Functional interactions between interneurons and the pyramidal cell population in the hippocampal CA1 area

Wierenga, C.J.

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Chapter 1

Introduction
The mammalian central nervous system consists of millions of neurons with even more connections between them. About 90% of the neurons in the brain are excitatory neurons, the rest are inhibitory interneurons. Neurons have highly complex dendritic trees at which thousands of synaptic inputs arrive. Individual neurons can perform complicated computations, but the essential computational units in the brain are small networks of neurons. Synaptic connections between neurons in these networks are not static, but their strength depends on the activation history of the connection. The identity and activity of the pre- and postsynaptic neurons determine the precise properties of a synapse, making each synapse in the brain unique. The brain comprises many interconnected small networks of sophisticated neurons with dynamic synaptic connections. This unique structure makes it the wonderful machine that can invent, remember, love, appreciate music and has a sense of humor.

**THE HIPPOCAMPAL CA1 NETWORK**

The hippocampus is a brain area that is involved in memory processing, probably contributing to the transition from short-term memories to long-lasting memories. Its role in memory is proposed to be most important in episodic memory (Eichenbaum et al. 1999; Wallenstein et al. 1998).

The hippocampal network is usually described as consisting of three main networks that are interconnected: dentate gyrus (DG), CA3 and CA1 (fig. 1.1). The main information flow is from higher cortical areas, such as the entorhinal cortex, to DG and then via CA3 to CA1. The CA1 area projects back to cortical areas. This simple loop is a highly simplified picture as precise anatomical and physiological studies have shown a much more complicated connectivity (Witter et al. 2000). In this thesis I will only consider the CA1 area.

The principal neurons of the CA1 area are pyramidal neurons. This type of neuron is very common in the central nervous system and is the principal neuron in most cortical areas. The somata of the pyramidal cells in the CA1 area are located in the stratum pyramidale and their vertically oriented apical dendrites run more or less parallel to each other into stratum radiatum and lacunosum–molecular (fig. 1.2). Their basal dendrites ramify in stratum oriens and alveus. An important input of the pyramidal cells is the input from the CA3 area, via the Schaffer collaterals. These collaterals make synapses predominantly on apical dendrites in stratum radiatum. Another important input to pyramidal cells is coming from the perforant path. These fibers come directly from the entorhinal cortex, without the DG–CA3
loop. Perforant path synapses are located at the distal dendrites of the pyramidal cells in stratum lacunosum–moleculare.

Figure 1.1. The hippocampal network
Transverse slice of the rat hippocampus. Input from the entorhinal cortex or other cortical areas arrives at the granule cells in the dentate gyrus (DG). These cells transmit the information to the pyramidal cells in the CA3 area (CA3), which project to the CA1 area (CA1). The axons of the CA1 pyramidal cells project back to entorhinal cortex and other brain areas.

Many other input pathways to the CA1 area exist, for instance from the contralateral hippocampus and from the septum. Furthermore, the CA1 network is innervated by fibers from other brain areas containing neurotransmitters such as serotonin, dopamine, noradrenaline or acetylcholine. Many of these modulatory inputs have a differential effect on Schaffer and perforant path inputs and can provide a mechanism by which one specific input pathway can be selected over the other (Otmakhova and Lisman 1998). This might be important for the role of the hippocampus in memory processes (see chapter 5).

In the CA1 area about 10% of the cells are inhibitory interneurons. Their somata are located in all layers of the CA1 area and their dendritic trees show a large variation in size and shape. Inhibitory synapses are located all over the pyramidal cell surface and originate from many different interneurons. With respect to a specific input pathway to the CA1 pyramidal population interneurons can be coupled in a feedforward or a feedback manner. In figure 1.2 these two couplings are shown for Schaffer collateral activation of the CA1 network. Feedforward input to the interneurons is input coming directly (monosynaptically) from the Schaffer collaterals (Buzsáki and Eidelberg 1982; Lacaille 1991; Sah et al. 1990).
Interneurons receiving feedback input get input from CA1 pyramidal cells that are activated by the Schaffer collaterals (Andersen et al. 1963; Andersen et al. 1964; Kandel et al. 1961).

**INTERNEURONS**

Interneurons form a highly heterogeneous group of cells and many attempts have been undertaken to distinguish different groups of interneurons with specific properties (for review see Freund and Buzsáki 1996). However, this turned out to be complicated, as different classifications are not in agreement with each other (Parra et al. 1998). I will not try to give a complete overview of the different interneurons and their possible classifications, but I will briefly describe the two most commonly used classifications and some of their merits. Other classifications are made on the basis of the firing properties of interneurons (fast spiking, regular spiking, burst spiking, etc.) (Gupta et al. 2000) or the presence of specific neurotransmitter receptors and/or subunits (metabotropic glutamate receptors, acetylcholine receptors, opiate receptors, etc.) (Hájos et al. 1998; Poncer et al. 2000; van Hooft et al. 2000).

**Classification of interneurons**

The most common classification is based on axon projection. The main interneuron types of this classification are basket cells, bistratified and axo–axonic cells. Basket cells make synapses preferentially on the soma and proximal dendrites of their target cells, bistratified cells make synapses onto dendrites slightly more distal and axo–axonic cells innervate the axon hillock (Buhl et al. 1994a). All these interneurons innervate mostly pyramidal cells, but interneurons are not avoided as targets. This classification is physiologically meaningful since somatic inhibition (provided by basket and axo-axonic cells) can block action potential firing by the postsynaptic cell, whereas dendritic inhibition (provided by bistratified cells) may function to prevent dendritic calcium spikes (Miles et al. 1996). Many subclassifications exist in which different interneuron subtypes are distinguished based on dendritic morphology or firing patterns. However, for these subclassifications it is hard to obtain stringent definitions of the specific subtypes.
Figure 1.2. Schematic view of the CA1 network

CA1 pyramidal cells (P) have somata in stratum pyramidale (pyr). They receive input from the Schaffer collaterals in stratum radiatum (rad). Perforant path afferents make synapses in stratum lacunosum–moleculare (LM). Upon activation of the Schaffer collaterals, interneurons (In) can receive monosynaptic input (feedforward input). Alternatively, Schaffer inputs activate pyramidal cells, which evoke synaptic input in the interneurons (feedback input) via their axons running in the Alveus (Alv) and stratum oriens (or). The way an interneuron is coupled to the CA1 network determines when it gets activated relative to the pyramidal cell population. This has important functional consequences. For clarity only the excitatory synapses made by the Schaffer collaterals and subsequent feedback synapses are indicated (black circles). Arrows indicate the information flow.

Another commonly used classification is based on the protein content of the interneurons. CA1 interneurons are classified based on their immunoreactivity for calcium–binding proteins such as parvalbumin (PV), calbindin D28k (CB) and calretinin (CR), or for neuropeptides such as somatostatin (SS), vasoactive intestinal polypeptide (VIP), cholecystokinin (CCK) or for a combination of these (Freund and Buzsáki 1996). The main advantage of this classification is that it is relatively easy to study connections between the different groups by double-staining brain slices. An interesting finding from such a study is that there are interneurons (containing CR) that are specialized to inhibit other interneurons and that avoid making contact with pyramidal cells (Gulyás et al. 1996). The big disadvantage of this classification is that the functional significance of the differences in expression patterns between interneurons is not clear. An interesting recent development is that genetically altered mice can be bred that express GFP (Green Fluorescent Protein) in cells expressing specific peptides or calcium–binding proteins (Caputi et al. 2000; Meyer et al. 2000; Meyer and Monyer 1999). In brain slices of these
animals interneurons expressing for instance PV are easily identified (as they are green fluorescent) and this provides a unique opportunity to unravel possible physiological and functional differences between the different types of interneurons in the near future.

It would be a great leap forward and a lot of discussions would be ended if one unambiguous classification of interneurons based on functional meaningful parameters could be made. However, the main problem remains that we do not know whether the differences between interneurons are a consequence or an indication of their function. Therefore, the big challenge is not to make the ultimate classification, but to unravel the different functions of interneurons in the brain.

Function of interneurons

Interneurons in the CA1 area of the rat hippocampus are thought to have several functions. The most important function of interneurons is to provide inhibition that restricts and shapes the activity in the pyramidal cell population. Furthermore, inhibition may modulate information processing in individual pyramidal cells in the network.

Interneurons are densely connected among themselves, forming interneuron networks. Coupled inhibitory neurons that can fire at high frequencies may lead to oscillations in these networks (Banks et al. 2000; Galarreta and Hestrin 2001; Gibson et al. 1999). Another intriguing finding is that a single interneuron is able to synchronize pyramidal cells by diverging inhibitory synaptic contacts (Cobb et al. 1995). A substantial number of pyramidal cells synchronously fired an action potential at the end of the inhibitory synaptic current (rebound excitation). Network oscillations and synchronization have been proposed to provide the precise temporal structure or context for the pyramidal neurons that is necessary for memory processing or sensory binding (Buzsáki 1997; Castelo-Branco et al. 2000; Jensen and Lisman 1996; Lisman 1999).

Inhibitory synaptic input, when overlapping with excitatory input, may veto specific excitatory inputs or parts of the dendritic tree (Staley and Mody 1992). Model studies have suggested that inhibitory synapses are most effective at dendritic shafts and their efficacy increases with proximity to the soma (Qian and Sejnowski 1990). Indeed, inhibitory synapses are largely found on dendritic shafts, in contrast to excitatory synapses, which are often present on dendritic spines (Megías et al. 2001). Inhibitory synapses are also more abundant at the soma and proximal dendrites compared to excitatory synapses (Gulyás et al. 1999; Megías et al. 2001). It was recently found that somatic inhibition on CA1 pyramidal cells shortens the integration window of these cells and therefore increases their ability
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as coincidence detector (Pouille and Scanziani 2001). Inhibitory synapses thus have a direct influence on the integration of synaptic signals in pyramidal cells.

In this thesis I focus on how interneurons participate in the CA1 network with respect to the pyramidal cells and how the specific connectivity of the interneurons and the dynamic properties of their synaptic connections influence their function in the network.

**DYNAMICS OF THE CA1 NETWORK**

The hippocampal CA1 network is not a static entity. In vivo, the neurons are activated in ever changing patterns by a varying number of afferent fibers (Dobrunz and Stevens 1999). The strengths of synapses between the neurons are continuously changing as a function of previous activity. New synapses are being formed and others disappear (Goldin et al. 2001; Maletic-Savatic et al. 1999; McKinney et al. 1999). To some extent, the network is even resistant to the loss of neurons. Its resistance against degradation and its dynamic structure are very important features of a neuronal network.

The strength of synaptic connections can change. Hebbian plasticity depends on the correlation between pre- and postsynaptic activity and is thought to be crucial for memory storage (Bliss and Collingridge 1993). Much research has been done to unravel the mechanisms underlying the Hebbian forms of plasticity long-term potentiation (LTP) and its counterpart long-term depression (LTD) (Bliss and Lømo 1973). Recently, another correlation–based type of plasticity, spike–timing dependent plasticity (Bi and Poo 1998; Markram et al. 1997; Zhang et al. 1998), has received much attention since its properties make it even more suitable to provide a cellular basis for learning and memory processes (Song et al. 2000; van Rossum et al. 2000). Hebbian plasticity can be induced at a time scale of several minutes. It lasts for hours or even longer. Recently it became clear that besides Hebbian plasticity, the dynamic properties of synapses (i.e. changes in synaptic strength at much shorter time scales) may also be very important in information processing (Abbott et al. 1997; Buonomano et al. 1997; Markram et al. 1998). In this thesis I focus on changes in the CA1 network on two different time scales, e.g. days and hundreds of milliseconds.
Epilepsy

At a time scale of days or longer, pathologies can develop that permanently change parts of the brain. Epilepsy is an example of such a disease. It is a very common neurological disorder that has many different causes, such as stroke, brain tumors or genetic predisposition. The induction of epilepsy probably takes several years in humans. In an epileptic brain subtle changes in neuronal networks have occurred that hardly interfere with normal brain function (McNamara 1994; McNamara 1999). Characteristic of the disease is the re-occurrence of seizures during which the patient loses consciousness and all muscles contract heavily. The hippocampus is often used for studies in epilepsy research, since this brain area is specifically prone for developing seizures. Many animal models for epilepsy or epileptogenesis have been developed to study several aspects of the disease. In the kainate and pilocarpine model, epilepsy is evoked by injection in the brain of that chemical substance (Ben-Ari 1985; Cossar et al. 2001; Fisher 1989). Epilepsy can also be evoked by electrical stimulation, which is done in the Status Epilepticus (SE) model (Gorter et al. 2001; Lothman et al. 1989). In these chronic models, the animals get spontaneous (and sometimes progressive) seizures and the brain damage, such as massive cell death and axonal sprouting, is comparable to human patients with temporal lobe epilepsy. The kindling model is a model for epileptogenesis. In this model normally no spontaneous seizures occur, but seizure susceptibility is permanently enhanced (Goddard et al. 1969).

In the hippocampal kindling model, epilepsy is evoked by repetitive high frequency stimulations (typically 50 Hz for 2 seconds), in our experiments at the Schaffer collaterals in rats. These stimulations initially evoke a small epileptic seizure, which can be measured by recording field potentials. By repeating the stimulations twice a day, convulsions develop that progressively increase in duration and severity. After 3–4 weeks of these stimulations, a stimulus that evokes little reaction in the naive rat will evoke a generalized seizure with tonic–clonic convulsions in the kindled rat. When the kindled rats are not stimulated they behave normally and do not appear different from normal rats. Without interfering much with normal functioning of the brain, the threshold for inducing an epileptic seizure is permanently decreased. Kindling induces several changes in the CA1 network, all resulting in a shift of the balance between the excitation produced by the many principal cells and the inhibition provided by the interneurons. The excitability of the pyramidal cells is enhanced by a small, but effective shift in the voltage dependence of its sodium current (Vreugdenhil et al. 1998) and an increase in its calcium current (Faas et al. 1996). The loss of a specific group of GABAergic interneurons (Kamphuis et al. 1989) and a subtle change in the GABAergic
innervation of CA1 pyramidal cells (chapter 2 of this thesis) lead to a reduction of the inhibition in the network. The shift in the balance between excitation and inhibition permanently enhances seizure susceptibility in this area.

SYNAPTIC DYNAMICS

The second time scale of focus in this thesis is much shorter. In tens to hundreds of milliseconds use–dependent changes in synaptic strength occur in the CA1 network. As pointed out above, the computational power of a neuronal network is not only determined by the different types of neurons in the network. Their mutual connections and especially the dynamic properties of these connections are highly important (Maass and Zador 1999), as they determine how the network translates an incoming spike train into a series of action potentials fired by the CA1 pyramidal cells.

Underlying mechanisms

The strength of the synapse between two neurons can change rapidly depending on its recent activity. The probability of releasing a vesicle from the presynaptic terminal upon the arrival of an action potential is highly dependent on the (local) presynaptic calcium concentration. Short–term facilitation of release probability is thought to be mainly due to a build–up of free calcium ions in the presynaptic terminal during repetitive arrivals of action potentials (Fisher et al. 1997; Zucker 1989; Zucker 1999). This build–up depends on several factors such as the presence of presynaptic calcium channels, calcium buffers and calcium pumps (Dittman et al. 2000; Rozov et al. 2001) and the release of calcium from or the uptake of calcium by internal stores and mitochondria (Emptage et al. 2001; Rose and Konnerth 2001).

Depression of release probability may result from a restriction of the number of vesicles that can be released. Only a small percentage (~5%) of the total number of presynaptic vesicles is normally available for release (often referred to as the readily releasable pool). During repetitive activation of a synapse this vesicle pool can be depleted and the probability for releasing a vesicle upon arrival of an action potential decreases. The rates at which this pool of vesicles is depleted and can be replenished are important factors for determining the depression rate at an individual synapse. These rates might depend on presynaptic calcium concentration (Dittman et al. 2000). Other factors contributing to the depression of synaptic strength during repetitive activity can be desensitization of the postsynaptic
receptors (Hunter and Milton 2001; Jones and Westbrook 1996) or calcium depletion from the synaptic cleft (Borst and Sakmann 1999).

Receptors that are present in the presynaptic membrane may modulate release probability by inhibiting presynaptic calcium channels, by activating potassium channels or by directly interfering with the release machinery, often through G-protein mediated pathways (Wu and Saggau 1997). Examples of such receptors are GABA_B (Brenowitz et al. 1998; Wu and Saggau 1995), kainate (Schmitz et al. 2001), metabotropic glutamate (Poncer et al. 2000; Töth et al. 2000), muscarinic acetylcholine and adenosine A1 receptors. Metabotropic glutamate and GABA_B receptors can be activated by a spill-over of neurotransmitter from the synaptic cleft (providing a feedback signal). Activation of presynaptic receptors can occur through the activity of other neurons in the local network (indirect local modulation) or through the release of hormones or other neuromodulatory substances from long-range axons originating from other brain areas.

Possible functions

The dynamic properties of the synapses between neurons strongly influence network activity. This type of synaptic plasticity was shown to be essential in the cellular basis for learning of simple behavior in Aplysia (Fisher et al. 1997). In the mammalian central nervous system only recently more attention is given to the functional implications of dynamic synapses. For instance, depression of excitatory synapses between neocortical pyramidal cells is proposed to function as a gain control mechanism, enabling the postsynaptic neuron to detect a change in the presynaptic firing rates of an input irrespective of the activity levels of other inputs (Abbott et al. 1997; Markram et al. 1998). The dynamic properties of both inhibitory and excitatory synapses in the hippocampus are proposed to increase the ability of pyramidal cells to detect specific intervals in presynaptic spike trains. In this way, populations of neurons can be generated that code for specific spike intervals. This may be important in transforming temporal information into a spatial code segregated over different populations of neurons (Buonomano 2000; Buonomano et al. 1997). Furthermore, differences in synaptic dynamics of inhibitory and excitatory connections result in a dynamic balance between inhibition and excitation in neuronal networks (Galarreta and Hestrin 2000; Varela et al. 1999).
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OVERVIEW OF THIS THESIS

The CA1 network translates spike trains that arrive via afferent fibers into a series of action potentials fired by the CA1 pyramidal cells. Interneurons participate in this network and they shape the output of the CA1 network. The central question in this thesis is how individual interneurons interact with the pyramidal cell population in the CA1 network. We have split this central question into three subquestions that are considered in chapter 2, 3 and 4:

A. Are inhibitory synapses different in the epileptic and normal hippocampus?
B. What is the relation between the input to individual interneurons and the population activity?
C. And what are the dynamic properties of the input to interneurons?

In the epileptic brain the balance between excitation and inhibition is permanently distorted. Inhibition is provided by interneurons that make synapses all over the membrane surface of the CA1 pyramidal cells. In chapter 2 of this thesis we have examined possible changes in these inhibitory synapses after kindling epileptogenesis by studying miniature inhibitory postsynaptic currents (mIPSCs) in CA1 pyramidal cells. Miniature IPSCs are small synaptic currents that can be measured in the postsynaptic cell. These currents are due to the spontaneous fusion of vesicles at the presynaptic inhibitory terminals in the absence of an action potential. Studying mIPSCs provides information about the number and strengths of the inhibitory synapses that are present (from mIPSC frequency and amplitude respectively). Changes in the inhibitory synapses can have important consequences for the stability of the CA1 network.

In the next chapters we focused on the role of interneurons in the CA1 network in the normal brain. I have investigated how interneurons participate in the local network and how their participation depends on their specific connectivity with respect to the pyramidal cells. In chapter 3 the input to interneurons in the CA1 network is examined after stimulation of the Schaffer collaterals. This stimulation evoked monosynaptic Schaffer input (feedforward input) in some interneurons, while in other interneurons bisynaptic input originating from axons of CA1 pyramidal cells (feedback input) was evoked. The way interneurons are coupled with respect to the pyramidal cell population in the CA1 network has important consequences for their contribution to the network activity.
Synapses have dynamic properties. The response of a synapse depends on its previous activity. The dynamic properties of synapses are important for determining the signal transfer of the CA1 network. I have examined the dynamics of excitatory input to CA1 interneurons and pyramidal cells in chapter 4, by measuring synaptic responses during repetitive activation. With dynamic synapses, the connections between neurons change as a function of input frequency. Knowledge of the dynamic properties of the synapses is therefore essential for understanding the computations performed by the CA1 network.

In the last chapter, chapter 5, a summary is given of the main findings of this thesis. These findings are discussed with respect to the present knowledge of information processing in the hippocampal CA1 network.