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# Cholinergic neuromodulation of an anatomically reconstructed hippocampal CA3 pyramidal cell

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## Abstract

We investigate the effects of cellular-level cholinergic neuromodulation on the physiological properties of a realistic, anatomically reconstructed CA3 pyramidal cell model. The model has 385-compartments, contains a wide variety of ion channels ( $\text{Na}$ ,  $K_{\text{DR}}$ ,  $K_{\text{C}}$ ,  $K_{\text{A}}$ ,  $K_{\text{M}}$ ,  $K_{\text{AHP}}$ ,  $\text{Ca}_L$ ,  $\text{Ca}_N$ ,  $\text{Ca}_T$ ), calcium diffusion, buffering and pumping, and represents an updating of a model of Migliore et al. (*J. Neurophys.* 73 (1995) 1157–1168) to reflect more recent biological data. The simulated application of acetylcholine resulted in several observed changes in single-cell physiology: (1) a transition from bursting (“complex-spiking”) to regular spiking, (2) an increased speed of action potential backpropagation, (3) an increased amplitude of back-propagating action potentials and (4) a decrease in dendritic calcium influx. These results confirm some earlier studies of ours in a simpler pyramidal cell model and are consistent with the “two-stage” memory model proposed by Buzsáki (*Neuroscience* 31 (1989) 551–570). The implications for this model of hippocampal function as well as for Alzheimer’s disease are discussed. © 2000 Published by Elsevier Science B.V. All rights reserved.

**Keywords:** Hippocampus; CA3; Acetylcholine; Neuromodulation; Alzheimer’s disease

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## 1. Introduction

Neuromodulation plays a critical role in cortical function and dysfunction through alterations in ionic conductances, membrane properties, and synaptic transmission

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(see [9] for a recent review). These changes in single-cell physiology and network properties regulate information processing, behavioral states, and the sleep-wake cycle. Perturbations of neuromodulatory systems, as occurs in Alzheimer's disease (AD) and other neurological disorders, have profound effects on cognitive function but may also represent a promising avenue for therapeutic intervention. While there is a wealth of data on in vitro cholinergic modulation of single cells, the functional roles of these processes in cellular and memory function remain unclear. Here we focus on the physiological consequences of cholinergic neuromodulation of a highly-detail biophysical model of a single pyramidal cell in the CA3 region of the hippocampus and consider implications for Alzheimer's disease.

## 2. Methods

We have ported the 385-compartment hippocampal pyramidal cell model originally used by Migliore and colleagues [19] to GENESIS and modified its channel distributions and kinetics to reflect more recent biological data. The model contains a fast sodium channel, two HVA calcium channels (L- and N-type), one LVA calcium channel (T-type), two calcium-dependent potassium channels ( $K_{AHP}$  and  $K_C$ ), four calcium-independent potassium channels ( $K_M$ ,  $K_{DR}$ , and two forms of  $K_A$ ), radial calcium diffusion, immobile calcium buffering, and, membrane-bound calcium ATPase pumping.

We have chosen this particular model because, to our knowledge, it is the most accurate biophysical representation of a hippocampal pyramidal cell yet reported in terms of its ability to mimic the firing characteristics and calcium dynamics of biological cells. For the most part, the conductances and the kinetics are the same as those previously described [13,19]. Our modifications of this cell consisted of replacing the  $K_A$  channel with two more recent models derived from patch-clamp studies of CA1 pyramidal cells as well as a somatodendritic distribution reflecting the increased number of such channels as a function of distance from soma [11]. Similarly, the distribution of calcium channel subtypes and sodium channels as well as their maximal conductances were altered to reflect more recent data [17]. A summary of the resulting model is provided in Table 1.

Neuromodulation of the single-cell model by acetylcholine was implemented as previously described [18] with the exception that the local concentration of ACh at any point along the cell was given by a sum of Gaussian functions. Consistent with the fact that cholinergic afferents to CA3 are found most densely in the stratum oriens and stratum lacunosum-moleculare [1], the Gaussians had means of  $-100 \mu\text{m}$  (negative values are relative to the soma, reflecting a position in the basal arbor) and  $700 \mu\text{m}$  with variances of  $100 \mu\text{m}$  for both distributions.

The cell was driven by 0.1 nA of current injection to the soma, however, we have confirmed the results using random synaptic stimulation of model AMPA, NMDA, and GABA<sub>A</sub> receptors in the stratum radiatum. This approach complicates the examination of backpropagating spikes and calcium transients in the dendrites as

Table 1  
Parameters for the anatomically reconstructed pyramidal cell model

Channel	$\bar{g}$ (mS/cm <sup>2</sup> )	Distribution and comments	Reference for kinetics
Na	50	With the introduction of the modified $K_A$ distribution, a slightly larger Na conductance was required, however, unlike Migliore et al. [19] we distributed the Na <sup>+</sup> channels with equal density throughout the cell as indicated by dendritic patch-clamp data [17]	[31]
Ca <sub>T</sub>	1.0	Distribution (throughout cell) and maximal conductance based on [17]	[13]
Ca <sub>L</sub>	1.2	Distribution (< 50 μm from soma) and maximal conductance based on [17]	[13]
Ca <sub>N</sub>	1.5	Distribution (throughout cell) and maximal conductance based on [17]	[13]
$K_{DR}$	36	The larger Na required a greater conductance for repolarization of the action potential	[19]
$K_A$	7 + 11* (distance from soma /100 μm)	Two forms of the channel exist; the more distally distributed channels (> 100 μm) activate at more hyperpolarized voltages.	[11]
$K_C$	2.4	Uniformly distributed throughout cell	[19]
$K_{AHP}$	0.4	Uniformly distributed throughout cell	[19]
$K_M$	0.1	Uniformly distributed throughout cell	[19]

$\bar{g}$ , maximal conductance. The electrotonic parameters of the model were  $R_m = 30,000 \Omega \text{ cm}^2$ ,  $C_m = 1 \mu\text{F cm}^{-2}$  in the somatic compartments and raised to  $1.6 \mu\text{F cm}^{-2}$  in the dendrites to compensate for spines. The axial resistivity  $R_i$  was  $150 \Omega \text{ cm}$ .

EPSPs and IPSPs bombard the arbor and occasionally produce dendritic spikes. For the sake of simplicity, the synaptic stimulation data are not presented here.

### 3. Results

#### 3.1. Modulation of membrane potential by acetylcholine

The major effect of simulated cholinergic neuromodulation is a change in firing mode similar to that which we found in a previous study [18] using the pyramidal cell model of Traub and colleagues [30]. As in the Traub cell, increasing concentrations of

ACh resulted in a transition from intrinsic bursting to regular spiking as shown in Fig. 1.

While this transition is understandable from the effects of ACh on single-channel conductances, a detailed investigation of the basis for this transition demonstrated a surprisingly complex interaction between the various currents. Briefly,  $I_{AHP}$  underlies the long latency between hippocampal bursts and so direct muscarinic inhibition of this current results in a shorter interburst interval. The direct, but partial inhibition of HVA calcium channels undermines the slow depolarization that contributes to burst formation. Indirectly, this reduction in calcium influx affects the calcium dependencies of both  $I_C$  and  $I_{AHP}$ . At relatively high concentrations of ACh (on the order of  $100 \mu\text{M}$ ) the direct inhibition of  $K_{AHP}$ ,  $Ca_L$ , and  $Ca_N$  was sufficient to change the cell from bursting to regular spiking, as we had previously observed in the Traub pyramidal cell [18] and reasoned intuitively as above. We were surprised to find, however, that inhibition of  $K_A$  alone was powerful enough to change bursts to spike doublets (*data not shown*). This

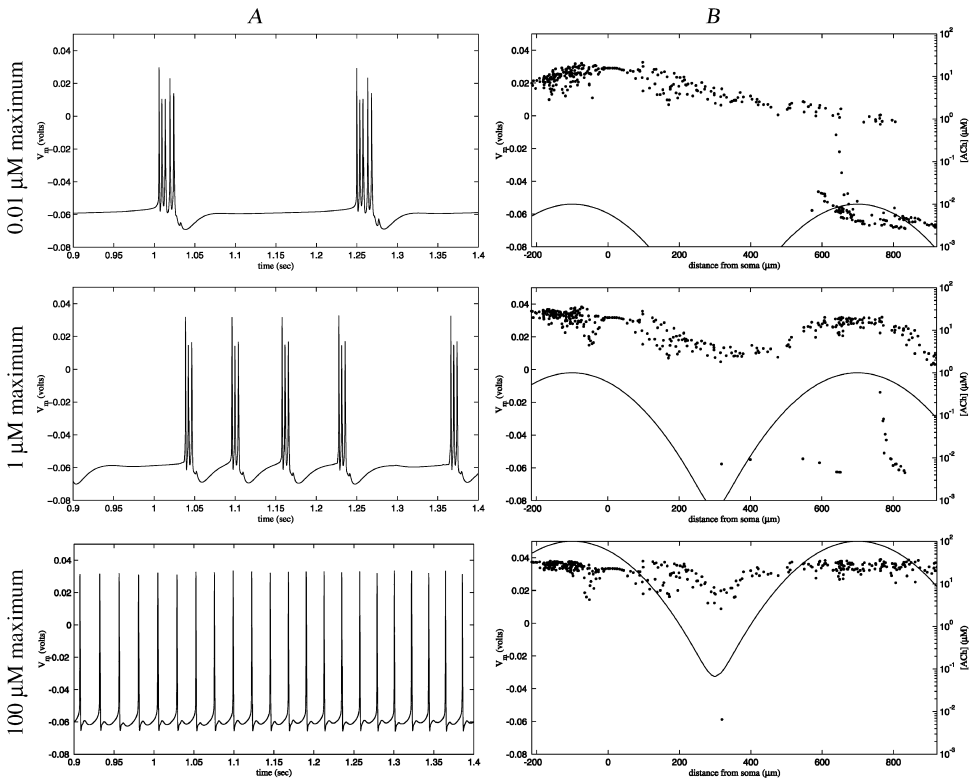


Fig. 1. Dependence of spiking mode (column A) and action potential amplitude (column B) upon the local concentration of acetylcholine. The solid lines in column B give the actual concentration of ACh as a function of distance. At low concentrations of ACh, the pyramidal cell demonstrates bursting (“complex spiking”) behavior with strong attenuation of action potential amplitudes and branch point failures as a function of distance from the soma. With increasing concentration of ACh, the firing mode switches to regular spiking while action potentials backpropagate strongly into the apical and basal dendrites.

is in contrast to the study by Migliore et al. [19] where the authors claim that the opposite effect occurs, i.e. increasing  $K_A$  should promote regular spiking. Here, the inhibition of  $K_A$  together with inhibition of  $K_{AHP}$ ,  $Ca_L$ , and  $Ca_N$  switched the cell from bursting to spiking at much lower concentrations of ACh (on the order of 5–10  $\mu\text{M}$ ) than observed with  $K_{AHP}$ ,  $Ca_L$ , and  $Ca_N$  alone. In addition, the inhibition of only  $K_A$  and  $K_{AHP}$  was sufficient to induce regular spiking at 100  $\mu\text{M}$  of ACh. We have subsequently learned of an in vitro demonstration of this cholinergically induced switch in physiological firing mode in the hippocampus [3] with [ACh] of 5  $\mu\text{M}$ .

In addition to assisting the transition to regular spiking, the muscarinic inhibition of  $K_A$  channels had two other noticeable effects. First, it increased the speed of backpropagation for single action potentials. Second, this inhibition boosted action potential amplitudes in the dendritic arbor (*data not shown*), which may relate to a recent report of muscarinic boosting of backpropagating action potential amplitudes [32].  $K_A$  channels do not account for the calcium-dependency of this muscarinic effect described by Tsubokawa and Ross, but their report makes no mention of  $K_A$  channels as a possible partial target of the muscarinic modulation. Given the important role these channels may play in dampening backpropagating spikes [7,11], their possible modulation by ACh requires further study especially given the recent report of their modulation by protein kinase A and protein kinase C [10].

### 3.2. Modulation of intracellular free calcium by acetylcholine

Less obvious but of significance for plasticity and intradendritic signaling pathways is the cholinergic modulation of intracellular free calcium demonstrated in Fig. 2. At low levels of ACh, intrinsic bursting in the pyramidal cell results in a large influx of calcium via the voltage-gated calcium channels (VGCCs) along the dendritic arbor. As ACh rises, the pyramidal cell is still in burst mode but the resulting calcium transients in the stratum oriens and stratum radiatum are dampened by the progressive inhibition of VGCCs. When ACh reaches even higher levels and the pyramidal cell has changed to its regular-spiking mode, the more complete blockage of VGCCs limits the level of calcium influx even as the inhibition of  $K_A$  allows single spikes to backpropagate strongly to the distal dendrites. While we found similar results in the Traub pyramidal cell, its simple model of calcium dynamics, the single form of HVA calcium channel, and its ad hoc distribution in the cell made us question the validity of the results. These results for the more detailed pyramidal cell model are not only reassuring, but also can provide quantitative estimates of free calcium concentrations which can be correlated with studies of plasticity and excitotoxicity.

## 4. Discussion

### 4.1. Regulation of memory function by ACh

The effects of cholinergic modulation that we have demonstrated are consistent with the “two-stage” model of memory formation and consolidation proposed by

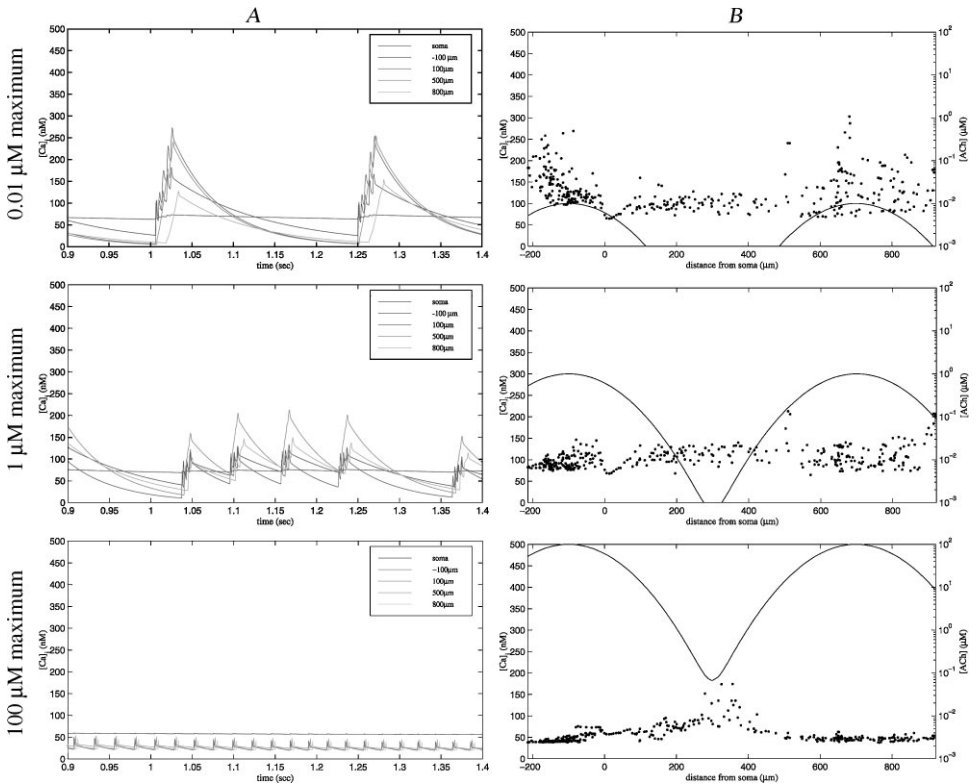


Fig. 2. Dependence of calcium dynamics upon the local concentration of acetylcholine. At low concentrations of ACh, bursting in the pyramidal cell results in large transients of calcium influx with considerable variability across the dendritic arbor. As the concentration of ACh rises and the firing mode switches to regular spiking, calcium transients are markedly attenuated across the length of the cell.

Buzsáki [6]. His model suggests that memories are formed in one stage and consolidated in the other. The first stage, hypothesized to occur during exploratory activity or REM sleep, is characterized physiologically by theta- and gamma-band oscillations in both intracellular and population recordings. It is during this state that pyramidal cells are relatively hyperpolarized and tend to fire regular spikes at low frequencies [8,16,27,35]. In contrast, the second stage, proposed to occur during awake immobility, consummatory behaviors, and slow-wave sleep, is characterized by predominantly bursting (“complex-spiking”) pyramidal cells [23,28], the presence of sharp waves [5,29] and 200 Hz ripple oscillations [34]. Our results fit well into this model of memory function, and the correlation between ACh levels and the sleep wake cycle [14] lend further support.

#### 4.2. Alzheimer’s disease and the perturbation of single-cell physiology

Often the early memory impairment in AD is attributed to hippocampal cell death from the presumed neurotoxicity of neuritic plaques and/or neurofibrillary tangles

[12,15,22,26], but this view runs counter to theoretical considerations and computational studies which suggest that a significant number of neurons must die before memory function is seriously impaired [24], far more than the 10% seen in the latest stages of AD [15]. In contrast, an early and more insidious manifestation of the disease is the neuromodulatory denervation that accompanies the death and/or dysfunction of specific subcortical neurons.

Our results suggest that acetylcholine has at least several regulatory actions on hippocampal pyramidal cells. Many, if not all, of these roles may be compromised as choline uptake decreases [25], ACh production is impaired [21], ACh release declines [20], cholinergic varicosities disappear [4], and subcortical cholinergic neurons die [2,33], all in relatively early stages of Alzheimer's disease. Our results suggest that these manifestations of AD can have significant consequences at the cellular level as switching between mnemonic stages is impaired, synaptic plasticity is altered by perturbations in dendritic calcium dynamics, and cells possibly die from excitotoxic calcium accumulation.

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