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Προσομοίωση των Μεταβολών στη διεγερσιμότητα ενός Πυραμιδικού Νευρικού Κυττάρου του Ιπποκάμπου κατά τη Γήρανση

Μαρία Μαρκάκη

Μεταπτυχιακή Εργασία

Ηράκλειο
Μάρτιος 2005

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Εργασία που υποβλήθηκε
στο Τμήμα Επιστήμης Υπολογιστών
ως μερική εκπλήρωση των απαιτήσεων για την απόκτηση
Μεταπτυχιακού Διπλώματος Ειδίκευσης

28η Φεβρουαρίου, 2005

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Ηράκλειο, Μάρτιος 2005

Στο Γιώργο, το Μανόλη, τον Κωστή, τους γονείς μου,
τους συμφοιτητές και τους καθηγητές μου στο Πανεπιστήμιο Κρήτης,
στον επόπτη μου Κ. Στέλιο Ορφανουδάκη.

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Περίληψη

Αντικείμενο της παρούσας εργασίας είναι η διερεύνηση των λειτουργικών μεταβολών στον ιππόκαμπο κατά τη γήρανση, οι οποίες φαίνεται να ευθύνονται για τη εξασθένηση της ¨δηλωτικής' μνήμης, της συνειδητής μνήμης για γεγονότα, πρόσωπα ή πράγματα. Τα ηλικιωμένα άτομα αντιμετωπίζουν ιδιαίτερα δυσκολία στο συσχετισμό μεταξύ των διαφόρων χαρακτηριστικών ενός γεγονότος, και όχι στην μεμονομωμένη απομνημόνευσή τους. Η προσομοίωση ενός κυτταρικού μοντέλου της γήρανσης, θα μπορούσε πιθανόν να συμβάλλει στην κατανόηση του ρόλου του ιππόκαμπου στην κωδικοποίηση της σχετικιστικής μνήμης.

Σε αυτή την εργασία μοντελοποιήθηκε λεπτομερειακά ένα πυραμιδικό νευρικό κύτταρο στο αμμώνειο κέρας του ιπποκάμπου (περιοχή CA1), με βάση δύο δημοσιευμένα υπολογιστικά μοντέλα του ίδιου κυττάρου. Οι σημαντικότερες αλλαγές στο νέο μοντέλο, αφορούν τους ασβεστιοεξαρτώμενους μηχανισμούς στο σώμα και τους κοντινούς δενδρίτες, οι οποίοι παρουσιάζουν σημαντικές μεταβολές κατά τη γήρανση, προκαλώντας μείωση της διεγερσιμότητας του κυττάρου. Επιλέχτηκε η σύζευξη της ενεργοποίησης συγκεκριμένων διαύλων ασβεστίου με ασβεστιοεξαρτώμενους διαύλους καλίου στο κυτταρικό μοντέλο. Κριτήριο για αυτή την επιλογή ήταν η ενεργοποίηση ή απενεργοποίησή τους απο τις ίδιες κυματομορφές εισόδου, καθώς και η ίδια χρονική εξέλιξη της ροής ιόντων (ασβεστίου και καλίου) διαμέσου των διαύλων. Η αντίθετη επίδραση (διεγερτική και ανασταλτική) των αντίστοιχων ρευμάτων στο δυναμικό της μεμβράνης, δημιουργεί πολλαπλά κυκλώματα θετικής και αρνητικής ανάδρασης μέσα στο κύτταρο. Τα κυκλώματα αυτά επιτρέπουν τη διαμόρφωση της απόκρισης σε κάθε διέγερση, ανάλογα προς τη διάρκεια και την ένταση της πρόσφατης ηλεκτρικής δραστηριότητας. Η ρύθμιση των παραμέτρων του μοντέλου έγινε με κριτήριο την εξομοίωση πειραμάτων ηλεκτροφυσιολογίας στα οποία καταγράφεται η απόκριση του κυττάρου σαν συνάρτηση της έντασης και της χρονικής διάρκειας της διέγερσης (καθήλωσης σταθερού ρεύματος στο σώμα του κυττάρου). Στις εξομοιώσεις, η απόκριση του κυτταρικού μοντέλου στις διάφορες τιμές ¨εισόδου¨, αναπαράγει τη μείωση της

διεγερσιμότητας εξαιτίας της μεταβολής των ιοντικών ρευμάτων που σχετίζεται με τη γήρανση.

Το μοντέλο χρησιμοποιήθηκε στη συνέχεια για τη μελέτη της επίδρασης του νευροδιαβιβαστή ακετυλοχολίνη, η οποία αυξάνει τη διεγερσιμότητα του κυττάρου σε καταστάσεις που απαιτούν αυξημένη προσοχή, και, επιπλέον, εμφανίζει μειωμένα επίπεδα κατά τη γήρανση. Συγκεκριμένα, στις προσομειώσεις, η επίδραση της ακετυλοχολίνης αναστέλλει την ενεργοποίηση των διαύλων καλίου στο σώμα και τους κοντινούς δενδρίτες, οι οποίοι προκαλούν αργή - κυρίως - υπερπόλωση της μεμβράνης μετά από μία ακολουθία δυναμικών ενέργειας και μειώνουν τη διεγερσιμότητα του κυττάρου. Το μοντέλο μελετήθηκε σε φυσιολογικές συνθήκες δραστηριότητας, οι οποίες παρατηρούνται στα κύτταρα του ιπποκάμπου των τρωκτικών στη διάρκεια εξερεύνησης ενός χώρου. Επαναλαμβανόμενη διέγερση σε συχνότητα ϑ (5-12 Hz, κι ένταση ικανή να προκαλέσει ριπές από 2-6 δυναμικά ενέργειας στα 100 Hz, αποτελεί ικανή και αναγκαία συνθήκη για την μακρόχρονη ενδυνάμωση των συναπτικών συνδέσεων. Σε ηλικιωμένα άτομα με προβλήματα μάθησης, η ίδια διέγερση δεν είναι σε θέση να προκαλέσει μακρόχρονη συναπτική ενδυνάμωση. Επιπλέον, η επαναλαμβανόμενη εκπυρσοκρότηση ενός κυττάρου κατά ριπές, σηματοδοτεί την συσχέτιση μεταξύ διαφορετικών χαρακτηριστικών: τη σύνδεση νέων αισθητηρίων ερεθισμάτων και αποθηκευμένης προϋπάρχουσας πληροφορίας - σε ένα ¨αντικείμενο' της μνήμης. Στη διάρκεια σταθερής περιοδικής διέγερσης το ¨νεανικό' κύτταρο εμφανίζει αυξανόμενη δραστηριότητα, η οποία συνεχίζεται και μετά το τέλος της διέγερσης, με ρυθμό περίπου 30-60 Hz (γ-ζώνη συχνοτήτων). Στις προσομειώσεις με το ¨γηρασμένο' κύτταρο, η ικανότητα αυτή παρουσιάζεται μειωμένη.

Κατά την κωδικοποίηση νέας πληροφορίας, στα κύτταρα του ιππόκαμπου παρατηρούνται ταλαντώσεις στη γ-ζώνη συχνοτήτων, υπερτιθέμενες σε ταλαντώσεις μικρότερης συχνότητας (στη ϑ-ζώνη). Η επίδραση της ακετυλοχολίνης έχει συσχετισθεί με τη δημιουργία αυτών των ταλαντώσεων, οι οποίες έχουν καταγραφεί και σε άλλες περιοχές του εγκεφάλου σε συνθήκες επιλεκτικής προσοχής. Επιπλέον, η ταυτόχρονη ρυθμική δραστηριότητα μίας ομάδας κυττάρων θεωρείται ότι σηματοδοτεί τη δημιουργία ενός νέου ¨αντικειμένου' στη μνήμη, μέσω της συνένωσης διαφορετικών χαρακτηριστικών ενός συμβάντος. Πιθανόν, ένας μηχανισμός εκκίνησης αυτής της κωδικοποίησης, να εντοπίζεται στα πυραμιδικά κύτταρα στην περιοχή CA1 του ιπποκάμπου: Αν ένα τέτοιο κύτταρο εκδήλωνε παρατεταμένη ηλεκτρική δραστηριότητα μετά το τέλος ενός ερεθίσματος, ο ρυθμός του ϑα μπορούσε να μεταδοθεί επιλεκτικά διαμέσου των συναπτικών συνδέσεων σε άλλα κύτταρα του δικτύου τα οποία έχουν ¨συντονιστεί' στην ίδια συχνότητα. Ενδεχέται λοιπόν, τα προβλήματα μάθησης κατά τη γήρανση να οφείλονται εν μέρει στην αδυναμία των πυραμιδικών κυττάρων της περιοχής ¨A1 του ιπποκάμπου να ωθήσουν το δίκτυο σε ρυθμική δραστηριότητα.

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Computational role of modulation of excitability in a CA1 hippocampal pyramidal neuron during aging

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Master Thesis
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Abstract

This modeling study focuses on the investigation of the functional alterations in hippocampus, which underlie the long-term episodic memory decrements during aging. 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.

In CA1 hippocampal pyramidal cells, the successful association of selected features of an event, is signified by repeated postsynaptic bursting activity in θ frequency band (5-12 Hz). In rodents, θ-bursting is commonly observed 'in vivo' in states of selective attention during exploratory behavior, and reliably induces long-term potentiation (LTP) of stimulated synapses in CA1 cells. In learning-impaired aged animals, however, θ-frequency synaptic stimulation fails to induce LTP.

During early aging, the intrinsic, calcium-dependent, membrane mechanisms of pyramidal neurons, are the first to be disrupted, before a significant change in the number of synapses or the dendritic morphology occurs. Moreover, these alterations induce an excitability reduction which has been correlated to the learning impairments in aged subjects.

In this work, the effects of these active membrane properties on neuronal excitability, have been investigated "in silico". A multi-compartmental model of a hippocampal CA1 pyramidal cell has been built, mainly based on two biophysically detailed compartmental models of the same cell. Main novel features are the simple coupling mechanisms between specific types of Ca^{2+}-channels and functionally colocalized, Ca^{2+}-dependent potassium channels. The coupled channels exhibit concurrent activation (or inactivation) by specific voltage waveforms, and the ionic currents through them exhibit the same time course. The competing - excitatory and inhibitory - feedback loops arising from their coupled activity, enable the fine-tuning of neuronal excitability.

Model parameters have been calibrated using physiological data. Computer simulations reproduce the decreased excitability of aged CA1 cells as a result of increased internal calcium accumulation, subsequently larger post-burst slow after-hyperpolarization, and enhanced spike frequency adaptation in the "aged" cell model.

The cell model has been further tested in simulations of a physiological pattern of activity, θ-bursting, using antidromic (non-synaptic) stimulation at θ-frequency. Relevant electrophysiology experiments have revealed a graded increase in postsynaptic spiking in successive bursts, which is thought to depict physiologic temporal integration at the cellular level. This ability of the postsynaptic neuron to integrate over time the effects of recent stimuli, is thought to be a short-term, working memory mechanism which is critical for the association of multiple stimuli ("features" of a memory object).

In simulations, θ-frequency antidromic stimulation could induce bursts with a gradually increasing number of spikes - *only* when the slow and medium afterhyperpolarization potassium currents, were sufficiently suppresed, imitating cholinergic-like influence. Moreover, self-sustaining oscillatory firing in the γ frequency band was induced in the "young", but not in the "aged" cell model.

It has been suggested that the intrinsic frequency preferences of neurons, enable the selective communication between resonant subsets of cells within a network. When a single cell fires persistently under physiological temporal patterns of stimulation, it could intrinsically drive a specific cellular assembly in synchronous rhythmic activity. Coherent network oscillations in the brain have been correlated with different behavioral and mental states; persistent hippocampal γ oscillations have been implicated in feature binding and short-term memory. Perhaps that, during aging, reduction in excitability impairs CA1 cell's pacemaking ability, delaying onset of network oscillations, hence inducing memory binding deficits.

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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 [99]. 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 [74]. 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 [99].

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, 90]. Aging-induced alterations of L-type Ca^{2+} channels have been correlated to sAHP enhancement and learning impairments in aged mammals [102, 13, 78, 81, 101, 20]. Disrupted calcium homeostasis during aging has also been reported, mainly as a result of disrupted Ca^{2+} handling, buffering, sequestration and efflux mechanisms [105]. 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 [99, 22]. Cholinergic muscarinic activation suppresses medium and slow AHP currents in states of selective attention, resulting in membrane depolarization and enhancement of firing activity [89, 92, 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 [78], supporting the role of the hippocampus as an intermediate storage buffer during learning [99]. 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 [77].

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) [86]. A model of the HPC is useful for studying many excitatory cortical neuron types

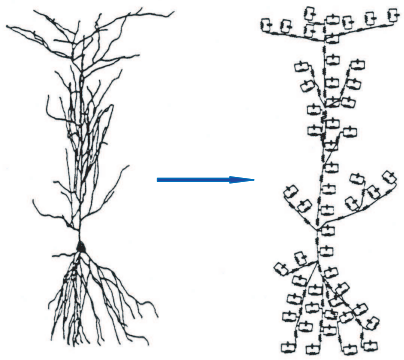


Figure 1.1: **Modeling neurons with electrical circuits** Left: Two-dimensional projection of the anatomy of a CA1 pyramidal neuron. Right: equivalent multi-compartmental model. Adapted from [41].

(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, 111, 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 [78]. Main changes, then, to the model of reference [86], 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], [113]).

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 modeling 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 [99]. 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 [99]. 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, 82, 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 [80]. The preferential activation of postsynaptic N-methyl-D-aspartate receptor (NMDA) channels by the coincidence of pre- and post-synaptic activity, satisfies both the

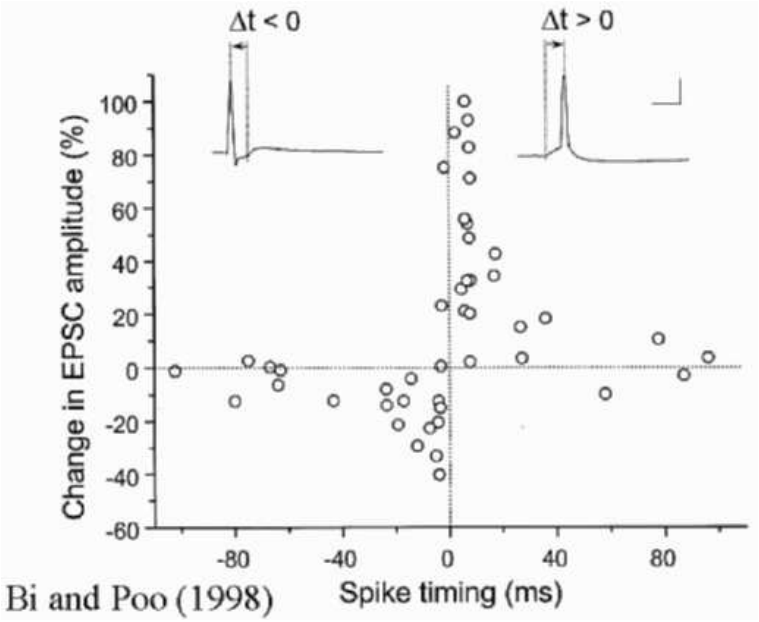


Figure 2.1: **Action potential (AP) backpropagation regulates synaptic plasticity**. The timing between incoming EPSPs and backpropagating APs determines whether LTP or LTD is induced in cultured hippocampal neurons (spike-timing dependent synaptic plasticity). Adapted from [6].

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, 99]. 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].

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 [100, 98, 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 2.1).

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 [82]. The

extent of this influence is subject to physiological control by ion channels [98, 39, 107], and synaptic inhibition [82]. 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 [82]. 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 [103].

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, 98]. Synaptic transmission failure is less likely to occur when 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 2.2, 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].

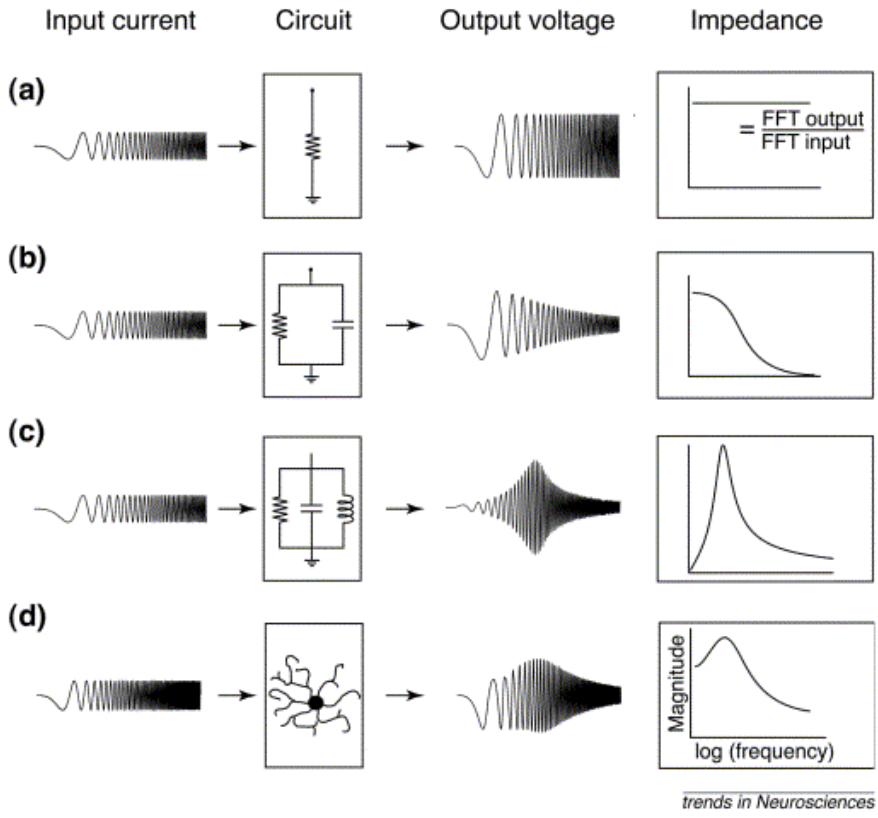


Figure 2.2: **Frequency-dependent properties of electronic circuits and neurons: detection and analysis.** The relationship between the current input (first input) and the voltage output (third column) of electrical circuits or neurons (second column) enables the calculation of impedance as a function 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].

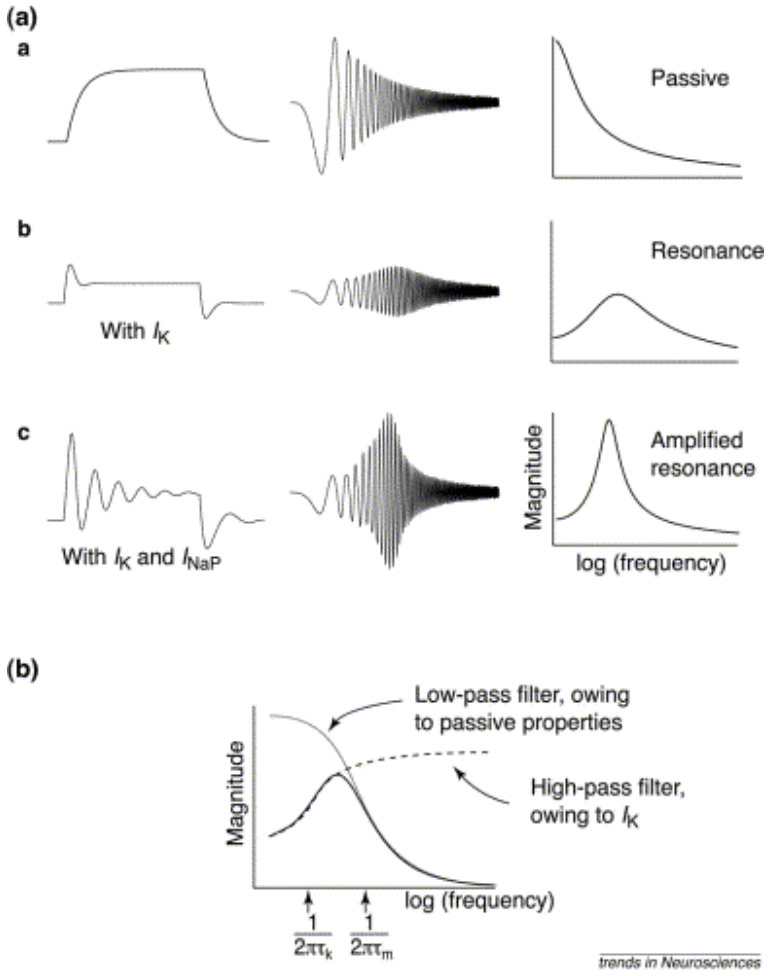


Figure 2.3: **Resonance is formed by the interaction of active and passive properties in a neuron. (a)** Properties of three models that have passive properties only (part a), passive properties plus a resonant current, I_K (part b), and passive properties, a resonant current and an additional amplifying 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. Adapted from [46].

2.2 θ-Burst Stimulation

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 [103]. 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 [99].

The fact that synaptic inputs active during complex spike bursting, undergo robust LTP [103], 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 [99, 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 2.4).

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 [95]. 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

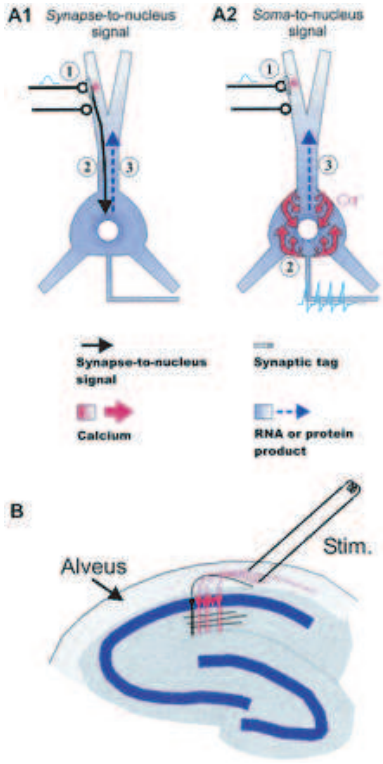


Figure 2.4: **Schematic diagram outlining two contrasting scenarios for LTP-related nuclear signaling**. Adapted from [25]

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 2.5). ERK, then, regulated neuronal excitability in hippocampal CA1 neurons during TBS. Moreover, [95] suggested that the observed increase in AP firing might be a form of physiologic temporal integration which depended on ERK MAPK activity.

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], [108], [106].

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

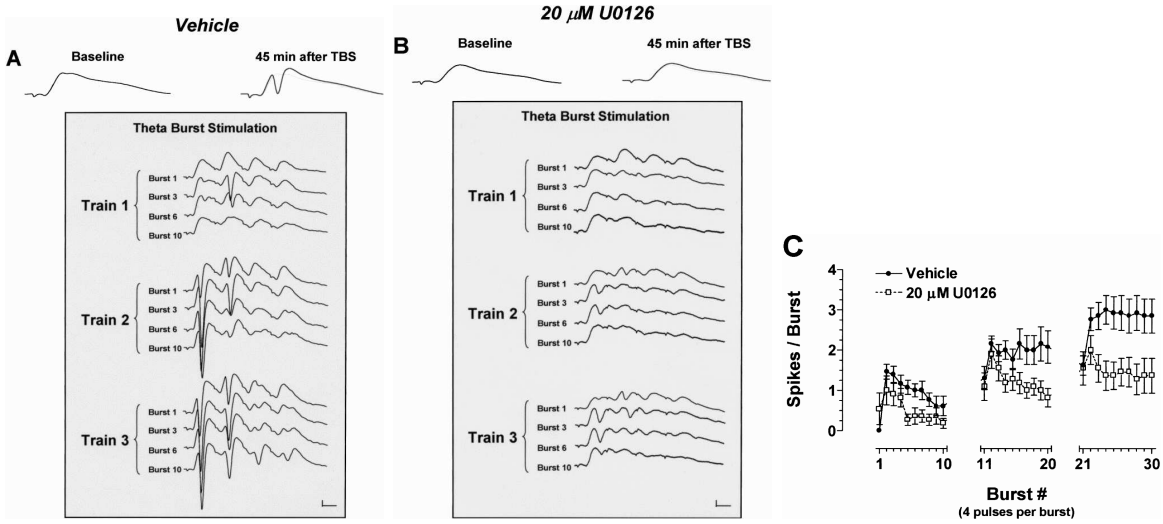


Figure 2.5: **Increased action potential firing over the course of θ-burst stimulation (TBS).** (A) Representative traces in response to TBS from a vehicle-treated slice. There is profound difference in spiking between the first and last bursts of the stimulation paradigm. Scale bars (bottom right corner 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 [95].

aged humans [104].

Although the putative mechanisms for theta-frequency LTP have been already described in [103, 108], 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 [105]. 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 [104]. 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, 78, 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 ([104] 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], [112], [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. 2.6). When neurons fire, information is relayed down the axon to the synaptic ending, activating the secretion of neurotransmitters - chemicals that excite neighboring neurons. Elementary

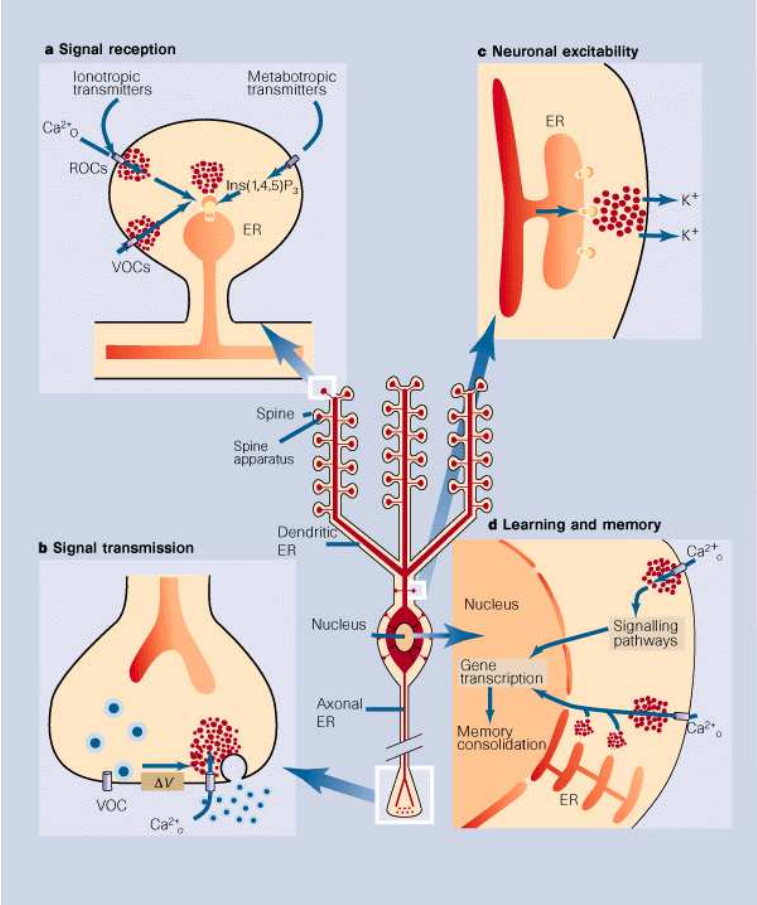


Figure 2.6: **Compartmentalization of Ca^{2+} signals in neurons**. a, External Ca^{2+} enters through receptor-operated, or voltage-gated Ca^{2+}-channels (VGCCs). The signal can be amplified by activating receptors for Ins(1,4,5)P_3 on the spine apparatus. By integrating separate inputs, these receptors 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].

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. 2.6).

The voltage-gated Ca^{2+} channels mediate Ca^{2+} entry into cells in response to membrane depolarization. Electrophysiological studies have revealed 6 different Ca^{2+} currents, of 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 2; an intracellular subunit; and in some cases a transmembrane subunit. Ten 1 subunits, four 2 complexes, four intracellular subunits, and two transmembrane subunits are known. The Cav1 family of 1 subunits conduct L-type Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} currents, which are activated and inactivated more rapidly and at more negative membrane potentials than other Ca^{2+} current types. The distinct structures and patterns of regulation of these three families of Ca^{2+} channels, provide a flexible array of Ca^{2+} entry pathways in response to changes in membrane potential and a range of possibilities for regulation of Ca^{2+} entry by second messenger pathways and interacting proteins [15]. Also, these channels are differentially distributed in somato-dendritic compartments, affecting the integration of either distal or proximal inputs.

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 [102], 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 [78].

Multiple functional kinds of L-type channels (LTCs) exist, and they differ in their conductance and kinetic properties [110], [94]. 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 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 [94]. 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 [94].

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 [112] 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 [112], [26]. The signalling capabilities of CaM seem to generalize to numerous biological systems, such as other high-voltage activated Ca^{2+} channels [75],[57], and small conductance K^+ (SK) channels (in which CaM affects gating by interacting with domains in analogous positions) [109].

2.3.2 N-type Ca^{2+} 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 [87] - 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 [96] (Fig.2.7). 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 [92, 37, 28], and transmitter release [28].

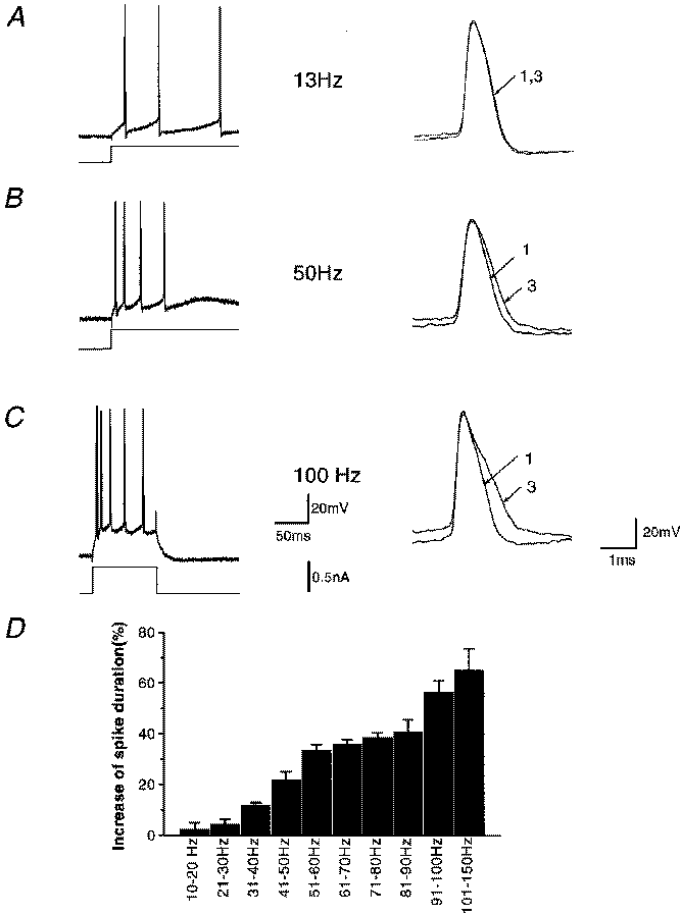


Figure 2.7: **Frequency dependence of the spike broadening**: A, during low-frequency repetitive firing, there was no detectable broadening from the 1st spike to the 3rd and no clear decline of the fAHP amplitude. B and C, during higher-frequency repetitive firing, in response to stronger current injections, there was increasing broadening of the 3rd spikes and decline of the fAHP amplitude. D, summary of the spike broadening at different average discharge frequencies. Adapted from [96].

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, 78, 77, 90]).

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 [92, 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 [109], 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, 78, 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 [90]. 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 [110].

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 s_{AHP} 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 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 2.8. 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 [99]. Furthermore, the persistence of the ACh levels in the hippocampus is correlated with the duration of this structure's involvement in memory consolidation [89].

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 ([90] 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 of further plasticity ([54]. Accordingly, the enhanced sIAHP in aging can hamper the formation of further plastic alterations important for learning and memory ([92], [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 [78], [77] 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 [78]. The time course of increased excitability may represent a critical

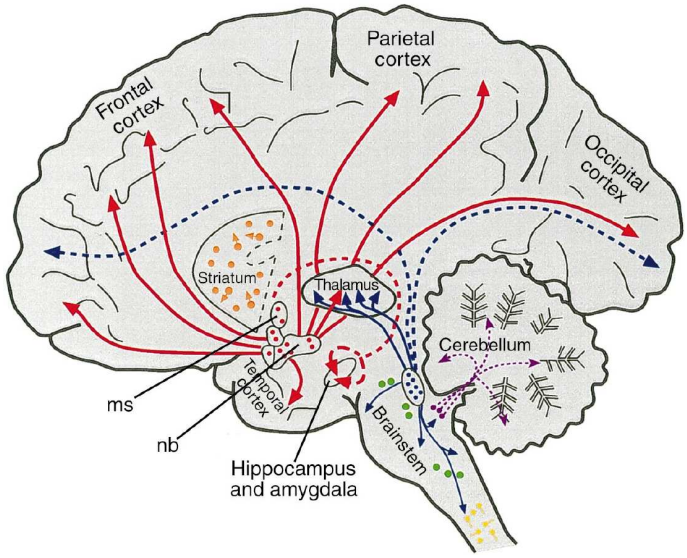


Figure 2.8: **Cholinergic systems in the human brain**: Two major pathways project widely to different brain areas: basal-forebrain cholinergic neurons [red, including the nucleus basalis (nb) and medial septal nucleus (ms)] and peduluncopontine-lateral dorsal tegmental neurons (blue). Other cholinergic neurons are included, too. Adapted from [84].

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. 2.9). Also, drugs that reduce the AHP in vitro have also been shown to improve learning in aging animals ([77, 90] 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 mechanism [36].

In Fig 2.10, 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.

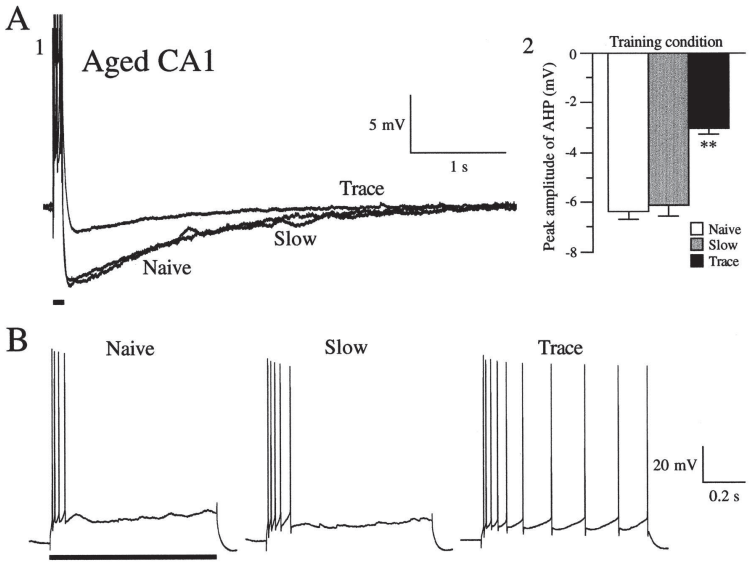


Figure 2.9: **Acquisition of hippocampally dependent trace eyeblink conditioning increased excitability of aging rabbit hippocampal CA1 pyramidal neurons**. (A) Effects of trace conditioning on the size of the postburst AHP. (1) Overlay of voltage recordings of the postburst AHP in CA1 neurons from an aging naive rabbit (Naive), a slow learning aging rabbit, and an aging trace-conditioned rabbit (Trace). (2) Mean effects of trace eyeblink conditioning on postburst AHP amplitude in aging rabbit CA1 neurons: 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 [77].

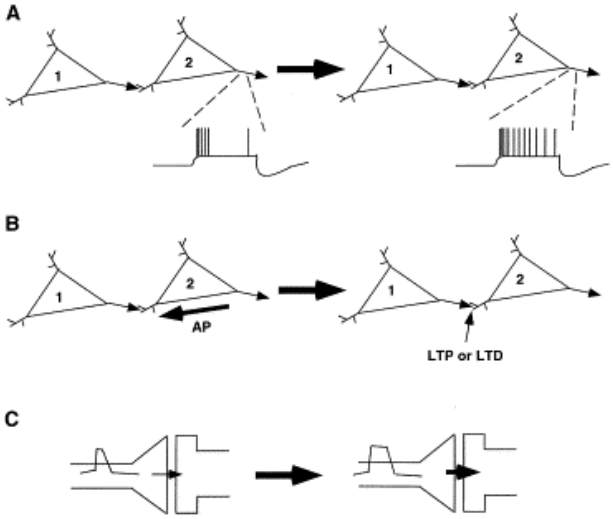


Figure 2.10: **Modulation of excitability as an L&M mechanism**. Three different scenarios illustrate how modulation of excitability could be involved in L&M. In each case the situation before and after learning is shown. (A) The excitability of neurone 2 changes (e.g., due to signalling from acetylcholine) 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].

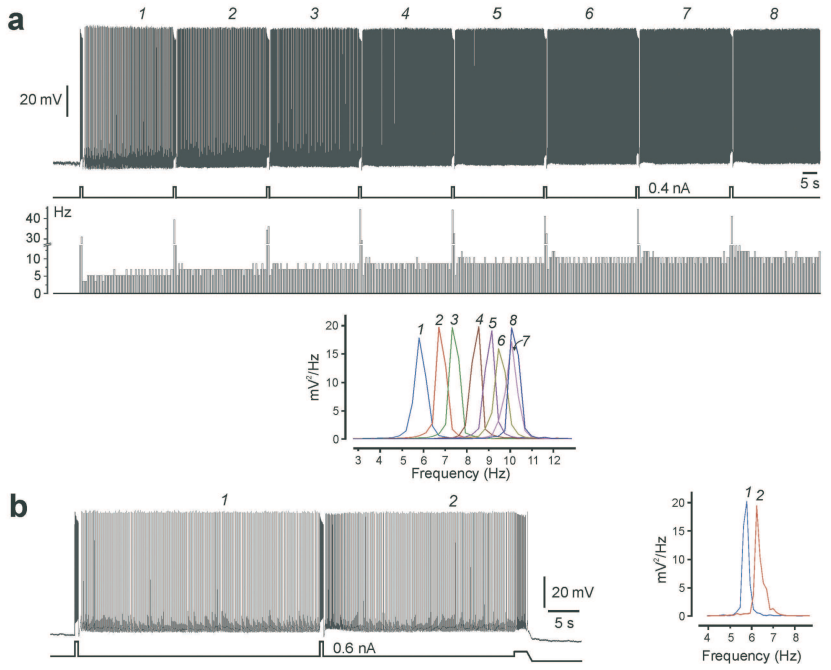
2.5 Persistent Activity and Working Memory

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 [19].

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 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



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 2.11: **Graded persistent activity with short stimulus**. Adapted from [27].

short stimulus gets it started. In [27], persistent firing is observed after cholinergic actions that block K^+ currents and increase spike frequency (Fig. 2.11). 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 ef-

fects 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

Experimental findings suggest a direct connection between the aforementioned types of calcium and potassium channels, which are mainly concentrated at the soma and proximal dendrites. This modelling work attempts to investigate, then, the effects of this coupling on the cell's electrical activity, and how these might be related to the aging-induced functional alterations. The model has been mainly based on two published, biophysically detailed, hippocampal pyramidal cell models: a multi-compartmental model by Poirazi et al, 2003 [86], and a "working model" of a CA1 pyramidal neuron by Borg-Graham, 1998 [10].

3.1 Theoretical Model

The compartmental biophysical simulations for the CA1 pyramidal neuron were developed using the NEURON simulation package [41]. The Crank-Nicholson method for numerical solution to differential equations and a variable time step were used for the simulations. The biophysical model, the morphology of which is shown in fig. 1.1A, consists of 183 sections (1830 compartments). Although dendritic compartments are not necessary to reproduce normal spiking activity, they are important for matching the shape of each action potential (AP), the membrane hyperpolarization after a train of APs, and the firing rate adaptation.

A uniform membrane resistance $Rm = 40k\Omega cm^2 = 4\Omega m^2$, a uniform intracellular resistivity $Ra = 70\Omega cm = 0.7\Omega m$, and a specific membrane capacitance of $1.0\mu F cm^{-2} = 0.01F/m^2$ were assumed. The resting membrane potential of the model neuron is $E_m = -70mV$.

Active mechanisms include two types of Hodgkin-Huxley-type Na^+ currents (somatic and axonic I_{Na}^{sa}, and dendritic I_{Na}^{d}), 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}.

Except for I_{Na}, I_{Kdr}, and I_{Nap} channels which are uniformly distributed in the cell [64, 67], the rest ionic channels are non-uniformly distributed (Figure 3.1). At the somatic and proximal dendritic compartments, there is a high concentration of N- and L-type calcium channels [65], s_{AHP} [93] and f_{AHP} potassium channels [87, 96], and M-type muscarinic K^+ channels [45]. Among these channels, L-type and s_{AHP} channels, are "open" at resting

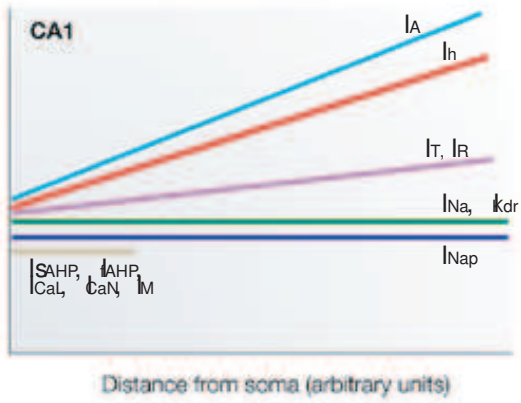


Figure 3.1: **Distribution of ionic conductances in a CA1 neuron**. Adopted from [73].

membrane potentials, contributing to leak current. At the distal dendrites, there is an increasing concentration with distance of h-type mixed kation [62, 80], T- and R-type calcium channels [65], and A-type potassium channels [43] T-type and I_h channels, among them, are "open" at resting membrane potentials. The calcium influx through T-type Ca^{2+} channels in distal dendrites, and through L-type Ca^{2+} channels in proximal dendrites and soma, contributes to a resting calcium concentration of $\sim 50nM$ at the respective cellular areas.

3.1.1 Non-synaptic ionic currents

The model has used Hodgkin-Huxley representations of the intrinsic currents that underlie changes in the membrane potential of these neurons. The following form of kinetic equations for current activations and inactivations are described in [10, 41, 51]:

$$\frac{dz}{dt} = \alpha_z(V)(1-z) - \beta_z(V)z, \tag{3.1.1}$$

where z denotes the dimensionless activation or inactivation variable, or 'particle', and V is the membrane voltage of each compartment. An equivalent equation is:

$$\frac{dz}{dt} = \frac{z - z_{inf}}{\tau} \tag{3.1.2}$$

where

$$z_{inf} = \frac{\alpha_z(V)}{\alpha_z(V) + \beta_z(V)}, \tag{3.1.3}$$

, is the steady-state value of activation or inactivation variable, and

$$\tau = \frac{1}{\alpha_z(V) + \beta_z(V)}, \tag{3.1.4}$$

is the respective time constant of activation or inactivation.

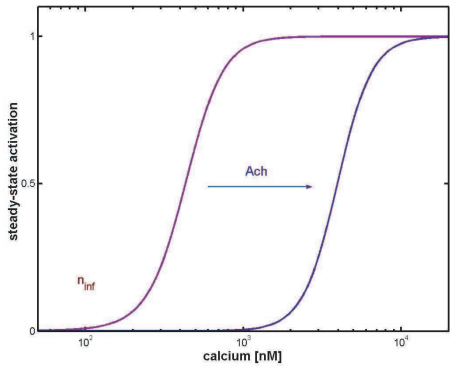


Figure 3.2: **Steady state activation of sAHP channels; shift of EC_{50} of the Ca^{2+}-response curve due to Ach**

Current equations, distributions and densities of I_{Na}, I_{Kdr}, and I_A, are described in [86]. I_{fAHP} has been modelled according to [111] and depends on calcium influx through N-type channels. I_{sAHP} has been modelled according to a standard formalism, using Hill equation [10], but it is driven by L-type channels' activation. This approach has been based on the same voltage activation protocol and time course of the respective currents, as well as their colocalization in somatic and proximal dendritic compartments [16, 71, 96]. More specifically, the current equation for I_{sAHP} is given by:

$$I_{sAHP} = g_{sAHP} * m^2 * (V - E_K) \tag{3.1.5}$$

where the steady state activation

$$m_{inf} = \frac{1}{1 + \left(\frac{EC_{50}}{[Ca^{2+}]_i}\right)^4} \tag{3.1.6}$$

is described by the Hill equation for SK channels [83]. $[Ca^{2+}]_i$ accounts for the local, spike driven Ca^{2+}-influx through LTCs. EC_{50}, the half-activation Ca^{2+} concentration of I_{sAHP}, has been set to 480 nM according to [53, 83, 109]. The time-constant of activation is Ca^{2+}-dependent, according to [10], varying between $\tau_{min} = 100ms$ and $\tau_{max} = 500ms$. The somatic conductance value is: $g_{sAHP} = 0.025S/cm^2$.

During TBS simulations, Ach has been assumed to suppress sAHP current by shifting the Ca^{2+}-response curve to higher EC_{50} values.

The remaining mechanisms have been described according to an extended Hodgkin-Huxley formalism, whose parameters have specific biophysical interpretations and are more closely related to the physiological behavior of each channel [10]. The parameters of I_h, I_M and I_Nap have been modified according to [45], where it is shown that they account for resonance at theta frequencies at hyper- and de-polarized potentials, close to the resting membrane potentials (without action potential triggering).

Two types of LTCs have been included in the model, with different activation kinetics and sensitivity to dihydropyridines, the common pharmacological blockers of LTCs [110, 94].

In neurons, 20 % of LTCs are of $Ca_v1.3_{\alpha_1}$ (or, Lp) type, and account for facilitation in the model, whereas 80% are of $Ca_v1.2_{\alpha_1}$ (or, "Ls") type [20]. Both seem to display an age-dependent up-regulation of their activity ([20] and references therein), accounting for the reported increase in spike-driven Ca^{2+} influx during aging [102].

The kinetics of "classic" (voltage-dependent and Ca^{2+}- inactivated) $Ca_v1.2_{\alpha_1}$ LTCs, as well as of N- and R-types of Ca^{2+} channels, have been described using the following current equation:

$$I_{Ca} = g_X * m^2 * h * h_i([Ca^{2+}]_i) * (V - E_{Ca}) \tag{3.1.7}$$

where V is the membrane voltage of each compartment, $E_{Ca} = 140mV$ the reversal potential of the calcium currents, g_X, where $X \in \{L, N, R\}$ denotes the channels' conductance to the appropriate current (in mS/cm^2), m and h are the voltage-dependent activation and inactivation levels, respectively, and h_i is a calcium-dependent inactivation level [111, 60]:

$$h_i([Ca^{2+}]_i) = \frac{k}{[Ca^{2+}]_i + k}. \tag{3.1.8}$$

The somatic conductance values are: $g_N = 0.2mS/cm^2$, $g_L = 0.24mS/cm^2$, $g_R = 0.02mS/cm^2$. The rate equations of the state variables m and h for all of these channels are described in [10].

$Ca_v1.3_{\alpha_1}$ LTCs have been modeled according to [112]: they display two opposing forms of autoregulatory feedback, Ca^{2+}-dependent inactivation and facilitation. These processes coexist, having different half-activation Ca^{2+}-concentrations values, and different kinetics of onset and decay: facilitation is much slower than inactivation, and is emphasized when the channels are repeatedly activated by trains of action potentials, according to [112]. In the equations for $Ca_v1.3$ LTCs' kinetics shown below, m and h are the activation and inactivation levels .

$$I_L = 0.25 * g_L * m * h(V - E_{Ca}) \tag{3.1.9}$$

Autoregulatory positive feedback (facilitation) has been described with the same formalism to SK channels (eq. 3.1.6), in order for respective currents to exhibit similar calcium and frequency dependence [16, 94, 78]. Also, both channels are "open" at relatively hyperpolarized potentials [110]. Inactivation of Cav1.3 L-type channels has a large value of $IC50 = 1500nM$ and a smaller time-constant $\tau = 30ms$ [112, 85]:

$$h_{inf} = 1 - \frac{1}{1 + \left(\frac{IC_{50}}{[Ca^{2+}]_i}\right)^2} \tag{3.1.10}$$

3.1.2 Ca^{2+} dynamics

Two distinct, exponentially decaying Ca^{2+}-pools exist in the model, accounting for spike-driven Ca^{2+} influx through functionally different Ca^{2+} channel types in the cell [21]. This simplistic description of Ca^{2+} dynamics is valid as long as the mechanisms of interest are:

i the coupling between the functionally co-localized Ca^{2+}-, and Ca^{2+}-dependent potassium channels [71],

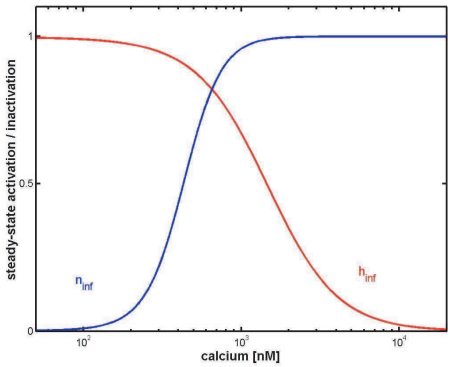


Figure 3.3: **Steady state activation and inactivation of $Ca_v1.3$ - L type channels.**

ii the Ca^{2+}-dependent inactivation of high-voltage activated Ca^{2+} channels [57], and

iii the autoregulatory feedback of L-type Ca-channels [112, 113].

In order to depict the local interactions reported among the above channels, then, the kinetics of local sub-membrane Ca^{2+} concentration should be identical to the kinetics of the respective channels. A small value of the decay time constant in the pool models ($1/\beta = 1ms$) has been adopted, resulting in $[Ca^{2+}]_i$ closely following spike-driven ionic influx through Ca^{2+}-channels, with minimal accumulation of $[Ca^{2+}]_i$. Calcium efflux from buffering/clearance mechanisms - which are not explicitly modelled - is described by a single parameter, the decay rate β:

$$\frac{d[Ca^{2+}]_i}{dt} = \Phi I_{CaL} + \beta([Ca^{2+}]_{i_0} - [Ca^{2+}]_i) \tag{3.1.11}$$

where the term $[Ca^{2+}]_i$ denotes the local, activity-driven Ca^{2+} concentration in each compartment (in mM), $\beta = 1/ms = 1000/s$ is the decay rate of calcium concentration, Φ denotes a factor to convert units of current to units of concentration, and $[Ca^{2+}]_{i,0} = 0.05\mu M$ denotes the baseline resting Ca^{2+} level [10].

The L-type Ca^{2+}-channels largely contribute to the baseline resting Ca^{2+} levels in somatic and proximal apical dendritic compartments, since they can start activating at very low potentials; the low-threshold, voltage-gated T-type Ca^{2+}-channels, on the other hand, seem to set a similar resting level of $[Ca^{2+}]_{i,0}$ in the distal apical dendritic compartments [10]. For these reasons, a distinct $[Ca^{2+}]_i$ pool has been specifically assigned to L- and T- types of Ca^{2+}-channels. The influx through them, modulates in turn the Ca^{2+}-dependent activation and/or inactivation of L- and N- type Ca^{2+}-channels in the soma and proximal dendrites, and R- type channels in distal dendrites [57]. A large increase in Ca^{2+} concentration - after prolonged, high-frequency, ongoing activity - will result in enhanced influx through $Ca_v1.3$-LTCs, and decreased influx through $Ca_v1.2$-LTCs, NTCs and RTCs. According to recent experimental findings, complex voltage waveforms, during tetanic stimulation for example, actually have such opposite effects on high-voltage activated Ca^{2+} channels [60, 94]. Also, similar effects could be observed 'in silico' by increasing the decay time constant of pool

model; such an increase is assumed to depict a slower free Ca^{2+} removal from the cytosol of aged CA1 cells.

In order to model the selective coupling of sAHP channels to LTCs, in the soma and proximal dendrites [71], the LT-type calcium pool ($[Ca^{2+}]_i$) also affects the rate parameters of sAHP channels. The current through $Ca_v1.3$-LTCs increases incrementally with each spike, its exact amount of increase being positively correlated to the firing rate [94, 112]. $[Ca^{2+}]_i$ closely follows the current through LTCs in these compartments, and modulates the current underlying sAHP (I_{sAHP}). I_{sAHP}, then, also increases incrementally with each spike, following the time-course of "delayed facilitation" of LTCs [16].

Fast AHP channels are also functionally colocalized to NTCs, in the soma and proximal dendrites [71]. A separate pool, $[NR - Ca^{2+}]_i$, which describes the Ca^{2+} influx through N- and R- types of Ca^{2+} channels (NTCs, RTCs), regulates the activity of fAHP channels. Ca^{2+}-dependent inactivation of NTCs, due to a large increase in Ca^{2+} concentration, would down-regulate the activity of fAHP (BK) channels. According to [96] and [60], this is actually the case for these channels' activity during high-frequency stimulation.

Furthermore, model parameters have been "tuned" using antidromic, non-synaptic stimulations, in order to reproduce:

- the distance and time-dependent attenuation of back-propagating axonal action potentials into dendrites,
- the reduction in cell's excitability due to the aging-induced alterations in calcium-dependent membrane mechanisms, and
- the role of acetylcholine on excitability's increase during a physiological pattern of activity, θ-bursting, which normally induces long-term modifications in stimulated synapses (LTP). Moreover, θ-burst LTP in CA1 neurons distinguishes cognitively impaired from unimpaired aged subjects [104].

The related simulations are presented in the following section.

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 [98] (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 [98] 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

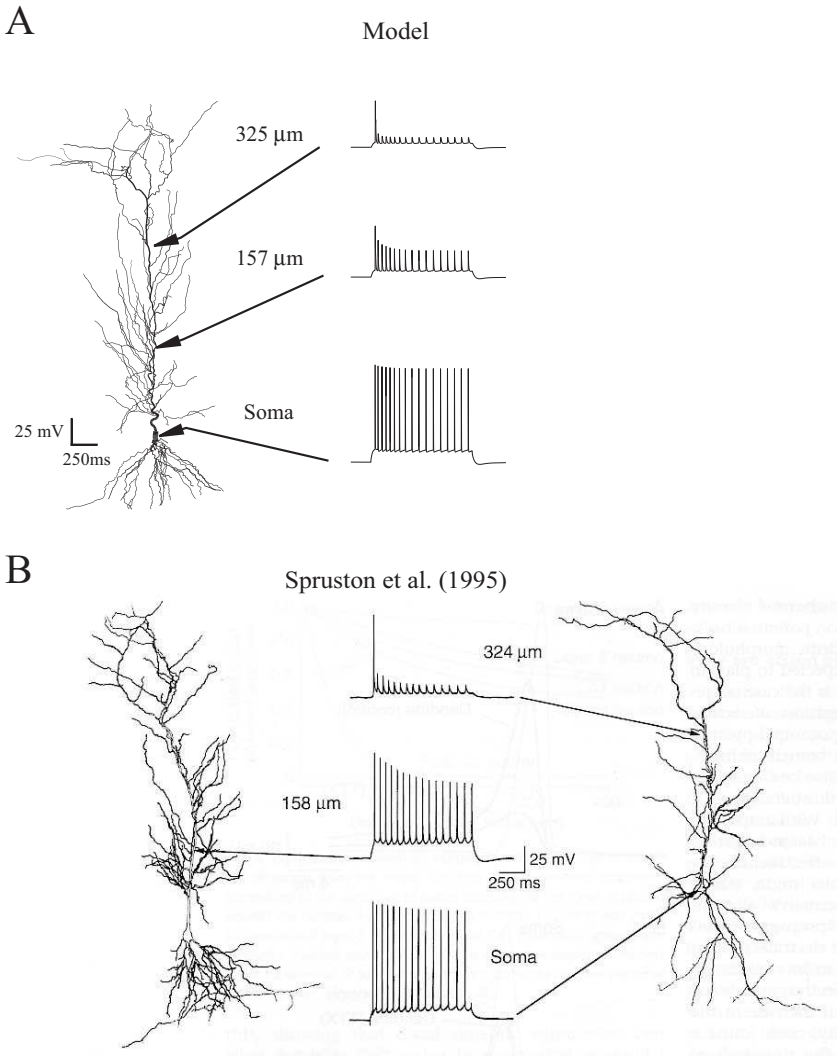


Figure 4.1: **Typical pattern of attenuation of backpropagating action potentials.** A. Backpropagating action potentials evoked by somatic current injection (220 pA, 700ms) in the model, show typical pattern of distance and time-dependent attenuation of spike height; the attenuation pattern is very similar to experimental traces from [98] shown in B.

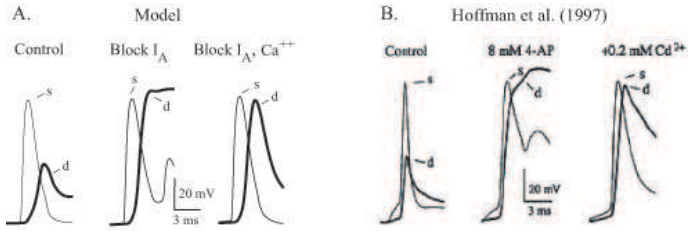


Figure 4.2: **Effect of block of I_A and Ca^{2+} currents on backpropagating action potentials**. Initial somatic and dendritic spikes are shown in response to somatic current injection (300 pA, 50 ms) in control conditions (left), with block of I_A (middle) and with block of Ca^{2+} currents (right).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

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.

4.2 Effect of Aging-induced Increase in Ca^{2+} Influx on Excitability

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 [78]. 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),

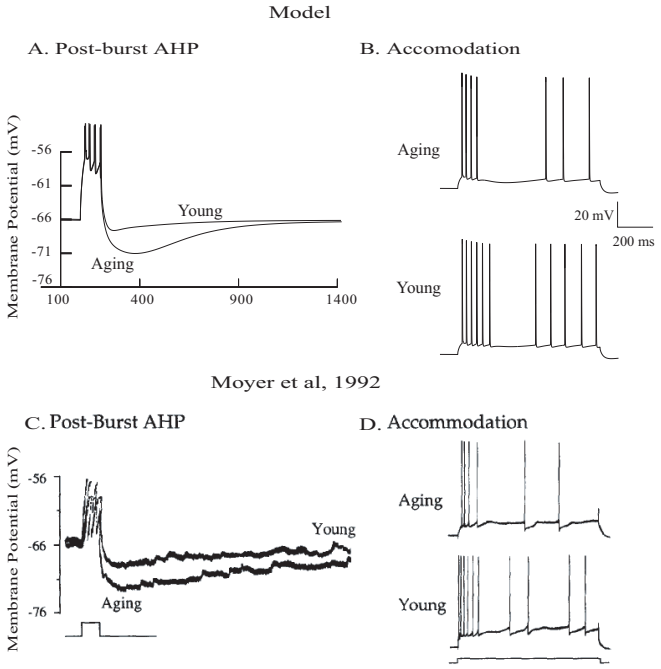


Figure 4.3: **Comparison of model and experimental traces of young vs. old CA1 cells**. Experimental recordings from [78].

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.

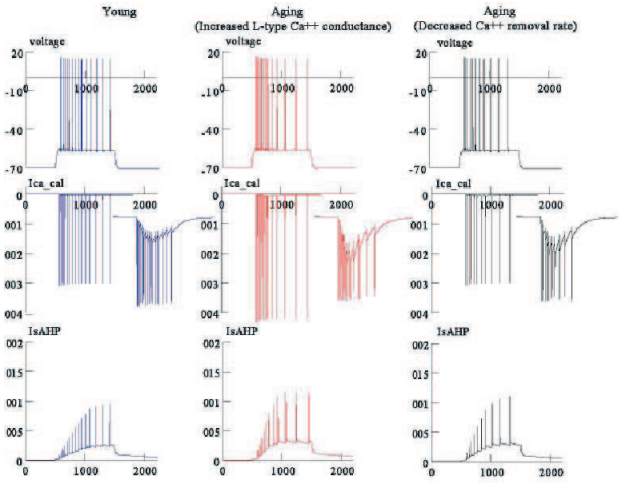


Figure 4.4: **Calcium influx through L-type channels in "young" and "aged" cell models**. Either an increase in LTCs' conductance or a decrease in decay rate, could lead to enhanced calcium influx through LTCs

4.3 Simulation of TBS under Suppresion of AHP Currents

θ-bursting is simulated by 10 antidromic (non-synaptic) identical stimuli at 5 Hz, each lasting for 40ms, with amplitude initially adjusted to induce 4 spikes at 100 Hz. Even in the "young" cell model, the number of spikes in each burst could be maintained and even increase as reported in [95], provided that the Ca^{2+}-dependent afterhyperpolarization K^+ currents were sufficiently suppressed, imitating cholinergic-like influences. Cholinergic actions 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 [80]), 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 Ca^{2+}-dependent AHP currents, its effects on the other currents remain controversial. Perhaps, the reason of this discrepancy is that these effects could be indirectly induced by changes in membrane voltage, Ca^{2+} concentration, or both of them.

4.3.1 TBS in the "young" cell model

In the simulation with the "young cell", depicted in Figure 4.5, cholinergic influences have been modelled as an 8-fold increase of the Ca^{2+} concentration for half-activation of s_{AHP} channels (EC_{50}). Other conductances explicitly suppresed - although of less impact in the long-term - include M-type K^+ (90% blockade), and h-type mixed cation conductance. A-type K^+ channels were indirectly inactivated, due to prolonged membrane depolarization. Also, the enhanced spike-driven Ca^{2+} influx induced inactivation of high-voltage activated

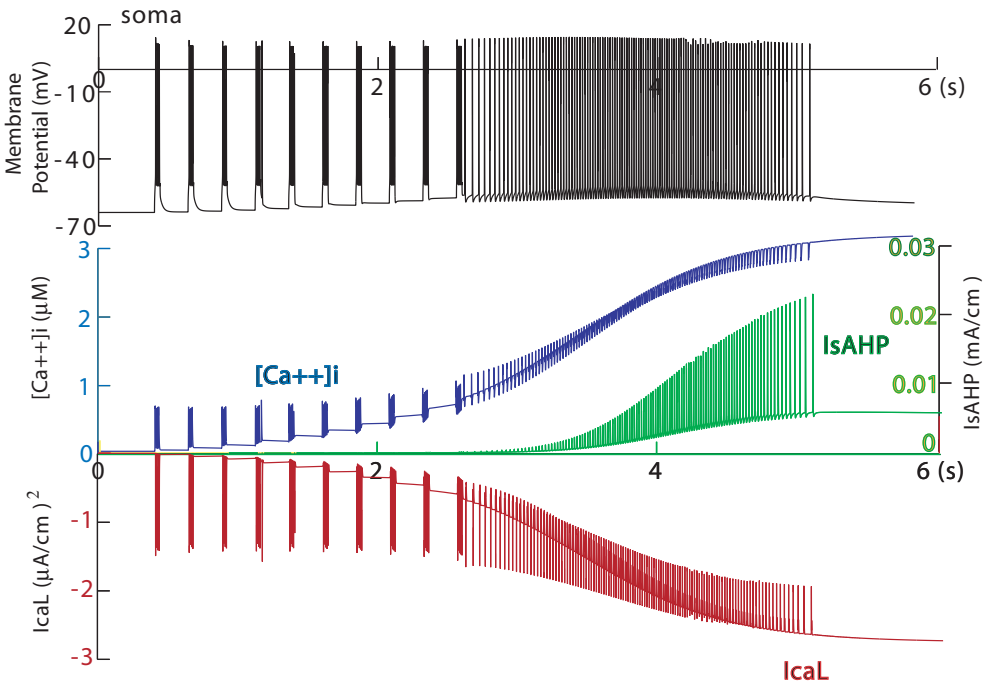


Figure 4.5: **θ-burst antidromic stimulation of the "young" cell, under cholinergic-like suppresion of medium and slow AHP currents**. Voltage trace at soma (black), Ca_i^{2+} accumulation due to LTCs' activity (blue), I_{Ca_L} (red), and I_{sAHP} (green).

Ca^{2+} channels, and fast AHP channels in the model (results not shown). After the end of the stimuli train, self-sustained firing activity arose, and lasted for a few seconds. The instantaneous frequency of spiking, varied between 30-60 Hz (gamma-frequency band), and depended on the competing effects of I_{CaL}, and the $I_{s_{AHP}}$ left unblocked, and gradually building up. Eventually, I_{sAHP} could hyperpolarize the membrane again. Firing frequency was gradually reduced, until spiking ended.

4.3.2 TBS in the "aged" cell model

Increase of $Ca_v1.2$-component of LTCs

An increase in $Ca_v1.2$ phosphorylation has been reported during aging (> 2-fold) [20]. It has been depicted with an increase of $Ca_v1.2$ conductance in the model. EC_{50} of sAHP channels should be increased more than 10-fold, for the number of spikes to be maintained in succesive bursts. Due to enhanced Ca^{2+} - dependent AHP currents, membrane potential is less depolarized, and firing activity does not outlast the end of stimuli train.

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

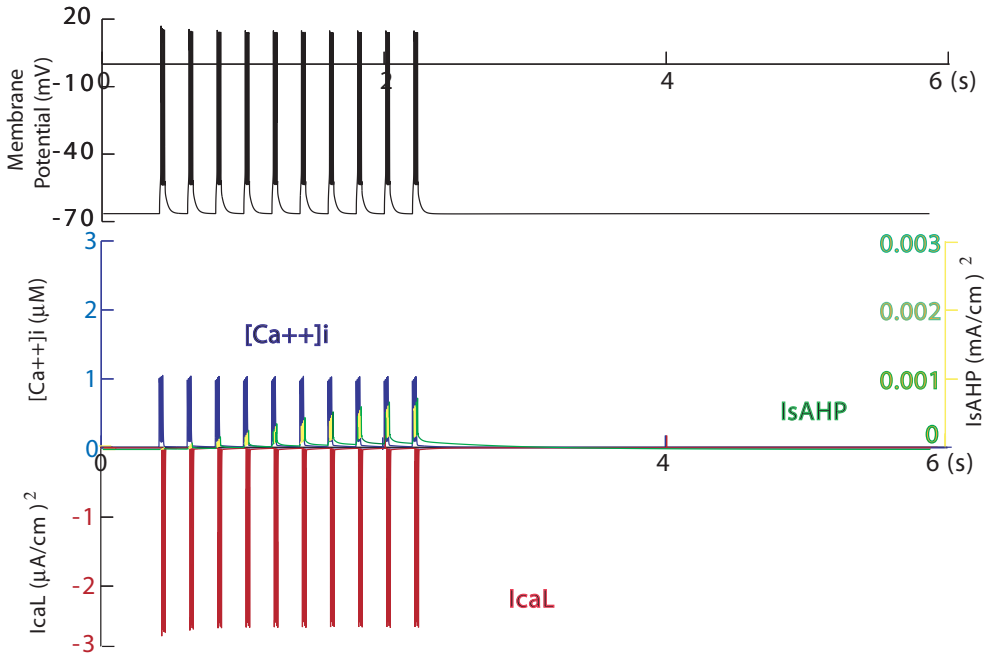


Figure 4.6: **TBS in the "aged" model, simulated with an increase in $Ca_v1.2$ LTCs**. Voltage trace at soma (black), Ca_i^{2+} accumulation due to LTCs' activity (blue), I_{Ca_L} (red), and I_{sAHP} (green)

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.

Increase of $Ca_v1.3$-component of LTCs

Cav1.3 expression is upregulated by 25% during aging (references in [20]). This could be depicted either through a 25% increase in its conductance, or a shift in EC_{50} (increase in facilitation), or an opposite shift in IC_{50} (decrease of inactivation). Although $Ca_v1.3$ account for only 20% of LTCs, their relative contribution in LTCs' activity is increased with succesive stimuli [94]. 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 slightly below threshold. In Figure 4.7, self-sustained rhythmic firing is super-posed on the theta-burst pattern, and outlasts it for $> 1sec$. Frequency of persistent activity increases over successive intra-stimuli intervals. After the end of the stimuli-train, firing persists for several hundreds of ms; the gradual buildup of inhibitory I_{sAHP} current decreases the rate of firing and eventually terminates it. If K^+-currents' blockade was large enough, "epileptiform" - like activity could be induced (results not shown).

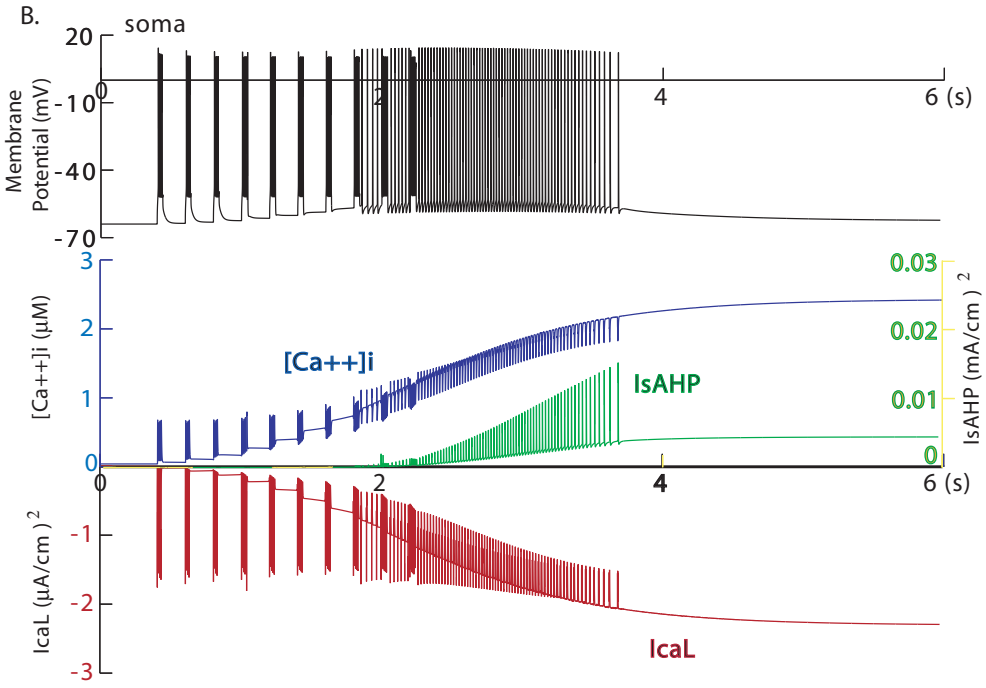


Figure 4.7: **TBS when aging is simulated by an increase in autoregulatory positive feedback of $Ca_v1.3$ LTCs**. Voltage trace at soma (black), Ca_i^{2+} accumulation due to LTCs' activity (blue), I_{Ca_L} (red), and I_{sAHP} (green).

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 [99]. 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 [91], 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 seem to compare direct sensory information (from layer III of the entorhinal cortex via the perforant path), with inputs from dentate/CA3 region (via the Schaffer collateral pathway), carrying predictions based on previously stored information [80] (Fig.5.1). A match or mismatch between these functionally separated inputs, might signify either redundant or irrelevant sensory stimuli which should be prevented from being re-encoded. 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 [80]. Successful association of these functionally separated inputs in a CA1 pyramidal neuron, is eventually signified by bursts of action potentials [59, 56].

In this study, we 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 things as simple as possible, in order to investigate the role of somatic mechanisms during physiological patterns of activity, such as θ-bursting, as well as the postsynaptic effects and the kind of information they might encode [59]

L-type channels control gene transcription in neurons, and seem to be implicated in various forms of synaptic plasticity [94, 110, 112]. Their dysregulation probably contributes to aging-induced learning and memory impairment [20, 77]. The dihydropyridines' antagonist nimodipine reverses learning deficits in aged subjects [77], but cannot effectively inhibit the activity of $Ca_v1.3$ (or, Lp) subtype of LTCs [20, 94, 110]. In fact, if clinical doses of dihy-

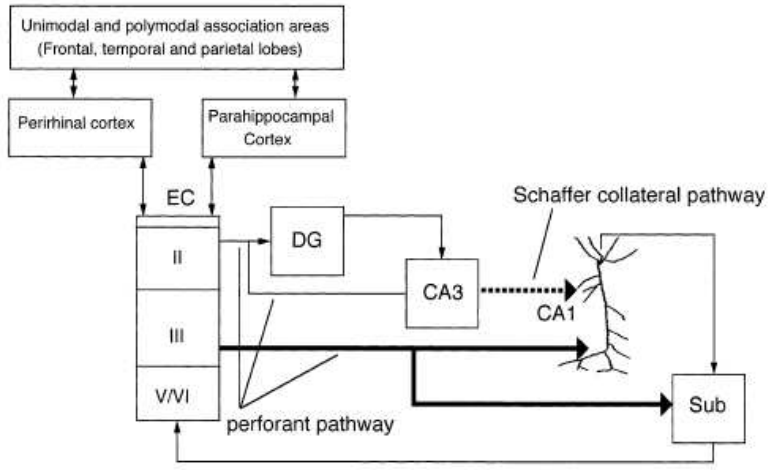


Figure 5.1: **The Organization of Medial Temporal Lobe Circuits**. Information flows through the hippocampal trisynaptic circuit from layer II of the entorhinal cortex (EC) via the dentate gyrus (DG) to CA3 and then to CA1. The input from CA3 to CA1, via the Schaffer collateral pathway, targets the proximal 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. Adapted from [80].

dropyridines affected $Ca_v1.3$ LTCs, various forms of arrhytmias would occur (see discussion in [20]). $Ca_v1.3$ channels have been modeled according to [112]: they display two opposing forms of autoregulatory feedback, Ca^{2+}-dependent inactivation and facilitation. These processes coexist, having different half-activation Ca^{2+} concentration values, and different kinetics of onset and decay (Fig 3.3): their Ca^{2+}-dependent facilitation is much slower than inactivation, and is emphasized when the channels are repeatedly activated by trains of action potentials [112]. According to [46], the coexistence of inactivation and facilitation in $Ca_v1.3$ channels, meet the criteria to produce an amplified resonance which depends on spike-driven Ca^{2+} influx through them, and hence, frequency of firing. Under conditions of suppresed AHP currents, membrane potential becomes depolarized, and firing rate is increased. When the amplifying facilitation process becomes strong enough, spontaneous oscillations can occur. The kinetics of $Ca_v1.3$ inactivation determine the frequency of these spontaneous oscillations: a 30 ms time-constant of inactivation, implies a cutoff frequency of $\sim$30 Hz (high-pass filtering favoring γ band frequencies, 30-70 Hz). Since $Ca_v1.3$ are higher at soma and proximal dendrites, they can make the model prone to generating intrinsic oscillatory firing in the absense of inputs [46]. This behavior is under the modulatory control of colocalized slow AHP channels.

Self-sustaining oscillations induced during TBS *in silico*, imply that a single CA1 cell

could act as a pacemaker under cholinergic influence (increased attentional demands), and intrinsically drive the network at the rhythm of its firing activity [46]. Oscillations in γ-frequency band are observed in the hippocampus, during states of focused attention (in vivo), 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 [30, 31]).

Neurotransmitters, like acetylcholine, acting to suppress I_{sAHP} during attentional states, regulate the ability of CA1 neurons to encode information. An enhanced I_{sAHP} and/or insufficient cholinergic neurotransmission during aging, might inhibit the association of novel sensory inputs, resulting in the "feature binding deficit" of learning-impaired aged subjects [74].

The model presented, is a phenomenological, rather qualitative description of the actual mechanisms involved. Activation and inactivation kinetics of $Ca_v1.3$ channels should be more accurately modelled using more complex Ca^{2+}-response curves, for exact quantitative predictions to be feasible. 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. Perhaps, it would be possible then to quantitatively describe how these channels' coupled activity dynamically adjusts the frequency content of spike output, according to recent spiking history; and how their neuromodulation might induce pacemaking properties to CA1 neurons [44, 33, 30, 31, 46].

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 "connect" resonant or pacemaker model neurons, with "tunable" synaptic connections, 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 of memories performed in hippocampal circuits [38, 63].

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