1 Introduction

1.1 The Hippocampal Formation

The hippocampus is part of the allocortex, a group of brain regions that are evolutionally older than most of the other cortex areas. It was named after its curved crescent shape, which reminded 18th century anatomists to the shape of a seahorse (Latin: hippocampus). The hippocampus resides in the medial temporal lobe (Figure 1.1) and forms part of the limbic system, a circuit closely associated with emotion, motivation, learning and memory. The physiological purpose of the hippocampal region is still a matter of debate. Current research follows mainly two classical concepts, which might not necessarily exclude each other (Stella et al., 2012).

Lesions studies indicated that the hippocampus plays a crucial role in the formation of episodic memories (Scoville and Milner, 1957; Zola-Morgan and Squire, 1986, 1990). This function involves active neurogenesis (Clelland et al., 2009; Creer et al., 2010; Sahay et al., 2011), which is otherwise rarely found in brains of adult mammals (Altman and Das, 1965; Eriksson et al., 1998). Additionally, hippocampal neurons were found to fire at specific locations within a given environment, thereby encoding spatial and navigational information into cellular activity (O'Keefe and Dostrovsky, 1971).

The following sections introduce some current concepts on hippocampal function, provide the basic architecture of the hippocampal formation, portray the lamination of the sub-area CA1, and explore some typical features of its pyramidal cells (Amaral and Lavenex, 2008).

1.1.1 Proposed Functions

The first functional models associated the hippocampal structure with the formation of episodic/autobiographic memory. It started with the medical case of patient Henry Gustav Molaison, better known in literature as »HM« (Scoville and Milner, 1957). He sought medical advice for his severe epileptic seizures. The neurosurgeons identified the left and right medial temporal lobes as focus of his seizures and therefore removed both of his hippocampi and most of the neighboring structures. After surgery, HM could still perform tasks that required short term and procedural memory, but he was unable to store new declarative information for more than some minutes. However, events which lay before his surgery remained largely

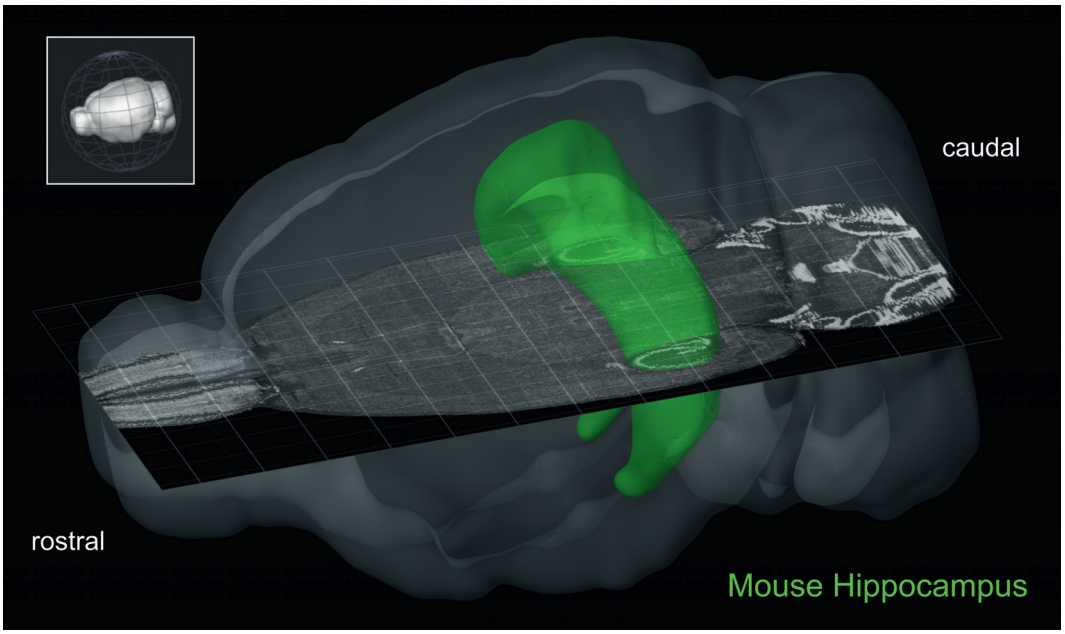

Figure 1.1. Location of the hippocampus in the rodent brain (mouse). The rodent hippocampus (green) lies in the medial left and right temporal lobes. Horizontal sections reveal the typical crescent of the pyramidal cell layer. Modified from Allen Brain Explorer (http://www.brain-map.org)

intact, as well as his ability to learn new motor skills such as golfing. This symptomatic pattern demonstrated that encoding and retrieval of long term memory is processed in distinct areas of the brain, in which only the first one seems to be hippocampus dependent. One popular line of ideas proposes the hippocampus as an area where cortical information converges (Eichenbaum, 2000; Marr, 1971; Rolls, 2013). New experiences, encoded in higher sensory, motor and prefrontal cortices might be bound together by their connections within the hippocampal formation to establish a temporary memory. Over time, the higher cortex areas may build connections directly between each other and the memory becomes independent of the hippocampus. Old memories could therefore be retrieved from cortical areas, whereas the formation of new memories would depend on initial hippocampal binding of multimodal representations associated with the new event.

The year 1971 brought a renaissance in hippocampal research that shifted its focus and was recently awarded with the Nobel Prize in Medicine 2014. O'Keefe and his student Dostrovsky discovered that some hippocampal cells fire preferentially when the animal resides at a specific location in its environment (O'Keefe and Dostrovsky, 1971). These cells were later named place cells and are since candidates for the neuronal representation of spatial information. Recordings during immobility and slow wave sleep revealed that place cells get reactivated in the same sequence the animal passed their respective fields during former exploration (Ji and Wilson, 2007; Louie and Wilson, 2001; Skaggs and McNaughton, 1996). This replay might reflect a neuronal correlate of memory consolidation during sleep. Furthermore, during resting immobility, the path an animal might take through its environment

can be predicted by a fast sequence of place cell activity following this trajectory (Diba and Buzsáki, 2007; Dragoi and Tonegawa, 2011; Foster and Wilson, 2006). The evidence that the hippocampus provides a cognitive map of space became so profound that some scientists consider it now the primary purpose of this region (O'Keefe, 1999).

Recent approaches combine both lines of observations and interpret the hippocampus as a temporal map of memories, in which spatial (locations) as well as non-spatial experiences, e.g. odors (MacDonald et al., 2013), can be ordered based on their chronological sequence (MacDonald et al., 2011; Rolls, 2013). Recent innovations, such as the broad availability of optogenetic tools as well as advanced *in vivo* recording conditions, made it possible to manipulate memory engrams directly (e.g. selective creation or deletion of memories; Liu et al., 2012; Ramirez et al., 2013). Thus, the current technical capabilities raise some hope that we can someday uncover the primary purpose of the hippocampal formation and understand its deeper operational principles.

1.1.2 Anatomical Architecture

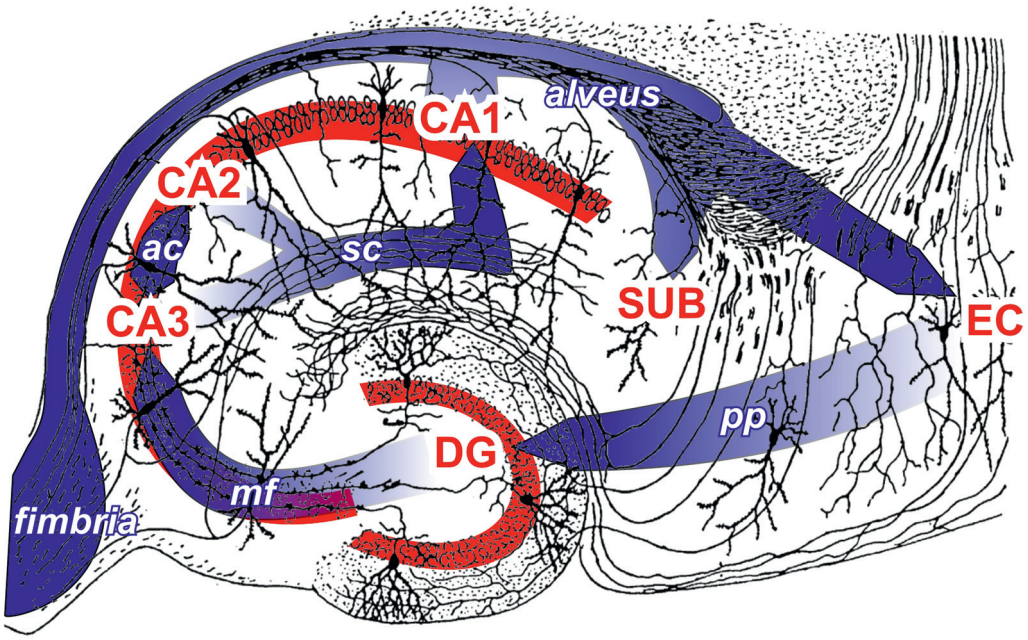

Figure 1.2. Basic circuit of the hippocampal formation in rodents. The entire hippocampal formation includes the entorhinal cortex (EC), dentate gyrus (DG), cornu ammonis areas CA1-CA3, subiculum (SUB), perforant path (pp), mossy fibers (mf), associational collaterals (ac), Schaffer collaterals (sc), alveus, and fimbria (f). Cell bands and sub-regions are labeled in red and main fiber projections in blue. Modified from Cajal, 1911.

The basic structure of the hippocampus was first described by Ramón y Cajal who used Golgi stainings to separate single neurons and define their projection patterns (Cajal, 1911). Subsequent neuroscientists subdivided the hippocampus proper, the cornu ammonis, into three sub-regions (CA1-CA3) according to cell anatomy and fiber projections (Blackstad, 1956; Lorente de Nó, 1934). However, due to their tight association with input and output areas it became conventional to speak rather of a hippocampal formation. Along with hippocampus proper (CA1-CA3), it incorporates neighboring cortical structures such as: entorhinal cortex, dentate gyrus, and subicular areas (subiculum as well as pre- and para-subiculum), the major input and output regions of the hippocampus (see Figure 1.2).

The best described signal flow through the hippocampal formation follows the trisynaptic pathway (entorhinal cortex → dentate gyrus → CA3 → CA1). Projections from layer II of the entorhinal cortex enter the hippocampal circuit through the performant path and make synapses to granule cells in the dentate gyrus. Granule cells are aligned in a C or V shape around the end of the CA3 cell band. They are principal cells of small elliptical shape with a cone-shaped dendritic tree stretching towards superficial layers (away from CA3). Typically, they carry no basal dendrites. Their axons, the mossy fibers, project towards the pyramidal neurons of the CA3 region.

CA3 pyramidal neurons receive input not only from granule cells but also from recurrent collateral connections within ipsilateral CA3 (associational projections) as well as contralateral CA3. Projections from CA3 towards CA1 are named Schaffer collaterals. These fibers also cross the hippocampal commissure, thus targeting pyramidal neurons of ipsi- and contralateral CA1.

CA2 constitutes a small and often neglected area between CA1 and CA3. It is not part of the classical trisynaptic pathway, but its pyramidal neurons largely resemble their neighbors in CA3 in shape and connectivity. Their main inputs derive from CA3 collaterals, but also from the performant path (entorhinal cortex layer II; Chevaleyre and Siegelbaum, 2010) and as recently shown from dentate gyrus (Kohara et al., 2014). They project preferentially to CA1 pyramidal cells.

Pyramidal cells in CA1 receive their main input from CA3 (Schaffer collaterals) and CA2, but are also targeted by the entorhinal cortex layer III via the temporoammonic pathway. In contrast to former believes, they also form numerous associational connections between each other along the longitudinal axis of the hippocampus (Yang et al., 2014). Outputs from CA1 target mainly neurons of the subiculum and deep layers of the entorhinal cortex (layer V and VI) as well as medial prefrontal cortex and amygdala (Cenquizca and Swanson, 2007).

The subiculum, in turn, is regarded as the final output structure of the hippocampal circuit (Amaral and Lavenex, 2008). It receives information mainly from CA1 and entorhinal cortex layer III and sends projections to a large range of brain regions, namely pre- and para-subiculum, entorhinal cortex, prefrontal cortex, amygdala, basal forebrain (septal nuclei, nucleus accumbens), hypothalamus (mammillary nuclei), and thalamus (nucleus reuniens and others).

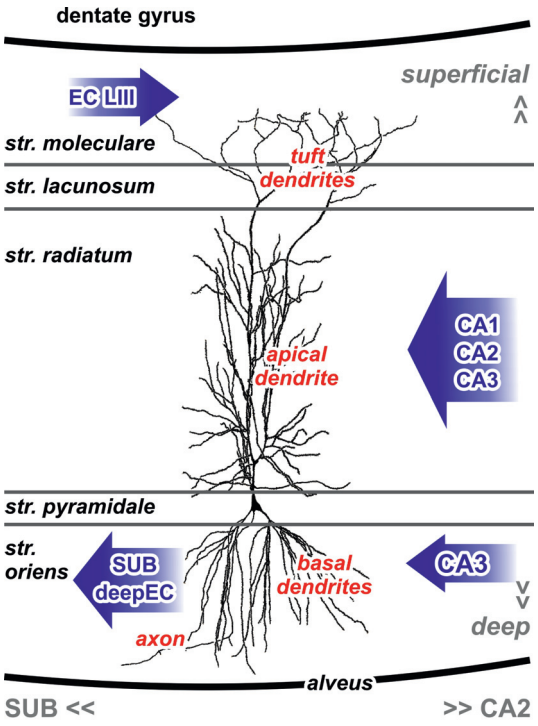

Figure 1.3. Laminar profile and main input/output projections in CA1. Reconstructed morphology of a CA1 pyramidal cell. Somata are located in stratum pyramidale. Apical dendrites span through stratum radiatum where they receive inputs from CA2, CA1 (associational collaterals), and mainly CA3 (Schaffer collaterals). The dendritic trunks bifurcate in stratum lacunosum and moleculare to multiple tuft dendrites, which receive input from entorhinal cortex layer III (EC LIII). Basal dendrites reach into stratum oriens where they receive mostly contralateral CA3 inputs. The axons stretch through stratum oriens and join the alvear tract to innervate targets in subiculum (SUB) and deep layers of entorhinal cortex (deepEC). Cellular compartments are labeled in red, fiber projections in blue. Compare to Figure 1.2. Modified from Amaral and Lavenex, 2008.

1.1.3 Laminar Organization of CA1

Hippocampal areas are segmented into several layers (Latin: strata, sing.: stratum). Most of them are present across all regions of the hippocampus proper. The following paragraphs focus on the anatomical layers of CA1 and how inputs and outputs are distributed along the vertical axis of its principal neurons. The cell bodies of principal neurons reside in stratum pyramidale in the center of CA1. Their apical dendrites rise into the superficial layers of stratum radiatum, lacunosum and moleculare, whereas basal dendrites and axons branch from the base of the cell into the deep layer of oriens, towards the alvear fiber tract (see Figure 1.3).

The alveus forms the deep border of the hippocampus to the lateral ventricle and is composed of efferent and afferent axons of hippocampus proper and subiculum. It extends from subiculum towards CA3, where it fuses with the fimbria and merges later to the fornix.

The stratum oriens constitutes the deepest layer of CA1. It contains axon collaterals and basal dendrites of CA1 pyramidal cells as well as a large variety of inhibitory interneurons (e.g. axo-axonic cells, basket cells, OLM cells; Klausberger, 2009). The axons of pyramidal cells emerge at their basal surface, transverse stratum oriens, and finally merge with the fiber bundle of the alveus. Axo-axonic cells in stratum oriens target the axon initial segment (AIS) of pyramidal cells to regulate output generation and back-propagation of action potentials (Dugladze et al., 2012; Howard et al., 2005). The basal dendrites receive their inputs largely from contralateral CA3 and ipsilateral CA2 (Ishizuka et al., 1990; Shinohara et al., 2012) as well as local interneuron networks (Klausberger, 2009). Further input sources

encompass the amygdala (Pikkarainen et al., 1999), entorhinal cortex (Deller et al., 1996) and recurrent associational collaterals of pyramidal cell themselves, which extend along the longitudinal axis of CA1 (Yang et al., 2014).

Stratum pyramidale contains the tightly packed somata of pyramidal neurons with little presence of other neuron types. CA1 pyramidal cells are discriminated into several subgroups that show particular spatial distributions within the call band. Neurons located near the subiculum exhibit a higher probability to fire in bursts (Graves et al., 2012; Jarsky et al., 2008). They receive more inputs from distal CA3, whereas neurons close to CA3 receive inputs mainly from neighboring CA2 and CA3 areas. CA1 neurons also differ according to their position vertical to the cell band. Superficial neurons (border to stratum radiatum) are generated 1-2 days before deep pyramidal cells and contain higher levels of calbindin and the synaptic modulator zinc (Baimbridge et al., 1991; Slomianka, 1992). Neurons deep in the pyramidal layer (border to stratum oriens) fire at higher rates, are more likely to have place fields, and are more strongly modulated during slow wave sleep (Mizuseki et al., 2011). They are also stronger targeted by CA2 projections compared to their superficial neighbors (Kohara et al., 2014). Genomic studies disclosed several additional surface and channel genes with sub-layer-specific expression patterns (Dong et al., 2009; Thompson et al., 2008). The increasing evidence of a superficial to deep segregation in CA1 might indicate a primitive form of »cortical lamination« in the hippocampus (Slomianka et al., 2011).

The somatic surface of CA1 pyramidal neurons elongates at their apex to a long thick apical dendrite, which crosses stratum radiatum and forms multiple oblique site branches. These receive inputs mainly from Schaffer Collaterals of CA3 but also from local interneurons (Klausberger, 2009).

The trunks of apical dendrites extend up into stratum lacunosum and moleculare, the most superficial hippocampal layers (mostly summarized as stratum lacunosum-moleculare), where they branch out into an arbor of tuft dendrites. These receive their inputs directly from entorhinal cortex layer III via the temporoammoniac path, from O-LM interneurons residing in stratum oriens, as well as from other sources such as the thalamus. Excitatory inputs from entorhinal layer III target mainly branches in the moleculare layer. Recently found additional projections from entorhinal layer II target specifically GABAergic interneurons in stratum lacunosum, which in turn inhibit excitation of tuft dendrites of pyramidal cells (Kitamura et al., 2014). This micro-circuit in stratum lacunosum and moleculare controls the input efficiency of entorhinal inputs reaching the apical tuft of pyramidal cells (see also section 1.3).

1.2 Neuronal Signal Processing

The brain processes information on a variety of scales ranging from single molecules up to complex networks. At the cellular level, neurons integrate converging inputs to elicit action potentials, which are transmitted as output signals towards succeeding targets. Pyramidal neurons represent the most common principal cells of the cortex and provide the majority of long range projections between different brain areas. Thus, a lot of knowledge has accumulated in the last decades about its computational properties. The canonical sequence of neuronal integration is summarized by a three-stage model: dendritic input, somatic integration, and axonal output. Each stage comprises its own specific anatomical compartment, molecular machinery, and computational principles. The following sections give an overview about the current knowledge regarding neuronal integration, of which some escape the stated classical order (see Figure 1.4).

1.2.1 Dendritic Input Integration

Dendrites constitute the primary input compartment of pyramidal cells. They sprout from the somatic membrane, bifurcate into multiple branches and collect inputs from selective axonal projections around them. Neuronal signals cross the boundaries between axon and dendrites of individual cells at the synapses (Figure 1.4B). In case of excitatory input, these are located on the tip of small globular spines protruding the dendritic branches. Depolarization of the presynaptic axonal membrane releases the neurotransmitter glutamate into the synaptic cleft, transforming the electrical into a chemical signal. The transmitter diffuses to the postsynaptic membrane, where it binds and opens ligand-gated ion channels. An influx of cations (usually sodium and calcium) from the extracellular space into the synaptic spine, causes a depolarization of the postsynaptic membrane. Thus, the extracellular chemical signal is converted back into an intracellular electrical signal. Multiple excitatory postsynaptic potentials (EPSPs) from neighboring synapses sum up in the local branch segment thereby performing the fist stage of electrical computation. These compound EPSPs then spread along the dendritic tree towards the somatic compartment (London and Häusser, 2005; Magee, 2000).

Formation and propagation of compound EPSPs along the dendritic tube was classically considered as a mere passive process guided by the cable theory of electric current. However, more and more evidence accumulated over the years that this concept poses an oversimplification. Stimulation of local dendritic segments revealed that dendrites are not only passive cables but allow active non-linear signal amplification. This is possible due to high densities of voltage gated ion channels (e.g. sodium and calcium), which can modify local EPSP generation and propagation along the dendritic tree (Spruston, 2008). Some dendritic branches are able to trigger local action potentials, called dendritic spikes according to their location (Losonczy and Magee, 2006; Remy et al., 2009).

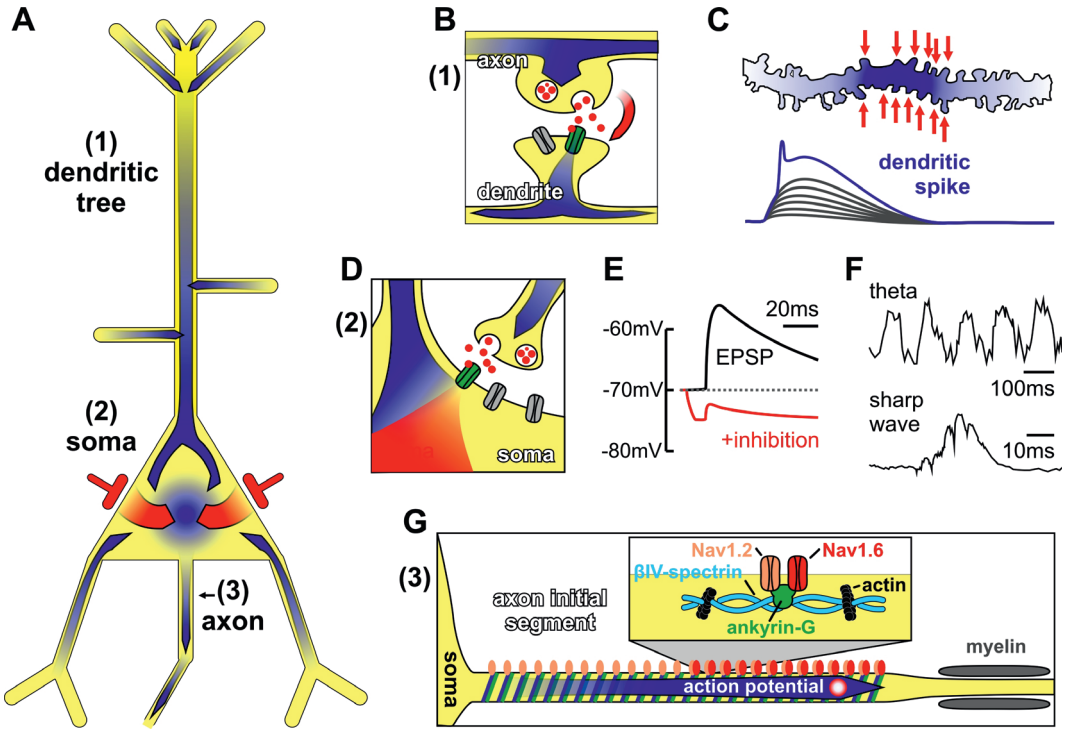

Figure 1.4. Signal flow through neuronal compartments (A) Schematic drawing of pyramidal neuron. The three classic stages of signal integration are numbered 1-3. Excitatory currents are labeled in blue, inhibitory in red. (B) Sketch of a glutamatergic synapse. Presynaptic deploraization releases glutamate into the synaptic cleft. It binds and opens sodium channels on dendritic spines, which causes a depolarization of the postsynaptic membrane. (C) Several excitatory postsynaptic potentials (EPSPs) summate linearly (grey traces). Synchronous activation of co-localized synapses (red arrows) activates voltage gated ion channels and triggers a super-linear voltage response (dendritic spike, blue trace). (D) Sketch of a GABAergic synapse. Interneurons target the perisomatic membrane and release the neurotransmitter gamma-aminobutyric acid (GABA) into the synaptic cleft. It opens chloride channels, which reduce excitability of the somatic compartment. (E) Effect of GABAergic inhibition on EPSPs. Influx of negative ions hyperpolarizes the cell and open ion channels shunt the amplitude of passing EPSPs. (F) Example traces of theta and sharp wave ripple oscillations in the extracellular field of CA1. (G) Molecular composition of the axon initial segment (AIS). The cytoskeletal proteins βIV-spectrin and ankyrin-G anchor ion channels towards the AIS membrane. Low voltage threshold Nav1.6 sodium channels concentrate at the distal portion of the AIS, whereas Nav1.2 channels are expressed across its whole length. Action potentials are triggered at AIS rear and propagate into the myelinated axon.

Weak and decorrelated unitary EPSPs integrate in a linear manner and propagate passively towards the soma thereby undergoing electrotonic attenuation. In case of tuft dendrites, they are usually incapable to reach the soma altogether (Williams and Stuart, 2002). However, if neighboring synapses are activated simultaneously and depolarize the dendritic membrane across a certain threshold, they might summate super-linearly by the opening of local voltage gated ion channels. The

resulting voltage response of these dendritic spikes is not only larger as expected via linear integration; it also activates channels in adjacent dendritic segments, thereby providing an active propagation of the signal along the dendritic tree.

There are several subtypes of dendritic spikes. The fastest and briefest are generated by sodium channels (Ariav et al., 2003; Gasparini et al., 2004; Losonczy and Magee, 2006). Others spikes feature calcium currents with larger and broader responses (Schiller et al., 1997; Schwartzkroin and Slawsky, 1977). NMDA spikes, for instance, are triggered by regenerative activation of NMDA receptors. They cannot propagate through the dendritic tree, but are restricted to the area of glutamate release (Polsky et al., 2009; Rhodes, 2006; Schiller et al., 2000).

Super-linear responses to spatially and temporally synchronized input have large implications for neuronal integration (London and Häusser, 2005). Dendritic spikes act as coincidence detectors of inputs converging at single branches and allow precise timing of action potential output with low temporal jitter (Ariav et al., 2003). Furthermore dendritic spikes were shown to resist the attenuating influence of shunting inhibition (Müller et al., 2012) and to increase the efficiency of local synapses (Branco et al., 2008). The physiological role of active dendrites was already demonstrated for the visual cortex, where dendritic spikes enhance the selectivity of neurons for visual cues (Smith et al., 2013). In addition to amplification of synaptic inputs, dendritic spikes also allow an active back-propagation of somatic action potentials into the dendritic tree (Stuart and Sakmann, 1994), which modifies spike time dependent plasticity and the firing mode of pyramidal cells (Larkum et al., 1999; Magee and Carruth, 1999).

Although dendrites are primarily responsible to gather excitatory input, they are themselves capable to perform a variety of signal transformations on their own, preceding the somatic integration. This enhances the computational power of individual pyramidal neurons and might even constitute a general operation principle of cortical structures (Larkum, 2013).

1.2.2 Somatic Summation and Recurrent Inhibition

All dendritic branches converge towards the somatic compartment. Consequently, EPSPs generated at individual branches eventually reach the soma where they unite to one somatic membrane potential. However, the cell body itself constitutes a synaptic target of a large variety of interneurons that modulate neuronal excitability (Klausberger and Somogyi, 2008). Typical inhibitory synapses release the neurotransmitter GABA, which activates chloride channels at the postsynaptic membranes (Figure 1.4D). The influx of negative chloride ions hyperpolarizes the somatic membrane thus reducing the amplitude of dendritic signals. Furthermore, active channels reduce the electrical resistance of the membrane. Incoming excitatory inputs are therefore shunted by the leaky membrane (Figure 1.4E). Several types of GABAergic interneurons, e.g. basked cells, target predominantly somatic membranes. Their input is therefore termed »perisomatic inhibition«.

Interneurons activity is highly orchestrated into certain temporal patterns. These can be measured in extracellular voltage recordings due to the strong laminar organization of input and output sources in many brain structures including the hippocampus. Recurrent phases of inhibitory inputs appear in these measurements as characteristic voltage oscillations in the extracellular field (Buzsáki and Draguhn, 2004).

Several of these oscillatory states were found to correlate with certain behavioral states of an animal. The theta rhythm is defined by large regular waves recurring at 3 to 10 Hertz (Figure 1.4F, top). This state is typical for attentive and active waking behavior (mainly movement), but it is also expressed during rapid eye movement (REM) sleep. In terms of hippocampal function, it is believed that theta oscillations synchronize converging inputs between different sub-regions and play an important role for the encoding of ongoing experience into memory (Buzsáki, 2002; Mizuseki et al., 2009).

Another prominent network condition features so-called sharp wave-ripple complexes (SPW-R; Figure 1.4F, bottom). These 50-100 ms long waves recur at random intervals and carry a high frequency oscillation of 150-200 Hz (ripples) on top of them. They appear mainly during resting immobility or slow wave sleep and are thought to reinforce and consolidate recently acquired memories (Buzsáki, 1998; Buzsáki et al., 1992).

All physiological network conditions involve recurrent phases of perisomatic inhibition. Yet, pyramidal cells are still able to fire action potentials during all phases of these oscillations. *In vivo* recordings of mice investigating new paths through a given environment demonstrated that phase-locked selection and reactivation of certain pyramidal cells is critical for the successful procession of new information (O'Neill et al., 2010). It is thus not only strength and precision of dendritic inputs which determines neuronal output. Tonic and phasic modulation of cellular excitability, via perisomatic inhibition, add another significant stage of neuronal signal processing.

1.2.3 Axonal Output Generation

All inputs entering from dendritic branches as well as perisomatic inhibition unite at the somatic surface into a single voltage potential. However, the actual output generation occurs within the axonal compartment (Debanne et al., 2011). The first $\sim$30 μm of the axon forms a molecularly defined compartment, the axon initial segment (AIS; Figure 1.4G). It comprises a highly specialized set of cytoskeletal anchoring proteins such as ankyrin-G and βIV-spectrin. They bind and concentrate high densities of voltage gated ion channels to the AIS (mainly sodium and potassium), which are closely involved in output generation (Gasser et al., 2012; Rasband, 2010).

Action potentials are triggered at the distal portion of the AIS. This sub-region features low threshold sodium channels (Nav1.6) as well as a favorable electrotonic

anatomy for voltage alterations. Both characteristics render the rear of the AIS the position of highest excitability within the whole neuron (Baranauskas et al., 2013; Hu et al., 2009b; Royeck et al., 2008). Certain potassium channels (Kv7.2 and Kv7.3) colocalize with sodium channels at the ankyrin-G scaffold and regulate excitability of the initial segment. They create a non-inactivating potassium current (M-current), which determines resting membrane potential, thresholds for action potential generation, as well as the sparse inherent firing pattern of CA1 pyramidal cells (Shah et al., 2008; Storm, 1990).

The machinery of the AIS emerges typically at somatic envelope, at the root of the axonal branch. After a variable length of 20-40 μm the molecular structure of the AIS ceases and gives way to the myelinated axon (Duflocq et al., 2011; Galiano et al., 2012). Once an action potential is generated at the AIS, it traverses actively along the axonal tree to transmit neuronal output signal further to subsequent cells of the network.

Recent studies reported considerable heterogeneity in length and location of the AIS along the axonal tube and suggested that these properties undergo activity-dependent plasticity, thereby regulating cellular excitability (Grubb and Burrone, 2010; Gutzmann et al., 2014; Kuba, 2010). In addition, there are axo-axonic cells that target directly initial segments of pyramidal neurons (Somogyi, 1977). They do not only modulate output generation (Howard et al., 2005; Viney et al., 2013) but also stop reverse propagation of action potentials from distal axonal segments back into the somato-dendritic compartment (Dugladze et al., 2012).

Consequently, initial segments do not simply react to the somatic membrane potential. Their morphology and excitability undergoes plastic changes and is controlled by external (interneurons) and internal activity.

Conclusion

In summary signal processing at the cellular scale is highly complex and comprises several stages of neuronal compartments with individual computational abilities. It is thus not only the amount of input reaching the neuron which determines the output signal. It is furthermore the complex interaction between input timing, synapse location, morphology, and molecular environment that exerts a strong influence on the generation of output.