University of Crete
Computer Science Department

Modelling aging-induced alteration of excitability in a CA1 hippocampal pyramidal cell - computational role of excitability's modulation in working memory

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Master Thesis

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Extended Abstract

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"An episodic memory is a memory that includes details that can
be used to attribute it to a source (e.g., the time, place, and
modality of its acquisition)"
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p. 527 [73],

Various neurophysiological studies have established the significant role of hippocampus in declarative memory, the memory for facts and events that can be readily brought to conscious recollection [96]. Specific functional alterations in hippocampus seem to underlie long-term episodic memory decrements during aging, i.e., an inability to form lasting memories of events [96]. Although aged subjects may have intact memory for individual features of an event, they have a difficulty to perform associations between them. The *feature binding* deficit probably arises from deficits at the encoding processes that support working memory tasks, i.e., the short-term maintenance or manipulation of information [73].

During early aging, the dendritic morphology of hippocampal neurons and the number of synaptic connections between them, remain almost unchanged [20]. The intrinsic calcium-dependent, membrane properties of CA1 hippocampal pyramidal neurons, instead, are the first to be disrupted. Specifically, an age-related increase in the L-type Ca^{2+} channel (LTC) activity has been reported [99, 98, 20], which contributes to the enhancement of the slow membrane afterhyperpolarization (sAHP) [13, 77]. This alteration induces further an excitability reduction which has been correlated to impaired synaptic plasticity and learning in aged subjects [55, 98, 87].

Various neuromodulators implicated in learning and memory [103, 105, 82, 86], regulate the intrinsic, non-synaptic neuronal properties which change the firing characteristics of neurons [89, 53, 33, 44, 85], and enable the emergence of complex temporal dynamics, like oscillations in characteristic frequencies [68, 30, 31]. Coherent network oscillations in the brain have been correlated with different behavioral and mental states [12]. During states of focused attention (*in vivo*), oscillations in the γ-frequency band (30-70 Hz) are observed in the hippocampus, most commonly superimposed on θ-waves (5-12 Hz) [68]. In vitro, persistent hippocampal γ oscillations are induced by muscarinic cholinergic receptor activation [30]. These oscillations have been implicated in feature binding and short-term

memory [31]. Notably, both cholinergic neurotransmission and γ oscillations are disrupted in cases of impaired memory formation (e.g., in neurodegenerative diseases as Alzheimer's, references in [30, 31]).

In order to study "in silico" the change of the firing characteristics of neurons via modulation of the various ionic conductances, I have modified a published multi-compartmental model of a hippocampal CA1 pyramidal cell (PC) [83]. Main features of the present model are the simple coupling mechanisms between specific types of Ca^{2+}-channels, and the co-localized, Ca^{2+}-dependent potassium channels which control the membrane afterhyperpolarization [2, 69]. Except for the standard, $Ca_v1.2$-type, high-voltage-activated LTCs [10], a $Ca_v1.3$-type has been included in the somatic and proximal dendritic compartments [91, 107]. It generates an activity-dependent, regenerative current which builds up with each spike, providing a record of recent spiking activity analogous to the one provided by the slow afterhyperpolarization current [51]. The competing - positive *and* negative - feedback loops that arise from the coupling of these channels, increase the range and precision of neuronal firing rate. Furthermore, when the model is presented with identical inputs, the frequency content of spike output might be dynamically adjusted, depending on recent spiking activity as well as selective neuromodulation of specific channels' properties (induced by cholinergic [89, 53, 31, 30, 18], or β-adrenergic neurotransmission [16, 44]).

Model parameters (describing conductance and distribution of various somatic and dendritic ion channels) have been calibrated using published physiological data [95, 43, 77]. Computer simulations reproduce the decreased excitability of aged CA1 cells as a result of an increased post-burst L-type Ca^{2+} channel activity, and enhanced slow afterhyperpolarization and spike frequency adaptation in the "aged" cell model.

"Young" and "aged" cell models have been further tested in simulations of selective attention during exploratory behavior [96]. Under these conditions, a specific temporal pattern, repeated postsynaptic bursting in θ frequency, is observed in the CA1 PCs in the hippocampus of adult animals [23, 25, 100, 105, 81]. Over successive θ-bursts, spiking activity has been reported to increase [100], a phenomenon that probably expresses physiological temporal integration in the postsynaptic CA1 neuron [92]. Somatic action potentials in θ-bursts initiate gene transcription and protein synthesis, mainly through selective activation of L-type Ca^{2+} channels [23]. Spike backpropagation through dendrites, in turn, induces protein buildup at recently active, "tagged" synapses [34, 25], but might also affect synapses in the whole cell, increasing or even *decreasing* their strength (LTP or LTD) [81]. θ-bursting is a necessary and sufficient condition for the induction of long-term potentiation (LTP) of stimulated synapses [25, 100]. Moreover, the activity-dependent "tuning" of synaptic connections ("metaplasticity") has been proposed to subserve at the same time plasticity and stability in cortical neural networks, and, eventually, long-term formation of complex episodic memories [1]. In aged animals with learning deficits, θ-burst induced LTP is selectively impaired [101].

TBS then, is simulated "in silico" by 10 antidromic (non-synaptic) stimuli in θ frequency, with identical, complex-spike-inducing amplitude and duration, as in [23]. Even in the "young" cell model, postsynaptic bursting activity could be maintained and gradually increase, only if the medium- and slow- AHP K^+ currents were sufficiently suppressed, imitating cholinergic influences during attentional states. Moreover, after several bursts (at least five in the simulations), self-sustaining oscillations arose, superposed on the θ-burst pattern. They increased in frequency over successive intra-stimuli intervals, but remained in the γ-frequency band, and decayed several hundreds of ms after the end of TBS. The time-course of their onset and decay depended on the competing effects of the Ca^{2+}-current I_{CaL}, and the medium and slow AHP K^+ currents left unblocked, and were influenced by the mixed, Na^+/K^+, current I_h (which also regulates AHP [45], and is influenced by cholinergic actions [18], [75], [85], [31], Fig. 4.5-4.6).

Graded persistent activity represents the ability of neurons to integrate over time the effects of past, appropriately "spaced" - in time and location - synaptic stimuli. The temporal integration observed during TBS has been proposed to be a short-term, working memory mechanism which is critical for the association of recent synaptic inputs, that have been strong enough to evoke bursting [92]. Self-sustaining γ oscillations induced during TBS "in silico", might be one of the mechanisms underlying the ability of a single cell to act as a pacemaker "in vivo", intrinsically driving the network at γ-frequency band [46].

The same percentage of K^+-currents blockade couldn't induce neither increased bursting, nor persistent oscillations in the "aged" cell model; an even greater suppression of K^+ conductances was required. Alternatively, a selective increase in the facilitation property of LTCs could act as a compensatory excitatory mechanism in the "aged" model, with the percentage of K^+-currents blockade kept unaltered (results not shown). Although counter-intuitive, the latter result implies that the age-related increase in Ca^{2+} influx through LTCs ([20] and references therein), could be in fact a compensatory mechanism. This hypothesis might be justified by the preferential activation of LTCs during complex spiking activity [60, 91, 107], and their ability to couple Ca^{2+} influx to downstream signaling cascades [23]. Interestingly, the selective LTCs blocker nimodipine which ameliorates learning impairment in aged subjects [77, 80], cannot effectively block the activity of $\mathrm{Ca}_v1.3\alpha_1$ type of LTCs, which activate at relatively hyperpolarized potentials and seem to induce oscillatory properties in neurons as well as other types of excitable cells ([107] and references therein).

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Chapter 1

Introduction

Cortex is the largest neural network in the brain, highly organized in order to perform sensory processing, motor control and higher-level processes like perception, learning and memory. It consists of a huge number of functionally diverse neuronal cells, connected through synapses in modular circuits [49]. As in any biological system [50], cortical functions depend both on the properties of the network, and of its neuronal elements. For example, the various mental and behavioral activities are correlated to coherent network oscillations in characteristic frequency bands [12, 68]. The generation of this oscillation-based neural code in the brain is supported by the co-operativity of the intrinsic neuronal properties with synaptic recurrent feedback loops [45, 46]. Yet, it is still unclear what computations are performed by selective, nonlinear interactions at the synaptic or the cellular level, and what computations can emerge only at the network level (Cosyne05: `www.cosyne.org`). Because of the inherent complexity of the brain systems, a combination of experimental and computational approaches has been suggested to offer novel insights in the mechanisms underlying their functioning [50].

A number of ion channels span the neuronal membrane and enable the influx or efflux of different ions from the cell, whereas pumps actively maintain the ionic concentration gradients across the membrane [49]. Active properties of the ionic channels' conductances account for the generation of electrical activity, such as oscillatory behavior and resonance [46]. Compartmental modelling involves building a neuronal model as an equivalent electrical circuit, using equations, in order to reproduce "in silico" certain electrophysiological experiments [51, 41] (Fig. 1.1). The level of morphological or biophysical details included in the model, depends both on data availability and the hypothesis to be tested. Computer simulations permit to modify and explore quantitatively selected model's parameters, a task which might be impossible to accomplish in biological preparations. Eventually, we wish to provide predictions for the behavior of mechanisms of interest, to be tested by in vitro and in vivo studies [10, 14].

The main focus of this computational study lies on the age-related deficits in hippocampus-dependent mechanisms of memory, that have been observed across a variety of species. Similar to animal models, computational models of aging might help us in better understanding the functioning of the hippocampus, not just the aging process [4]. It has been proposed that hippocampus is critical for consolidation processes. It is the site where new facts and events are encoded, and their features are bound together, in order for long-term episodic memory to be established [96]. Deficits at the encoding processes that support working memory tasks in hippocampus (i.e., the temporary maintainance or manipulation of information), have been assumed to result in the feature binding deficit of aged subjects [73]. Disruption of hippocampal function affects recent, rather than remote, memories, in a temporally graded manner that seems to depend on the spatial extent of this damage [32]. Hippocampus, then, probably has a time-limited role in the storage and retrieval of

declarative memories which, over time, might be permanently stored elsewhere [96].

Experimental and theoretical studies investigating the neurobiological basis of mammalian Learning and Memory (L&M) have focused mainly on the role of synaptic plasticity, i.e., the changes in the transmission properties of synapses [1]. However, as pointed out in [36], processes additional to those confined to the synapse, are also involved in hippocampal learning tasks. Specifically, it has been argued that the modulation of neuronal excitability might be an essential cellular mechanism of L&M [36]. Modulation of excitability is directly correlated with postsynaptic firing rate, globally regulating the levels of neuronal activity; however, total excitation can be distributed in different ways across the synapses of a network by Hebbian processes [1].

Alterations of neuronal properties such as the postburst afterhyperpolarization (AHP), control the excitability of neurons. Hippocampal pyramidal neurons in learning-impaired aged rodents, exhibit enhanced slow AHP and reduced excitability [55, 87]. Aging-induced alterations of L-type Ca^{2+} channels have been correlated to sAHP enhancement and learning impairments in aged mammals [99, 13, 77, 80, 98, 20]. Disrupted calcium homeostasis during aging has also been reported, mainly as a result of disrupted Ca^{2+} handling, buffering, sequestration and efflux mechanisms [102]. These calcium-related alterations have been reported to occur early during aging, before any significant change in synaptic parameters or dendritic morphology occurs (references in [20]).

On the other hand, aging has been shown to disrupt various neuromodulatory systems which are important for learning and memory, like cholinergic system [96, 22]. Cholinergic muscarinic activation suppresses medium and slow AHP currents in states of selective attention, resulting in membrane depolarization and enhancement of firing activity [86, 89, 53]. The reduction in the slow AHP and, hence, accommodation lasts for $\sim$7 days, as long as it takes for the new stimuli to be consolidated in memory [77], supporting the role of the hippocampus as an intermediate storage buffer during learning [96]. During this typical time-window, hippocampal pyramidal neurons from aged animals that accomplish a hippocampus-dependent task, also display a reduced postburst AHP, similar to the one of young subjects [76].

Experimental studies then, suggest a dynamic adjustment of slow AHP, both "directly", by neuromodulators related to learning [53], and "indirectly", through regulation of LTCs' activity (induced by specific voltage waveforms [60], second-messenger pathways [20], or β_2-adrenergic neuromodulation [16, 44]). The "sAHP hypothesis of aging" proposes that, an enhanced sAHP and/or a reduced capacity to down-regulate it, may be responsible for learning impairments in aged subjects [22].

To study "in silico" the role of these mechanisms in the age-related memory deficits, I have modified a published multicompartmental model of a hippocampal CA1 cell (HPC) [83]. A model of the HPC is useful for studying many excitatory cortical neuron types (pyramidal cells as well as interneurons) with qualitatively similar intrinsic firing properties. Also, experimental data on the HPC are widely available and well documented [10]. Almost all new mechanisms have been adopted from the "working model" of HPC [10]; the modelling formalism for additional modifications, follows suggestions in [10, 21, 108, 41].

The objective was to model the aging-induced alterations of calcium-dependent, non-synaptic, membrane mechanisms, in order to reproduce "in silico" the related excitability reduction due to enhanced L-type Ca^{2+} channels activity [77]. Main changes, then, to the model of reference [83], concern the processes related to calcium dynamics (accounting both for Ca^{2+}-influx and efflux), as well as the potassium channels accounting for the action potential afterhyperpolarization. Simple coupling mechanisms connect specific types of Ca^{2+}-channels to functionally co-localized Ca^{2+}-dependent potassium channels in the soma and proximal apical dendrites. In addition, the mechanisms of high-voltage activated Ca^{2+}-channels include Ca^{2+}-dependence, according to recent experimental findings ([57],

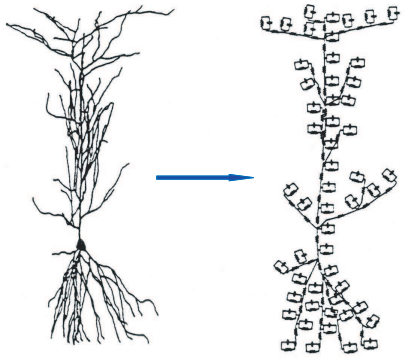


Figure 1.1: **Modelling neurons with electrical circuits** Left: Two-dimensional projection of the anatomy of a CA1 pyramidal neuron. Right: equivalent multi-compartmental model. Adapted from [41].

[110]).

In the subsequent sections, a rather brief overview of the literature related to hippocampus-dependent learning and memory mechanisms is presented, in order to justify the modelling approach adopted in this work.

Chapter 2

Background Theory

The hippocampus is a paleocortical region involved in transferring information during learning from short- to long-term memory storage. Pyramidal cells from CA1 area, have been involved in laying down the "memory trace" in the hippocampus [96]. Memory appears to be encoded by activity-dependent, persistent modifications of the synaptic connections between neurons. These can either enhance or depress synaptic transmission, and are commonly referred to as Long-Term Potentiation (LTP) or Depression (LTD) [49]. It is critical to understand the mechanisms that account for the stability *and* the plasticity of these changes [72].

2.1 Potentiation and Depression of Synapses

LTP in hippocampus has been shown to have distinct phases that correspond to successive stages of memory: an early phase (E-LTP) that lasts 1-2 hours, and does not require new protein synthesis, and a late phase (L-LTP) that lasts longer than 3 hours, and does require local protein synthesis to be maintained [96]. The various forms of synaptic plasticity, then, seem to operate over a wide range of timescales; in addition, they can be synapse-specific or distributed across synapses, and might involve both pre- and post-synaptic mechanisms [1, 81, 58]. Several of these properties have been stated in the well-known Hebb's postulate [40]. Hebb originally proposed how memories could be permanently encoded as changes in synaptic "weights":

```
When an axon of cell A is near enough to excite a cell B and
repeatedly or persistently takes part in firing it, some growth
process or metabolic change takes place in one or both cells such
that A's efficiency, as one of the cells firing B, is increased.
```

LTP of stimulated synapses has been the first physiological mechanism discovered to confirm Hebbian model of memory [8, 7]. LTP is associative in the Schaffer collateral pathway, where the input from CA3 pyramidal neurons, targeting the proximal dendrites of CA1 neurons, has been suggested to provide predictions on the basis of previously stored information [79]. The preferential activation of postsynaptic N-methyl-D-aspartate receptor (NMDA) channels by the coincidence of pre- and post-synaptic activity, satisfies both the associativity and the input specificity of LTP [49]: when the postsynaptic neuron is substantially depolarized by the presynaptic neuron, the Mg^2 plug is removed from the "mouth" of the NMDA receptor channel [7, 96]. Calcium influx through NMDA channels in turn, contributes to the induction of immediate-early genes in the activated synapse, creating a "synaptic tag" that is essential for conferring input specificity of synaptic modification [34].

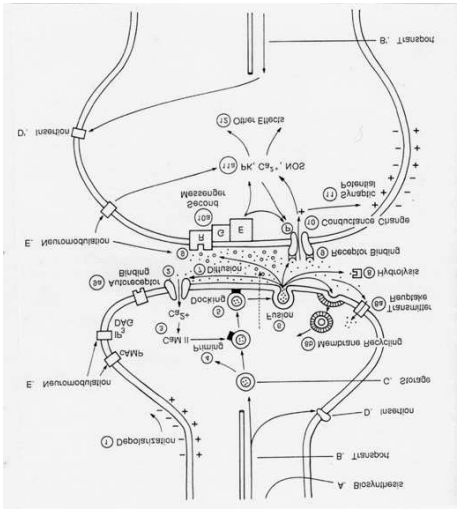


Figure 2.1: **The chemical synapse**. Adapted from [94].

2.1.1 Role of back-propagating Action Potentials

The strong postsynaptic depolarization required for NMDA receptor activation and LTP induction, was thought to arise primarily from the temporal and spatial summation of Excitatory Post-Synaptic Potentials (EPSPs) during high-frequency stimulation of multiple presynaptic fibers [7]. Later experiments revealed that action potentials (APs) initiated near the cell body, actively backpropagate into the dendritic tree, signaling back to the synapse that the postsynaptic cell has fired [97, 95, 66, 70]. Moreover, during natural patterns of presynaptic activity, postsynaptic cell firing was shown to be necessary for the long term modification of recently active "tagged" synapses; and the relative timing of presynaptic activity and postsynaptic spiking (over a $\sim$10 ms timescale), could determine whether "tagged" synapses strengthened, weakened, or remained the same [70, 66, 6, 52], in accordance to the constraints for postsynaptic firing and temporal order in Hebb's postulate (Fig 2.2).

These findings also suggested that postsynaptic, back-propagating APs provided a global associative signal which might influence almost all of the synapses onto the neuron [81]. The extent of this influence is subject to physiological control by ion channels [95, 39, 104], and synaptic inhibition [81]. The active properties of voltage-gated dendritic channels also determine the efficacy of synaptic potentials' integration and orthograde propagation to the soma [48, 62, 43, 35]. The exact timing of neuronal discharge then, is determined not only by synaptic input properties, but also by the intrinsic properties of pyramidal cells which influence their excitability [54].

The above observations about spike-timing dependent plasticity, mainly concern experiments in cultured hippocampal neurons [81]. However, as recent studies in hippocampal neurons from adult animals have shown, repeated pairings of synaptic inputs with single postsynaptic action potentials are not sufficient to induce LTP; instead, pairing presynaptic single spikes with postsynaptic bursting activity, is a necessary and sufficient condition for LTP to be induced [100].

Indeed, stimuli can more efficiently depolarize dendrites and contribute to synaptic modifications if they induce the same number of postsynaptic spikes in short bursts, as opposed to long trains of single spikes [58, 59]. The effectiveness of bursting activity might be explained by the remarkably slow recovery from inactivation of dendritic Na^+ channels, which prevents the later spikes from backpropagating in the distal dendrites of postsynaptic CA1 cells [17, 95]. Synaptic transmission failure is less likely to occur when

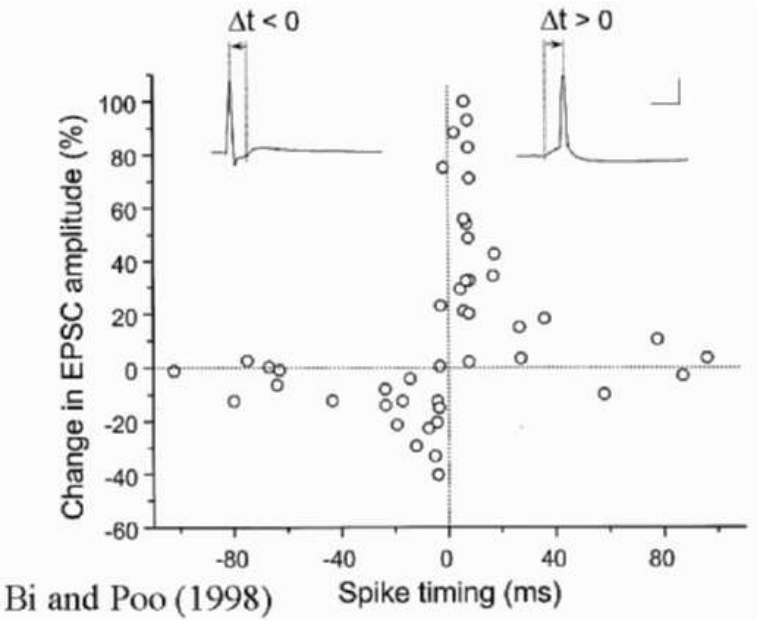


Figure 2.2: **Action potential (AP) backpropagation regulates synaptic plasticity**. The timing between incoming EPSPs and backpropagating APs determines whether LTP or LTD is induced in cultured hippocampal neurons (spike-timing dependent synaptic plasticity). Adapted from [6].

spikes are presented in a short burst: at least one of them will be reliably transmitted [59].

It is worth mentioning at this point that the active properties of ion channels, along with the passive membrane properties, induce frequency preferences in neurons [46], Figures 2.3, reffig:resonance2. Individual neurons respond as bandpass filters and amplifiers, selectively enhancing inputs within specific frequency bands (resonance [45]), or even producing spontaneous membrane potential oscillations (pacemaking activity) [46, 31]. Individual synapses also display a frequency preference due to an interplay between short-term synaptic facilitation and depression [47].

The frequency preference at the synaptic and cellular level, implies that postsynaptic cells will selectively respond to bursts with a specific frequency content, presented in specific inter-burst intervals, as well [47]. The selectivity in the timing of spikes within a burst, and the timing of bursts themselves, might enable the selective, reliable communication between neurons [47]. Furthermore, natural resonance frequencies are of particular biological significance, since they are associated with characteristic brain rhythms, coherent rhythmic activation of large number of neurons [12, 38, 30, 63].

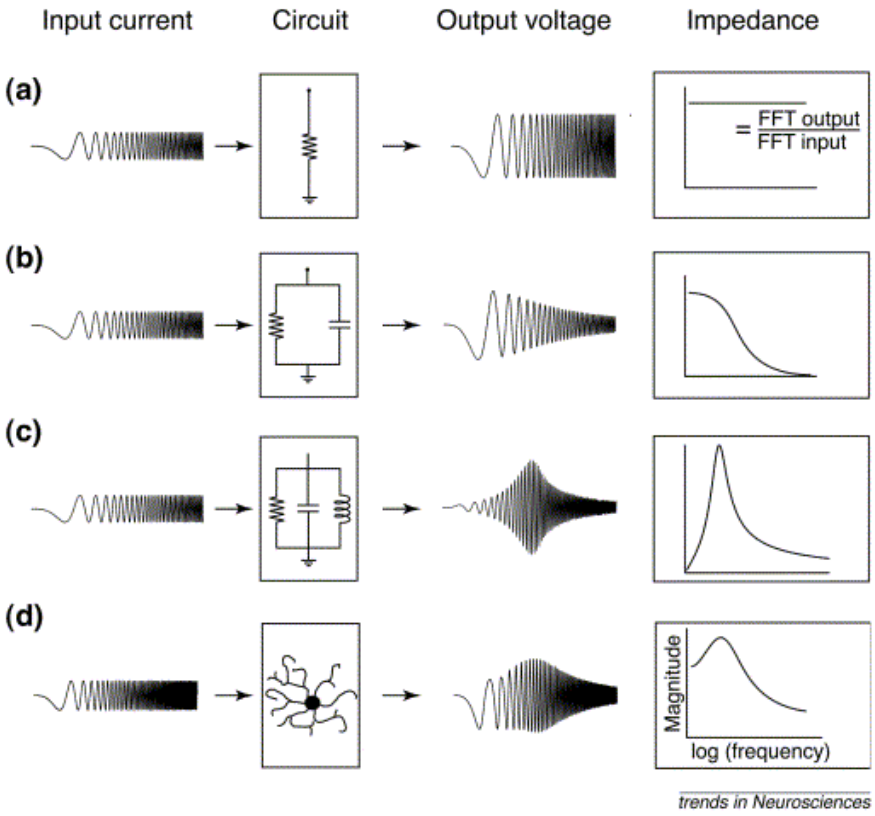


Figure 2.3: **Frequency-dependent properties of electronic circuits and neurons: detection and analysis.** The relationship between the current input (first input) and the voltage output (third column) of electrical circuits or neurons (second column) enables the calculation of impedance as a function of frequency (fourth column). The use of a ZAP input function (a signal that sweeps through many frequencies over time) concentrates the analysis within a specific range of frequencies. Adapted from [46].

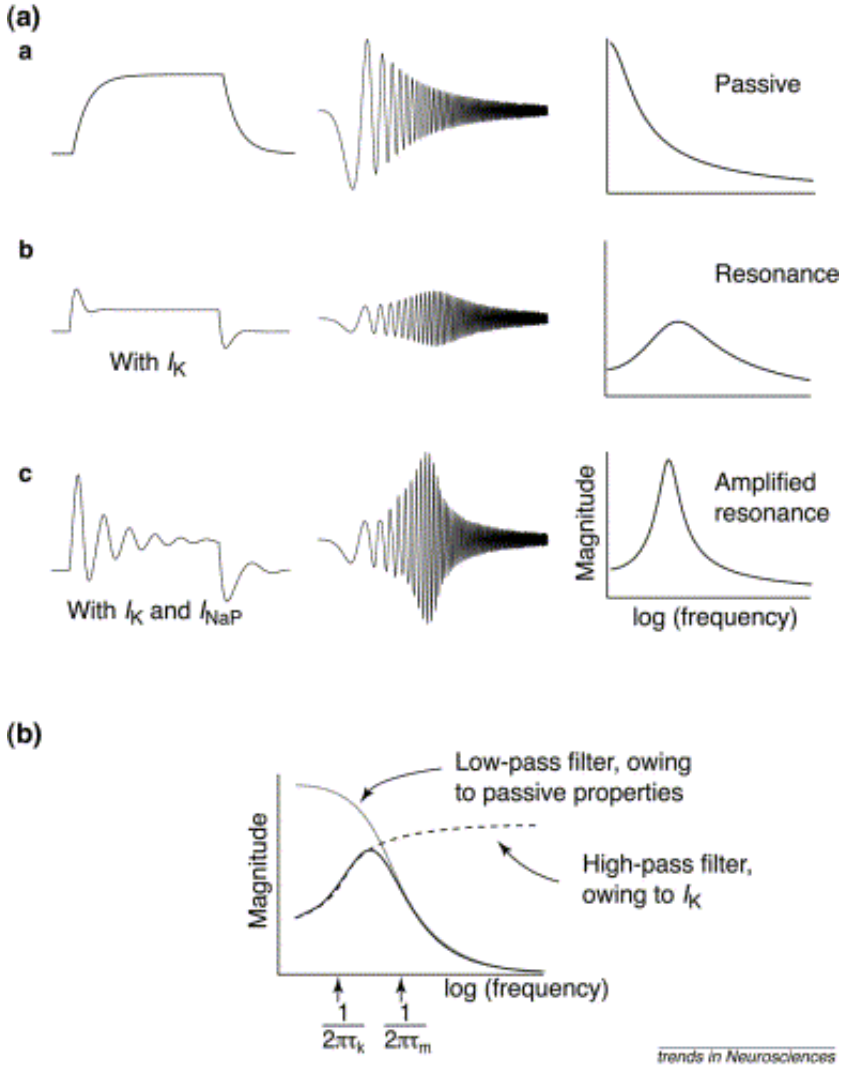


Figure 2.4: **Resonance is formed by the interaction of active and passive properties in a neuron. (a)** Properties of three models that have passive properties only (part a), passive properties plus a resonant current, I_K (part b), and passive properties, a resonant current and an additional amplifying current, I_{Nap} (part c). For each model, the response to a pulse of current is shown on the left, the response to a 'ZAP' input in the middle and the corresponding impedance magnitude on the right. The amplified resonance results in oscillations, and an enlargement and narrowing of the resonant peak in the impedance magnitude. If the conductance of the amplifying current is increased much beyond the value shown, the oscillations become self-sustaining and the model acts as a pacemaker. **(b)**. Demonstration of the separate contributions of the resonant current and passive properties to resonance in the impedance (unbroken line). The broken line shows the contribution of the resonant current (I_K) to the impedance. At low frequencies, the effectiveness of I_K at counteracting voltage changes is high, resulting in a small impedance. This effect is reduced at frequencies above $2\pi/\tau_k$, where τ_k is the time constant for activation of I_K. On the other hand, the passive properties of the membrane (gray line) dominate the impedance at frequencies above $2\pi/\tau_m$, where τ_m is the membrane time constant. The resonant peak occurs between these two frequencies. The figure is adopted from [46].

2.2 θ-burst stimulation inducing LTP

In vivo, hippocampal CA1 neurons generate action potentials either as single, isolated spikes or in high frequency bursts of two or more APs that progressively decline in amplitude and increase in duration during the burst. This second mode of firing, known as the complex spike burst, is a defining electrophysiological signature of HPCs and may represent an important form of information coding in the hippocampus [100]. A detailed internal representation, a cognitive map of space, can be encoded in rodents' hippocampus by individual neurons firing in characteristic patterns. It is thought that these patterns of spiking activity, underlie an animal's ability to remember a given space. Burst firing in CA1 occurs at θ frequencies (5-12 Hz) during exploratory behavior, and it's place specific [96].

The fact that synaptic inputs active during complex spike bursting, undergo robust LTP [100], underscores the contribution of θ-frequency complex spike bursting to memory formation. Specifically, early phase of LTP (E-LTP) can be induced by 5-10 bursts, of 4 pulses at 100 Hz each, delivered at 5 Hz (20-40 pulses total). The late phase of LTP (L-LTP) (which requires protein and RNA synthesis to be maintained) is induced by more than 10 θ-bursts (usually 20-30 in 2-3 trains of 10 bursts each separated by 30 seconds interval), and lasts longer than 3 hours [96, 25].

The key protein-synthetic events involved in L-LTP occur very early after the synaptic stimulation (within 15-30 min). If new RNA synthesis occurs, it takes place nearly coincident with the time of induction, whereas protein synthesis from the pre-existing mRNA in the dendrites is not sufficient to support L-LTP (references in [25]). In [25], it was proposed that action potential firing in the postsynaptic neuron might suffice to activate gene expression, via calcium influx through voltage-gated Ca^{2+}-channels, mainly located at the soma and proximal dendrites ([25] and references therein) (Fig 2.5).

Indeed, it was shown that antidromic stimulation producing somatic action potentials in a θ-burst pattern, although not producing LTP itself without synaptic activation could activate some signaling pathways previously associated with late LTP, such as the Extracellular-signal Regulated Kinase (ERK) pathway [24]. The expression of specific genes induced by the rise in somatic and/or nuclear calcium by action potentials, could rescue early LTP from decay, promoting long-term changes in synaptic strength in stimulated synapses. Nuclear RNA and protein synthesis was largely due to Ca^{2+}- influx through L-Type voltage gated Calcium channels (LTCs), since blocking LTCs with nimodipine, prevented antidromically stimulated phospho-ERK staining [25].

2.2.1 Temporal integration during LTP-inducing TBS

In another series of experiments in hippocampal slices, spike production in the cell body layer was monitored during specific patterns of stimulation mimicking the endogenous θ-rhythm [92]. θ-burst, LTP-inducing stimulation at Schaffer collateral synapses, consisted of three trains of 10 pulses of four 100-Hz bursts delivered at 5 Hz with an intertrain interval of 20 sec between trains. TBS produced a significant amount of postsynaptic spiking, with the likelihood of spike production increasing progressively over the course of the three trains, without any increase in EPSP magnitude. Inhibition of ERK Mitogen-Activated Protein Kinase (MAPK)-activity (i.e., inhibitors of the dedicated upstream regulator of ERK Mitogen and Extracellular signal regulated Kinase, or MEK) dampened this TBS-associated increase in spiking, and blocked LTP induction (Fig 2.6). ERK, then, regulated neuronal excitability in hippocampal CA1 neurons during TBS. Moreover, [92] suggested that the observed increase in AP firing might be a form of physiologic temporal integration which depended on ERK MAPK activity.

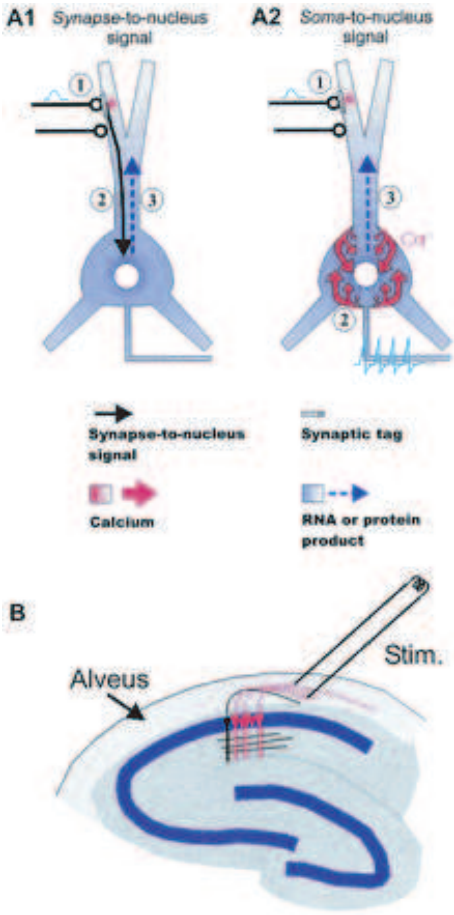


Figure 2.5: **Schematic diagram outlining two contrasting scenarios for LTP-related nuclear signaling**. Adapted from [25]

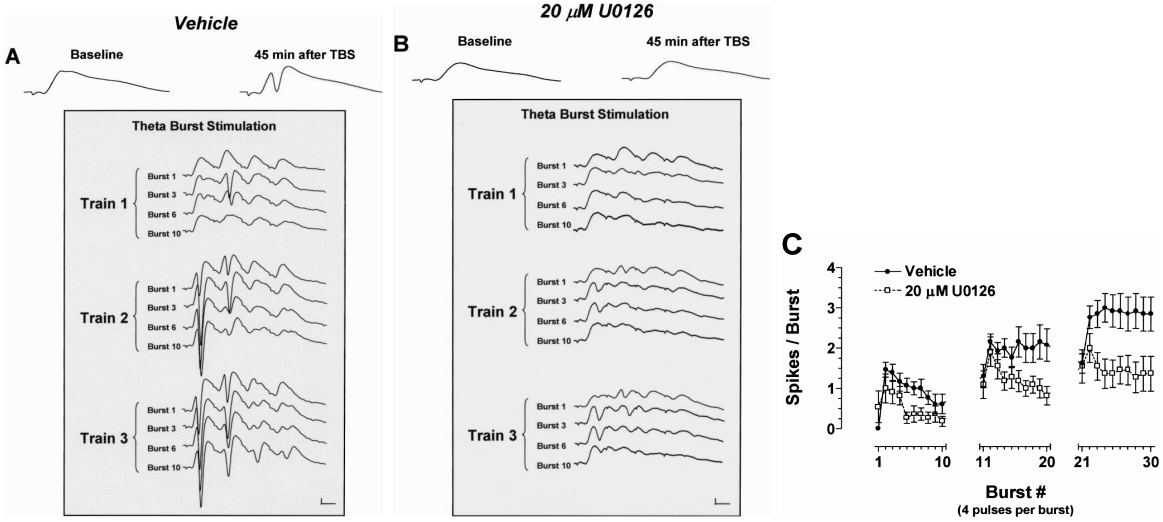


Figure 2.6: **Increased action potential firing over the course of θ-burst stimulation (TBS) is blocked by MEK inhibitors** (U0126) (LTP induction is significantly impaired, as well). (A) Representative traces in response to TBS from a vehicle-treated slice. There is profound difference in spiking between the first and last bursts of the stimulation paradigm. Scale bars (bottom right corner of shaded box) are 1 mV by 5 msec. (B) Representative traces in response to TBS from a slice treated with U0126. Compared with controls, there is much less difference in spiking between the first and last bursts of the stimulation paradigm. Scale bars are 1 mV by 5 msec. (C) Increased spiking during TBS is modulated by ERK. Population spike counts recorded in stratum pyramidale of hippocampal area CA1 during TBS in slices pretreated with vehicle (n = 13 slices) or U0126 (n = 11 slices). Slices exposed to vehicle showed a progressive increase in spike generation during TBS; administration of U0126 impaired this enhanced spiking. Adapted from [92].

It is worth-reminding at this point, the relation of LTCs to ERK MAPK activity [24, 25]. An LTC-calmodulin complex has been reported to signal to the nucleus through the MAP kinase pathway [23]. On the other hand, indirect evidence comes from experimental observations, where both LTCs and ERK/MAPK activation were shown to be facilitated by the activation of β_2 adrenergic receptors in the hippocampal CA1 region [44], [105], [103].

2.2.2 Aging-induced alterations in TBS-induced LTP

LTP induced in the CA1 region using theta frequency stimulation, is selectively impaired in aged rats that exhibit poor spatial learning (Aged-Impaired, AI). Also, 5 Hz LTP amplitude strongly correlates with individual learning performance among aged rats, being almost intact in aged rats that exhibit spatial learning (Aged-Unimpaired, AU). This defect in the ability to store spatial memory is consistent with the spatial memory deficits observed in aged humans [101].

Although the putative mechanisms for theta-frequency LTP have been already described in [100, 105], the basis for the AU-AI difference in 5 Hz LTP remains speculative. Synaptic and dendritic parameters (such as the length and degree of arborization of dendrites) have been reported to remain almost unchanged during early aging [20]. Internal Ca^{2+} accumulation, instead, increases early, before a significant reduction in the number of synapses occurs. Specifically, an elevation in the free cytosolic Ca^{2+} concentration has been reported, most likely resulting from a combination of disrupted Ca^{2+} handling, buffering, sequestration, influx and efflux mechanisms [102]. One possible scenario then for this difference in θ-frequency synaptic potentiation between cognitively impaired from unimpaired aged rats, involves a difference in Ca^{2+}-channels signaling [101]. Calcium influx through L-type Ca^{2+}-channels (LTCs) in rat CA1 neurons increases with age [55], and has been linked to the activation of the slow afterhyperpolarization (sAHP) channels ([71, 11]). CA1 neurons in aged rodents exhibit both larger sAHP and decreased membrane excitability, which vary inversely with hippocampal-dependent learning ability ([55, 77, 22]). Reduced cell excitability in turn, might eliminate complex spike probability normally observed in 5Hz TBS, and spike propagation into the dendrites, which is critical for the induction of 5 Hz LTP ([101] and references therein).

This scenario supports the hypothesis that the control of neuronal excitability might be an indirect mechanism for L&M. In subsequent sections, calcium channel signaling, and sAHP hypothesis of aging are reviewed in more detail.

2.3 Calcium dynamics and intracellular pathways

In order to understand the behavior of single neurons on time scales of more than a few milliseconds, it is often necessary to take the dynamics of calcium into account. Ca^{2+} acts as an intracellular messenger, relaying information within cells to regulate their activity. A combination of elementary and global Ca^{2+} signals have been adapted to regulate a range of processes in a single neuron. For example, Ca^{2+} is pivotal in receiving and transmitting neuronal signals, as well as in regulating excitability and the changes that underlie learning and memory [5], [65], [15], [109], [57].

Ca^{2+} signalling depends on increased levels of intracellular Ca^{2+}, derived either from sources outside the cell (Ca_o^{2+}) or from internal calcium stores within the endoplasmic reticulum. Ca_o^{2+} may enter through (1) voltage-gated Ca^{2+} channels (VGCCs) in excitable cells such as neurons or muscle cells, or (2) receptor-operated Ca^{2+} channels (ROCs) in response to neurotransmitters. The neuronal endoplasmic reticulum network contributes

to the dynamics of Ca^{2+} signaling by acting either as a source or as a sink of signal Ca^{2+}. The existence of this internal reservoir of Ca^{2+} can have a profound effect on shaping neuronal Ca^{2+} signals. Elevations in Ca^{2+} can be highly localized within compartments such as the spines or the terminals or they can spread through neurons as global Ca^{2+} waves [5].

Spatially restricted Ca^{2+} signals also control neuronal excitability (Fig. 2.7). When neurons fire, information is relayed down the axon to the synaptic ending, activating the secretion of neurotransmitters - chemicals that excite neighboring neurons. Elementary Ca^{2+} signals are used to produce brief, highly localized transients that trigger release of vesicles containing the neurotransmitter. In the cell body itself, elementary Ca^{2+} signals can modulate neuronal excitability by activating Ca^{2+}-dependent K^+ channels. These channels allow an efflux of K^+ ions through the plasma membrane, hyperpolarizing the membrane and inhibiting subsequent electrical activity [5].

To create more permanent memories, the short-term modifications described above have to be consolidated by information from the nucleus. Ca^{2+} is involved in this case, as well, recruiting additional signalling components that migrate into the nucleus and activate genes there. In addition, the Ca^{2+} signals themselves, derived from either the entry or release of Ca^{2+}, can also activate genes in the nucleus [5] (Fig. 2.7).

"Voltage-gated Ca2+ channels mediate Ca2+ entry into cells in response to membrane depolarization. Electrophysiological studies reveal different Ca2+ currents designated L-, N-, P-, Q-, R-, and T-type. The high-voltage-activated Ca2+ channels that have been characterized biochemically are complexes of a pore-forming 1 subunit of 190250 kDa; a transmembrane, disulfide-linked complex of 2 and subunits; an intracellular subunit; and in some cases a transmembrane subunit. Ten 1 subunits, four 2 complexes, four subunits, and two subunits are known. The Cav1 family of 1 subunits conduct L-type Ca2+ currents, which initiate muscle contraction, endocrine secretion, and gene transcription, and are regulated primarily by second messenger-activated protein phosphorylation pathways. The Cav2 family of 1 subunits conduct N-type, P/Q-type, and R-type Ca2+ currents, which initiate rapid synaptic transmission and are regulated primarily by direct interaction with G proteins and SNARE proteins and secondarily by protein phosphorylation. The Cav3 family of 1 subunits conduct T-type Ca2+ currents, which are activated and inactivated more rapidly and at more negative membrane potentials than other Ca2+ current types. The distinct structures and patterns of regulation of these three families of Ca2+ channels provide a flexible array of Ca2+ entry pathways in response to changes in membrane potential and a range of possibilities for regulation of Ca2+ entry by second messenger pathways and interacting proteins.[15]"

2.3.1 L-type Ca^{2+} channels

Experimental findings suggest that the increase in internal Ca^{2+} accumulation can be explained by a substantial increase in the functional density of single L-type Ca^{2+} channels [99], or, by increased phosphorylation of the neuronal L-type Ca^{2+} channel Cav1.2 during aging [20]. This increase can in turn be linked to an enlargement of somatic calcium action potential width and a subsequent enhancement of Ca^{2+}-dependent afterhyperpolarization (AHP) and spike frequency adaptation [77].

Multiple functional kinds of L-type channels (LTCs) exist, and they differ in their conductance and kinetic properties [107], [91]. The biophysical diversity of the subtypes of LTCs means that they probably have different functional roles. Pharmacological antagonists used to block L-type channels, mostly dihydropiridines like nimodipine, do not discriminate between the recently identified Lp (or, D-class, or $Ca_v1.3$) and Ls (or, C-class, or $Ca_v1.2$) subtypes of L-type calcium channel; moreover, dihydropiridines do not

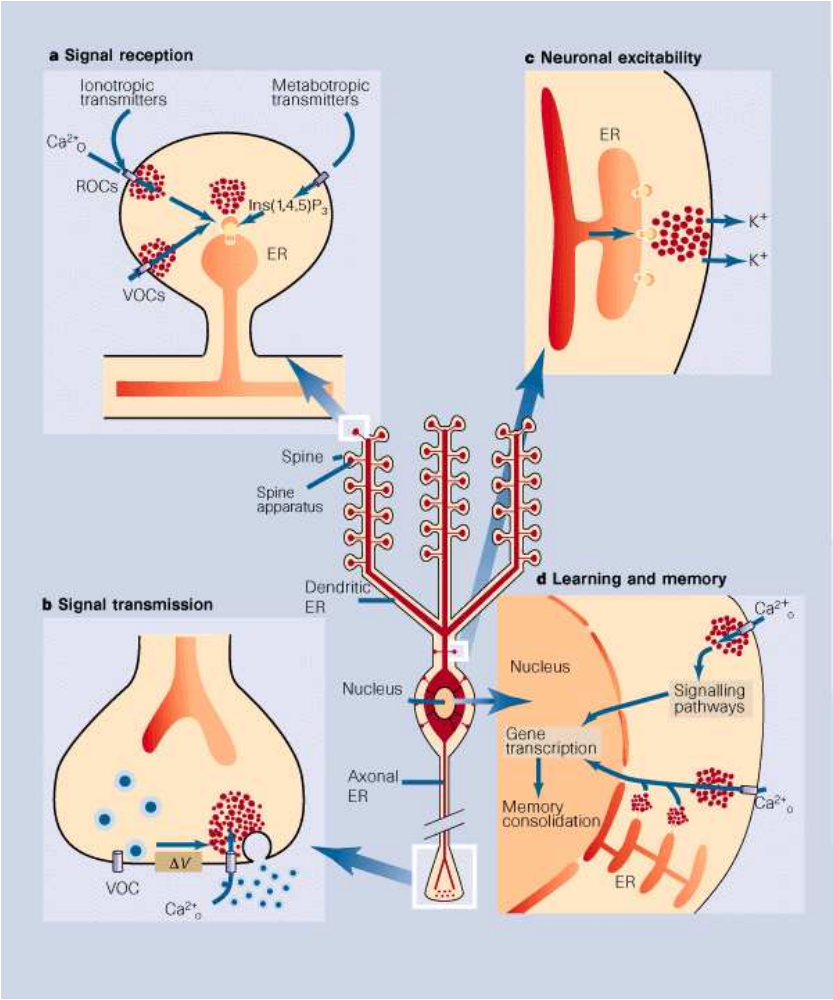


Figure 2.7: **Compartmentalization of Ca^{2+} signals in neurons**. a, External Ca^{2+} enters through receptor-operated, or voltage-gated Ca^{2+}-channels (VGCCs). The signal can be amplified by activating receptors for Ins(1,4,5)P_3 on the spine apparatus. By integrating separate inputs, these receptors could detect coincident signals. b, Action potentials (V), cause the entry of Ca^{2+} through VGCCs stimulating neurotransmitter release. c, Localized Ca^{2+} signals open K^+ channels which regulate neuronal excitability. d, Possible role of Ca^{2+} in memory consolidation. Entry of external Ca^{2+} can act locally by co-opting other signalling pathways, or it can act globally by flooding directly into the nucleus. This global signal can be amplified by release of Ca^{2+} from the internal stores. Adapted from [5].

consistently block Lp-type channels. Thus,the criterion used to distinguish the types of L-type channels should be the presence of repolarization reopenings (openings that occur subsequent to a depolarization and return to the holding potential). Lp channels are preferentially activated by the same kinds of stimuli known to elicit changes in synaptic strength [91]. Both Ls and Lp channels are effectively activated when simulating postsynaptic responses to a burst of presynaptic action potentials, but Lp channel activity is more sustained, whereas Ls channel activity terminates after the end of the action potential train. It has been suggested then, that Lp channels might be critically involved in the induction of NMDA receptor-independent forms of synaptic plasticity [91].

Interestingly, Ca^{2+} entry through L-type Ca^{2+} channels, except for driving AHP, triggers transcriptional events with major downstream consequences for learning and memory [23]. L-type channels are examples of molecular signal-transduction units that regulate themselves through their own activity: they display inactivation and facilitation, both of which are closely linked to the earlier entry of Ca^{2+} ions. Calmodulin (CaM) is the critical Ca^{2+} sensor linking the Ca^{2+} regulation of L-type Ca^{2+} channels [109] to the local Ca^{2+} trigerring of nuclear gene transcription [23]. The same calmodulin molecule seems to act as a Ca^{2+} sensor for both positive and negative modulation. Both forms of autoregulation have a significant impact on the amount of Ca^{2+} that enters the cell during repetitive activity [109], [26]. The signalling capabilities of CaM seem to generalize to numerous biological systems, such as other high-voltage activated Ca^{2+} channels [74],[57], and small conductance K^+ (SK) channels (in which CaM affects gating by interacting with domains in analogous positions) [106].

2.3.2 N-type calcium channels

In hippocampal neurons, a fast afterhyperpolarization (fAHP) immediately follows an action potential, lasting up to ten milliseconds. It contributes to cell membrane repolarization, controlling thus the spike width. The fAHP is mediated by a Ca^{2+}- and voltage-activated K^+ current, I_C, which likely results from the activity of large conductance potassium channels (BK channels) [28]. The dependence of BK channels' activation both on calcium and membrane depolarization, allows them to operate as coincidence detectors, a role of great significance to their physiological function ([28] and references therein).

According to recent experimental findings, BK channels are selectively activated by co-localized high-voltage activated N-type Ca^{2+} channels (NTCs), their activation being nearly coincident [71]. BK channels are located mainly in the soma and proximal dendrites of CA1 pyramidal neurons [84] - and NTCs are located there as well [64]. NTCs display both Ca^{2+}- and voltage-dependent inactivation [57], particularly in response to complex voltage waveforms [60]. On the other hand, BK channels inactivate rapidly during repetitive firing, accounting for the frequency-dependent decline of the fast AHP and subsequent spike broadening [93] (Fig.2.8). A simplified modeling approach to the functional coupling between the above conductances, could exploit the facts that: (a) intracellular Ca^{2+} concentration is an indicator of the cell's electrical activity [51]; and (b) local rather than global rises in calcium are involved in the coupling of calcium-dependent processes [71]. Thus, it seems reasonable to describe BK channels' calcium dependence using a phenomenological, calcium pool model to account for influx through NTCs [21].

The time course of spike re-polarization is of particular significance since action-potential duration determines the amount of activity-dependent Ca^{2+} influx, affecting numerous downstream Ca^{2+}-dependent processes, such as postburst afterhyperpolarization [89, 37, 28], and transmitter release [28].

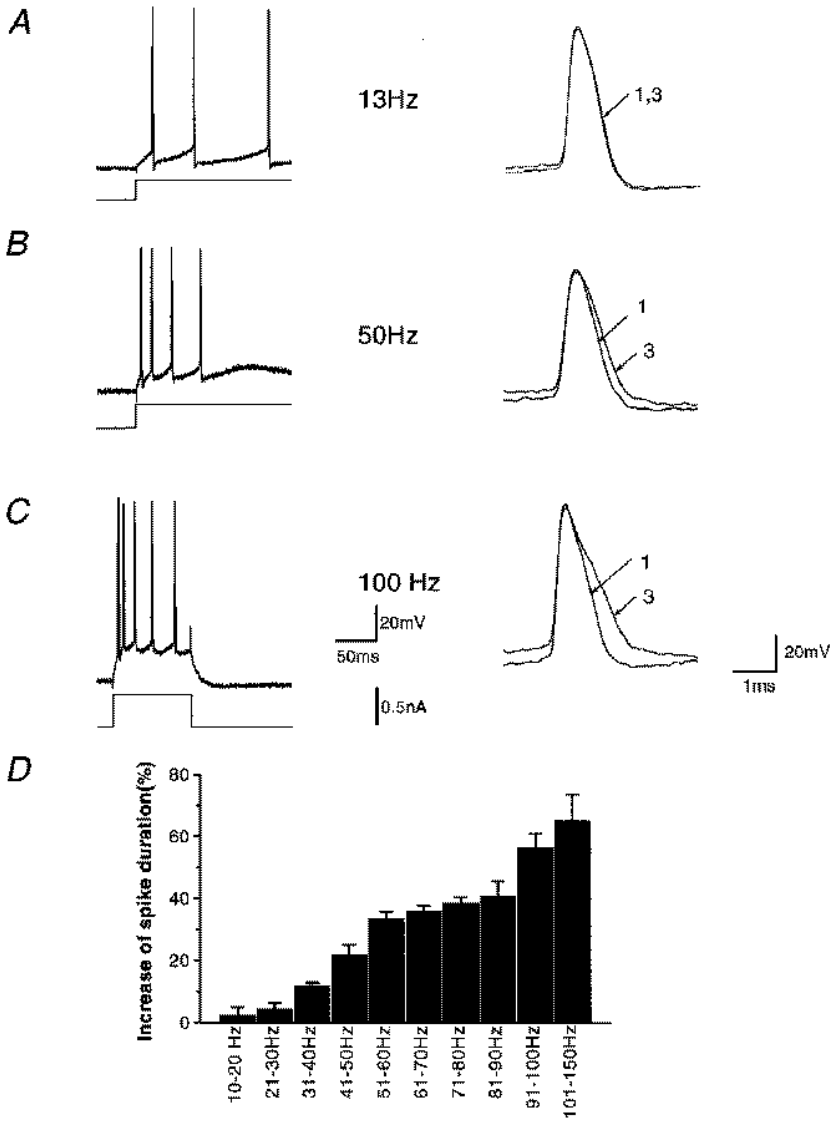


Figure 2.8: **Frequency dependence of the spike broadening**: A, during low-frequency repetitive firing (average frequency 13 Hz), there was no detectable broadening from the 1st spike to the 3rd and no clear decline of the fAHP amplitude. B and C, during higher-frequency repetitive firing (average frequencies, from the 1st to the 3rd spike: 50 and 100 Hz), in response to stronger current injections, there was increasing broadening of the 3rd spikes and decline of the fAHP amplitude. A-C are from the same cell. D, summary of the spike broadening at different average discharge frequencies (means minus-plus S.E.M., n = 10). Adapted from [93].

2.4 Medium and Slow afterhyperpolarization channels

Afterhyperpolarization (AHP) is mediated by four K^+ currents (I_C, I_M, I_{AHP}, and slow I_{AHP}) [28].Its time course is modulated by the hyperpolarization-activated current, I_h[62]. Given the kinetics of these currents, previous experiments showing prolonged AHP and enhanced accommodation in aging neurons strongly implicate alterations in the slower currents, particularly the sIAHP, in aging ([55, 77, 76, 87]).

Recordings from hippocampal slices have shown that the slow AHP follows after a train of action potentials [28], and likely results from the activation of small-conductance calcium-activated potassium (SK) channels [89, 28], although recent findings report that sAHP channels are not of SK type [9]. SK channels sense local calcium transients driven by a train of action potentials and allow an efflux of K^+ ions through the plasma membrane that tend to hyperpolarize it. They serve to reduce the firing rate of action potentials during a long period of membrane depolarization caused by current injection directly into the neuron or by high frequency sustained excitation - a phenomenon called spike-frequency adaptation (accommodation). SK channels are activated rapidly in response to a rise in calcium [106], independent of the transmembrane voltage. SK channel subtypes exhibit an open probability (P_o) of 0.5 at a calcium concentration of 0.5-0.7 μM, predicting a requirement for an intracellular calcium concentration of 1 μM [42]. However, bulk increases of intracellular calcium of only 30 nM have been measured during the slow AHP. For SK channels to experience 1 μM calcium, micro-domain models predict that they must be within 150 nm of a calcium channel [71].

In addition, SK channel kinetics alone are not sufficient to explain the time course of the slow AHP [42]. An alternative explanation is that the calcium channel kinetics might account for the slow AHP dynamics. It has been reported that a train of action potentials that would evoke the slow AHP, induces an enhanced activity of L-type calcium channels at membrane potentials negative to -50 mV. This behavior - termed delayed facilitation - has been proposed to provide a prolonged source of calcium entry at negative membrane potentials. Both the time course and modulation of delayed facilitation closely resemble those of the slow AHP [16]. In addition, L-type Ca^{2+} channels in rat CA1 neurons seem to be functionally colocalized to the slow afterhyperpolarization channels [71], [11]. Therefore, the observed co-localization of L-type Ca^{2+} channels and SK channels, together with the delayed facilitation of L-type channels, might account for the AHP slow dynamics [16, 77, 11].

In aging neurons, saturating concentrations of L-type channel pharmacological blocker nimodipine cause quantitatively greater reductions in the sAHP current in aging neurons than in young neurons, suggesting that the contribution of the L-type Ca^{2+} influx to the sAHP activation, is enhanced in aging. However, after blocking the L-type Ca^{2+} influx, the residual sAHP current from aging neurons remained significantly larger than that of the young neurons, indicating that the enhanced L-type Ca^{2+} influx alone is not sufficient to account for the aging-related enhancement of the sIAHP [87]. On the other hand, the reported incomplete blockade of $Ca_v1.3$ (or, D-class) L-type channels by dihydropyridines, might explain this residual sAHP current [107].

The sIAHP is a Ca^{2+}-dependent K^+ current that is modulated by many neurotransmitters. Therefore, its amplitude also depends on the degree of neuromodulation it receives.

2.4.1 Altered neurotransmission and sAHP hypothesis of aging

The brain has a number of modulatory systems that are important for attention, learning, and memory. With aging, there is a loss of modulatory input into the hippocampus

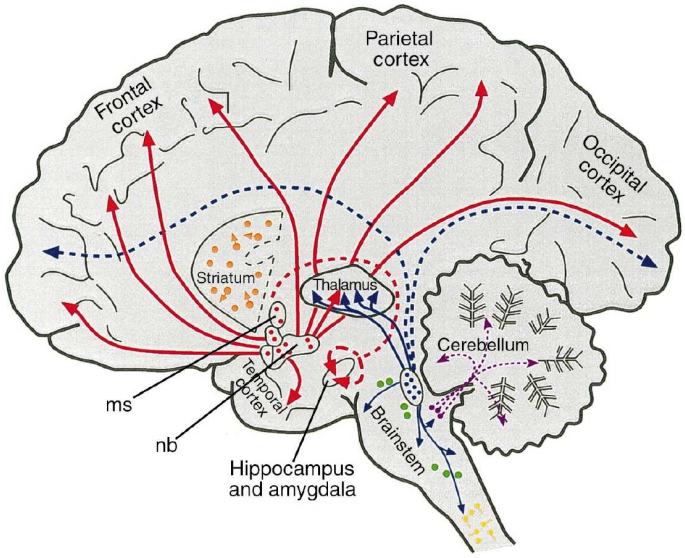


Figure 2.9: **Cholinergic systems in the human brain**: Two major pathways project widely to different brain areas: basal-forebrain cholinergic neurons [red, including the nucleus basalis (nb) and medial septal nucleus (ms)] and pedunculopontine-lateral dorsal tegmental neurons (blue). Other cholinergic neurons are included, too. Adapted from [82].

from brain systems that release the neurotransmitters acetylcholine (ACh), norepinephrine, serotonin, and dopamine. Cholinergic muscarinic influences, in particular, seem to be vital to hippocampal-dependent memory processes 2.9. Activation of the cholinergic system has been shown to modulate processing in sensory and visual cortex. The cholinergic innervation of the cerebral cortex and the hippocampus originates primarily from the cholinergic basal nuclear complex. Lesions of these basal forebrain neurons have been reported to result in impairment in memory, learning, and attention, whereas cholinergic agonists facilitate learning and memory [96]. Furthermore, the persistence of the ACh levels in the hippocampus is correlated with the duration of this structure's involvement in memory consolidation [86].

The hippocampal sIAHP can be reduced by many neurotransmitters that are implicated in L&M: metabotropic glutamate agonists, acetylcholine, serotonin, histamine, dopamine, noradrenaline, corticotropin releasing factor, vasoactive intestinal peptide, and calcitonin gene-related peptide. Many of these molecules were shown to suppress the sIAHP through protein kinase activities. Changes in many of these neurotransmitter systems, as well as their effector kinases, have been implicated in aging. Conceivably, altered neurotransmission in aging, coupled with altered kinase functions, can shift the balance between kinase and phosphatase activities that normally maintain the sIAHP and alter this current. The enhanced sIAHP in aging neurons is a postsynaptic phenomenon and does not reflect changes in basal neurotransmission ([87] and references therein).

Molecules that affect the sIAHP have also been implicated in other forms of plasticity. For example, kinases known to modulate the sIAHP (PKC, PKA, and calcium-calmodulin kinase II) are also important for the induction of long-term potentiation (LTP), suggesting a role for the sIAHP in controlling dendritic excitability and synaptic integration. Consistent with this hypothesis, activation of the sIAHP dampens temporal summation of the EPSPs as well as speeds up their decay rate. Pharmacological manipulations that facilitated LTP have also been shown to reduce the AHP, suggesting that the AHP and its underlying currents can serve as an adjustable gain control, variably hyperpolarizing and shunting synaptic potentials arising in the distal dendrites and controlling the induction

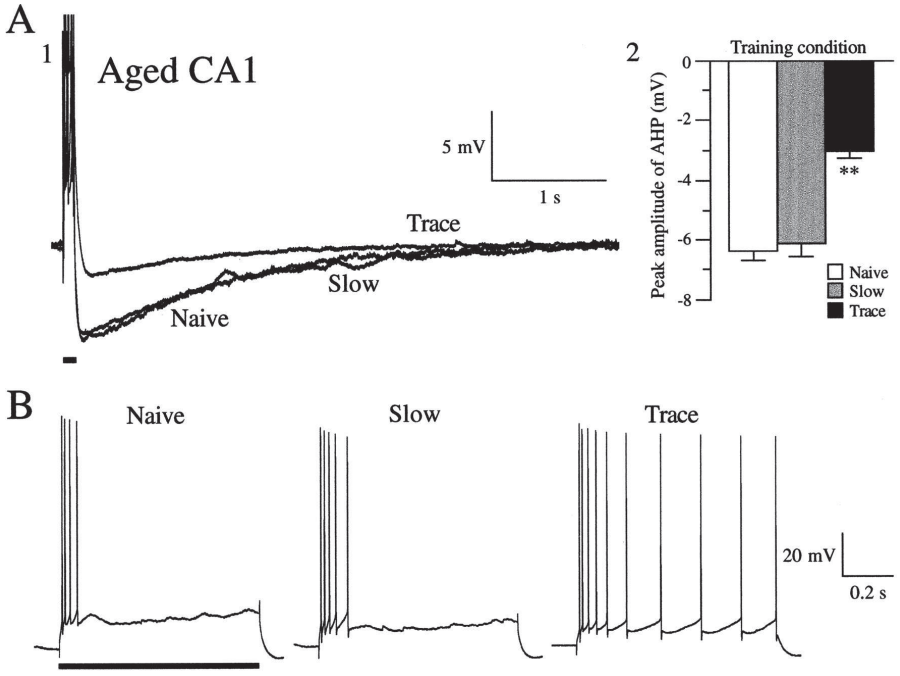


Figure 2.10: **Acquisition of hippocampally dependent trace eyeblink conditioning increased excitability of aging rabbit hippocampal CA1 pyramidal neurons**. (A) Effects of trace conditioning on the size of the postburst AHP. (1) Overlay of voltage recordings of the postburst AHP in CA1 neurons from an aging naive rabbit (Naive), an aging rabbit that showed $< 15\%$ CRs after 15 sessions (Slow), and an aging trace-conditioned rabbit (Trace). (2) Mean effects of trace eyeblink conditioning on postburst AHP amplitude in aging rabbit CA1 neurons. Notice that, after learning, the AHP was significantly reduced compared with naive and slow-learning aging controls. (B) Typical examples of accommodation responses in CA1 pyramidal cells from aging naive (Naive), aging slow-learning (Slow), and aging trace-conditioned (Trace) rabbits. Adapted from [76].

of further plasticity ([54]. Accordingly, the enhanced sIAHP in aging can hamper the formation of further plastic alterations important for learning and memory ([89], [36] and references therein).

Learning can be associated with an alteration of excitability. Reductions in the AHP and accommodation have been observed in neurons from animals trained in various hippocampus-dependent [77], [76] and non-hippocampus-dependent tasks. These biophysical changes are most likely learning induced, because they are not observed in neurons of pseudoconditioned controls (which receive the same but unpaired stimuli), naive controls, and animals that failed to acquire the task. Furthermore, reductions in the AHP and accommodation return to baseline in 7 d [77]. The time course of increased excitability may represent a critical window during which learning-specific alterations in postsynaptic excitability of hippocampal neurons are important for consolidation of the learned association elsewhere in the brain. [22] (Fig. 2.10). Also, drugs that reduce the AHP in vitro have also been shown to improve learning in aging animals ([76, 87] and references therein).

A working hypothesis has been proposed that the sAHP and accomodation of CA1 neurons from aged animals are potentially plastic and reducible; and that aging rabbits that fail to acquire the task have neurons with too large an AHP to allow learning to occur and/or reduced capacity for reducing AHP [22]. The 'sAHP hypothesis of aging' [22] is linked to the assumption that modulation of the hippocampal sAHP is an L&M

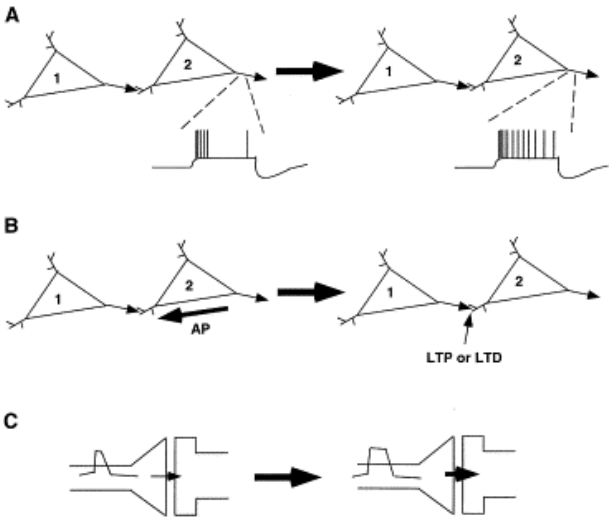


Figure 2.11: Modulation of excitability as an L&M mechanism. Three different scenarios illustrate how modulation of excitability could be involved in L&M. In each case the situation before and after learning is shown. (A) The excitability of neurone 2 changes (e.g., due to signalling from acetylcholine) with no lasting synaptic implications. (B) AP backpropagation regulates synaptic plasticity. The timing between incoming EPSPs and backpropagating APs determines whether LTP or LTD is induced. (C) Alteration of excitability is responsible for synaptic plasticity. Adapted from [36].

mechanism [36].

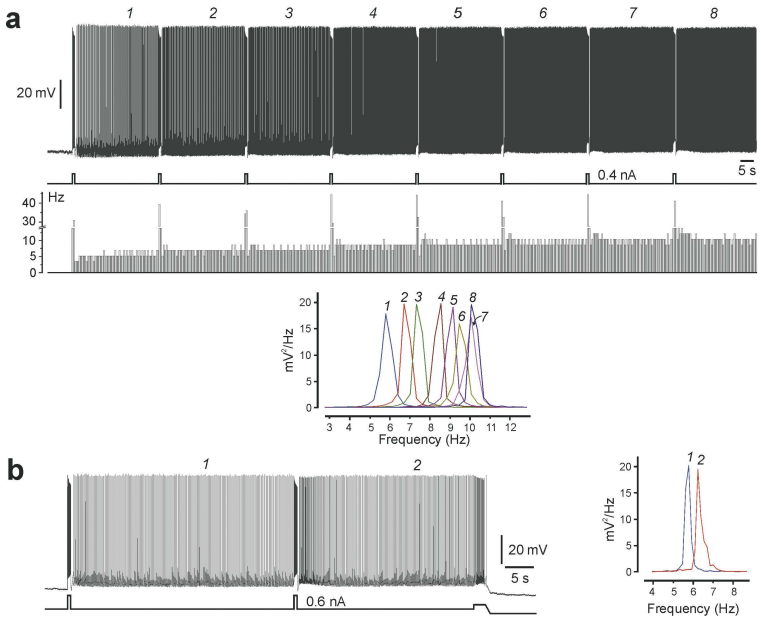
In Fig 2.11, the different scenarios on the role of excitability in learning and memory are illustrated. Cases (B) and (C) are related to post- or pre- synaptic mechanisms of memory, already mentioned briefly above. Case (A) refers to the non-synaptic, neuronal level; according to [36], an altered firing behavior does not seem to be specific for different inputs and may not be long-lasting in order to be considered as an additional learning mechanism. However, recent experimental evidence has added another point of view in this topic.

2.5 Persistent activity and working memory

In some brain areas, neurons show sustained spiking activity after a transient stimulus, with firing levels that vary with the stimulus parameters in a graded manner. The sustained firing rates are proportional to the time integral of the previous stimuli, making them candidate neuronal integrators (Fig. 2.12).

In [27] muscarinic cholinergic actions were simulated in a single, isolated neuron in layer V of entorhinal cortex (EC), using the cholinergic agent carbachol (CCh). When stimulated

Figure 2.12: Memory and the single neuron. Adapted from [19].



Supplementary Figure A. Graded persistent activity with short stimulus. **a**, Repetitive stimulation with a 1 sec long depolarizing step gives rise to seven distinct increases of discharge rate (CCh, 10 μM). The middle diagram corresponds to the peristimulus histogram (bin of 580 ms) and the plot at the bottom illustrates the Fourier analysis for the corresponding numbered segments. **b**, Graded persistent firing induced by a 600 ms stimulus. The right plot illustrates the Fourier analysis for the corresponding numbered segments. Initial membrane potential in **a**, **b**: –62.8 mV, -60 mV.

Figure 2.13: Graded persistent activity with short stimulus. Adapted from [27].

briefly, either by step depolarizations or by repetitive activation with a synaptic train, the neuron could generate sustained increases in its electrical activity that were graded in intensity and readily reversible. A non-synaptic spike- and Ca^{2+}-induced potential was sufficient for the generation of the prolonged persistent activity. In addition, graded decreases in stable frequency could also be produced by synaptic inhibition. In other words, this neuron could quickly "remember" (and "forget") multiple bits of information.

Working memory - already mentioned above - represents the ability of the brain to hold externally or internally driven information for relatively short periods of time. The above mentioned persistent neuronal activity is thought to be the elementary process underlying working memory [27], [3], [61].

Persistent activity for working memory can directly encode dimensions of input or output signals since it can maintain stable analogue values of activity [61]. The feature that is essential for persistent firing is an excitatory feedback that maintains neural activity after a short stimulus gets it started. In [27], persistent firing is observed after cholinergic actions that block K^+ currents and increase spike frequency (Fig. 2.13). Moreover, the triggering stimulus must cause Ca^{2+} ions to enter the neuron, probably through Ca^{2+}-selective membrane channels; internal Ca^{2+} seems to activate Ca^{2+}-dependent nonspecific cation (CAN) channels, which provide an inward ionic current that further excites the neurons. By this scheme, Ca^{2+} is the trigger stimulus and the CAN current underlies the persistent activity [19].

Yet, this scheme does not fully explain what physiological mechanisms might account for the graded nature of the persistent activity, or how it could be reversed. In [19], it is mentioned that each pulse of Ca^{2+} (spike-induced Ca^{2+} influx) triggers (directly or indirectly) a change of state in just a fraction of the CAN (Ca^{2+}-dependent nonspecific cationic, or some other) channels, slightly enhancing the neuron's excitability. During brief periods of inhibition, internal Ca^{2+} levels might fall, and the CAN channels would relax

towards their resting state.

In this work, it is suggested that the Ca^{2+}-dependent nonspecific cation (CAN) channels, could be L-type Ca^{2+} channels. They provide an inward, regenerative ionic current that further excites the neuron and might lead to persistent activity when the inhibitory effects of medium and slow AHP currents are sufficiently blocked by neurotransmission. I_{sAHP} gradually builds up, following the time-course of LTC current, and can quickly reverse persistent activity. Eventually, the excitatory and inhibitory feedback loops that arise due to the selective coupling of these Ca^{2+} and Ca^{2+}-dependent K^+ channels, seem to maintain a delicate, sustained balance of excitability subject to neuro-modulatory control. Indeed, in [27], L-type Ca^{2+}-channel blocker nifedipine curtailed the ability of the cells to generate persistent activity, in all cases.

Chapter 3

Methodology

3.1 Theoretical model

Although experimental findings suggest a direct connection between the aforementioned calcium-potassium channel types, no modelling work has been done to investigate the effects of such a coupling on the cell's electrical activity. In this work, a previously developed detailed compartmental model of a CA1 pyramidal neuron [83] is modified to include sAHP and fAHP K^+ channels which are coupled to L- and N-type Ca^{2+} channels, respectively.

The compartmental model used in this work implements the variable time step method of the NEURON simulation environment [41]. The biophysical model, the morphology of which is shown in fig. 1.1A, is a refinement of a previous model described in [83]. The model consists of 183 compartments and includes a variety of passive and active membrane mechanisms known to be present in CA1 pyramidal cells. A uniform membrane resistance $Rm = 40k\Omega cm^2$, a uniform intracellular resistivity $Ra = 70\Omega cm$, and a specific membrane capacitance of $1.0\mu F cm^{-2}$ were assumed. The resting membrane potential of the model neuron is $-70mV$. Active mechanisms include two types of Hodgkin-Huxley-type Na^+ currents (somatic and axonic I^{sa}_{Na}, and dendritic I^{d}_{Na}), three voltage-dependent K^+ currents (I_{Kdr}, I_A, I_m), a fast Ca^{2+}- and voltage-dependent K^+ current, I_{fAHP}, a slow Ca^{2+}-dependent K^+ current, I_{sAHP}, a hyperpolarization-activated non-specific cation current (I_h), a low-voltage activated calcium current I_{CaT}, three types of Ca^{2+}- and voltage-dependent calcium currents, I_{CaN}, I_{CaR} and I_{CaL}, and a persistent sodium current I_{Nap}. Channel equations, distributions and densities of I_{Na}, I_{Kdr}, and I_A are described in [83]. I_{fAHP} is modeled according to [108] and is driven by NTCs activation, whereas I_{sAHP} has been modelled according to a standard formalism [10] but is driven by LTCs activation. This approach has been based on the same activation protocol and time-course of respective currents ([16], [71], [93]).

More specifically,the half-activation [Ca^{2+} concentration of I_{sAHP} has been taken from [53], [106]. The time-constant of its activation is faster, but it still exhibits slow dynamics since it is driven by the slow, "delayed" facilitation of LTCs [16]. There are two types of LTCs in the model, with different activation kinetics and sensitivity to dihydropyridines, the common pharmacological blockers of LTCs [107], [91]. In neurons, 20 % of LTCs are of $Ca_v1.3_{\alpha_1}$ type (or, "D-class"), and account for facilitation in the model, whereas 80% are of $Ca_v1.2_{\alpha_1}$ type (or, "C-type") [20]. Both types seem to display age-dependent up-regulation of their activity ([20] and references therein), accounting for the reported increase in Ca^{2+} influx through LTCs during aging [99]. The kinetics of "classic" (voltage-dependent and Ca^{2+}- inactivated) $Ca_v1.2_{\alpha_1}$ LTCs are adopted from [10], whereas $Ca_v1.3_{\alpha_1}$ LTCs have been modeled as Ca^{2+}-dependent, with a formalism similar to that of sAHP channels (so that they are "open" at relatively hyperpolarized potentials [107]).

Distinct, exponentially decaying Ca^{2+} pools account for the local submembrane calcium

accumulation: $[N - Ca^{2+}]_i$ and $[L - Ca^{2+}]_i$ account for influx through N- and L-types of Ca^{2+} channels (NTCs, LTCs), and $[Ca^{2+}]_i$ for all types of Ca^{2+}-channels. Calcium efflux from buffering/clearance mechanisms - which are not explicitly modelled - is described by a single parameter, the decay time constant. An increase in decay time constant is assumed to depict a slower free Ca^{2+} removal from the cytosol of aged CA1 cells. This simplistic modelling approach is valid as long as the mechanism of interest is the coupling between the reported as functionally co-localized calcium-, and calcium-dependent potassium channels [21]. ($[Ca^{2+}]_i$), which modulates calcium-dependent activation and/or inactivation of L-, N- and R- type calcium channels [57]. The pool accounts for influx from all types of Ca^{2+} channels, taking into consideration calcium efflux from buffering/clearance mechanisms which are not explicitly modelled. These mechanisms are lumped together in a single parameter, i.e. the decay time constant [21].

In order to model the coupling of sAHP with LTCs and fAHP with NTCs, two additional pools describe the Ca^{2+} influx through LTCs and NTCs. The L-type calcium pool ($[L-Ca^{2+}]_i$) modulates sAHP channels, while the N-type calcium pool ($[N-Ca^{2+}]_i$) regulates fAHP channels. These pools have the same large decay rate (equivalently, small decay time constant (2ms)) with the $[Ca^{2+}]_i$ pool, for the following reason: A small value of this parameter results in $[Ca^{2+}]_i$ closely following calcium influx, with minimal accumulation of Ca^{2+}. depict the local interactions reported among the above channels.

The mechanisms for I_h, I_M and $I_N ap$ channels are adopted from [45], where it is shown that they account for resonance at theta frequencies at hyper- and de-polarized potentials (but, close to the resting membrane potentials, without action potential triggering).

Chapter 4

Results

4.1 Model Validation

The model cell was tested using a constant depolarizing current injection at the soma while recording simultaneously at the soma and two locations in the apical trunk. Na^+ action potentials propagate into the dendrites of the CA1 neuron and they drive an influx of Ca^{2+} that seems to be important for associative synaptic plasticity.

A pattern of distance and time-dependent attenuation of back-propagating action potentials (BPAPs) is observed, (fig. 4.1A), similar to that reported by [95] (fig. 4.1B). The only difference is that somatic spikes produced by the model are a few milivolts smaller than experimental traces and this difference in height is propagated in the dendritic traces. Model deviations are more pronounced in middle traces. However, the respective experimental trace in [95] is taken from another cell.

During repetitive (15 Hz) firing, dendritic action potentials display a marked and prolonged voltage-dependent decrease in amplitude. Such a decrease is not apparent in somatic action potentials. In [17], the authors have investigated the mechanisms of the different activity dependence of somatic and dendritic action potentials in CA1 pyramidal neurons. They have found out that dendritic Na^+ currents recover from previous voltage-dependent inactivation much slower than somatic Na^+ currents. Also, in [43] it was shown that an increasing density of A-type K^+ channels along the dendrites accounts for distance-dependent attenuation of back-propagating action potentials. Therefore, the regional differences in Na^+ and K^+ channels determine the differences in the activity dependence of somatic and dendritic action potential amplitudes [17].

In fig.4.2A I_A is blocked by 90% throughout the cell; the initial BPAP which under control conditions was severely attenuated at the dendritic recording electrode (fig. 4.2, left), reached full height (fig. 4.2, middle) as was also observed by [43]. Given the cell is much more excitable in this condition than normal, the dendritic response in both data and model shows a failure to repolarize, as if the voltage were dominated by an unopposed dendritic Ca^{2+} current. When calcium currents were 75% blocked to mimick bath application of 200 mM Cd^{++}, the dendritic spike, though broader than in control conditions, was more fully repolarized (fig. 4.2A, right). Differences between model and experimental traces include slightly smaller and wider somatic spikes in the model, as well as a stronger repolarization of somatic spikes under both blockade conditions. These differences imply a slightly increased I_A and slightly reduced I_{fAHP} in the model, compared to the experimental cell, which can be improved with further tuning. Overall, model and experimental traces are very similar.

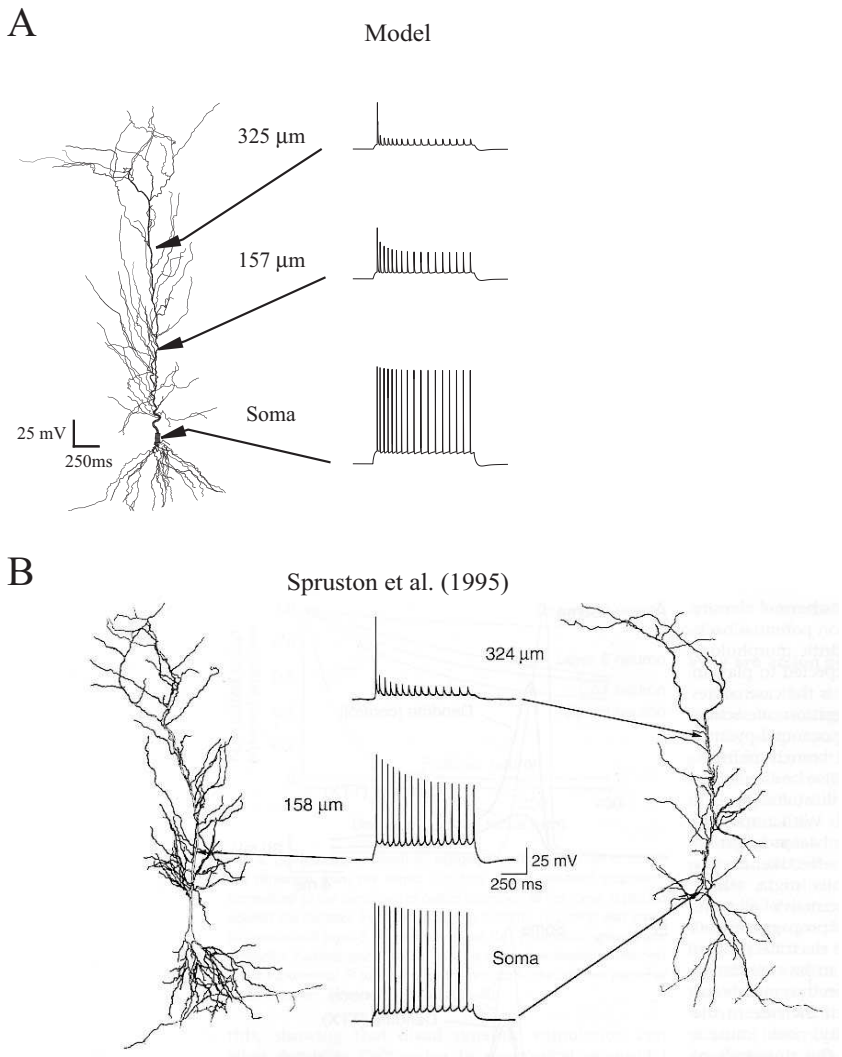


Figure 4.1: A. Backpropagating action potentials evoked by somatic current injection (220 pA, 700ms) show typical pattern of distance and time-dependent attenuation of spike height; the attenuation pattern is very similar to experimental traces from [95] shown in B.

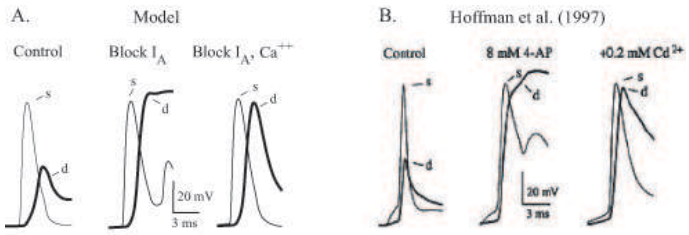


Figure 4.2: Initial somatic and dendritic spikes are shown in response to somatic current injection (300 pA, 50 ms) in control conditions (left), with block of I_A (middle) and with block of Ca^{2+} currents (right). Results are comparable to those of [43].

4.2 Effect of aging induced increase in Ca^{2+} influx on electrical activity

To study the effects of aging-induced increase in Ca^{2+} accumulation on the cell's electrical activity, I have performed simulations of electrophysiological recordings from young and aged CA1 pyramidal cells. Figures 4.3C and 4.3D show experimental traces from young and aged CA1 cells grouped to equate resting membrane potentials [77]. In fig. 4.3C, AHP was recorded following a burst of action potentials elicited by a 100-ms depolarizing pulse. The current was adjusted to a minimal level that reliably evoked a burst of four action potentials in both cells. In figure 4.3D, the same current was injected for 800-ms and the number of action potentials was recorded. Spike frequency adaptation is compared in young vs. aged neurons.

Model simulations are shown in figures 4.3A and 4.3B. A rather small increase in the 'young' model's LTC conductance (about 10%) was sufficient to replicate experimental traces for the 'aged' cell. The sAHP and spike frequency adaptation traces were induced using similar to the experimental somatic current injections. Both 'young' and 'aged' model traces are comparable to physiological recordings. Differences in model's sAHP time course and repolarization potential as well as spike frequency adaptation traces could be eliminated with further refinement of LTC kinetics (which drive the slow AHP), as well as I_{sAHP} kinetics. Ideally, a Markov-state formalism should be adopted for both channels' modelling; the present models are simple phenomenological models, however they capture important, qualitative aspects of these channels' properties.

Interestingly, the above results could be reproduced by reducing the decay rate of the global $[Ca^{2+}]_i$ pool model. Another set of experiments is shown in Fig. 4.4, where a small increase in the decay time constant - from 2ms to 2.2 ms - resulted in a marked increase in calcium influx from LTCs, presumably because of their positive feedback mechanism (CDF), mimicking the model response produced by increasing LTC conductance (Figure 4.4).

Fine-tuning of N-type calcium channels and associated fast AHP conductances has not been attempted, since - to my knowledge - there is no study on aging-induced alterations of these mechanisms. However, these channels are also affected by calcium influx alterations; according to the present modeling approach, an increase of intracellular calcium concentration would lead to an earlier calcium-dependent inactivation of NTCs and, hence, of fAHP channels. The subsequent action potential broadening then, could induce an enhanced calcium influx in somatic and proximal dendritic compartments, through LTCs mainly, since NTCs would be inactivated (RTCs inactivation is less influential in somatic calcium influx, since these are preferentially located in the distal dendrites, together with low-voltage activated T-type Ca^{2+} channels). This second coupling constitutes, then, an additional factor of sAHP enhancement, indirectly reducing cell's firing activity. In fact, [60] have reported that complex voltage waveforms observed during synaptic plasticity (γ and θ spiking frequency) result in selective inactivation of P/Q and N-type Ca^{2+}-channels and lead to a Ca^{2+} current mediated predominantly by LTCs.

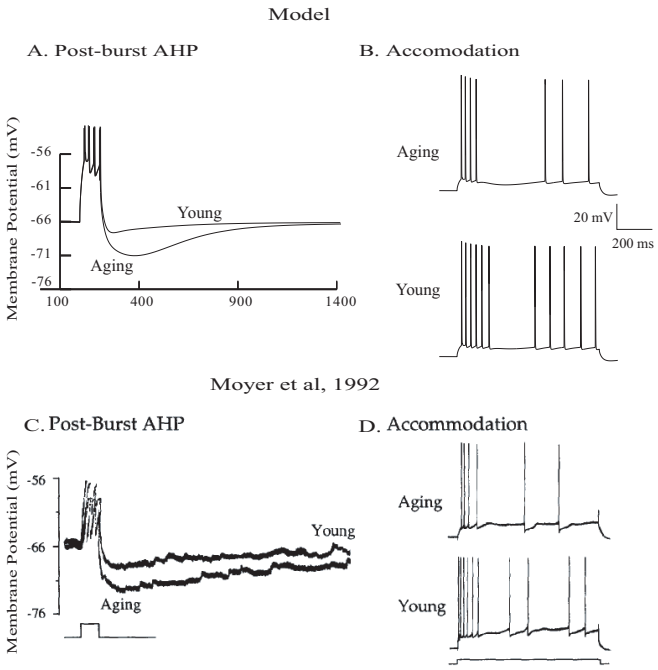


Figure 4.3: Comparison of model and experimental traces (from [77]) of young vs. old CA1 cells. Model traces are very similar to experimental recordings. A detailed comparison is provided in the text.

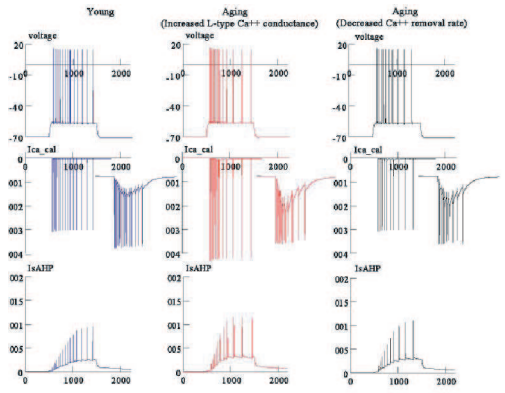


Figure 4.4: Calcium influx through L-type channels in "young" and "aged" cell models; in the latter case, either an increase in LTCs' conductance or a decrease in decay rate could lead to enhanced calcium influx through LTCs

4.3 Effects of aging induced reduction of excitability on persistent electrical activity

The existence of a balance between excitation and inhibition due to L-type calcium current and sAHP current, permits to the neuromodulation of these channels to move the point of equilibrium to less or more excitable neuronal states.

Cholinergic influences are mainly excitatory in the hippocampus. Acetylcholine (Ach) - acting via muscarinic receptors - modulates a number of ionic currents: it suppresses AHP K^+ conductances [53], modulates the mixed cation (Na^+, K^+) current I_h [18] (which contributes to the medium AHP [45] and mainly affects inputs to distal dendrites [79]), and might influence Na^+, Ca^{2+}, and unspecified cationic currents as well ([33] and references in [53]); However, except for Ach's suppressive action on calcium-dependent AHP currents, its effects on the other currents remain controversial; maybe, the reason of this discrepancy is that these effects are indirectly induced by changes in membrane voltage, calcium concentration, or both of them.

For example, the suppression of AHP currents in the model, permits the buildup of a graded persistent current due to calcium influx through L- type channels, I_{CaL}. I_{CaL} depolarizes membrane enough in order to continue firing after the end of stimulation. It represents then, an excitatory feedback that can maintain neural activity after a short stimulus gets it started. Eventually, I_{sAHP} that is still present (otherwise, epileptiform-like activity incurs) grows enough so that it hyperpolarizes the membrane again. Firing frequency is gradually reduced, and eventually spiking ends.

During the simulation, cholinergic influences have been modelled as a 90% blockade of I_M conductance, while $[Ca^{2+}]$ needed for half-activation of $\mathrm{I}_{s_{AHP}}$ is increased 10 times in (A) and 8 times in (B). In (A) and (B), different degrees of $I_{s_{AHP}}$ blockade, result in different timecourses of slow AHP, and eventually different patterns of persistent activity.

Due to differences in the time course of excitatory and inhibitory currents, persistent firing occurs after the end of the 10 bursts in Fig 4.5, and after the 6^{th} burst in Fig 4.6. Each successive burst further increases the intra-stimuli frequency in Fig 4.6. After the end of the stimuli-train, firing persists for several hundreds of ms in both cases; the gradual buildup of inhibitory I_{sAHP} current decreases the rate of firing and eventually terminates it.

Although the model presents an oversimplification of the actual cellular processes involved, still it might offer an insight in the effects of these ionic mechanisms. According to the "slow AHP hypothesis of aging", either sAHP is too large, or, the cell's ability to down-regulate it, is compromised in aged subjects that fail to learn [22]. Both cases might be depicted in the above simulations of TBS: when the blockade of slow and medium AHP currents was smaller than some threshold, even the "young" cell model was not able to generate persistent activity under the same stimulus conditions; as expected, this threshold was even higher in the "aged", less excitable, cell model.

Interestingly, a selective increase in the facilitation property of LTCs could act as a compensatory excitatory mechanism in the model, with the percentage of K^+-currents blockade kept slightly below threshold, for both models; whereas, if K^+-currents' blockade was large enough, "epileptiform" - like activity appeared (results not shown).

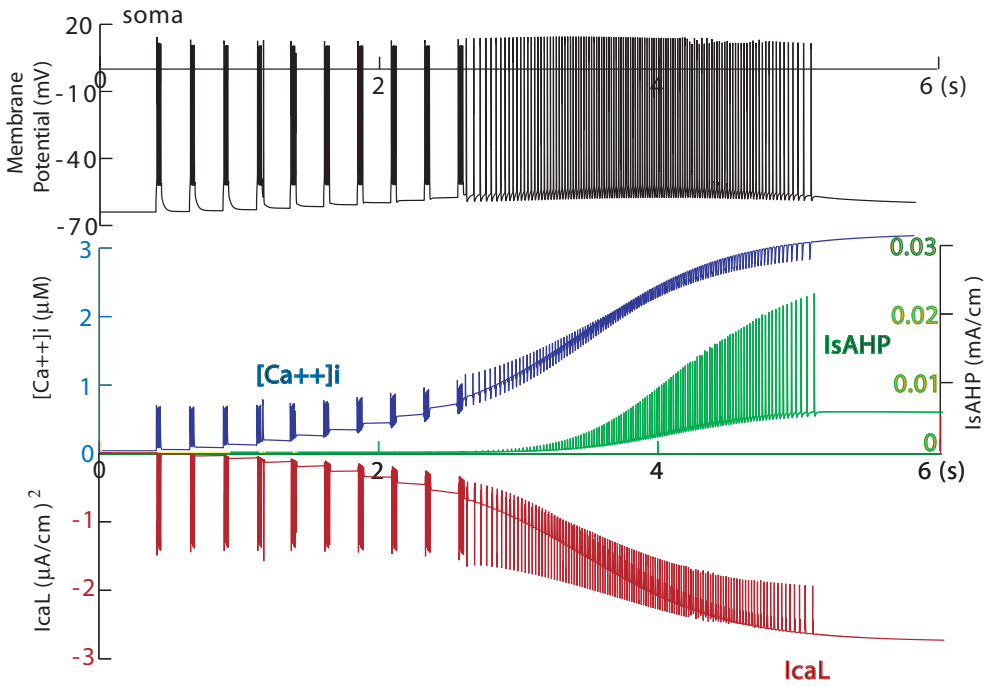


Figure 4.5: Model traces under θ-burst antidromic stimulation and a specific percentage of blockade of slow and medium AHP currents for the "young" cell. Increased spiking during TBS (and, subsequently, persistent activity) occured only if the percentage of blockade of slow and medium AHP K^+ currents exceeded a threshold, even in the young cell model. Voltage trace at soma (black), Ca_i^{2+} accumulation due to LTCs' activity (blue), I_{Ca_L} (red), and I_{sAHP} (green).

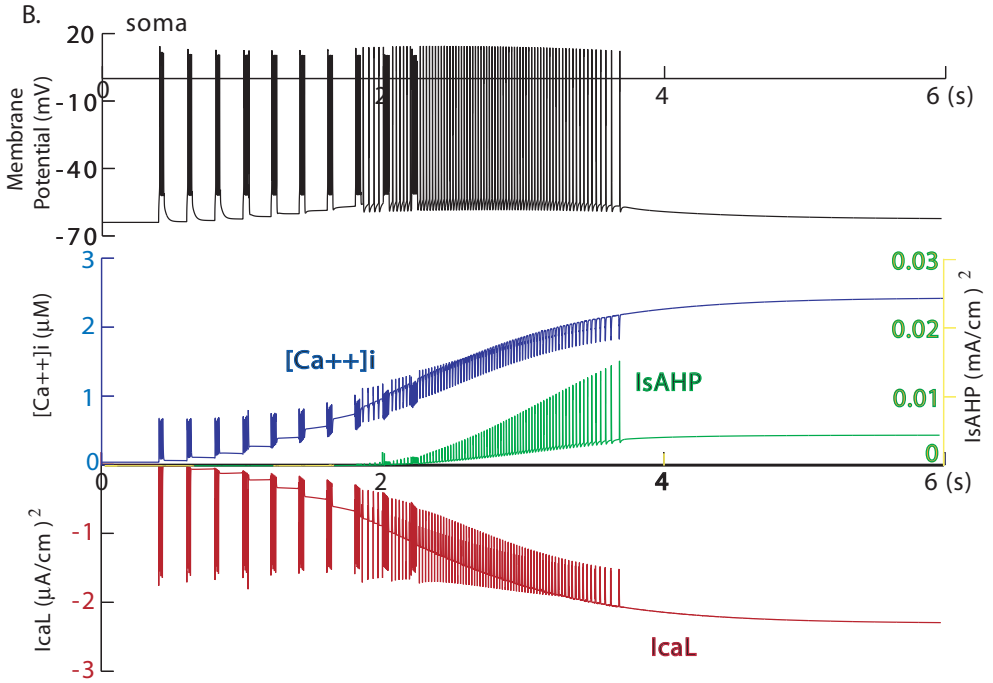


Figure 4.6: A different pattern of persistent activity arises for another percentage of blockade of medium and slow AHP currents. Model traces under θ-burst antidromic stimulation for the "young" cell: Voltage trace at soma (black), Ca_i^{2+} accumulation due to LTCs' activity (blue), I_{Ca_L} (red), and I_{sAHP} (green).

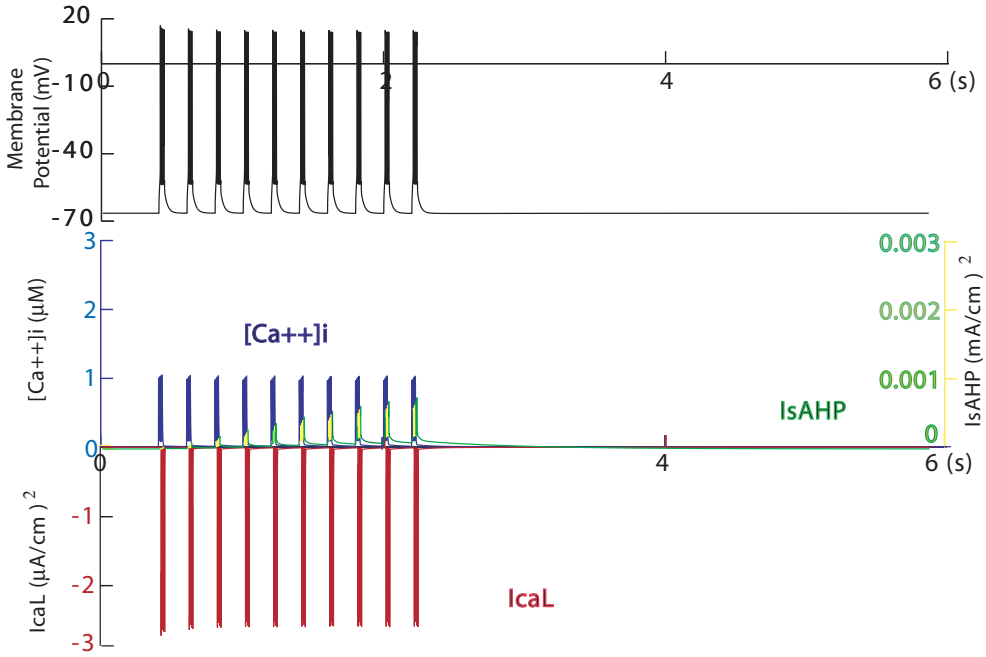


Figure 4.7: Model traces under θ-burst antidromic stimulation and same percentage of blockade of the (enhanced) medium and slow AHP currents in the "aged" cell model. Neither inscreased spiking (nor persistent activity) could be induced in that case. Voltage trace at soma (black), Ca_i^{2+} accumulation due to LTCs' activity (blue), I_{Ca_L} (red), and I_{sAHP} (green)

Chapter 5

Discussion

It has been suggested that the hippocampal formation has a time-limited, transitory role in memory storage ("encoding") and retrieval, and that memory traces might be gradually transferred elsewhere for permanent storage [96]. However, recent studies have proposed that an intact hippocampus is always necessary for episodic details, including contextual/perceptual and noncontextual/semantic ones [32]. Furthermore, in [88], it was shown that the left hippocampus was the only region in the entire brain to be activated during both encoding and retrieval of semantic and perceptual associations between the components of an event, highlighting its key role in relational memory.

CA1 hippocampal pyramidal neurons, in particular, seem to compare direct sensory information (provided from layer III of the entorhinal cortex via the perforant path), with inputs via the Schaffer collateral pathway, carrying predictions made by the dentate/CA3 region on the basis of previously stored information [79] (Fig.5.1). Various learning-related neurotransmitters such as acetylcholine, gate the ability of neurons to store information during different behavioral states: either local or global release can selectively modulate the activity of somato-dendritic ionic channels, and shift the relative importance of distal versus proximal synaptic inputs [79]. The successful association of these functionally separated inputs in CA1 pyramidal neurons, is eventually signified by bursts of action potentials [59, 56]. It is crucial then to understand the postsynaptic effects of bursting activity, and the kind of information they might encode [59].

In this study, I have assumed that the spatial and temporal distribution of synaptic inputs has succeeded to elicit bursts of axonal action potentials [56]. Many different mechanisms could facilitate bursting behaviour [59]; testing various spatial and temporal patterns of synaptic stimulation could address this issue. However, the objective has been to keep the things as simple as possible, in order to investigate the role of somatic mechanisms during this physiological pattern of θ-burst activity.

Long-lasting forms of memory appear to be encoded by molecular or structural changes in synapses which require protein synthesis and the expression of new genes [96]. Short-term memory, instead, is a dynamic, ephemeral process, assumed to be held by interconnected groups of neurons that fire persistently because they excite one another recursively ([19], and references therein). Persistent activity is though to subserve the short-term maintenance or manipulation of information; during this "working" memory process, the different stimuli can be eventually associated[27, 96]. However, recent studies in many different brain areas have shown that an isolated neuron, when stimulated briefly, can generate sustained, graded increases in its electrical activity which can be readily reversible; a single neuron, then, has the ability to quickly "remember" (and forget) numerous bits of information. [27, 3, 61].

According to the modelling study of θ-burst stimulation (TBS) presented in this work, the excitatory feedback that enables persistent activity in a single CA1 pyramidal cell, is

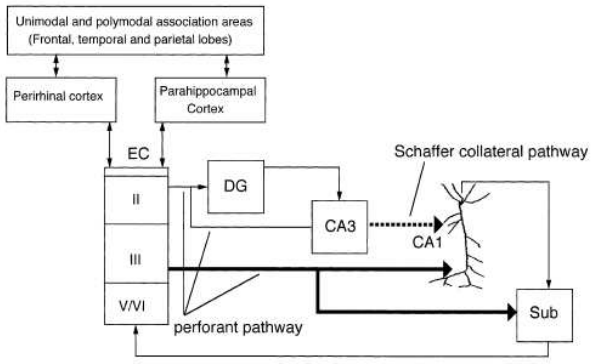


Figure 5.1: The Organization of Medial Temporal Lobe Circuits. Information flows through the hippocampal trisynaptic circuit from layer II of the entorhinal cortex (EC) via the dentate gyrus (DG) to CA3 and then to CA1. The input from CA3 to CA1, via the Schaffer collateral pathway, targets the proximal dendrites of CA1 pyramidal neurons and is indicated by the dashed line. In addition, CA1 neurons receive a direct input from the EC to their distal apical dendrites via the perforant path indicated by the bold line. The distal apical dendrites of CA1 pyramidal neurons contain the highest density of HCN1 channels. This figure was adopted from [79].

provided by the internal Ca^{2+} concentration, the graded, sustained L-type calcium current (I_{CaL}), and the cell's membrane voltage. Gradual buildup of slow AHP current (I_{sAHP}), reduces the probability of firing and eventually turns off persistent activity. Neurotransmitters, like acetylcholine, acting to suppress I_{sAHP} during attentional states, gate the ability of the neuron to generate burst firing. Perhaps, the inhibitory effect of the I_{sAHP} observed during TBS "in silico", might be an important regulatory mechanism which complements synaptic inhibition, in order to prevent irrelevant inputs from being associated [56], or even prevent re-encoding of redundant information [32]. The absence of regulatory mechanisms would lead to cells continuously bursting, presumably inducing instability in the interconnected cortical networks [56]. On the other hand, aging-induced enhancement of I_{sAHP} and insufficient cholinergic neurotransmission, might inhibit the association of memories in general, resulting in the "feature binding deficit" of learning-impaired aged subjects [73].

Acting in concert, I_{CaL} and I_{sAHP} channels seem to induce a kind of $[Ca^{2+}]_i$ clamp, either counteracting or reinforcing the spike-driven, local calcium accumulation $[Ca^{2+}]_i$ (Figs 4.5, 4.6, 4.7). L-type Ca^{2+}-channels preferentially respond during this complex spiking (or, bursting) activity [60]; subsequent Ca^{2+} influx through them, triggers intracellular cascades that lead to transcription of several genes important for learning [23, 25]. A recent model of late-LTP induction, suggests that proteins synthesized during this process, are broadly transported through dendrites to be incorporated in recently active "tagged" synapses [25]. Also, synapses other than the activated ones might be affected, depending on the extent of spike back-propagation through dendrites [81]. The alteration of synaptic plasticity thresholds ("metaplasticity") might concern the entire cell. This activity-dependent "tuning" of synaptic connections has been proposed to subserve at the same time plasticity and stability in cortical neural networks, and, eventually, long-term formation of complex episodic memories [1].

The single-cell behaviour exhibited during TBS stimulation *in silico*, is yet to be repli-

cated both *in vitro* and *in vivo* in the CA1 hippocampal area. Further theoretical and experimental analysis of the above-mentioned intrinsic cellular mechanisms, will be necessary to investigate their role in working memory for novel stimuli. The analysis of the biophysical properties of the different subtypes of LTCs, as well as of sAHP channels (whose identity remains controversial [9]), will enable a more accurate modelling of them, beyond a phenomenological qualitative description. In this work, the modelling of I_{sAHP} and $Ca_v1.3$-type LTC channels has been "tailored" to reproduce the identical time-course and activation protocol of them [16, 90]; due to their selective coupling, the slow kinetics and the gradual buildup of I_{CaL} drives in turn I_{sAHP}. Also, Ca^{2+}-dependent inactivation of high-voltage activated Ca^{2+}-channels ($Ca_v1.2$-type of LTCs, NTCs and RTCs), is described using a rather simplistic approach found in the literature [10, 108]. A Markov-state formalism might better describe the state transition of these channels, possibly taking into account their gating by the Ca^{2+}-sensor protein, calmodulin (CaM) [109], [110], [74], [57], [26], [106].

Perhaps, it would be possible in that case to quantitatively describe how these channels' coupled activity, assigns oscillatory properties to neurons, especially under conditions of cholinergic and β-adrenergic neurotransmission [44, 33, 30, 31, 46]. The dynamical adjustment of the frequency content of spike output according to recent spiking activity and the local or global action of neurotransmitters, seems to favor the communication between specific subsets of cells with the same frequency preference [47]. Multiple mechanisms of neuronal oscillations in specific frequency bands, seem to act synchronously at the synaptic, cellular and network level [12, 68, 47]. It would be interesting to incorporate realistic models of neurons, with resonance and pacemaking properties, into network models ([61], [19], and references therein). Maybe, these models might enable us to resolve the mechanisms acting at each level, and their functional roles in the temporal coding performed by hippocampal circuits [38, 63].

Chapter 6

Appendix: Model equations

Equations for LTCs' kinetics are shown below. The time course of delayed facilitation of LTCs is identical to that of I_{sAHP}.

$$I_L = 0.8 * g_L * \left(m^2 * h_i([Ca^{2+}]_i) * (v - eca)\right) + 0.2 * g_L * s_f * s_i(v - eca) \tag{6.0.1}$$

$$h_i([Ca^{2+}]_i) = \frac{k}{[Ca^{2+}]_i + k} \tag{6.0.2}$$

$$s_{f_{inf}} = \frac{1}{1 + \exp\left(\frac{b_f - [Ca^{2+}]_i}{k_f}\right)} \tag{6.0.3}$$

$$s_{i_{inf}} = 1. - \frac{1}{1 + \exp\left(\frac{b_i - [Ca^{2+}]_i}{k_i}\right)} \tag{6.0.4}$$

$$\tau_f = \tau_{min} + \left((\tau_{max} - \tau_{min})^{-1} + \exp(\frac{[Ca^{2+}]_i - b_f}{k_f})\right)^{-1} \tag{6.0.5}$$

$$\tau_i = \tau_{min} + \left((\tau_{max} - \tau_{min})^{-1} + \exp(\frac{[Ca^{2+}]_i - b_i}{k_i})\right)^{-1} \tag{6.0.6}$$

where $\tau_{min} = 10ms$ and $\tau_{max} = 100ms$ are the lower and upper limit for the time constant, $k = 0.025mM$ the slope factor, $b = 0.01mM$ the Ca^{2+}-concentration for half-inactivation of $Ca_v1.2$-LTCs, $b_f = 0.01mM$ and $b_i = 0.025mM$ the Ca^{2+}-concentrations for half-activation and inactivation of $Ca_v1.3$-LTCs, $k_f = 0.1mM$ and $k_i = 0.25mM$ the respective slope factors. The somatic conductance value is: $g_L = 0.3mS/cm^2$. The state variable m is described in [10].

The current equation for N-type (and R-type) Ca^{2+} channels is given by:

$$I_N = g_N * m^2 * h * h_i([Ca^{2+}]_i) * (v - eca) \tag{6.0.7}$$

where the function $h_i()$ is the same as in LTCs and the somatic conductance value is: $g_N = 0.2mS/cm^2$. The state variables m and h are described in [10]

Equations for I_{sAHP} are given by:

$$I_{sAHP} = g_{sAHP} * m^2 * (v - ek) \tag{6.0.8}$$

$$m_{inf} = \frac{1}{1 + \exp\left(\frac{b - [L - Ca^{2+}]_i}{k}\right)} \tag{6.0.9}$$

$$\tau_{inf} = \tau_{min} + \left((\tau_{max} - \tau_{min})^{-1} + \exp(\frac{[L - Ca^{2+}]_i - b}{k}) \right)^{-1} \tag{6.0.10}$$

where $\tau_{min} = 10ms$ and $\tau_{max} = 100ms$ the lower and upper limits of time constant, $b = 0.005mM$ the half-activation Ca^{2+}-concentration, and $k = 0.1mM$ the slope factor. The somatic conductance value is: $g_{sAHP} = 0.025S/cm^2$.

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