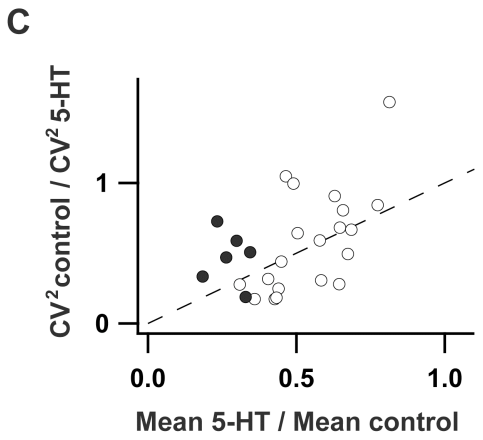

Fig.3.4C. Coefficient of variation (n=26). Filled circles indicate minimal stimulation experiments (n=6).

To provide further evidence for a presynaptic locus of action, we photolytically activated caged glutamate with a UV-laser flash. **Fig. 3.4D** shows experiments in which stimulus-evoked EPSCs and glutamate-evoked currents were measured in an alternating manner. Note,

that while stimulus-evoked EPSCs were clearly reduced by 5-HT, no such effect was found on glutamate-evoked currents.

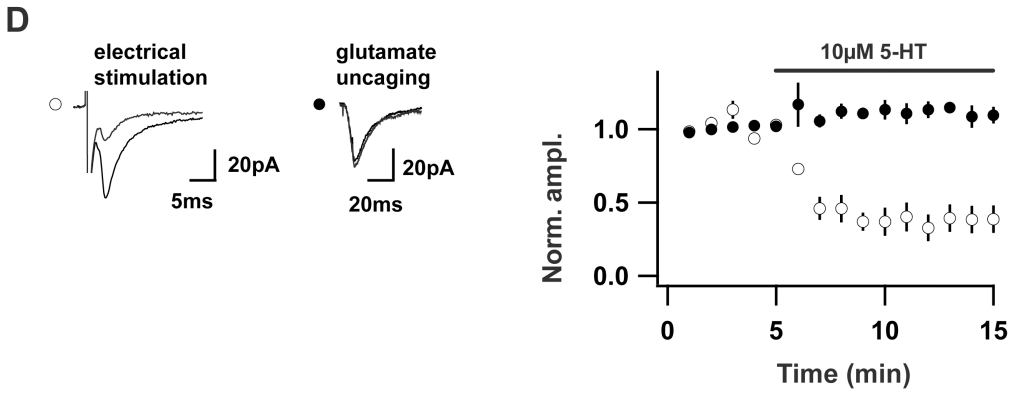

Fig 3.4D. Glutamate-evoked currents are insensitive to 5-HT application. Left, example traces for stimulus-evoked EPSCs and glutamate-evoked currents under control conditions and in 10µM 5-HT. Bottom, summary (n=5) of the time course of normalized and binned (1min) stimulus-evoked EPSCs (open circles) and glutamate-evoked currents (filled circles). Black bar indicates application of 10µM 5-HT.

In summary, cumulative evidence indicates that serotonin decreases glutamatergic transmission through presynaptically localized 5-HT receptors.

3.5 5-HT_{1B} receptors mediate the reduction in glutamatergic transmission

In hippocampal neurons, several subtypes of 5-HT receptors are differentially expressed. 5-HT_{1A} and $5HT_7$ receptors are expressed in pyramidal neurons of area CA3, 5-HT_{1A}, 5-HT_{1B} and 5-HT_4 receptors in pyramidal neurons of area CA1 and 5-HT_{1A} in granule cells of the dentate gyrus. In hippocampal interneurons 5-HT_2 and 5-HT_3 receptor expressions have been reported (Andrade, 1998). In order to identify the subtype of 5-HT receptor mediating the depression of glutamatergic transmission, we tested various specific receptor agonists and antagonists. 5-HT_{1A} receptors mediate several effects of serotonin on intrinsic properties and synaptic potentials/currents of CA1 pyramidal neurons (Colino and Halliwell, 1987; Andrade and Nicoll, 1987; Schmitz et al., 1995). 8-OH-DPAT, a potent agonist at 5-$HT_{1A/7}$ receptors, however, did not mimic the presynaptic effects of serotonin on interneuron EPSCs. Importantly, within the very same cells, *i.e.* those treated with 8-OH-DPAT, serotonin clearly depressed glutamatergic transmission (**Fig. 3.5A$_{1,2}$**).

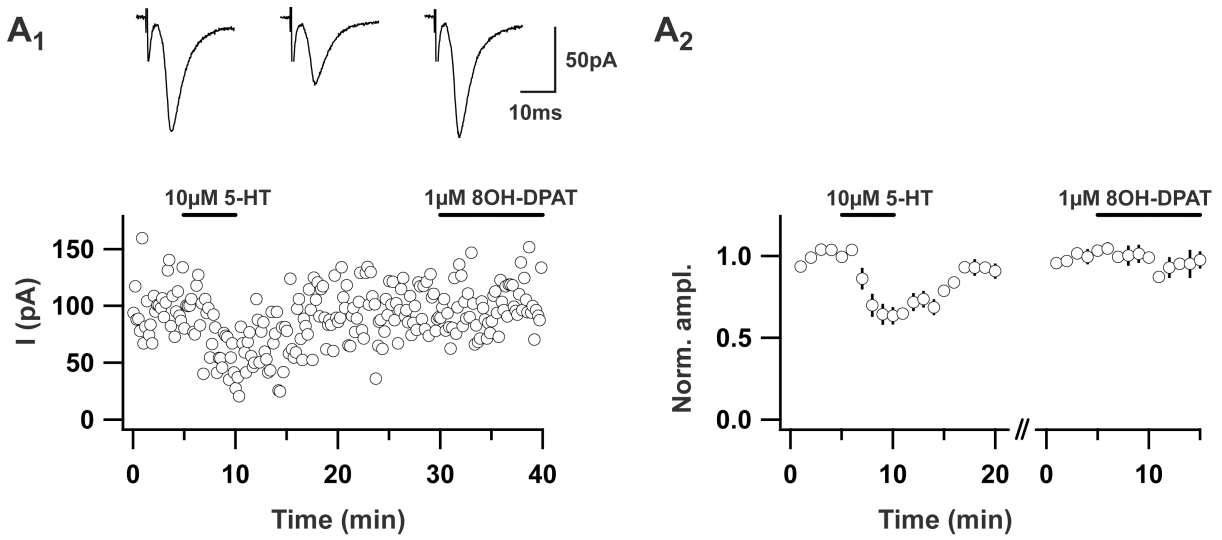

Fig.3.5A. The reduction in glutamatergic transmission is not mediated by 5-HT$_{1A}$ receptors. (A_1) Top, example traces of EPSCs in control conditions, in 10µM 5-HT and in 1µM 8-OH-DPAT. Bottom, time course of EPSCs in the same cell. Black bars indicate the application of 10µM 5-HT and the application of 1µM 8-OH-DPAT. (A_2) Summary of the time course of normalized and binned (1min) EPSC amplitudes (n=6).

Another subtype expressed in the hippocampal formation is the 5-HT$_{1B}$ receptor, which has been shown to mediate effects of serotonin on synaptic potentials within area CA1 and the subiculum (Boeijinga and Boddeke, 1996; Mlinar et al., 2003). Indeed, we found that the 5-HT$_{1B}$ receptor agonist CP93129 mimicked the effect of serotonin (**Fig. 3.5B$_{1, 2}$**).

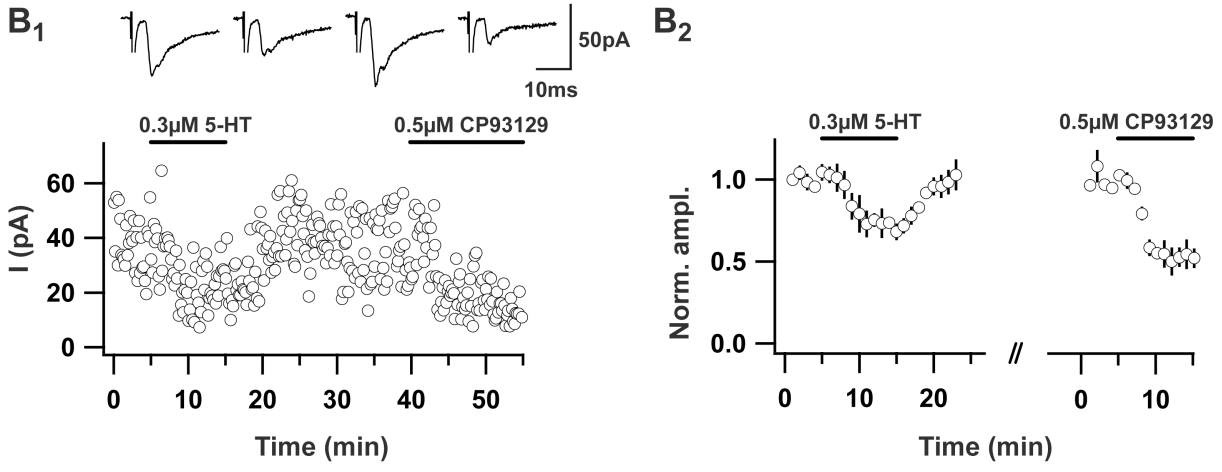

Fig.3.5B. The reduction in glutamatergic transmission is mediated by 5-HT$_{1B}$ receptors. (B1) Top, example traces of EPSCs in control conditions, in 0.3µM 5-HT, after wash out and in 0.5µM CP93129. Bottom, time course of EPSCs in the same cell. Black bars indicate the application of 0.3µM 5-HT and the application of 0.5µM CP93129. (B2) Summary of the time course of normalized and binned (1min) EPSC amplitudes (n=5).

Yet, in contrast to high serotonin concentrations, CP93129 had no effect on the input resistance of the recorded interneurons (mean value in control: 251.2 ± 44.5MΩ vs. mean value in 0.5µM CP93129: 244.9 ± 38.1MΩ; n=4; p=0.75 paired *t*-test; data not shown). Moreover GR127935, a potent and specific 5-HT_{1B} receptor antagonist, blocked the effect of serotonin on interneuron EPSCs after subsequent 5-HT co-application (see **Fig. 3.5C$_{1, 2}$**). In line with this finding, paired-pulse changes observed following the application of 5-HT were also blocked by the 5-HT_{1B} receptor antagonist GR127935 (paired-pulse ratio in GR127935: 1.35 ± 0.21, in GR127935 and 5-HT: 1.44 ± 0.27; n=5; p=0.39 paired *t*-test; data not shown).

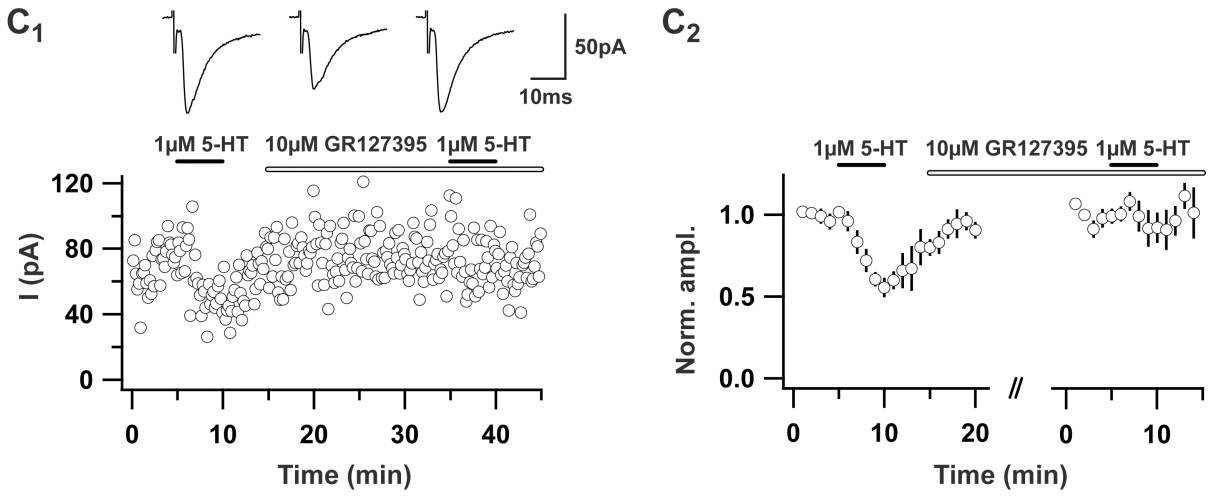

Fig.3.5C. The 5-HT_{1B} receptor antagonist GR127395 blocks the 5-HT effect. (C_1) Top, example traces of EPSCs in control, in 1µM 5-HT and in10µM GR127395 and 1µM 5-HT. Bottom, time course of EPSCs in the same cell. Black bars indicate the application of 1µM 5-HT. The application of 10µM GR127395 is indicated as open bar. (C_2) Summary of the time course of normalized and binned (1min) EPSC amplitudes (n=5).

We conclude that the activation of presynaptic 5-HT_{1B} receptors is most likely responsible for the effects of serotonin on glutamatergic transmission onto interneurons.

3.6 Fenfluramine mimics the effect of bath-applied 5-HT

So far we used bath application of 5-HT to elucidate the effects of 5-HT on excitatory synaptic transmission onto CCK-positive interneurons in the hippocampus. Nevertheless, the question remains, whether physiological release of 5-HT from serotonergic fibers in the hippocampus exhibits similar effects. One way to address this question is to make use of the properties of the compound fenfluramine. Fenfluramine is thought to provoke the release of serotonin by disrupting the vesicular storage of 5-HT and consecutively reversing the

serotonin transporter (SERT) (Schmitz et al., 1999). In the following sets of experiments we tested whether the effect of serotonin on EPSCs could be mimicked by fenfluramine-induced physiological release of 5-HT from hippocampal serotonergic fibers. Having confirmed that the recorded interneuron was sensitive to 5-HT, subsequent application of 200µM fenfluramine reliably mimicked the effect of 5-HT (**Fig. 3.6A, 3.6D$_2$**).

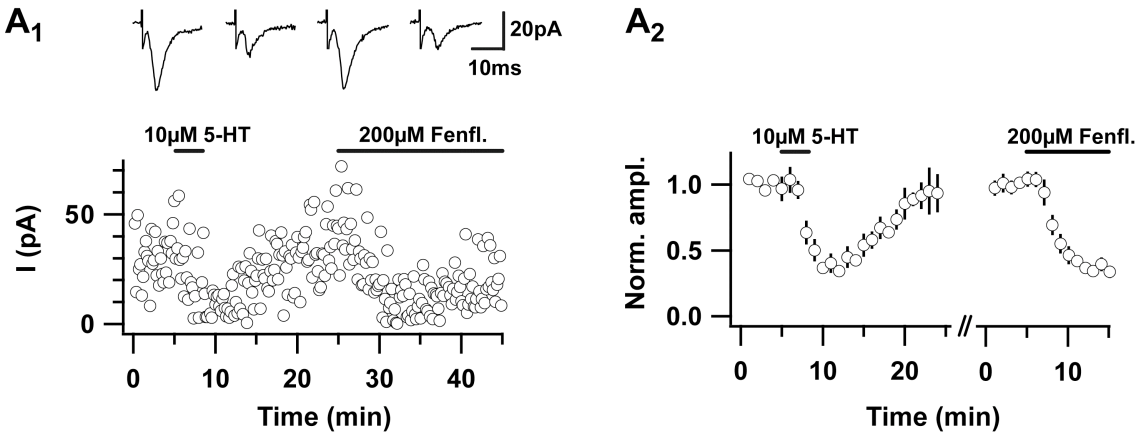

Fig.3.6A. Fenfluramine mimics the effect of 5-HT on interneurons. (A_1) Top, example traces of EPSCs in control conditions, in 10µM 5-HT, after washout and in 200µM fenfluramine. Bottom, time course of EPSCs in the same cell. Black bars indicate the application of 10µM 5-HT and the application of 200µM fenfluramine. (A_2) Summary of the time course of normalized and binned (1min) EPSC amplitudes (n=5).

To see if the target cell-specific effect of 5-HT is mimicked by bath application of fenfluramine, we furthermore tested the effect of fenfluramine on excitatory potentials onto CA1 pyramidal neurons and onto fast spiking interneurons.

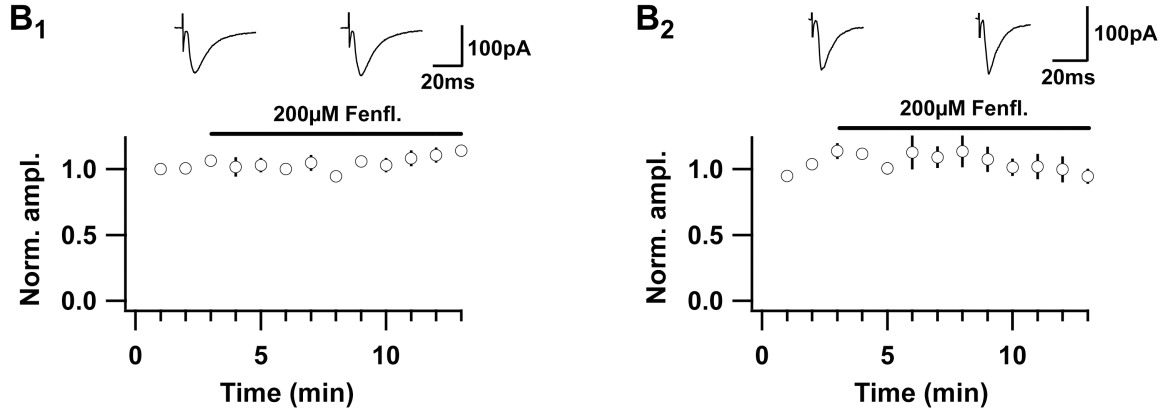

Fig.3.6B. Fenfluramine has no effect on EPSCs onto pyramidal cells and fast-spiking interneurons. (B1,B2) Top, example traces of EPSCs in control conditions and in 200µM fenfluramine. Bottom, summary of the

time course of normalized and binned (1min) EPSC amplitudes in (B_1) pyramidal cells (n=5) and (B_2) fast-spiking interneurons (n=5).

Indeed, excitatory inputs onto CA1 pyramidal neurons (**Fig. 3.6B$_1$, 3.6D$_2$**) as well as excitatory inputs onto fast-spiking interneurons were not affected (**Fig. 3.6B$_2$, 3.6D$_2$;** n=5 (4 basket cells, of which 2 were PV-positive, 1 could not be reconstructed)). Furthermore, the specific 5-HT$_{1B}$ receptor antagonist GR127935 blocked the effect of endogenously released 5-HT on interneuron EPSCs as well. After verifying that the recorded interneuron was sensitive to 5-HT (n=5; % of control in 10µM 5-HT: 61.8 ± 4.8; $p<0.05$; data not shown), application of fenfluramine did not affect EPSC amplitudes significantly during co-application of GR127935 (**Fig. 3.6C, 3.6D$_2$;** n=5; % of control in 200µM fenfluramine: 87.5 ± 9.4; p=0.24 paired *t*-test).

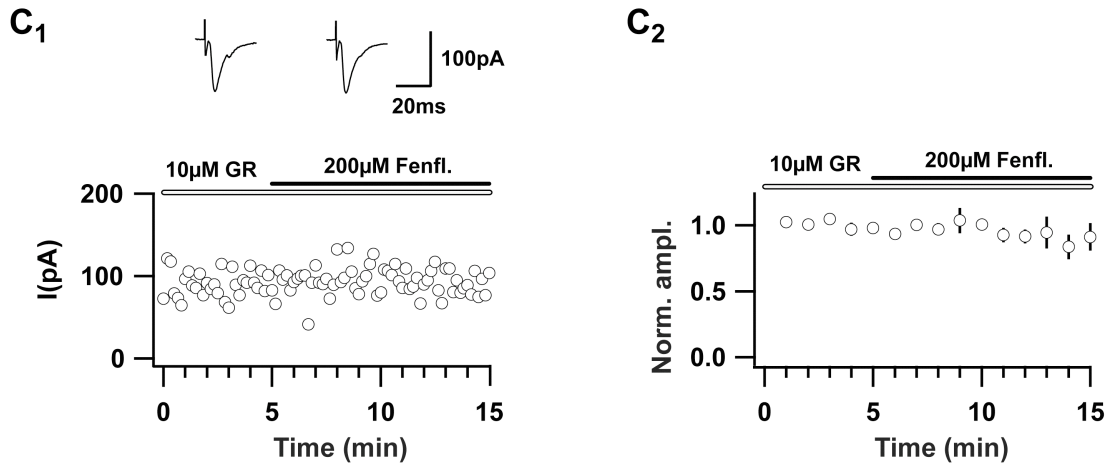

Fig.3.6C. GR127935 antagonizes fenfluramine. (C_1) Top, example traces of EPSCs in 10µM GR127935 and in 10µM GR127935 and 200µM fenfluramine. Bottom, time course of EPSCs in the same cell. (C_2) Summary of the time course of normalized and binned (1min) EPSC amplitudes (n=5). Black bar indicates the application of 200µM fenfluramine.

Corresponding to the changes in the paired pulse ratio after bath application of 5-HT, fenfluramine increased the paired-pulse ratio from 1.10 ± 0.10 to 2.41 ± 0.77 (normalized EPSC amplitudes after washout of 5-HT; **Fig. 5D$_1$**; n=4)

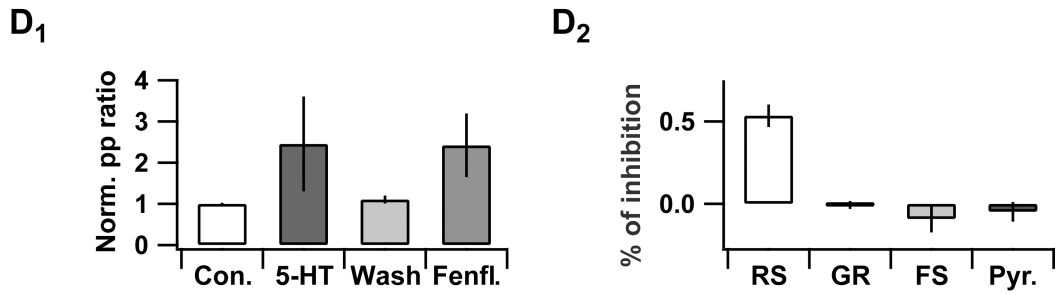

Fig3.6D. Effects of fenfluramine on paired pulse ratio and summary of fenfluramine effects on EPSCs. (D_1) Baseline: 1.00 ± 0.03 to 2.46 ± 1.15 in 10µM 5-HT and after washout of 5-HT from 1.10 ± 0.10 to 2.41 ± 0.77 in 200µM fenfluramine (n=4). (D_2) Summary graph of the % of inhibition of normalized EPSC amplitudes 5 min. after application of fenfluramine in regular-spiking interneurons (RS), in RS with the antagonist GR127935, in fast-spiking interneurons (FS) and in CA1 pyramidal neurons (Pyr.).

These data indicate that endogenous release of 5-HT provoked by fenfluramine as well is able to modulate excitatory inputs onto interneurons in a cell-type-specific manner.

3.7 Input-specific modulation by 5-HT_{1B} receptors

It has been shown by in situ-hybridization that in the cortex the highest expression levels of 5-HT_{1B} receptor mRNA can be detected in the pyramidal cell layer of area CA1 of the hippocampus (Voigt et al., 1991; Svenningsson et al., 2006). Furthermore, these studies revealed that there is no detectable expression of 5-HT_{1B} receptor mRNA in CA3 pyramidal cells and dentate gyrus granule cells. This striking expression pattern prompted us to test the hypothesis that serotonergic modulation by 5-HT_{1B} receptor signaling is restricted to glutamatergic transmission. We obtained whole cell recordings from CCK-positive interneurons and elicited monosynaptic inhibitory postsynaptic currents in the presence of the AMPA receptor blocker NBQX (10µM) and the NMDA receptor blocker APV (25µM). According to the pattern of 5-HT_{1B} receptor mRNA expression we observed that the 5-HT_{1B} receptor agonist CP93129 had no effect on stimulus-induced IPSCs onto CCK-positive interneurons (**Fig. 3.7A_2;** n=6; % of control in 1µM CP93129: 92.0 ± 7.1; p=0.38 paired *t*-test).

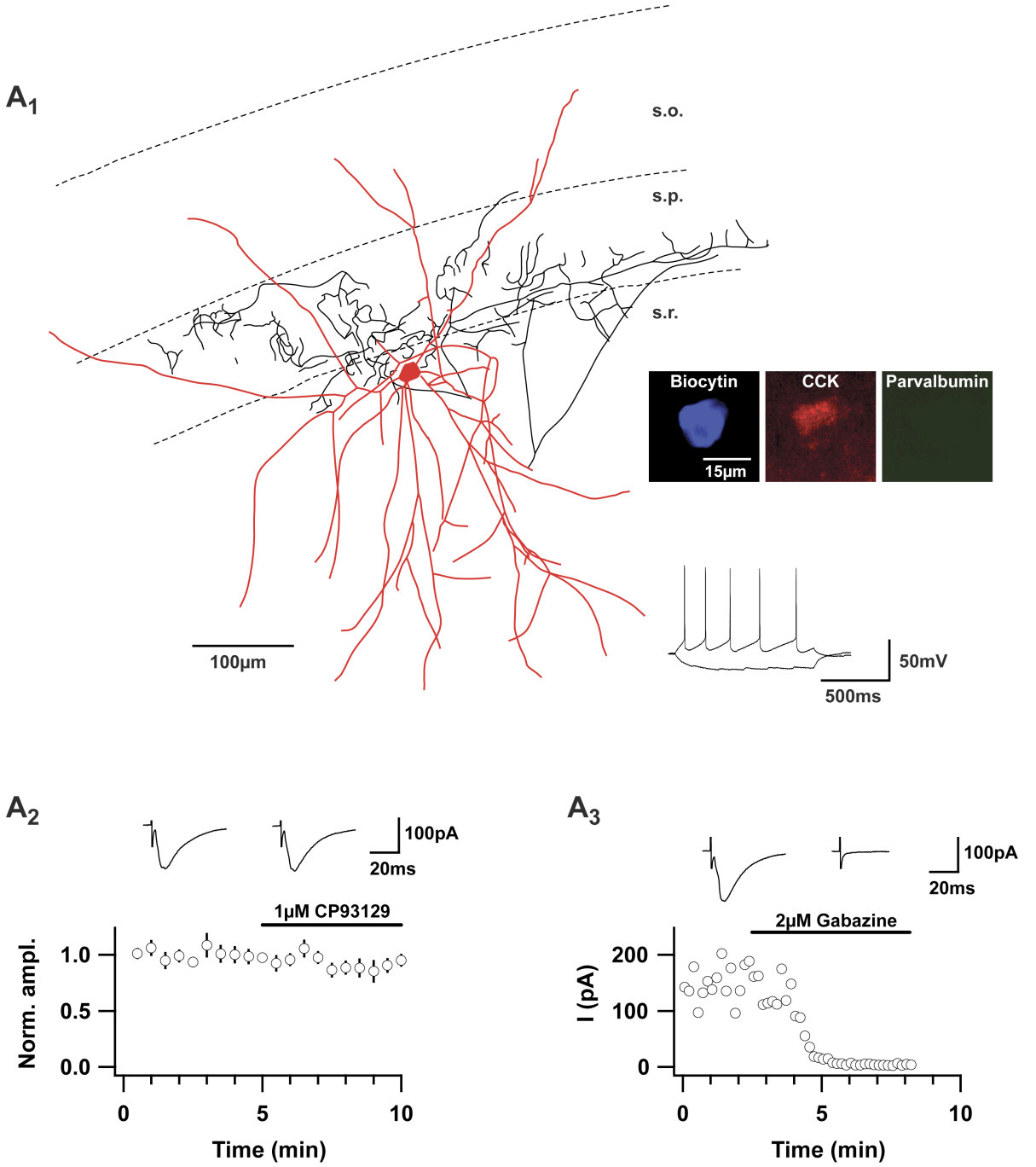

Fig.3.7A. 5-HT$_{1B}$ receptor signaling does not affect inhibition in CCK-positive interneurons. (A_1) Left, reconstruction of a CCK-positive basket cell. Scale bar: 100µm; dendrites in red, axons in black; s.o.: stratum oriens, s.p.: stratum pyramidale, s.r.: stratum radiatum. Right, immunohistochemistry of biocytin filled CCK-positive cell body and spike pattern of the reconstructed interneuron. (A_2) Top, example traces of stimulus induced monosynaptic IPSCs before and after application of 1µM CP93129 of the characterized cell. Bottom, summary of the time course of normalized and binned (1/2min) IPSC amplitudes (n=6). Black bar indicates the application of 1µM CP93129. (A_3) Top, example traces of stimulus induced monosynaptic IPSCs before and after application of 2µM Gabazine. Bottom, time course of IPSCs in a CCK-positive basket cell. Application of 2µM Gabazine confirms the inhibitory input.

The expression pattern of 5-HT_{1B} receptor mRNA implies that the neuromodulatory effect of 5-HT onto CCK-positive interneurons is input-specific for CA1 pyramidal neurons. To probe the input-specificity we aimed to differentiate between CA3- and CA1-inputs onto interneurons by focal release of caged glutamate with a UV-laser flash, in the pyramidal cell layer of either area CA3 or area CA1 of the hippocampus, thereby recruiting selectively the input of CA3 pyramidal neurons and the input of CA1, respectively. As 5-HT is known to modulate the intrinsic properties of hippocampal pyramidal cells via 5-HT_{1A} receptors (Colino and Halliwell, 1987; Andrade and Nicoll, 1987) and 5-HT_4 receptors (Torres et al., 1995; 1996), we made use of the 5-HT_{1B} receptor agonist CP93129 instead of 5-HT to ensure that the intrinsic properties of the stimulated pyramidal cells remained unaffected. We observed that CP93129 had no effect on EPSCs onto CCK-positive interneurons if the excitatory input originated from a CA3 pyramidal cell (mean inhibition: 5.3 ± 8.7%; p=0.79 paired *t*-test; n=6 (including 2 basket cells and 2 Schaffer-collateral associated cells, and 2 cells that could not be reconstructed); **Fig. 3.7B$_{1-3}$**). If, in contrast, the excitatory input derived from CA1 pyramidal cells, then the EPSCs were depressed (mean inhibition: 35.6 ± 5.9%; $p<0.05$ paired *t*-test; n=9 (4 basket cells, 3 Schaffer-collateral associated, 2 could not be reconstructed); **Fig. 3.7C$_{1-3}$**). Additionally, we tested whether fast-spiking basket cells were sensitive to the 5-HT_{1B} receptor agonist CP93129 when the excitatory input arose from CA1 pyramidal cell firing. Again, we did not detect a decrease in EPSC amplitude of fast spiking-basket cells (mean inhibition: -2.5 ± 11.5%; p=0.85 paired *t*-test; n=3; **Fig. 3.7D$_{1-3}$**).

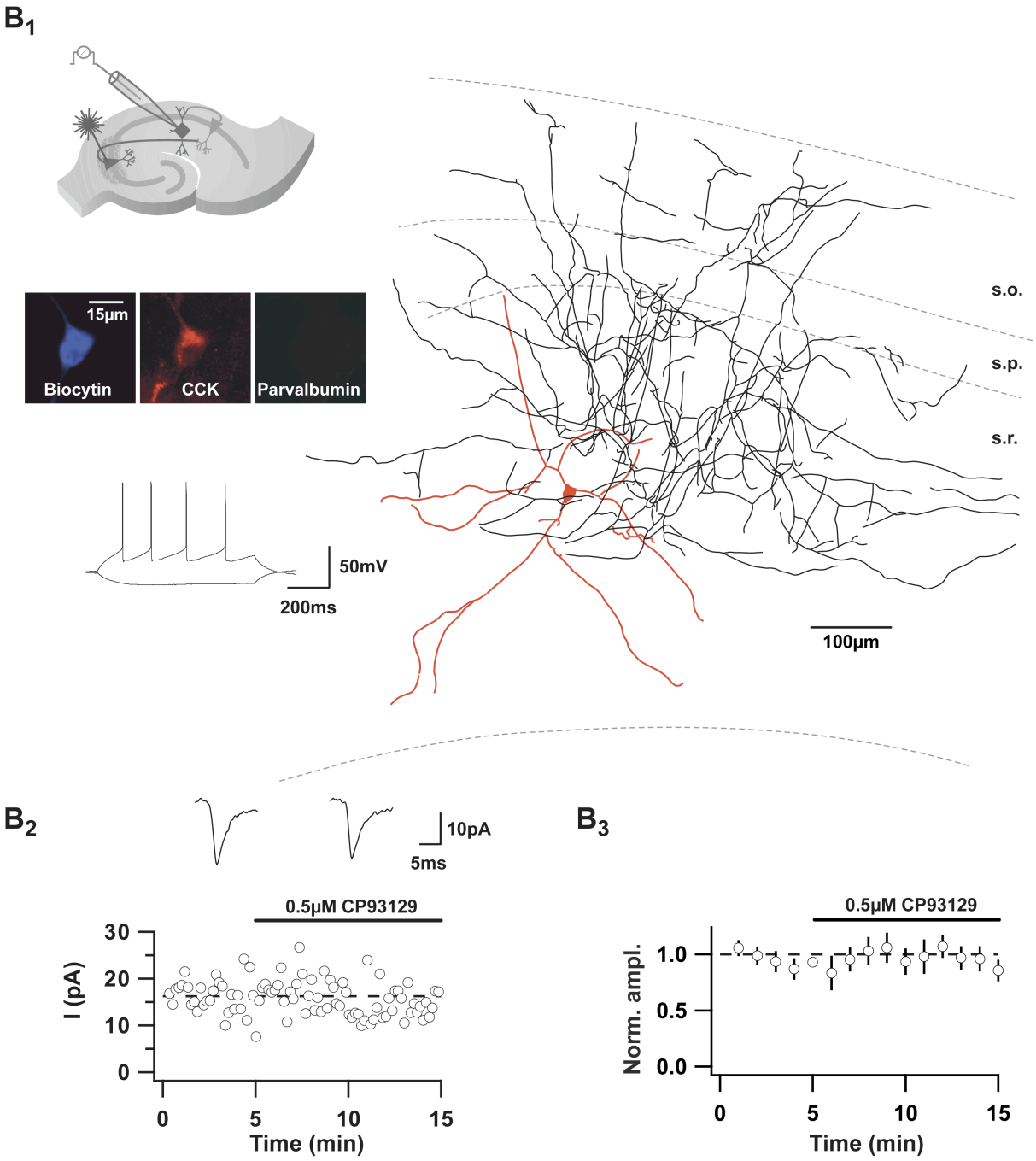

Fig.3.7B. CA3 input onto CCK+ interneurons is insensitive to 5-HT$_{1B}$ receptor activation. (B1) Left, top, schematic of the recording configuration. Right, reconstruction of a CCK-positive Schaffer-collateral associated interneuron. Scale bar: 100µm; dendrites in red, axons in black; s.o.: stratum oriens, s.p.: stratum pyramidale, s.r.: stratum radiatum. Left, middle, immunohistochemistry of biocytin filled CCK-positive cell body. Left, bottom spike pattern of a CCK-positive Schaffer-collateral associated interneuron. (B2) Left, example traces of EPSCs evoked by local uncaging of glutamate in area CA3 before and in 0.5µM CP93129 of the characterized cell. Bottom, time courses of EPSCs in the same cells. (B3) Summary of the time course of normalized and binned (1min) EPSC amplitudes for CCK-positive interneurons (n=6) evoked by local uncaging in CA3.

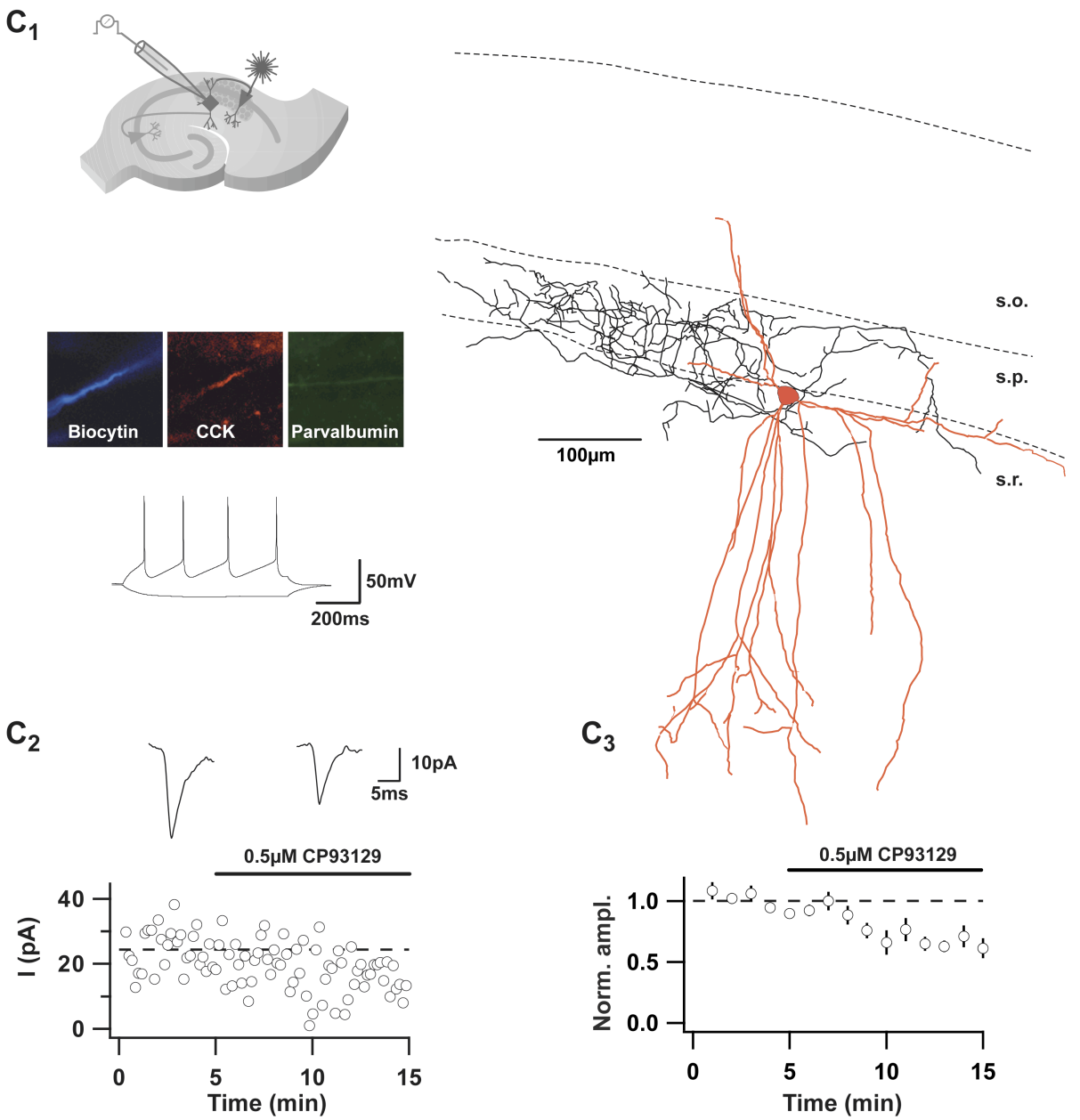

Fig.3.7C. CA1 input onto CCK+ interneurons is sensitive to 5-HT$_{1B}$ receptor activation. (C1) Left, top, schematic of the recording configuration. Right, reconstruction of a CCK-positive basket cell. Scale bar: 100µm; dendrites in red, axons in black; s.o.: stratum oriens, s.p.: stratum pyramidale, s.r.: stratum radiatum. Left, middle, immunohistochemistry of biocytin filled CCK-positive dendrite. Left, bottom, spike pattern of a CCK-positive basket cell. (C2) Top, example traces of EPSCs evoked by local uncaging of glutamate in area CA1 before and in 0.5µM CP93129 of the characterized cell. Bottom, time courses of EPSCs in the same cells. (C3) Summary of the time course of normalized and binned (1min) EPSC amplitudes for CCK-positive interneurons (n=9) evoked by local uncaging in CA1.

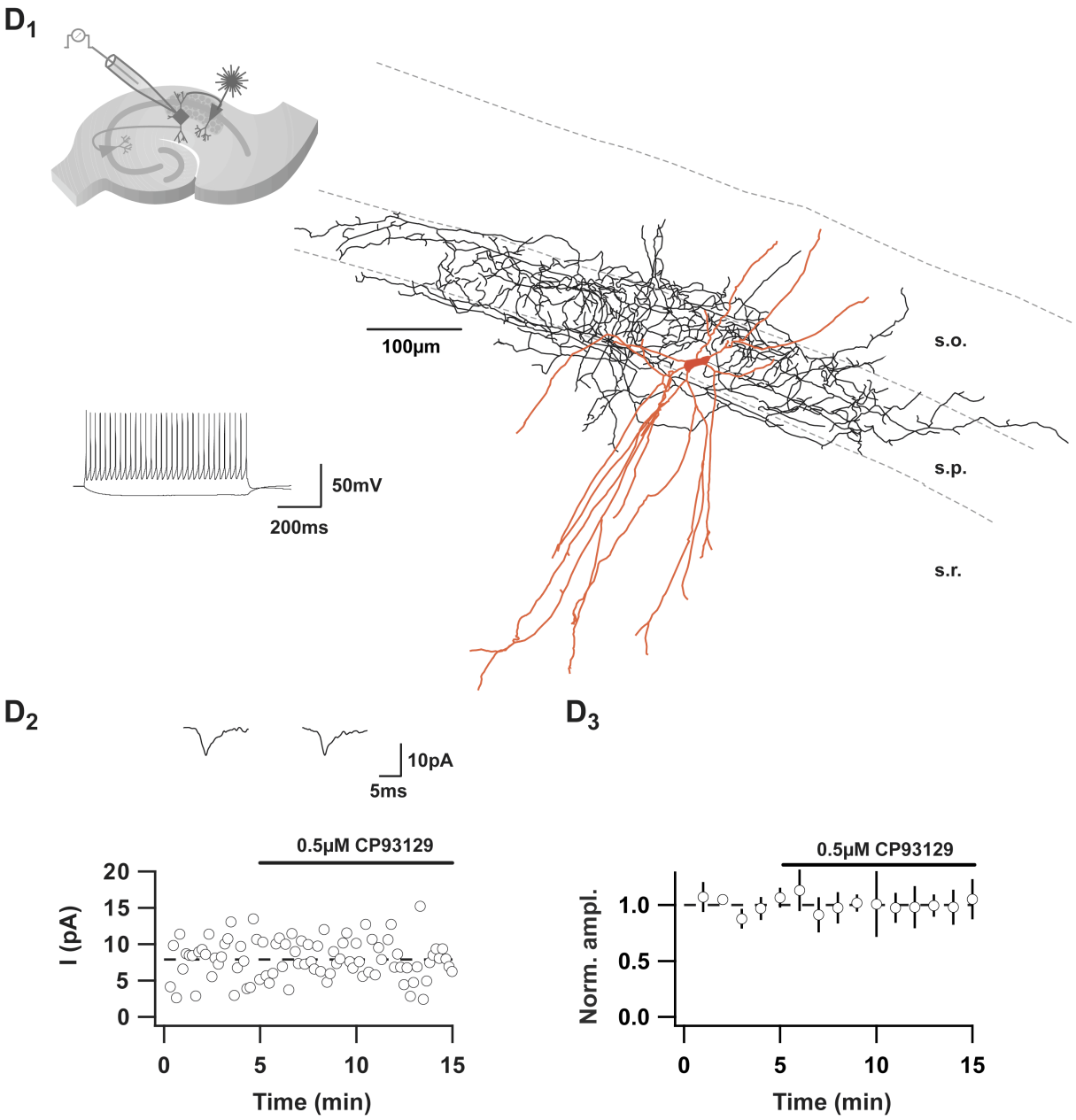

Fig.3.7D. CA1 input onto fast-spiking interneurons is insensitive to 5-HT$_{1B}$ receptor activation. (D1) Left, top, schematic of the recording configuration. Right, reconstruction of a fast spiking basket cell. Scale bar: 100µm; dendrites in red, axons in black; s.o.: stratum oriens, s.p.: stratum pyramidale, s.r.: stratum radiatum. Left, bottom, spike pattern of a fast-spiking basket cell. (D2) Top, example traces of EPSCs evoked by local uncaging of glutamate in area CA1 before and in 0.5µM CP93129 of the characterized cell. Bottom, time courses of EPSCs in the same cells. (D3) Summary of the time course of normalized and binned (1min) EPSC amplitudes for fast-spiking interneurons (n=3) evoked by local uncaging in CA1.

3.8 Serotonin reduces feedback inhibition in area CA1 of the hippocampus

So far we found that serotonin, via presynaptic 5-HT_{1B} heteroreceptors, reduces glutamatergic synaptic transmission onto CCK-positive interneurons in a target- and input-specific manner. The target cell specificity is given by the observation that only the excitatory input onto CCK-positive interneurons is affected. The input specificity relies on the expression of presynaptic 5-HT_{1B} receptors that is restricted to CA1 pyramidal neurons. But what is the functional role of this highly specific neuromodulation in area CA1 of the hippocampus? To determine whether this effect of serotonin has consequences for the target cells of the affected CCK-positive interneurons, we asked if 5-HT is able to reduce inhibition in CA1 pyramidal cells. We recorded EPSC-IPSC sequences in CA1 pyramidal cells elicited by electrical stimulation in the alveus, where activation of CA1 pyramidal cell axons is most likely. Indeed, a significant reduction of the IPSC component to 70.9 ± 3% following the application of 10µM 5-HT (**Fig. 3.8A$_1$**) could be observed, whereas the EPSC component was not significantly altered (data not shown).

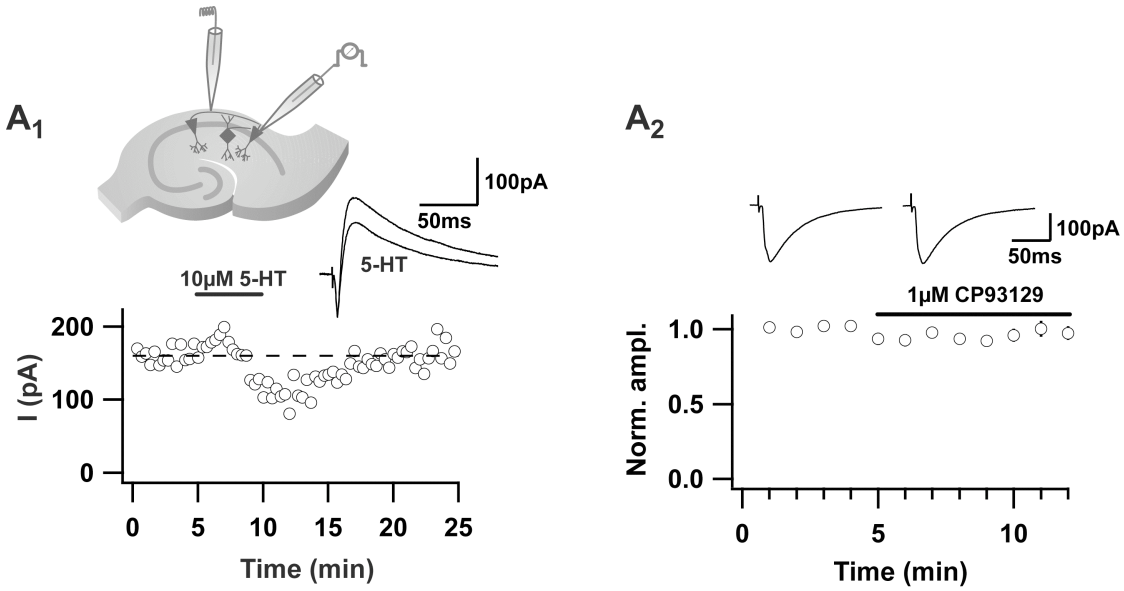

Fig.3.8A. 5-HT reduces feedback inhibition in CA1 pyramidal cells. (A1) Top, schematic of the recording configuration. Bottom, time course of IPSCs in an example cell (CA1 pyramidal neuron). Black bars indicate the application of 10µM 5-HT. Inset, example traces of EPSC-IPSC sequences stimulated in the alveus in control conditions and in 10µM 5-HT (scale bar: 100pA; 50ms). (A2) Top, example traces of monosynaptic IPSCs onto a CA1 pyramidal neuron, elicited in stratum radiatum in control conditions and in 1µM CP93129. Bottom, time course of the normalized and binned (1min) monosynaptic IPSCs (n=6). Black bar indicates the application of 0.5-1µM CP93129 (scale bar: 100pA; 50ms).

The reduction of the IPSC component might as well result from a direct activation of 5-HT_{1B} receptors on inhibitory synapses. To rule out this scenario we measured monosynaptic IPSCs in CA1 pyramidal cells elicited by electrical stimulation in stratum radiatum (in the presence of NBQX and D-AP5). However, we could not detect any effect of the 5-HT_{1B} receptor agonist CP93129 on stimulus induced IPSCs (**Fig. 3.8A$_2$**).

We have observed that disynaptic inhibition is reduced in CA1 pyramidal cells upon alveus stimulation (**Fig. 3.8A$_1$**) and that this reduction is not due to a direct effect of serotonin onto inhibitory fibers. Therefore, we hypothesized that activation of presynaptic 5-HT_{1B} receptors is sufficient to reduce the spike probability in serotonin-sensitive interneurons. In order to address this, we recorded from serotonin-sensitive interneurons, stimulated in the alveus and set the stimulation at an intensity generating a spike in about 60% of the stimuli in the recorded interneuron. After application of the 5-HT_{1B} receptor agonist CP93129 the spike probability was decreased to zero in all of the recorded interneurons (**Fig. 8C$_{1-3}$**, n=5; spike probability: 57.0 ± 0.09% under control vs. 0% in CP93129; $p<0.05$; 4 interneurons could be characterized and reconstructed as regular spiking basket cells, of which 3 were CCK-positive; one reconstruction failed).

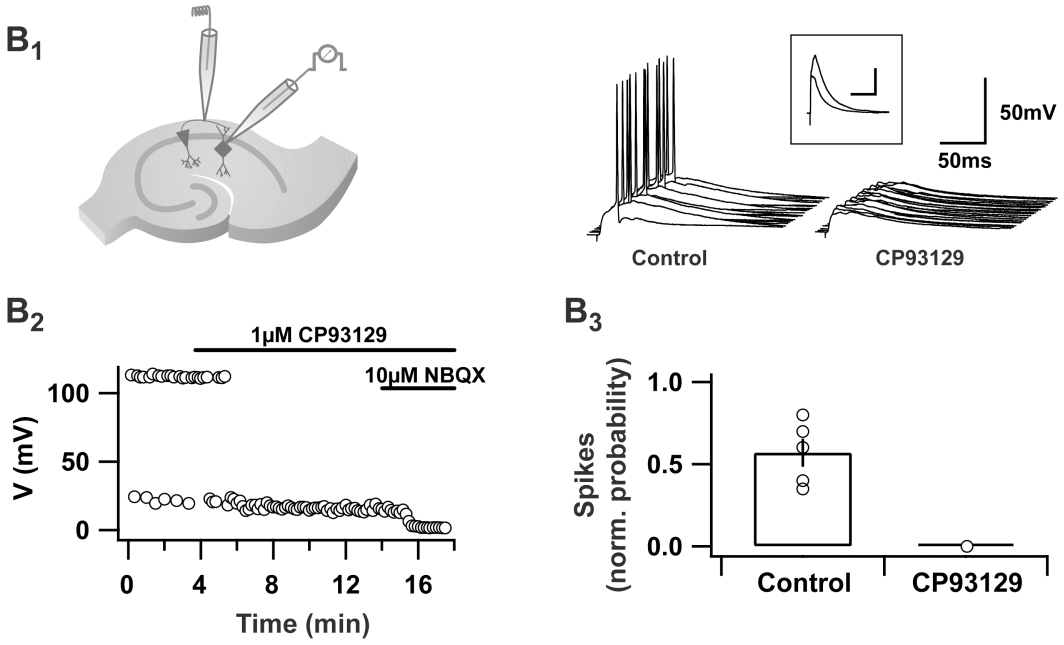

Fig.3.8B. Activation of 5-HT_{1B} reduces spike probability in serotonin sensitive interneurons. (B1) Left, schematic of the recording configuration. Right, example traces (single sweeps) of EPSPs stimulated in the alveus in control conditions and in 1µM CP93129 (scale bar: 50mV; 50ms). Inset, example traces of EPSPs without spikes (average of 6 sweeps) in control and in 1µM CP93129 (scale bar: 10mV; 200ms). (B2) Time

course of the same experiment. Black bar indicates the application of 1µM CP93129 and 10µM NBQX. (B3) Summary graph of the normalized spike probability in control conditions and in 1µM CP93129 (n=5, 4 interneurons could be reconstructed as regular spiking basket cells, of which 3 were CCK-positive, one reconstruction failed; spike probability in control conditions: 57.0 ± 0.09% vs. 0% in CP93129; $p<0.05$; paired t-test).

In agreement with this finding, the comparison of EPSPs without spikes in control and in CP93129 revealed a significant reduction in EPSP amplitude following drug application of CP93129 (**Fig. 8C$_1$** inset, n=5; EPSP amplitude in CP93129: 66.6 ± 7% of control; $p<0.05$; data not shown).

3.9 Serotonin broadens the integration time window for spike timing

The ability of CA1 pyramidal neurons to sum up independent inputs for spike generation relies on the timing of these inputs, i.e. only inputs that coincide in a relatively narrow time window of approximately two milliseconds can be integrated for spike generation (Pouille and Scanziani, 2001). This time window is determined by inhibition provided by perisomatically targeting interneurons: inputs that do not coincide fall under inhibitory control. As mentioned above, one type of perisomatically targeting interneurons are CCK-positive basket cells. They preferentially participate in feedback inhibition due to their long membrane time constant, which enables them to integrate independent inputs over a broad time window (Glickfeld and Scanziani, 2006). We have shown that the probability of spike generation in serotonin-sensitive interneurons is dramatically reduced by activation of the 5-HT_{1B} receptor. Consequently, feedback inhibition is substantially affected by 5-HT and by the 5-HT_{1B} receptor agonist CP93129. Therefore, we tested if serotonin can influence spike timing of CA1 pyramidal cells via modulation of CCK-positive basket cell recruitment.

We stimulated two independent pathways (alveus and Schaffer-collateral pathway) and measured the evoked EPSP-IPSP sequences in a CA1 pyramidal cell in current clamp mode. To determine the integration time window for spike generation, we set the stimulus intensity of the alveus and the Schaffer-collateral pathway at the threshold for spike generation, when the two pathways were stimulated simultaneously. By delaying the Schaffer-collateral stimulation in respect to the alveus stimulation, the probability of generating a spike at different inter-stimulus intervals was determined. Indeed, we observed a broadening of the integration time window for spike generation in CA1 pyramidal cells by application of the 5-

HT_{1B} receptor agonist CP93129 (**Fig. 3.9A**). At 5ms and 10ms inter-stimulus intervals, CP93129 increased the probability of spike generation from 16 ± 5.1% and 0% (under control conditions) to 70 ± 11.4% and 34 ± 9.8%, respectively, thereby demonstrating an effective expansion of the integration time window by 5-HT_{1B} receptor activation (**Fig. 3.9A$_3$**; n=5).

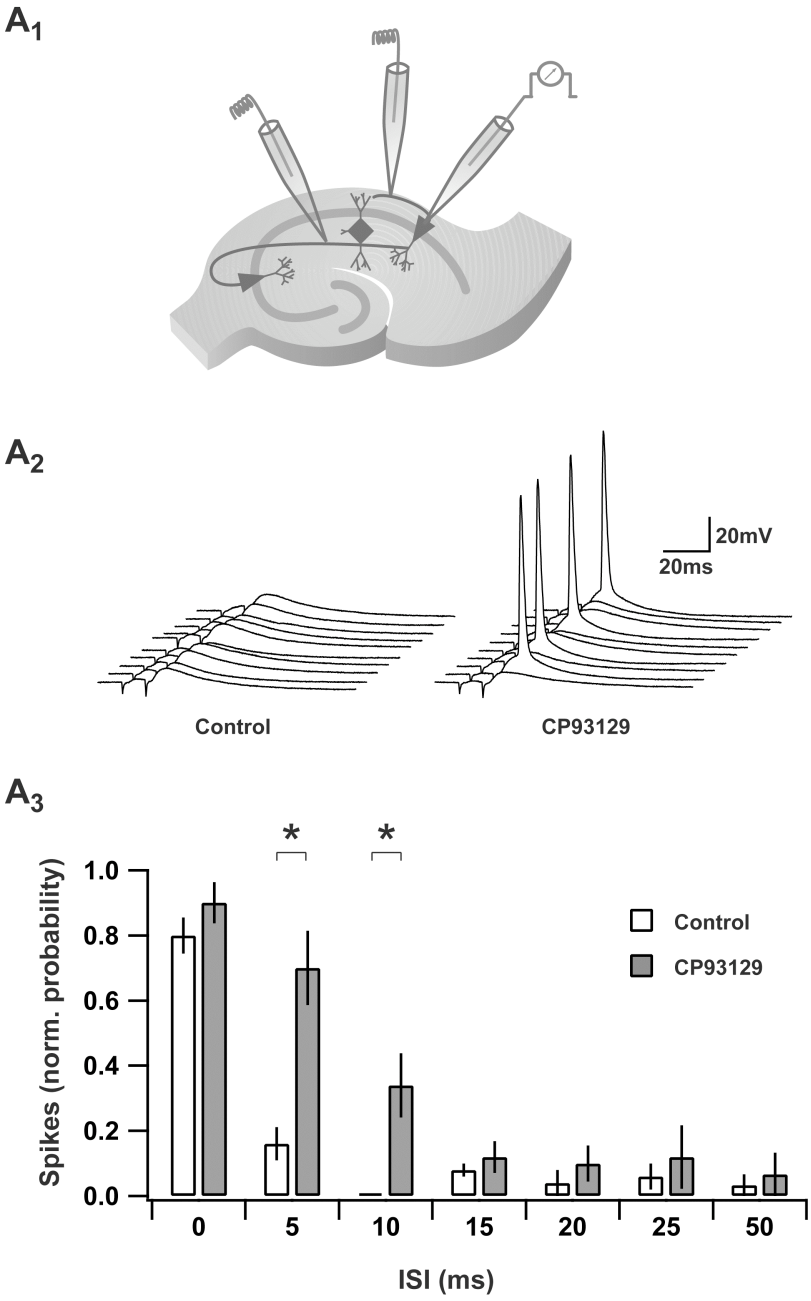

Fig.3.9A. CP93129 increases the integration time window for spike-timing in CA1 pyramidal cells. (A1) Schematic of the recording configuration. (A2) Example traces (single sweeps) of EPSP-IPSP sequences stimulated in the alveus followed by EPSP-IPSP sequences elicited by Schaffer-collateral stimulation in control and in 1µM CP93129 (inter-stimulus interval: isi: 10ms). (A3) Summary graph of the normalized spike probability at different inter-stimulus intervals (n=5; for isi: 5ms and isi: 10ms, $p<0.05$; paired *t*-test).

3.10 Serotonin reinforces hippocampal gamma activity *in vivo* and *in vitro*

During gamma oscillations, various types of interneurons contribute differentially to the temporal coordination of distinct subcellular domains of a single pyramidal cell (Klausberger and Somogyi, 2008). PV-positive basket cells fire phase-coupled to the ascending phase of extracellular gamma oscillations in the pyramidal cell layer and are thought to be involved in the generation of gamma frequency oscillations. In contrast, CCK-expressing interneurons fire earliest in the gamma cycle and CCK-positive basket cells display a highly asynchronous GABA release pattern upon gamma frequency bursts of action potentials, causing long lasting inhibition in pyramidal cell (Hefft and Jonas, 2005; Tukker et al., 2007; Klausberger and Somogyi, 2008). We hypothesized that a selectively impaired excitatory drive onto CCK-expressing interneurons following intraperitoneal (i.p) injection of the 5-HT_{1B} receptor agonist CP94253 may increase gamma oscillatory activity *in vivo*.

To identify the effects of CP94253 on oscillatory activity we carried out electroencephalogram (EEG) recordings from hippocampal area CA1 of freely moving mice. *In vivo* recordings during exploratory behavior showed two frequencies, theta waves and gamma oscillations (**Fig. 3.10A$_1$**), reflecting a nested theta-gamma rhythm associated with explorative behavior (Bragin et al., 1995). The analysis of the EEG revealed an increased gamma oscillatory activity from 158 ± 11μV^2/Hz under control conditions to 281 ± 47μV^2/Hz after i.p. injection of CP94253 (10mg/kg) (n=4, $p<0.05$, paired *t*-test, one-tailed, **Fig. 3.10A$_{1-3}$**), whereas theta activity was not affected significantly (n=4; $8.91x10^{-3}$ ± $2.34x10^{-3}mV^2$/Hz under control conditions vs. $7.75x10^{-3}$ ± $1.87x10^{-3}mV^2$/Hz after i.p. injection of CP94253; p=0.07, paired *t*-test, one-tailed; data not shown). As a control, we also performed experiments with i.p. injection of saline where we did not observe a significant alteration of gamma oscillatory activity (n=3, mean power under control: 157 ± 15μV^2/Hz vs. 165 ± 19μV^2/Hz after saline injection, p=0.17, paired *t*-test, one-tailed, **Fig. 3.10A$_3$).**

The *in vivo* and *in vitro* gamma recordings were performed in collaboration with the research group of Tengis Gloveli.

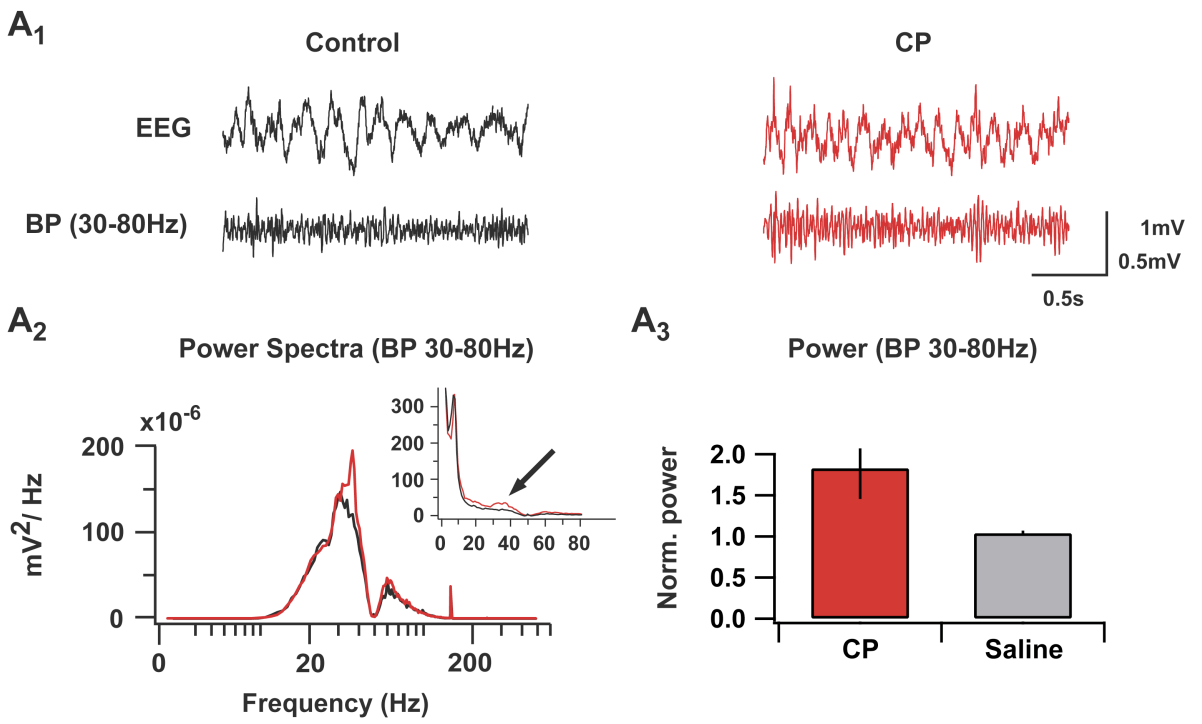

Fig.3.10A. Effects of the 5-HT$_{1B}$ receptor agonist CP94253 on hippocampal gamma oscillatory activity *in vivo*. (A1) Representative electroencephalogram recordings from area CA1 of the hippocampus before (left) and after (right) i.p. injection of CP94253. Top, typical theta activity in mice during exploratory behavior. Bottom, band-path filtered (30-80Hz) recordings unmask gamma-oscillatory activity. (A2, A3) Gamma frequency oscillations in hippocampal area CA1 have significantly higher power following i.p. injection of CP94253 (n=4). Inset in (A2): power spectra 1-1000Hz with pronounced theta and small gamma power (arrow).

Systemic administration of the 5-HT$_{1B}$ receptor agonist CP94253 most likely alters subcortical and cortical inputs to the hippocampus, thereby indirectly modulating hippocampal activity patterns. Thus, the observed effects on hippocampal gamma- oscillatory activity *in vivo* might be secondary to state transitions of the brain. In order to avoid this obstacle, we tested the effects of the 5-HT$_{1B}$ receptor agonist CP93129 on hippocampal gamma oscillations *in vitro* by performing extracellular recordings from acute transverse hippocampal slices, where gamma oscillatory activity was generated in area CA1 after bath application of low concentrations of kainate (KA, 200nM, n=10, **Fig. 3.10B$_1$**). The power spectrum of the oscillations showed a clear peak at the gamma frequency range (**Fig. 3.10B$_2$**). Bath application of CP93129 (1µM) significantly increased the power of gamma oscillatory activity from 485 ± 159µV^2/Hz to 1607 ± 431µV^2/Hz (n=10; $p<0.05$, paired *t*-test; **Fig. 3.10B$_3$**).

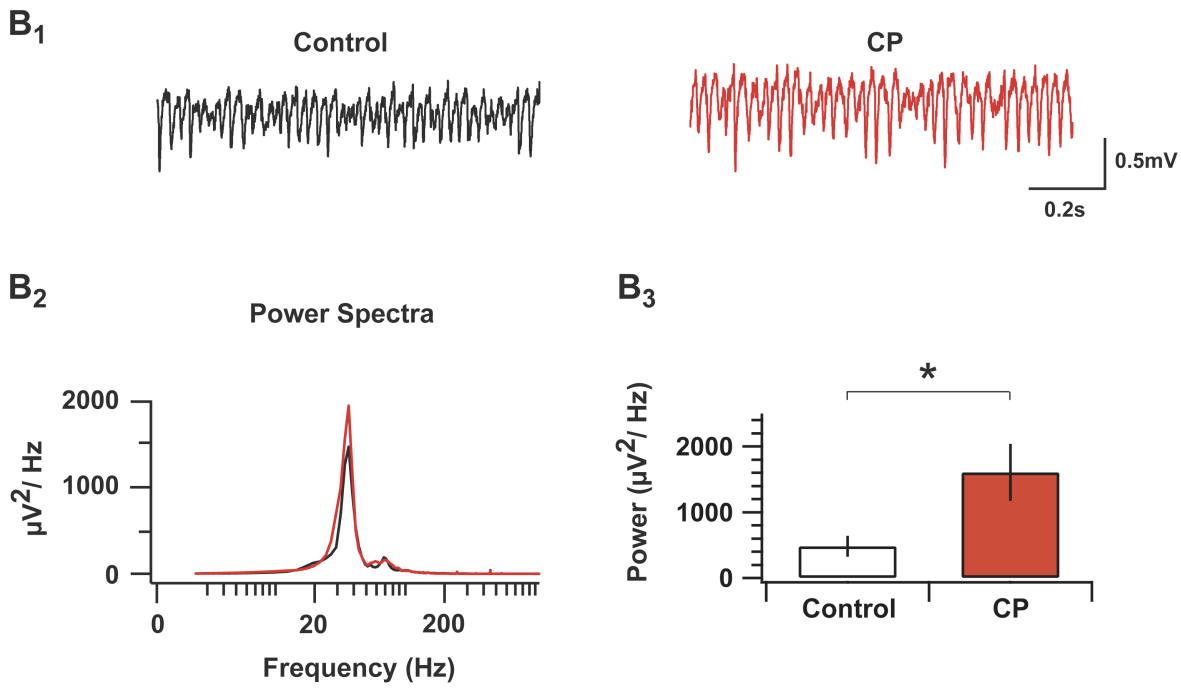

Fig.3.10B. Effects of CP93129 on network oscillations in area CA1 of the hippocampus *in vitro* in intact slices. (B1) Gamma frequency oscillations in area CA1 of the hippocampus after bath application of KA (200nM, left). CP93129 (1μM) significantly increases the power of the gamma oscillations (right). (B2) Corresponding power spectra. (B3) Network gamma oscillations in area CA1 display a significantly higher power following bath application of CP93129 (n=10).

In a more defined approach, we sought to assess the impact of 5-HT_{1B} receptor activation on gamma oscillatory activity in distinct hippocampal areas. We made use of hippocampal CA1 and CA3 mini slices and determined the effect of CP93129 on kainate-induced gamma oscillations (KA, 400nM). We observed a significantly increased power of gamma oscillatory activity from 128.7 ± 60.3μV^2/Hz to 197.1 ± 77.8μV^2/Hz in CA1 mini slices (n=7; $p<0.05$, paired *t*-test; **Fig 3.10C$_{1-5}$**).

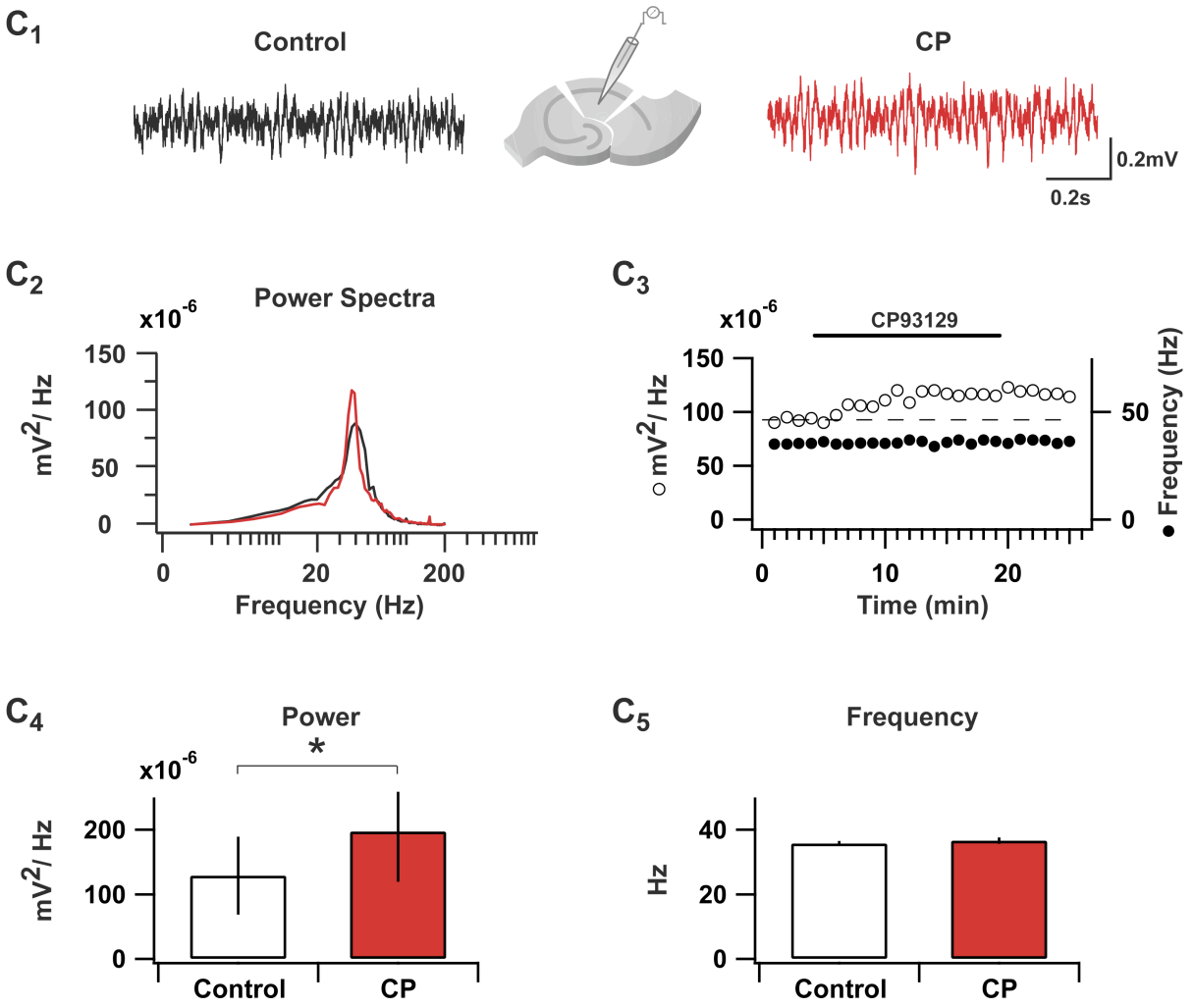

Fig.3.10C. Effects of CP93129 on network oscillations in CA1 mini slices of the hippocampus *in vitro*. (C1) Gamma frequency oscillations in area CA1 of the hippocampus after bath application of KA (200nM, left). CP93129 (1µM) significantly increases the power of the gamma oscillations (right). Recording configuration (middle). (C2) Corresponding power spectra. (C3) Example trace of time dependent, drug induced increase of gamma-power (open circles), but not frequency (filled circles). (C4) Network gamma oscillations in area CA1 display a significantly higher power following bath application of CP93129 (n=7). (C5) The 5-HT_{1B} receptor agonist does not affect the frequency of gamma oscillations (n=7).

In contrast, gamma power in CA3 mini slices was not affected significantly (control conditions: 175.8 ± 89.8µV^2/Hz vs. gamma power in 1µM CP93129: 152.6 ± 67.6µV^2/Hz; n=5; p=0.37; **Fig.3.10D$_{1,2}$**).

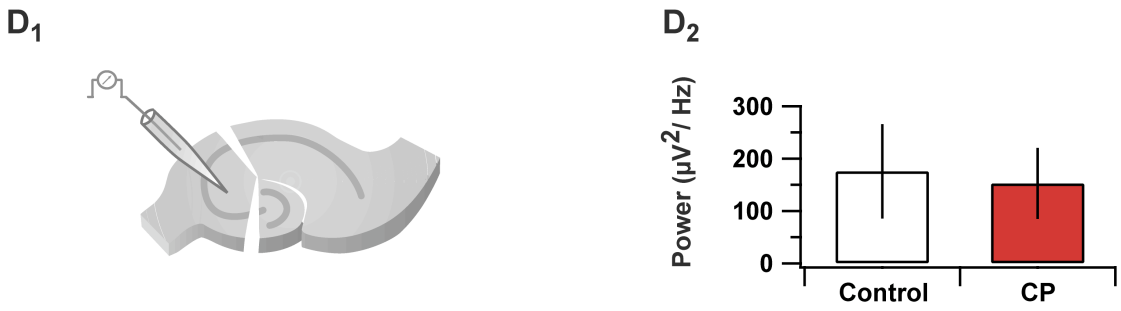

Fig.3.10D. Network gamma oscillations in hippocampal area CA3 are not affected by 1μM CP93129. (D1) Recording configuration. (D2) Summary graph of gamma oscillatory activity in mini slices of hippocampal area CA3 (n=5).

Our results demonstrate altered properties, specifically a reinforcement of gamma oscillatory activity in area CA1 of the hippocampus *in vivo* and *in vitro*, following the suppression of excitatory input to CCK-expressing interneurons by 5-HT_{1B} receptor activation.

4 Discussion

The work presented here describes a new and highly specific form of neuromodulation within hippocampal area CA1 by serotonin. We show that serotonin efficiently reduces glutamate release from hippocampal CA1 pyramidal cells onto CCK-positive interneurons via a presynaptic, 5-HT_{1B} receptor-dependent mechanism.

The following paragraphs will discuss the key findings of this work and address a number of questions regarding the functional consequences of the neuromodulatory effects of serotonin within area CA1 of the hippocampus: I will discuss (1) the presynaptic nature of the serotonin effect, (2) the 5-HT receptor subtype, mediating the serotonin effect and (3) the input as well as the target cell specificity of the serotonin effect. The finding that activation of 5-HT_{1B} heteroreceptors reduces the excitation of CCK-positive interneurons contrasts the reports of a direct excitatory effect of serotonin at this cell type via ionotropic 5-HT_3 receptors. I will thus also try to form a concept on (4) a possible functional role of the coexistence of both effects. Furthermore, I would like to discuss the results that (5) the serotonergic modulation of interneurons is transmitted to the output elements of the hippocampus: i.e. CA1 pyramidal neurons and finally that (6) serotonin modulates gamma oscillatory activity in hippocampal area CA1 *in vivo* and *in vitro*.

4.1 Presynaptic modulation

The presynaptic mode of action is supported by several findings: first, 5-HT application led to a reduction in EPSC amplitude while increasing the paired-pulse ratio. In a quantitative description, the postsynaptic signal depends on three parameters: the total number of vesicles ready to be released (n), their release probability (p), and the quantal content (q), where (n) and (p) are presynaptic parameters and (q) is postsynaptic (Zucker and Regehr, 2002). Synapses with a high probability of release tend to express short term depression while synapses with a low probability of release usually display short term facilitation (Dobrunz and Stevens, 1997). An increase in paired pulse ratio as observed upon the application of

serotonin is therefore indicative of a decrease in the probability of transmitter release and thus points to a presynaptic mode of action. Second, analysis of the coefficient of variation (CV) applied to multi-afferent and minimal stimulation revealed a presynaptic locus of serotonin action. Here, a simple binomial model of a synaptic connection between two neurons predicts that the CV of the EPSC amplitude is strongly dependent on the presynaptic parameters number of release sites (n) and probability of release (p) and mostly independent of the postsynaptic quantal size (q). An unchanged CV while synaptic efficacy is altered is thought to reflect a postsynaptic modulation, whereas a presynaptic change would result in an altered CV (Faber and Korn, 1991). As the changes in CV scaled linearly with the change of the mean response upon application of 5-HT a presynaptic modulation is most likely. Third, the observed increase of synaptic failures upon serotonin application is again thought to reflect a decrease in the probability of transmitter release. Finally, keeping the amount of released glutamate nominally constant and independent of the presynaptic release machinery by means of photolysis of caged glutamate we could additionally show that 5-HT did not alter glutamate-evoked currents. Notably, in the very same recordings stimulus-induced EPSCs were reduced in amplitude. In summary, none of the above-described experiments per se verifies a presynaptic mode of action for serotonin, but taken together, cumulative evidence clearly points to a presynaptic target that imparts the reduction of EPSC amplitudes in CCK-positive interneurons.

4.2 5-HT receptor subtype

The receptor most likely mediating the presynaptic depression of glutamatergic transmission is of the 5-HT_{1B} receptor subtype, as revealed by multiple pharmacological tools. Firstly, the potent and selective 5-HT_{1B} receptor agonist CP93129 mimicked the action of serotonin. In contrast, activation of the highly abundant 5-HT_{1A} receptor with the potent and selective agonist 8-OH DPAT had no effect on serotonin-sensitive interneurons. Secondly, the reduction of the EPSC amplitudes by serotonin could be prevented by co-application of the selective 5-HT_{1B} receptor antagonist GR127359. Additionally, we could show that the selective 5-HT_{1B} receptor antagonist GR127359 was able to impede the effects of endogenously released serotonin induced by the application of fenfluramine as well. 5-HT_{1B} receptors are coupled to inhibitory G proteins (G_i/G_o) and are thought to inhibit calcium channels via the G_o subunit $G\beta\gamma$. They act as auto- or heteroreceptors at serotonergic and non-

serotonergic neurons, respectively. In the hippocampus, activation of 5-HT_{1B} autoreceptors has been reported to inhibit 5-HT release, whereas 5-HT_{1B} heteroreceptors, predominantly localized to axon terminals (Boschert et al., 1994), control acetylcholine release in the septo-hippocampal pathway and modulate glutamatergic transmission in the subiculum (for review see Sari, 2004).

4.3 Input and target cell specificity

We provide evidence that the modulation of synaptic transmission on CCK-positive interneurons by serotonin is input-specific. In agreement with the patterns of 5-HT_{1B} receptor m-RNA expression in the hippocampus (Voigt et al., 1991; Svenningsson et al., 2006), serotonergic depression of synaptic transmission was restricted to the glutamatergic input derived from CA1 pyramidal cells. Neither local GABAergic synaptic transmission, nor glutamatergic synaptic transmission arising from CA3 pyramidal cells was affected by activation of the 5-HT_{1B} receptor as shown by extracellular electrical stimulation and by means of glutamate uncaging and the consecutive focal activation of presynaptic pyramidal neurons, respectively. In addition, this set of experiments supports the suggested target cell specificity of serotonin action. In our first attempt to classify the interneuron subtype being sensitive to serotonin application we found that all interneurons that displayed a reduction in EPSC amplitude after serotonin application were CCK-positive interneurons. But not all CCK-positive interneurons were serotonin sensitive. By means of focal uncaging of glutamate on CA1 pyramidal cell bodies we observed again that serotonin efficiently and selectively reduced glutamate release onto CCK-positive interneurons, *i.e.* Schaffer-collateral associated interneurons and basket cells. But contrary to the approach with extracellular electrical stimulation, now all recorded CCK-positive interneurons were sensitive to the 5-HT_{1B} receptor agonist CP93129. This suggests that the observation of a few serotonin-insensitive CCK-positive cells (see above) can be attributed to insufficient activation of CA1 fibers by electrical stimulation. In contrast, PV-positive basket cells were never affected by serotonin or CP93129 application, neither in the experiments with electrical stimulation, nor in the experiments where glutamate was uncaged on pyramidal cell bodies in area CA1. Therefore, we conclude that the effect of the presynaptic 5-HT_{1B} receptor activation in area CA1 of the hippocampus is input and target cell specific. The latter finding is intriguing, as CCK-positive interneurons could be contacted specifically by a subclass of CA1 pyramidal cells expressing

the 5-HT_{1B} receptor. Or, within the same axonal plexus of a single CA1 pyramidal cell, the expression of 5-HT_{1B} receptors could be restricted to terminals impinging exclusively onto CCK-positive interneurons. A target cell specific expression of the receptor at distinct terminals of the axonal plexus of a single CA1 pyramidal cell seems to be more likely, as there is no obvious mosaic-like expression of 5-HT_{1B} receptor m-RNA in the hippocampus (Voigt et al., 1991; Svenningsson et al., 2006), as would be expected if the expression of the 5-HT_{1B} receptor is specific for a subclass of CA1 pyramidal cells. Moreover, there are examples in the literature where a target-specific specialization of release properties within the same axonal arborization is described (Markram et al., 1998; Reyes et al., 1998; Pelkey and McBain, 2007). The difference of serotonergic action between the two major classes of perisomatic inhibitory cells, namely fast spiking, PV-positive basket cells and regular spiking, CCK-positive basket cells, adds a further argument to the concept of a functional dichotomy between these two types of perisomatic inhibitory interneurons in the hippocampus (Freund and Katona, 2007).

4.4 Serotonin in the perspective of a CCK-positive cell: reducing excitation or exciting?

The finding of a highly selective reduction of glutamate release from hippocampal CA1 pyramidal cells onto CCK-positive interneurons via presynaptic 5-HT_{1B} receptors contrasts the excitatory effect of serotonin at specific GABAergic interneurons in the hippocampus via ionotropic 5-HT_3 receptors (McMahon and Kauer, 1997). Although in this study a detailed classification of the interneurons was not accomplished, it is likely that the interneurons affected by serotonin application were CCK-positive as well. Furthermore, recently it has been shown that the selective activation of optogenetically labeled serotonergic fibers originating in the median raphe nuclei resulted in a fast synaptic activation of a subgroup of hippocampal interneurons, among them CCK-positive interneurons (Varga et al., 2009). This fast synaptic excitation is mediated by glutamate/serotonin cotransmission, as shown by its sensitivity to ionotropic glutamate receptor and 5-HT_3 receptor antagonism. How can this obvious discrepancy of modulatory effects mediated by serotonin, being excitatory at a specific interneuron subtype while on the other hand reducing excitation onto the same cell type, be reconciled in the light of the present work?

We found that serotonin concentrations as low as 0.3µM had significant effects on glutamatergic transmission onto CCK-positive interneurons, while the intrinsic properties of the targeted interneurons remained unaffected at this dosage. In other words, the presumable activation of the excitatory ionotropic 5-HT_3 receptors expressed by CCK-positive interneurons required higher serotonin concentrations than the reduction of glutamatergic drive onto these interneurons. This concentration dependency leads to the question of how much serotonin is actually released upon raphe-hippocampal fiber activation. Two indirect approaches might give further insight: Firstly, we provide evidence that endogenously released serotonin induced by the application of fenfluramine is able to mimic the specific effect of exogenously applied serotonin. These experiments highlight the fact that releasing serotonin by means of a reversal of the serotonin transporter expressed on serotonergic fibers is sufficient to activate heterosynaptic 5-HT_{1B} receptors. Secondly, it has been shown that in a slice co-preparation of the inferior olive and the brain stem the concentration of endogenously released serotonin, after stimulation of serotonergic brain stem nuclei, could be estimated to be around 0.5µM (Best and Regehr, 2008). Although the stimulated serotonergic neurons of the nucleus reticularis paragigantocellularis do not project to the hippocampus and therefore assumptions for the hippocampal projections have to be made with caution, the amount of serotonin released is comparable to the concentration range within which we could only detect significant effects on glutamate release from hippocampal CA1 pyramidal cells. Our findings suggest that the reduction of glutamatergic transmission is a distinctive mechanism for the regulation of GABAergic action by serotonin and that the amount of serotonin that is released determines the effect of serotonin onto CCK-positive interneurons.

Another possible scenario could be the actual coexistence of both effects. This is supported by the observation that activation of optogenetically labeled serotonergic fibers originating in the median raphe nuclei (MnR) is sufficient to induce action potentials in a subpopulation of hippocampal interneurons with a relatively high probability of ~ 30% (Varga et al., 2009). MnR fibers project to the hippocampus where they terminate in dense bands in the infragranular zone of the dentate gyrus, in stratum radiatum of area CA3 and in stratum lacunosum moleculare of area CA1 (Vertes et al., 1999). Anatomical evidence shows that some of the MnR fibers form classical chemical synapses onto CCK-positive and/or calbindin-positive interneurons expressing the ionotropic 5-HT_3 receptor (Freund et al., 1990; Freund, 2003). Activation of heterosynaptic 5-HT_{1B} receptors expressed on glutamatergic

fibers targeting CCK-positive interneurons might therefore be accomplished by MnR fibers that do not form synaptic contacts with CCK-positive interneurons, releasing serotonin by volume transmission. And/or, the activation of heterosynaptic 5-HT_{1B} receptors might result from fibers that do form synapses onto their targets. The latter possibility would imply synaptic spillover of released serotonin onto heterosynaptic 5-HT_{1B} receptors. At least in this respect it seems to be unlikely that the amount of serotonin released by the activation of these specific serotonergic fibers is as low to activate only heterosynaptic 5-HT_{1B} receptors while leaving synaptically localized 5-HT_3 receptors unaffected.

We have shown that the activation of 5-HT_{1B} receptors caused a highly efficient reduction of spike probability in CCK-positive interneurons. In case of an actual coexistence of both serotonin effects this would imply that CCK-positive interneurons are disengaged from the local hippocampal microcircuit through activation of heterosynaptic 5-HT_{1B} receptors while at the same time being driven by a subcortical neuromodulatory center through MnR fibers via the activation of ionotropic 5-HT_3 and glutamate receptors. It has been suggested that CCK-positive basket cells are preferentially driven in a feedback manner (Glickfeld and Scanziani, 2006; Glickfeld et al., 2008). In this respect, the input specific expression of the 5-HT_{1B} receptor on CA1 pyramidal cell terminals seems to control a critical input relay for the excitation of CCK-positive basket cells driven by the local hippocampal circuitry.

But coming back to the question whether the amount of serotonin released determines the effect on CCK-positive interneurons. Up to now, we disregarded the second raphe-hippocampal fiber tract in our discussion. Serotonergic fibers originating in the dorsal raphe nuclei (DnR) project to the hippocampus as well, and the axonal pattern of DnR fibers in the hippocampus is somewhat different from MnR fibers. They spread fairly uniformly throughout the hippocampal formation and do not show associated postsynaptic specializations (Vertes, 1991). Hence it remains to be determined whether DnR fiber activation can lead to a concentration-dependent, selective targeting of heterosynaptic 5-HT_{1B} receptors, too.

4.5 Impact on CA1 pyramidal neurons

We could show that the reduction of excitation of this inhibitory circuit is effectively transmitted to the output elements, i.e. pyramidal cells in the hippocampal circuit. While

direct, monosynaptic inhibition onto CA1 pyramidal neurons was not affected by application of the 5-HT_{1B} receptor agonist CP93129, we observed a significant reduction of disynaptic inhibition in CA1 pyramidal cells after application of 5-HT upon alveus stimulation. Thereby the reduction of excitatory drive onto CCK-positive interneurons must be strong enough to prevent the generation of action potentials in these cells and consequently reducing the amount of released GABA. Corroborating this and as mentioned above, we could demonstrate a highly effective reduction of the spike probability in serotonin-sensitive interneurons following the application of the 5-HT_{1B} receptor agonist CP93129. The latter experiments were stimulated in the alveus, where activation of CA1 axons is most likely. In terms of the local CA1 microcircuitry this recording configuration as well as the above mentioned patterns of 5-HT_{1B} receptor m-RNA expression in the hippocampus implies a selective effect of serotonin on feedback inhibition.

The reduction of feedback inhibition has functional implications for the integration time window of CA1 pyramidal cells. It has been shown that the narrow time window for two independent inputs to generate a spike in CA1 pyramidal cells is provided by perisomatically targeting interneurons rather than by interneurons that impinge on the apical dendrites (Pouille and Scanziani, 2001). In another set of experiments we could provide evidence that altering the recruitment of perisomatically targeting CCK-positive basket cells upon activation of 5-HT_{1B} receptors effectively increased the time window for spike generation and consequently enabled CA1 pyramidal cells to integrate independent inputs over broader time windows.

4.6 A functional readout for network activity: gamma oscillations

The activation of 5-HT_{1B} receptors and the subsequent reduction of excitatory drive onto CCK-expressing interneurons interfere with gamma synchronicity in area CA1 of the hippocampus *in vivo* and *in vitro*. Although it is generally accepted that fast spiking, PV-positive basket cells are essential for the generation of gamma rhythmic activity in multiple brain regions, a clear picture for the other basket cell type and in general for the role of CCK-positive interneurons in gamma oscillations has not yet been evolved. The lower abundance of CCK-positive basket cells compared to PV-positive basket cells might be a reason for this, as well as the observation that CCK-positive interneurons are coupled to *in vivo* gamma

oscillations in hippocampal area CA1 at a lower rate than PV-positive interneurons. We observed a reinforcement of gamma oscillatory activity upon a target- and input-specific neuromodulation of CCK-positive interneurons by serotonin. Different scenarios could account for this finding. First, if CCK-expressing interneurons are coupled to gamma oscillations, they fire earlier than pyramidal cells during the gamma cycle (Tukker et al., 2007). Therefore their participation in gamma-oscillatory activity might interfere with gamma synchronicity as a result of inhibition of pyramidal cells. Another feature of CCK-positive basket cells is a looser coupling of the Ca^{2+} source and the release machinery causing a highly asynchronous release of GABA with a long lasting inhibition as shown for CCK-positive basket cells in the dentate gyrus as well as in area CA1 of the hippocampus (Hefft and Jonas, 2005; Daw et al., 2009). Asynchronous release of GABA mediated by CCK-positive basket cells might as well result in a decreased gamma synchronicity. Finally, pyramidal cells show a more variable latency to the onset of the unitary IPSCs after presynaptic action potentials in CCK-positive basket cells, which might again interfere with gamma synchronicity (Neu et al., 2006). In each of these scenarios, the activation of 5-HT_{1B} receptors, the subsequent reduction of the excitatory drive onto CCK-expressing interneurons and the consequent reduction in feedback inhibition could lead to a significant reinforcement of gamma oscillatory activity.

At a first glance, this finding contrasts the effect of endocannabinoids, known to reduce GABA release from synaptic terminals of CCK-positive interneurons. Endocannabinoids have been shown to reduce gamma activity *in vitro* (Hájos et al., 2000). But as CB1 receptors that mediate the effect of endocannabinoids are not exclusively expressed on axonal terminals of CCK-positive interneurons and have been shown to modulate glutamatergic as well, the sole effect of a reduced inhibition from CCK-positive interneurons on gamma activity is hard to predict (Holderith et al., 2011).

In several studies, the role of serotonin on gamma oscillatory activity has been studied. Recently, it has been shown that serotonin reuptake inhibitors targeting the serotonin transporter on serotonergic terminals had no effect on gamma oscillations. In contrast, the application of serotonin could reduce kainate-induced gamma oscillations in hippocampal area CA1 (Wójtowicz et al., 2009). The differential effect of serotonin and of the 5-HT_{1B} receptor agonist CP93129 on gamma oscillations in hippocampal area CA1 could derive from the unspecific activation of 5-HT_{1A} receptors highly expressed in CA1 pyramidal neurons by serotonin. The resulting hyperpolarization of CA1 pyramidal neurons might explain the

reduction of gamma power and, as the serotonin concentration in this study was 30µM, excitatory effects of serotonin on CCK-positive interneurons and therefore opposite effects cannot be excluded. Interestingly, the latter point again highlights the concentration dependency of serotonin effects discussed above and would argue for a concentration-dependent, distinctive role of serotonin at CCK-positive interneurons in the hippocampus.

Taken together, this arrangement of serotonergic modulation of specific GABAergic action may constitute a key mechanism for subcortical control of hippocampal output.

In future studies, I would like to address the role of CCK-positive interneurons in gamma oscillatory activity. Although fewer in number than PV-positive basket cells, their target and input specific modulation by presynaptic 5-HT_{1B} receptors that results in a reinforcement of gamma oscillatory activity is indicative of an opposite role in comparison to PV-positive basket cells. By means of combining standard electrophysiological approaches with optogenetic manipulation of genetically targeted interneuron subtypes (Taniguchi et al., 2011), we hope to be able to decipher the role of CCK positive interneurons in gamma oscillatory activity and thus in higher brain functions.

5 Appendix

5.1 References

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5.2 Frequently used abbreviations

A	
AMPA	α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (specific agonist for subgroup of ionotropic glutamate receptors)
AP	action potential
B	
C	
Ca^{2+}	calcium
cAMP	cyclic adenosine monophosphate
CCK	cholecystokinin
CNS	central nervous system
D	
E	
EC	entorhinal cortex
EGTA	ethylene gylcol-bis(β -aminoethyl ether)-N,N,N',N'-tetraacetic acid
EPSC	excitatory postsynaptic current
F	
5-HT	5-hydroxytryptamine, serotonin
G	
GABA	gamma-aminobutyric acid
$GABA_AR$	GABA receptor (subtype A; ionotropic)
$GABA_BR$	GABA receptor (subtype B; metabotropic)
GIRK	G-protein-activated inwardly rectifying potassium (K^+) channel
GPCR	G-protein coupled receptor
G-protein	Guanine nucleotide-binding protein
GTP	guanosine triphosphate
H	
I	
K	
L	
LSD	d-lysergic acid diethylamid
LTD	long-term depression
LTP	long-term potentiation
M	
mGluR	metabotropic glutamate receptor
N	
NMDA	N-methyl-D-aspartic acid (specific agonist for subgroup of ionotropic glutamate receptors)
n.s.	not significant
P	
PV	Parvalbumin
PBS	phosphate buffered saline
PLC	Phospholipase C
R	
RT	room temperature
S	
T	
V	
VDCCs	voltage-dependent Ca^{2+} channels

5.3 Statement of contribution

The experiments described in this thesis resulted from a collaborative scientific project. In the following I state the respective contribution to the data presented:

Chapter 3.3: Differential modulation of basket cell types by serotonin.

> Dr. Csaba Földy (AG Ivan Soltesz, Department of Anatomy & Neurobiology, University of California, Irvine, CA, USA) contributed to the classification of interneurons sensitive to serotonin application described in this chapter. Dr Tamar Dugladze (AG Tengis Gloveli, Institute of Neurophysiology, Neuroscience Research Center at the Charité-Universitätsmedizin Berlin) performed the experiments for the characterization of serotonin sensitive interneurons in mice described in this chapter.

Chapter 3.6: Fenfluramine mimics the effect of bath-applied 5-HT.

> Dr. Aleksandar Zivcovic (AG Dietmar Schmitz, Neuroscience Research Center at the Charité-Universitätsmedizin Berlin) contributed to the characterization of the effect of bath-applied fenfluramine on fast spiking and regular spiking basket cells described in this chapter.

Chapter 3.8: Serotonin reduces feedback inhibition in area CA1 of the hippocampus.

> Vanessa Stempel (AG Dietmar Schmitz, Neuroscience Research Center at the Charité-Universitätsmedizin Berlin) contributed to the characterization of the spike probability in serotonin sensitive interneurons described in this chapter.

Chapter 3.10: Serotonin reinforces hippocampal gamma activity *in vivo* and *in vitro*.

> Nino Maziashvilli and Dr. Tamar Dugladze (AG Tengis Gloveli, Institute of Neurophysiology, Neuroscience Research Center at the Charité-Universitätsmedizin Berlin) performed the *in vitro* and *in vivo* recordings of gamma oscillatory activity described in this chapter.

I performed all other experiments.

5.4 Acknowledgments

The work for this thesis was supported and influenced by a number of people. First and foremost I want to thank Dietmar Schmitz. He gave me the opportunity to work in his research group and most importantly initiated our interneuron-serotonin project. His scientific enthusiasm, his deep insight and knowledge of the literature and his experimental experience and creativity have always been inspiring.

I'm grateful to the members of the "Schmitzlab"; it is a great lab with an extraordinary working atmosphere offering friendly and lively discussions. In particular I want to thank Vanessa Stempel and Christian Wozny for critically reading this thesis.

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I want to thank Ivan Soltesz for inviting me to visit his lab in Irvine. It turned out to be two intensive weeks of work resulting in a deeper insight into the electrophysiological and anatomical characteristics of interneurons.

Finally, I want to thank my family – my parents, my brother with his family, my cousin– and most of all Gamze, for their support, trust and love.

5.5 Curriculum Vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

5.6 Publications

Böhm C, Pangalos M, Schmitz D*, Winterer J* Serotonergic modulation of oriens-lacunosum moleculare interneurons in hippocampal CA1. In preparation.

Maier N, Tejero-Cantero A, Dorrn AL, Winterer J, Beed PS, Morris G, Kempter R, Poulet JFA, Leibold C, Schmitz D (2011) Coherent Phasic Excitation during Hippocampal Ripples. Neuron 72:137–152

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Schaefer M, Winterer J, Sarkar R, Uebelhack R, Franke L, Heinz A, Friebe A (2008) Three cases of successful tryptophan add-on or monotherapy of hepatitis C and IFNalpha-associated mood disorders. Psychosomatics 49:442–446

Zhang X-L, Zhou Z-Y, Winterer J, Müller W, Stanton PK (2006) NMDA-dependent, but not group I metabotropic glutamate receptor-dependent, long-term depression at Schaffer collateral-CA1 synapses is associated with long-term reduction of release from the rapidly recycling presynaptic vesicle pool. J. Neurosci. 26:10270–10280

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Alix P, Winterer J, Müller W (2003) New illumination technique for IR-video guided patch-clamp recording from neurons in slice cultures on biomembrane. J. Neurosci. Methods 128:79–84

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5.7 Erklärung an Eides statt

Hiermit erkläre ich, Jochen Winterer, geboren am 13.04.1970 in St. Georgen im Schwarzwald, dass ich die vorgelegte Dissertation mit dem Thema: *Cell-Type-Specific Modulation of a Hippocampal Microcircuit by Serotonin* selbst und ohne die (unzulässige) Hilfe Dritter verfasst, dass ich keine anderen als die angegebenen Quellen und Hilfsmittel benutzt und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe.

Berlin, den 29.02.2012

Jochen Winterer