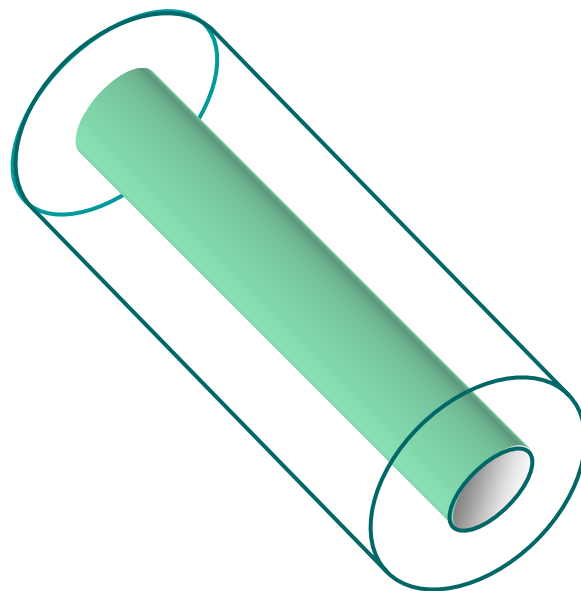


From Neuroscience
Karolinska Institutet, Stockholm, Sweden

**Extra- and Intracellular Electrotonic
Conductance of Signals and its
Significance for Synaptic Plasticity**

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**Karolinska
Institutet**

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ABSTRACT

Electrical signals play a pivotal role in the normal function of most if not all organisms. Understanding the electrophysiological principles that govern electrical signaling is essential for insights into normal- and pathological processes in cell biology.

The main aim of the current thesis is to provide a novel perspective on the way in which a passive electrical signal may participate in shaping the cellular response in the nervous system (e.g. for memory and learning) and in the cardiovascular system (cardiac contractility). A second aim is to demonstrate that some of these novel theoretical predictions may be of importance in living tissue under normal as well as pathological conditions. These aims have been obtained through a combination of theoretical and empirical studies in isolated cardiac muscle and brain slices.

The initial studies show that a theoretical model of cardiac muscle, which may initially appear counter-intuitive (virtual electrodes predicted by the bidomain model), may actually be used to modulate intracellular calcium dynamics and modify the contraction of the cardiac muscle in normal conditions as well as in pathological conditions (paper I).

I have then investigated whether similar principles could be applied to neuronal function, which has led to the development of the Cable-In-Cable (CIC) theory (paper II and paper III). The CIC theory represents a passive cable theory that is an extension of the conventional cable theory. The CIC theory suggests that post-synaptic potentials may be more accurately described by a system of cable within cable, where synaptic currents travel simultaneously in the medium between the cell membrane and the ER, and within the ER lumen. The CIC theory suggests a novel pathway by which the synaptic activity can modulate the activity of the cell nucleus (paper II). Additionally, the CIC theory suggests that synapses located on dendritic spines may code a second level of information (paper III), introducing a second dimension of synaptic plasticity.

Synaptic plasticity, a dynamic refinement of synaptic efficacy, is generally regarded as a cellular correlate for particular forms of learning and memory. The majority of the experimental evidence for synaptic plasticity in the cortex relates to excitatory connections onto pyramidal cells. The primary postsynaptic target for these connections is the dendritic spines.

The capability of dendritic spines to implement the Hebbian rule of synaptic plasticity has been addressed here through a combination of theoretical modeling and paired patch clamp recordings from cortical pyramidal neurons (unitary synaptic connections). Paper IV suggests that the Hebbian learning rule in a unitary synaptic connection is determined by free calcium dynamics within a temporal window of about 15-20 ms following the synaptic signal.

Finally, synaptic plasticity (LTP) in healthy brain slices and in a transgenic animal model of Alzheimer disease was studied using patch clamp recording from cortical pyramidal neurons (paper V). This study suggested that synaptic plasticity is impaired at the onset of the disease, when the microcircuits architecture is still intact but the levels of the soluble β amyloid are elevated. This study shows that soluble β amyloid impairs LTP by diminishing the increase in the AMPA fraction of the synaptic signal. Specifically, the study shows that selective amplification of the AMPA fraction of the synaptic signal is capable of rescuing synaptic plasticity *in vitro* at the onset of Alzheimer-like disease.

In summary, this thesis combines theoretical studies and empirical *in vitro* studies to introduce a new perspective to the way a passive electrical signal and intracellular calcium dynamics may participate in shaping cellular response in the cortical neurons and in the myocardium.

LIST OF PUBLICATIONS

- I. **SHEMER I***, Brunckhorst CB*, Mika Y, Ben-Haim SA, Burkhoff D. Cardiac contractility modulation by non-excitatory currents: studies in isolated cardiac muscle. *Eur J Heart Fail.* 2006 Jan;8(1):7-15.
- II. **SHEMER I**, Brinne B, Tegnér J, Grillner S. Electrotonic Signals Along Intracellular Membranes May Interconnect Dendritic Spines and Nucleus. *PLoS Comput Biol.* 2008 Mar 28;4(3):e1000036
- III. **SHEMER I** and Grillner S. Cable-In-Cable theory predicts a second level of synaptic plasticity in spiny neurons. *PLoS Computational Biology* (submitted)
- IV. **SHEMER I***, Zilberter M*, Holmgren H, Zilberter Y. Calcium Dynamics within a Short Temporal Window Determines Spike-timing-dependent Plasticity in Synapses between Layer 2/3 Pyramidal Cells. *Cerebral Cortex* (submitted)
- V. **SHEMER I***, Holmgren C*, Min R, Fülöp L, Zilberter M, Sousa KM, Farkas T, Härtig W, Penke B, Burnashev N, Tanila H, Zilberter Y, Harkany T. Non-fibrillar beta-amyloid abates spike-timing-dependent synaptic potentiation at excitatory synapses in layer 2/3 of the neocortex by targeting postsynaptic AMPA receptors. *Eur J Neurosci.* 2006 Apr;23(8):2035-47.

* Denotes equal contribution

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LIST OF ABBREVIATIONS

Aβ	Amyloid β protein
AD	Alzheimer's Disease
AP	Action Potential
CIC	Cable-In-Cable
ER	Endoplasmic Reticulum (<i>i.e.</i> the inner cable)
EPSP	Excitatory Post-Synaptic Potential
EPSP_{eff}	The actual potential generated by an EPSP at the position of a putative integrator
ER	Endoplasmic Reticulum
<i>I</i>	The ratio between currents <i>actively</i> injected into the ER lumen and cytosol at the synapse ($x=0$).
<i>I</i>_{ER}	The axial current inside the internal cable (ER lumen)
<i>I</i>_i	The axial current inside the external cable (Cytosolic lumen)
PM	Plasma Membrane (<i>i.e.</i> the external cable)
STDP	Spike-Timing-Dependent Plasticity
<i>V</i>_{o_{mP}}	Transmembrane potential across the PM, at the synapse
<i>VE</i>	Virtual Electrode (see Figure 1)
<i>V</i>_{ER}	The potential inside the ER lumen (see Figure 3)
<i>V</i>_i	The potential inside the cytosol (see Figure 3)
<i>V</i>_{mE}	Transmembrane potential across the ER
<i>V</i>_{mP}	Transmembrane potential across the PM

1 THESIS SUMMARY – MAIN SECTION

1.1 INTRODUCTION

The main aim of the current study was to consider new perspectives regarding the normal function of passive electrical signaling and its direct effect on intracellular calcium dynamics. For that purpose the thesis combines theoretical studies and experimental electrophysiological studies in two organ systems: the neocortex and the ventricular myocardium. In both systems transmembrane potentials carry signals that modulate intracellular calcium levels, which, in turn mediate the main cellular response: the contraction of the single cardiomyocyte in the heart and plasticity of a single synaptic connection between pyramidal cells in cortex.

The work presented here aims at putting the insights obtained theoretically into an applicable context. For that reason, the experimental work extended into the relevant pathologies of contractility and synaptic plasticity respectively, which are the hallmarks of two major diseases: chronic heart failure and (the onset of) Alzheimer's disease, respectively.

In this thesis, I will start by presenting the theoretical principals in cardiac electrophysiology, which form the conceptual basis for the experimental work, presented in paper I. Then, I describe how theoretical insights obtained from the field of cardiac electrophysiology may be applied to the brain, in a form that may affect a synapse-to-nucleus signaling (paper II) and synaptic plasticity (paper III). The summary ends with a brief description of theoretical and experimental work of synaptic plasticity which suggests a new perspective on the rules governing synaptic plasticity (paper IV) and the pathology during the onset of Alzheimer disease (paper V).

1.2 THE VIRTUAL ELECTRODE (VE) CONCEPT

1.2.1 VE in a simple cable

Conventional biophysics theory predicts that during a unipolar stimulation of a uniform fiber, a unipolar electrode generates an electrical field which polarizes the fiber according to the shape of the electrical field generated by that electrode. Figure 1b shows the extracellular potential produced along a fiber by a steady-state current applied through a single, extracellular electrode (illustrated in Figure 1a). If the electrode is a cathode (Figure, 1c), then near the cathode the fiber is strongly depolarized (shaded), but away from the cathode we observe two hyperpolarized regions, called *virtual anodes*. If the electrode is an anode (Figure 1d), then near the anode the fiber is hyperpolarized, but we observe two depolarized (shaded) areas, called *virtual cathodes* (see Roth BJ, 1994, for review).

Virtual electrodes can have significant electrophysiological effects. For example, during anodal stimulation, excitation that occurs at a virtual cathode (some times called anode-make stimulation) is fundamentally different from the anode-break stimulation predicted by Hodgkin & Huxley's model (Hodgkin & Huxley 1952). The latency of an action potential excited by anode-make stimulation depends on when the stimulation pulse is turned on (the "make" of the pulse), whereas the latency of an action potential excited by anode-break stimulation depends on when the stimulation pulse is turned off (the "break" of the pulse). The spatial dependence of V_m is

essential to anode-make stimulation; it does not occur if the fiber is space-clamped. Excitation of nerve fibers by virtual electrodes using anode-make stimulation has been observed experimentally (for a review see Ranck JB Jr, 1975).

1.2.2 Field stimulations of single cells

One of the simplest examples of electrical stimulation is an isolated, passive, spherical cell placed in a uniform, steady electric field. The transmembrane potential is

$$1.5E a \cos \theta \quad (1)$$

where a is the cell radius,
 E is the strength of the electric field, and
 θ is the angle from the direction of the electric field.
(Assuming that the cell is small enough, and the membrane resistive enough, to make the intracellular potential uniform.)

The factor $\cos \theta$ implies that one side of the cell is depolarized and the other side is hyperpolarized. Qualitative behavior of other cell shapes is similar to that of the spherical example, including individual cardiac cells, which are more accurately modeled as short cylinders, rather than as spheres.

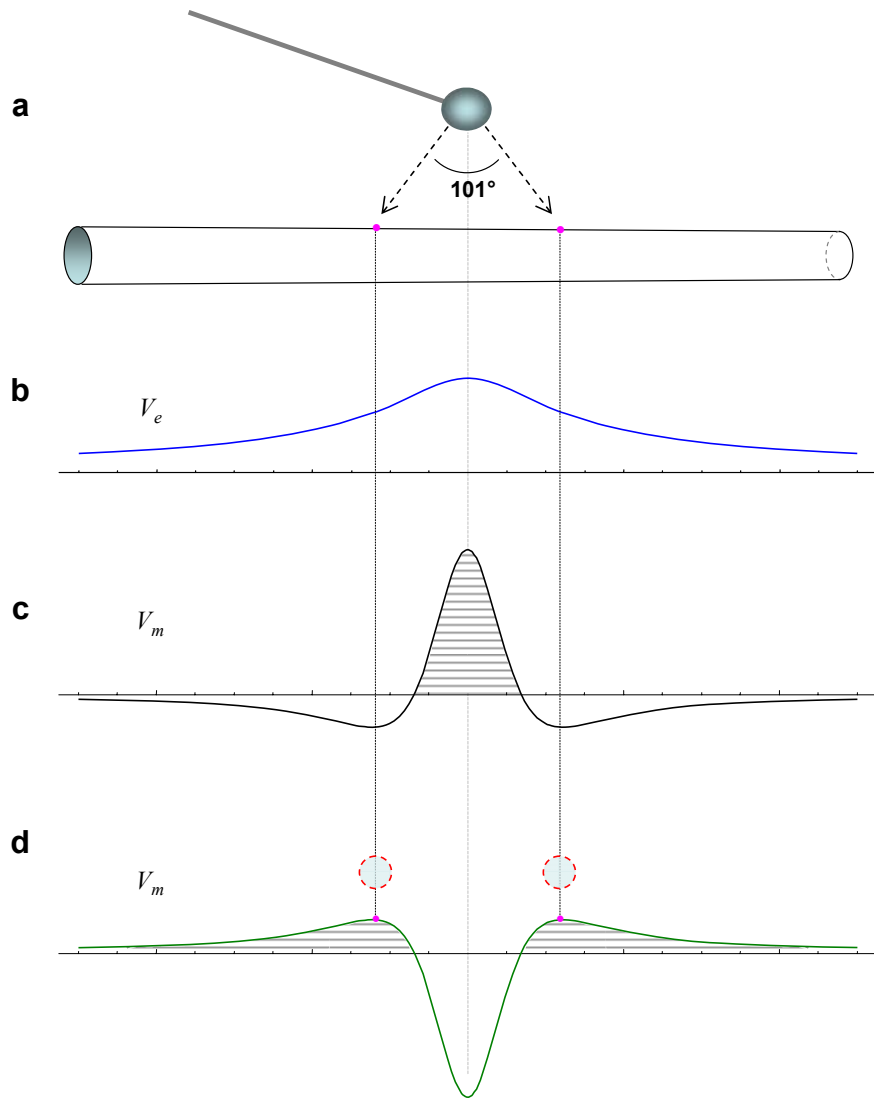


Figure 1. *The virtual electrode concept.*

(a) Stimulation of a uniform fiber by a steady-state current applied through a unipolar, extracellular electrode. (b) The calculated extracellular potential along a fiber produced by a spherical electrode, and the transmembrane potential for (c) cathodal and (d) anodal stimulation. The shaded areas indicate areas in which depolarization is expected.

Note: The two depolarized regions in (d) are termed *virtual cathodes*. The term originates from the fact that these depolarizations may appear to result from two imaginary extracellular cathodes (red dashed circles) positioned at 101° with respect to the actual anode.

1.2.3 The *Bi-domain* model of the myocardium & multicellular VE

Cardiac muscle is fundamentally different from nervous tissue because the heart is a *syncytium*. The intracellular space of each cardiac cell is coupled to its neighbors through intercellular channels (gap junctions; Figure 2): Current can flow from the interior of one cell to the interior of another without crossing a cell membrane. To a first approximation, therefore, the intracellular space of all cardiac cells acts like a “three-dimensional cable network”.

This “three-dimensional cable” is also anisotropic: Both the cylindrical geometry of the individual cardiac myocytes and the distribution of intercellular channels make the electrical conductivity of the tissue different when it is measured parallel to the myocyte orientation than when it is measured perpendicular to the myocyte orientation.

The interstitial medium that surrounds the different cardiac myocytes forms the interstitial space. The interstitial space is anisotropic, but not to the same extent as the intracellular space. (The ratio of conductivity parallel to- and perpendicular to the fibers in the intracellular space is about 10:1, whereas this ratio in the extracellular space is about 2:1. See Roth BJ, 1994, for review)

Cardiac tissue may be electrically approximated by two domains (representing intracellular and interstitial spaces) with unequal anisotropy ratios (Sepulveda NG, 1989; Wikswo JP Jr., 1995). Such an anisotropic, three-dimensional cable can be represented by use of the bidomain model (Sepulveda NG *et al*, 1989; Roth BJ, 1994; Wikswo JP Jr, 1995). This bidomain model predicts that a unipolar, point-source stimulus would result in adjacent areas of virtual anodes during cathodal stimulation or virtual cathodes during anodal stimulation, which is analogous to the creation of virtual electrodes along a one-dimensional fiber. The virtual electrodes predicted by the bidomain model of the myocardium have been verified experimentally (Knisley SB *et al*, 1994; Sambelashvili AT *et al*, 2003).

In the myocardium, the virtual electrodes are three-dimensional with diameter of 2-3 mm (Sambelashvili AT *et al*, 2003). Since the intracellular domain is a multicellular structure, composed of electrically coupled myocytes, the virtual electrodes extend over hundreds of cardiac myocytes. At the region of a virtual cathode the current flows from the intracellular space to the interstitial space. In other words, the cardiac myocytes in the virtual cathode will experience intracellular current injection which resembles intracellular current injected by patch clamp. The significance of that is the fact that the membrane depolarization of an individual cell would be homogenous under current injected by a patch clamp technique, whereas under external field stimulation one side of the cell would depolarized and the other side would be hyperpolarized (described in Figure 1). This effect is utilized in **paper I** to modulate intracellular calcium dynamics and modify muscle contractions.

1.3 MYOCARDIAL, INTRACELLULAR CALCIUM MODULATION BY SUBTHRESHOLD ELECTRICAL SIGNALING

1.3.1 Intracellular Calcium dynamics during contraction of a single cardiomyocyte

Figure 2 provides a schematic description of the key mechanisms which couples AP with muscle contraction in a cardiac myocyte (‘Excitation-Contraction coupling’). At the cellular level, the

excitation-contraction coupling commences with an increase in transmembrane potential of the sarcolemma and ends with the rapid rise in cytosolic calcium and the shortening of the contractile elements in the sarcomere. This process is comprised of a chain of cellular elements which can (separately or together) affect the shape and degree of contraction.

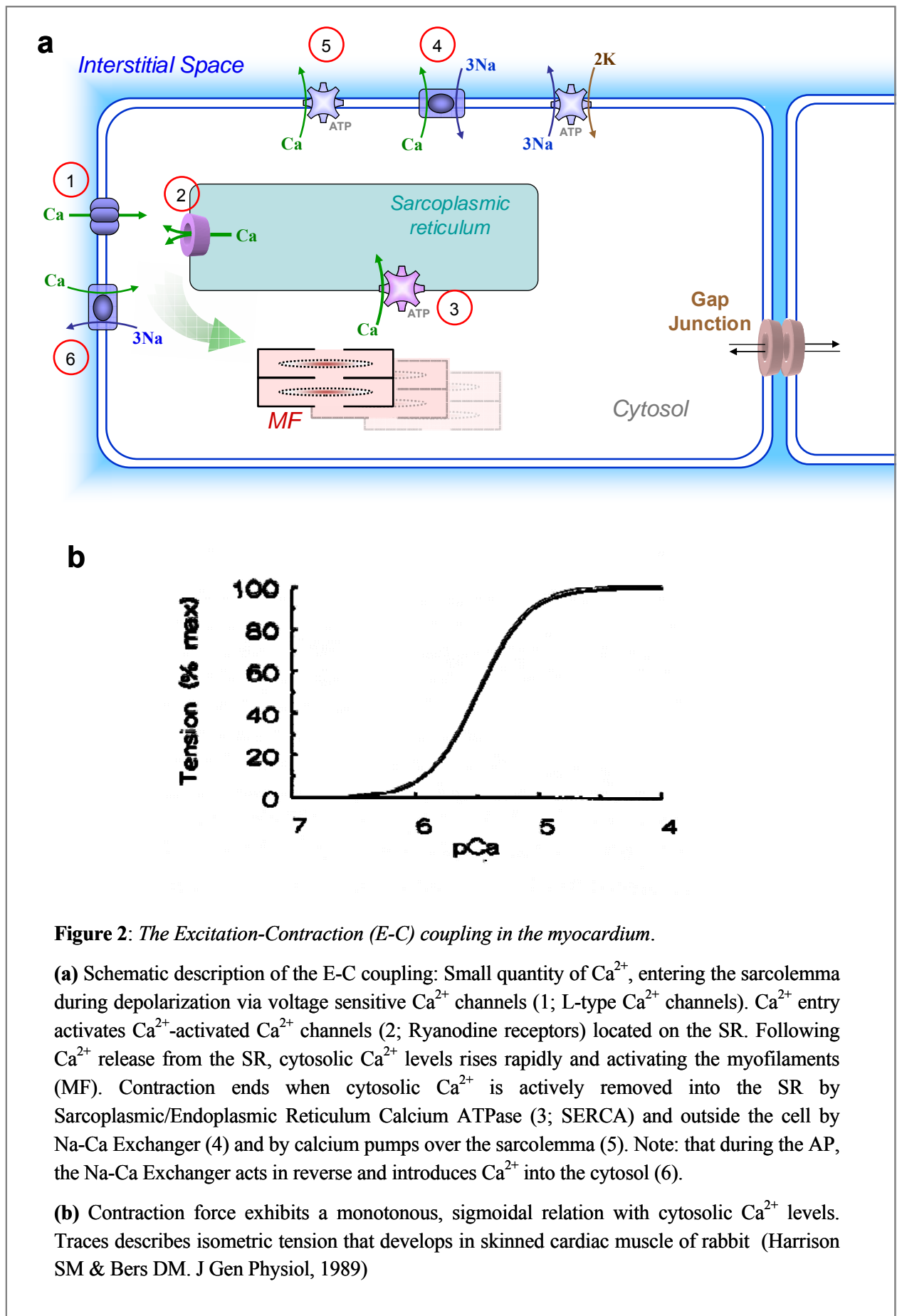
The sarcoplasmic reticulum (SR) is an intracellular system of tubes and sacs which function as the main intracellular calcium store. During depolarization of the sarcolemma, a relatively small amount of free calcium enters the cytosol (mainly through L-type calcium channels) and triggers calcium-induced calcium release from the SR via ryanodine channels. The amount of calcium released from the SR is proportional to the amount of free calcium that is stored in the SR (Adler D *et al*, 1985; Yue DT *et al*, 1985; Bers D, 1993). Accordingly, an increase in the amounts of calcium stored in the SR will result in an increase in calcium released from the SR to the cytosol. The concentration of free Calcium in the cytosol controls the strength of the contraction in a monotonous manner. (Figure 2b)

Thus, following depolarization of the sarcolemma the cytosolic levels of free calcium increases rapidly to levels which trigger contraction of the myofilaments (Bers D, 1993). Myocardial contraction is followed by muscle relaxation, which is mediated by the rapid uptake of Calcium into the SR by Calcium pumps (Sarcoplasmic/Endoplasmic Reticulum Calcium ATPase; SERCA), and clearance of calcium outside the myocytes through the Sodium Calcium Exchangers (SCX).

Calcium exchange between those three compartments (SR, cytosol, interstitium) maintains steady state that is dependent on the exchange rate between the compartments. Theoretical description of the dynamics of free calcium between these three compartment, have shown high correlation with experimental observation.

The AP in the myocard is significantly longer than AP in nerve fibers (around 270 ms in humans). The sodium calcium exchanger (SCX) uses the electro-chemical potential of sodium to pump out calcium in exchange of sodium influx. However, the electro-chemical potential that drives calcium out through the SCX during resting membrane potential is reversed during the AP and allows calcium influx (Bers D, 1993). Dependent on this mechanism, a prolongation or shortening of the AP would respectively increase or decrease the calcium influx through the SCX. Since cytosolic calcium levels are correlated with the strength of contraction, it would be expected that manipulation of AP duration would be followed by a modulation of the strength of contraction.

Indeed, artificial prolongation or shortening of AP duration, by patch clamp technique, have been shown to enhance or weaken the strength of muscle contraction, respectively.



1.3.2 CCM in the intact myocard: the problem, the hypothesized solution and the experimental support (paper I)

As elaborated above, prior studies demonstrated that current injections into cardiac cells using voltage clamp techniques can modulate the contractility by modulating AP duration (Cardiac contractility modulation; CCM). However, since voltage clamp techniques are not applicable for obvious reasons to the intact heart, and since external field stimulation would hyperpolarize and depolarize the single cell simultaneously at opposite sides (see above '1.2.2 Field stimulations of single cells'), this approach has not been pursued in a relevant manner. We have had hypothesized that virtual electrodes will be able to reproduce the patch clamp conditions and enable modulation of AP duration and CCM in the intact myocardium.

We tested this hypothesis in vitro on an intact myocardium (rabbit papillary muscle). We showed that (1) contractility modulation can be produced over an intact muscle, (2) the effect on contractility reverses with reversing the potential (3) enhancement and weakening of contraction was correlated with change in AP duration.

In summary, the concept of applying extracellular electrical impulses during the refractory period to modulate contractility in intact myocardium has been validated in this study of *ex-vivo* rabbit and the human myocardium. The mechanism appears to involve the direct modulation of the action potential duration with associated changes in trans-sarcolemmal calcium fluxes followed by modulation of the SR calcium content. These data indicate that further study of the possible therapeutic utility of CCM signals in the setting of heart failure is warranted.

1.4 THE CABLE-IN-CABLE (CIC) THEORY: BIDOMAIN MODEL & VIRTUAL ELECTRODES APPLIED TO NEURONS (PAPER II & III)

The cardiac bidomain model describes a three-dimensional network of passive cables (see above, 1.1.3) and its predictions have been verified experimentally. Inspired by the cardiac bidomain model, we aimed to describe the intracellular compartment in neurons (namely the endoplasmic reticulum) as a three-dimensional network of passive cables, and study the potential role of this inner compartment in processing of synaptic signals by the single neuron (**paper II** and **paper III**)

At the end of the 90's it was acknowledged that the endoplasmic reticulum (ER) forms a continuous network of tubes and sacs that extends from the nuclear envelope out to the cell periphery (Petersen OH *et al*, 2001; Spacek J *et al*, 1997; Subramanian K *et al*, 1997; Terasaki M *et al*, 1994). This was shown through EM reconstructions (Spacek J *et al*, 1997) and diffusion of dye along internal membranes (Terasaki M *et al*, 1994) to show the ER continuity in neurons, across the axon, soma, dendrites and the spine apparatus at the dendritic spines. Accordingly, the ER has been suggested to act as a 'neuron-within-neuron', as originally suggested by Berridge (1998). However, until now signal propagation and integration along the ER have been considered only in relation to regenerative Ca^{2+} waves.

Our hypothesis proposes that postsynaptic potentials incorporate a passive electrotonic potential along the ER lumen and across the ER membrane. (Figure 3a) This hypothesis is supported by reconstruction studies of spiny dendrites describing the ER as a continuous network of anastomosing tubes running parallel to the longitudinal axis of the dendrite (Martone ME *et al*, 1993) and extends, virtually, into all mature dendritic spines (Spacek J *et al*, 1997; Terasaki M *et al*, 1994). This hypothesis is further supported by direct recordings from ER in skinned

myocytes having an input resistance of $\sim 850 \text{ M}\Omega$ and a resting membrane potential around 0 mV between the ER lumen and the cytosol (Tang JM *et al*, 1989) (values of $\sim 7.5 \text{ k}\Omega/\text{cm}^2$ and 15- 20 mV were estimated earlier for ER membrane specific resistance (Vergara J *et al*, 1978) and membrane potential (Meissner G, 1983), respectively). Those studies provide the experimental grounds for suggesting that ER membrane can separate charges and that it exhibits a specific resistance that is similar in magnitude to the plasma membrane (e.g. a typical input resistance for L2/3 pyramidal cells is around $100 \text{ M}\Omega$ (**Paper II**) with $20 \text{ k}\Omega/\text{cm}^2$ specific resistance for plasma membrane (Rapp M *et al*, 1996).

In order to test the suggested hypothesis against realistic parameters, provide realistic predictions and enable analytic study of the theory, we developed a mathematical model of a cable-in-cable, thereby generalizing the classical cable theory developed by Rall (Rall W, 1989).

1.4.1 The CIC Model predicts synaptically-induced, intracellular VE where dendritic spines may play a novel role

The CIC model shows that current flow along a system of a cable within a cable would, essentially, follow the predictions of the classical cable theory along the *external* cable (i.e. the plasma membrane), but at the same time, would exhibit VE over the *internal* cable, which cannot be predicted by the classical cable theory.

We further show that the distance between the origin of the depolarization (synapse) and the position where VE appears, can be modified at the single spine head, by passive properties (such as the surface area of the spine apparatus) and by active properties (such as the Ca^{2+} -dependent current influx through SERCA pumps or CICR through the ryanodine and IP3 receptors).

Excitatory synaptic activation on a dendritic spine initiates Ca^{2+} influx into the spine mainly through glutamatergic receptors (i.e. N-methyl D-aspartate receptors; NMDAR) but also voltage dependent Ca channels. Approximately 30 % of the Ca^{2+} entering the spine is carried into the ER lumen by the electrogenic (Zimniak P *et al*, 1978) Ca^{2+} pump, SERCA (Sarcoplasmic Endoplasmic Reticulum Calcium-ATPase)(Sabatini BL *et al*, 2002). Additionally, Ca^{2+} influx into the spine has been suggested to induce Ca^{2+} -induced Ca^{2+} release (CICR) from the ER within the spine (Rose CR *et al*, 2001; Emptage N *et al*, 1999). Thus, excitatory synaptic activity onto the spine is coupled with positive and/or negative Ca^{2+} -mediated currents flowing into the ER lumen at the spine head. In the context of the CIC system, these two processes actively govern the position of the peak of the VE. Our study suggest that compartmentalization of free calcium by the dendritic spines is essential for maintaining synapse-specific tuning of signaling via VE along the internal membrane. This assumption is further supported by experimental evidence indicating that each dendritic spine head usually accommodates a single glutametric synapse (Elston GN *et al*, 2002; Hering H *et al*, 2001).

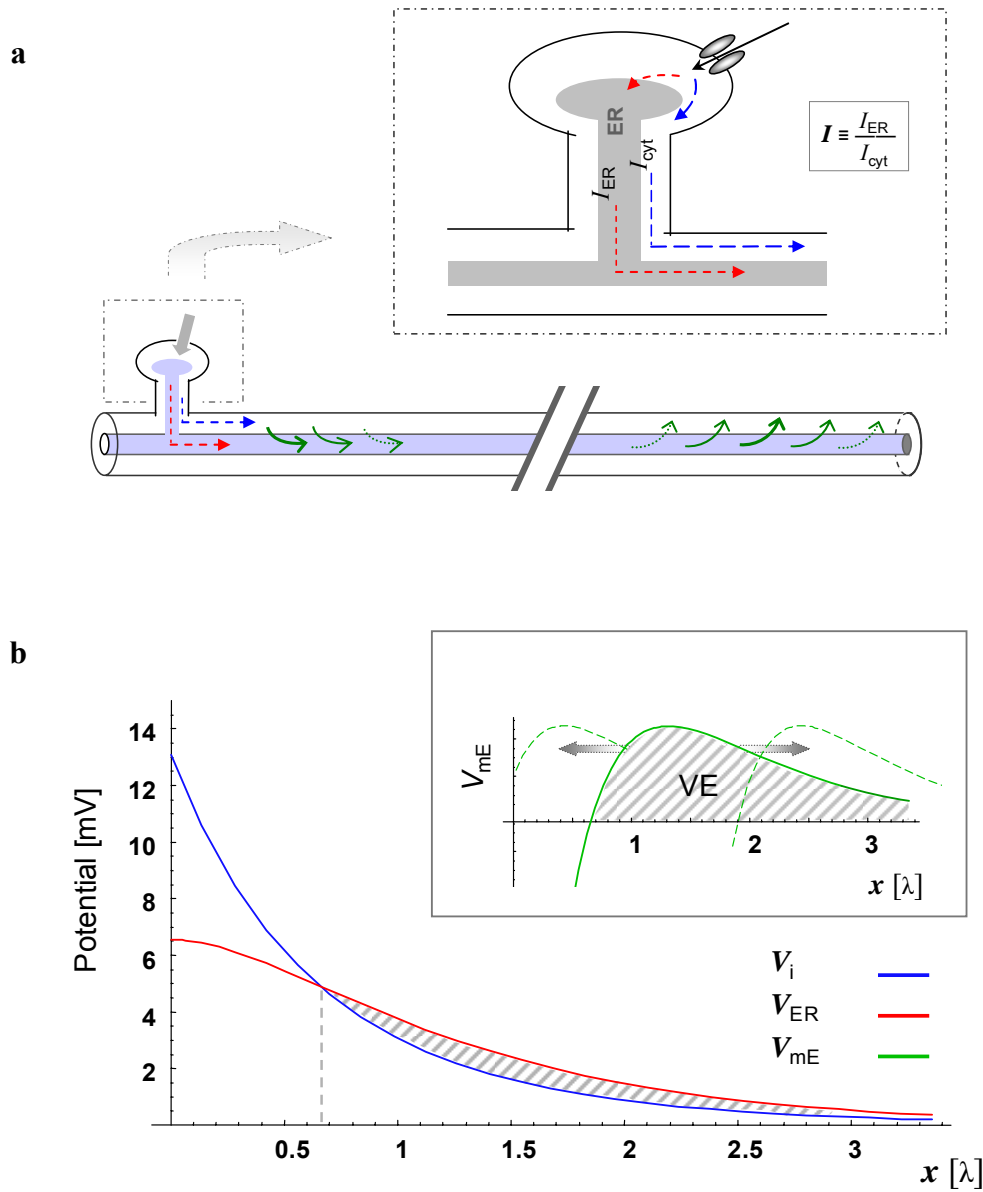


Figure 3: *The CIC hypothesis.*

(a) The principal hypothesis: synaptic activation onto a dendritic spine generates two simultaneous currents, along the cytoplasmic compartment and along the ER lumen. These currents generate two passive electrotonic signals, across the plasma membrane (outer cable) and across the ER membrane (the inner cable). Proximal to the synapse, the inner cable passively absorbs current from the outer cable (inward green arrows). At some distance from the synapse ($\sim 0.5 \lambda$), the inner cable gradually becomes a passive current source for the external cable (outward green arrows). (b) The potential along the cytosol (blue; V_i) and the potential along the ER lumen (red; V_{ER}) decay at different rates along distance ($x [\lambda]$) given in electrotonic units. The V_i attenuates faster than the V_{ER} and they cross each other. As a result, the ER transmembrane potential, V_{mE} , (given by the difference between V_{ER} and V_i ; plotted in B₁ by a green trace) is negative along ~ 0.65 space-constants from the synapse, where it crosses the zero line, reverses to reach a positive peak and then decay to zero. The reversal of the potential across the inner membrane is referred to as ‘Virtual-Electrode’ (VE; Dashed area). The VE position can be modulated (horizontal arrows) by a synapse-specific property, namely the ratio between the current entering the inner cable and the current entering the outer cable, at the synapse.

1.4.2 VE as a potential synapse-to-nucleus signal (Paper II)

All communication between the cell and its nucleus travels through the nuclear envelope. The nuclear envelope is a double membrane composed of two lipid bilayers separated by a gap of 20 to 40 nm (the perinuclear space). The outer nuclear membrane is continuous with the ER membrane and the lumen of the ER is continuous with the lumen of the perinuclear space (illustrated in Figure 4). The nuclear envelope allows continuity between the nucleoplasm and the cytosol through nuclear pores (P; about 9 nm in diameter, Figure 4). The nuclear pores allow non-selective flux of ions and therefore enable electrical continuity between cytoplasm and nucleoplasm. (For review see Prunuske AJ *et al*, 2006)

Using the CIC model we show that under realistic parameters the excitatory synaptic activity at the spine level can give rise to an EPSP-like depolarization across the nuclear envelope, whereas a depolarizing signal initiated at the soma (e.g. action potential) would result in hyperpolarization of the nuclear envelope.

This biophysical study provides a new explanation for the remarkable ability of the pyramidal cell nucleus to differentiate between orthodromic depolarizing signals and antidromic depolarizing signals (Deisseroth K *et al*, 1996; Mermelstein PG *et al*, 2000). It predicts that a depolarization at the dendrites level is capable of depolarizing the nuclear envelope (via a virtual cathode), whereas a depolarization of the soma would cause an opposite effect (hyperpolarization) of the nuclear envelope. Additionally, the hypothesized pathway of electrotonic signaling from synapse to nucleus, provides an explanation for the remarkable speed with which signals can be transmitted from the spine to the nucleus. The nuclear response (CREB phosphorylation) can be shown 15 sec after the beginning of the synaptic signal (Mermelstein *et al*, 2001), whereas a diffusion of a second messenger along a similar distance would take 10 times longer (see Table 1 in **paper II**).

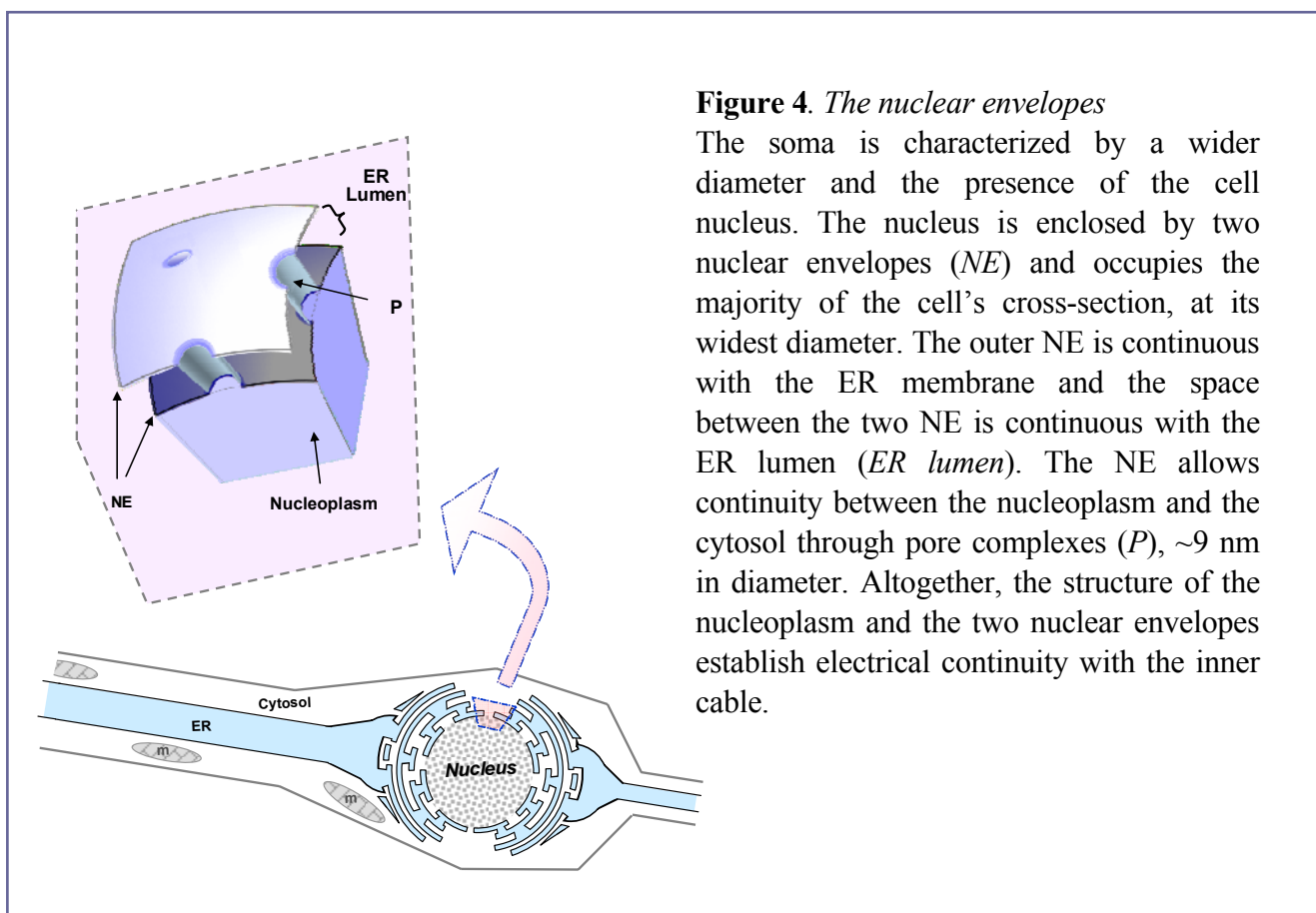


Figure 4. *The nuclear envelopes*

The soma is characterized by a wider diameter and the presence of the cell nucleus. The nucleus is enclosed by two nuclear envelopes (NE) and occupies the majority of the cell's cross-section, at its widest diameter. The outer NE is continuous with the ER membrane and the space between the two NE is continuous with the ER lumen (ER lumen). The NE allows continuity between the nucleoplasm and the cytosol through pore complexes (P), ~9 nm in diameter. Altogether, the structure of the nucleoplasm and the two nuclear envelopes establish electrical continuity with the inner cable.

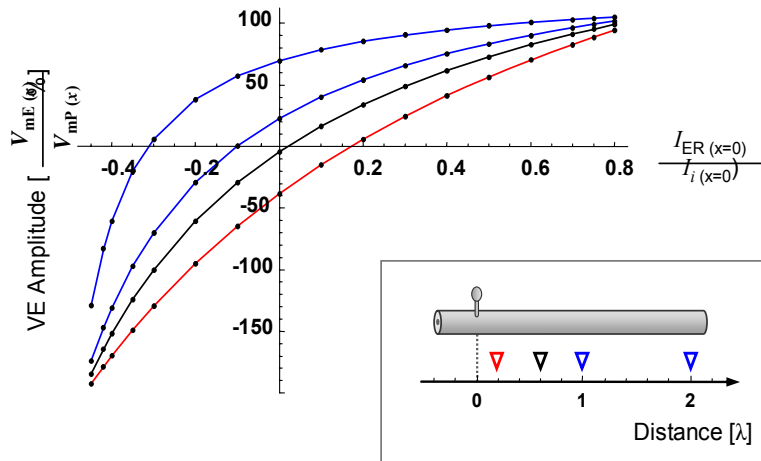


Figure 5. *Synaptic effect on the internal cable (e.g. the nuclear envelope).*

Synaptic input simultaneously initiates axial currents along the cytosol and along the ER lumen. The ratio between these currents is I . The graphs describe the effect of I on V_{mE} at several fixed distances from the synapse (0.2, 0.6, 1, 2 space constants from the synapse; red, black blue and blue, respectively). V_{mE} amplitude at each position is described as percent of V_{mP} amplitude (EPSP) at that specific distance from the synapse. **Inset:** Triangles depict the sampling position of traces with corresponding color. Note that at each target, VE amplitude can reach 100 % of EPSP level or drop below zero.

1.4.3 The CIC theory may introduce a second dimension of synaptic plasticity (Paper III)

1.4.3.1 Introduction & principal hypothesis

One intriguing prediction of the CIC model is that the VE along the internal cable is a *sink* for the current which entered the *internal* cable at the synapse (Figure 3a). At the same time, at the VE, the current which leaves the internal cable portrays a locally-distinct, internal *source* of current to the *external* cable. (illustrated in Figure 3a). In **paper II**, we showed that the position of the VE can be determined by a synapse-specific parameter (i.e. the **I parameter**). Taken together, the virtual cathode induced along the internal cable provides an internal current source, the position of which is determined by the synapse. In **paper III** we studied the effects that this may have on the integration of post-synaptic potentials.

Post-synaptic integration of transmembrane potentials is a fundamental step in neuronal processing, whereas the modulation of post-synaptic potentials is commonly conceived as a pivotal process mediating learning and memory in neuronal and neuronal-like systems (see example in **paper IV**). The main role of modulating post-synaptic potentials is conventionally

assumed to be modifying the effect an individual synaptic signal has on the decision to initiate AP. The distal part of the proximal segment of the axon is considered as the principle integration zone of the neuron, since the AP is initiated there (Palmer LM *et al*, 2006; Shu Y *et al*, 2007).

Taken together, we hypothesized that positioning a VE at the integration zone can affect the integration of post-synaptic potentials and hence, the generation of AP. Since the position of the VE is determined by the individual synapse (by the I parameter), the effect a VE may have on the integration processes would be, to some degree, synapse-specific.

In other words, the barrage of individual post-synaptic potentials is conventionally assumed to be summated momentarily at the proximal segment of the axon to initiate an AP. According to the CIC theory, each post-synaptic potential is accompanied by an internal, locally-distinct current source: a virtual cathode along the internal cable, the position of which is determined by the individual synapse. We hypothesize that if the VE can modify significantly the effect each individual post-synaptic potential will have on the integration process, then the VE could play a meaningful role in processing of synaptic inputs.

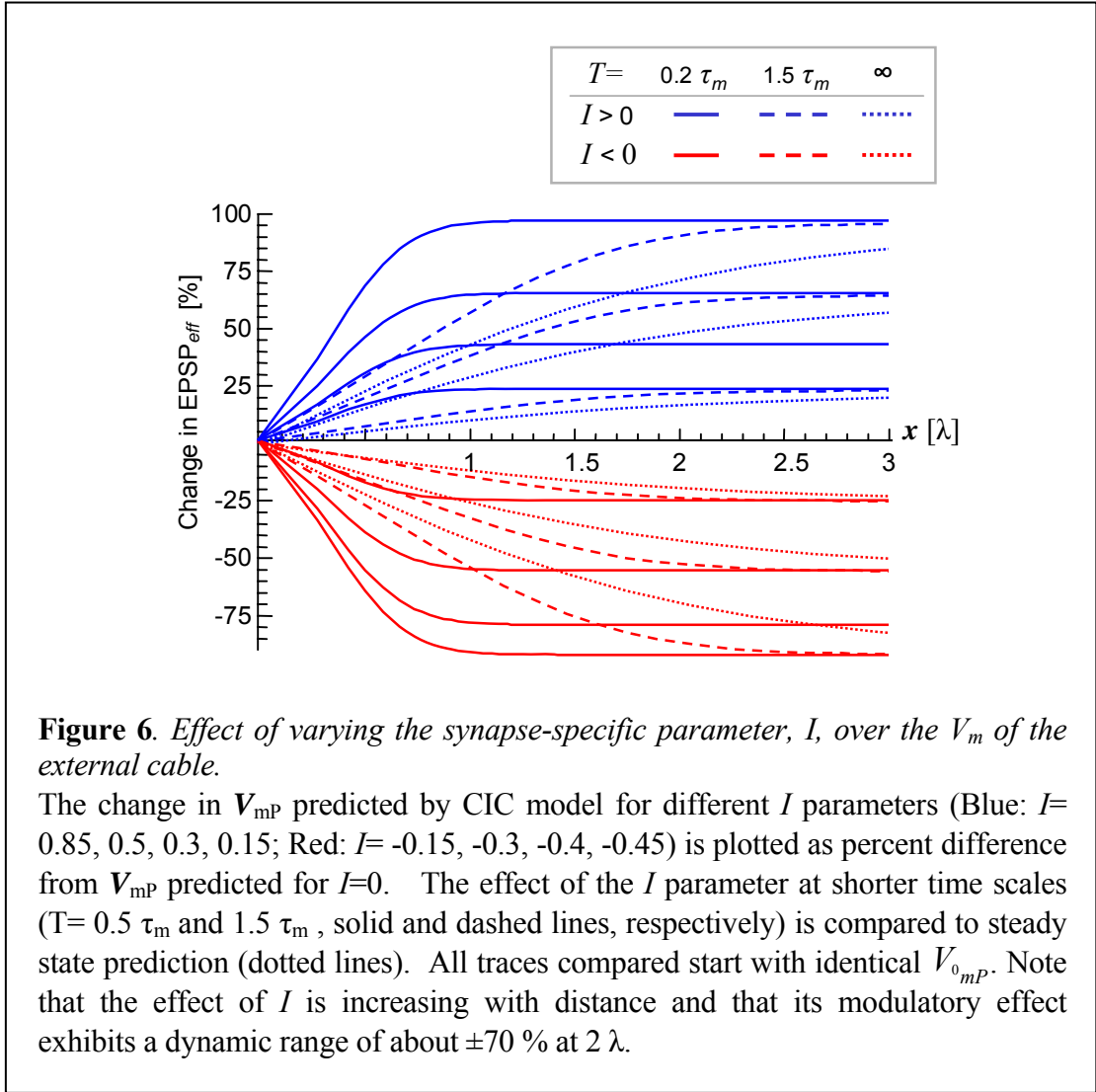
1.4.3.2 Main findings

We found it useful to define the term “*effective-EPSP*” (EPSP_{eff}), as the transmembrane potential, V_{mp} , induced at an arbitrary position (e.g. position of a putative voltage sensor at the integration zone), by a specific excitatory synaptic activity.

Modifying the synapse-specific parameter, I , results in a passive shift of the position of the VE (**paper II**), as illustrated in Figure 3b. Figure 5 shows that different I parameters can significantly affect the EPSP_{eff} , with a substantial dynamic range ($\pm 70\%$ change in EPSP_{eff}). It also shows that this ability of the synapses to modify the EPSP_{eff} without changing the EPSP at the synapse, can be produced at different magnitudes at any positions along the CIC system.

In **paper III**, we demonstrate that summation of two synchronous synaptic inputs entering at an identical position, will do not add up linearly. Their summation rule may be supra-linear, linear or sub-linear, depending on the individual I parameter of each synapse, as well as the specific position at which the effect is integrated. Next, we calculated the temporal dynamics of the ability of the I parameter to modulate EPSP_{eff} . Figure 6 shows that at a shorter time scale ($T = 0.2 \tau_m$ and $1.5 \tau_m$, equivalent to 10 and 72 ms, respectively; Table 1), the dynamic range of modulatory effect over EPSP_{eff} is amplified at shorter distances, while maintaining the dynamic range at longer distances.

In summary, paper III used realistic parameters to show theoretically that the VE along the ER membrane can affect the integration of synaptic activity at the integration zone, suggesting that VE may play a meaningful role in the processing of the synaptic input.



1.5 FREE CALCIUM KINETICS AND SPIKE-TIMING-DEPENDENT PLASTICITY (STDP) IN DENDRITIC SPINES

1.5.1 STDP and postsynaptic Calcium

The temporal relation between the pre- and post- synaptic activity plays a pivotal role in the induction of the synaptic plasticity in the cortex (Markram *et al.*, 1997a; Bi and Poo, 1998; Feldman, 2000; Froemke and Dan, 2002). When a presynaptic action potential (*i.e.* a post synaptic EPSP) occurs in a window of several tens of milliseconds before a postsynaptic AP, LTP is induced. In contrast, when a presynaptic action potential occurs after the postsynaptic EPSP, LTD is induced (Spike-timing-dependent plasticity; STDP). Both LTP and LTD typically, require N-methyl-D-aspartate subtype glutamate receptor (**NMDAR**) activation (Bliss and Collingridge, 1993; Bear and Malenka, 1994; Bi and Poo, 1998; Feldman, 2000; Sjöström *et al.*, 2001). Ca^{2+} influx through the NMDAR substantially increases when synaptic activity is shortly

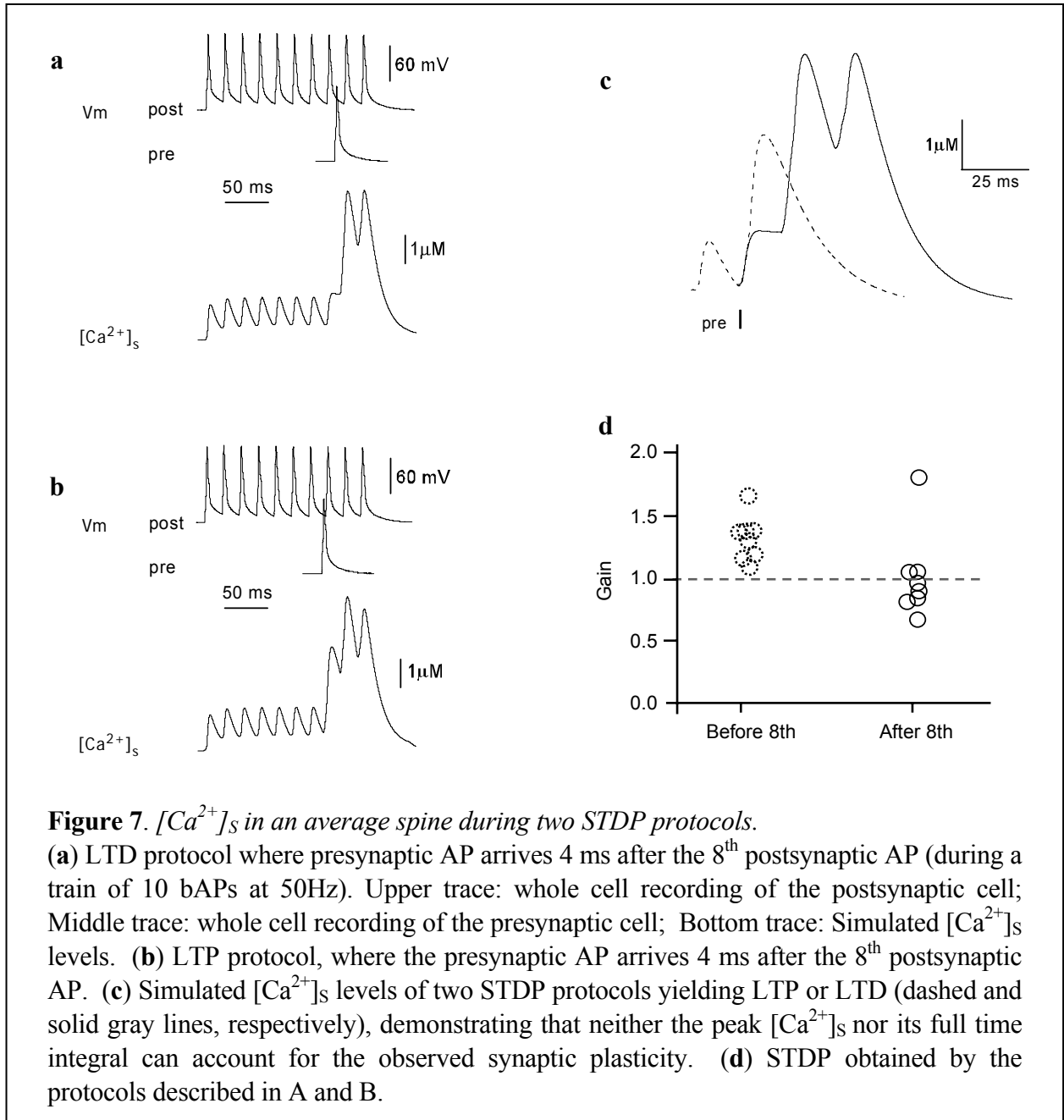
followed by a postsynaptic depolarization, such as a single postsynaptic AP (Köster and Sakmann, 1998; Nevian and Sakmann, 2004). Consequently, the temporal relations between pre- and post- synaptic activity are transformed into levels of postsynaptic Ca^{2+} , where moderate, slow elevation of calcium induces LTD, and larger rapid elevation of postsynaptic Ca^{2+} induces LTP (Yang *et al.*, 1999; Nishiyama *et al.*, 2000; Cormier *et al.*, 2001; Sjöström and Nelson, 2002).

The dendritic spine provides the primary postsynaptic target for the excitatory connections onto pyramidal cells. Its main role is assumed to be compartmentalization of fast postsynaptic Ca^{2+} kinetics from the dendritic shaft (Svoboda *et al.*, 1996; Yuste *et al.*, 2000). Ca^{2+} transients in a single spine subside rapidly, with time constants of ~ 12 ms (Sabatini *et al.*, 2002).

1.5.2 STDP through calcium dynamics in a short temporal window: the problem, the hypothesized mechanism and the experimental support (paper IV)

Previous studies describing the relation between postsynaptic Ca^{2+} -kinetics and plasticity in the cortex have focused on slower (seconds) Ca^{2+} -kinetics in the dendritic shaft induced by tetanic stimulation (Malenka *et al.*, 1988; Kimura *et al.*, 1990; Hansel *et al.*, 1996, 1997; Cormier *et al.*, 2001). The relationship between rapid Ca^{2+} dynamics (<1 sec) in the dendritic spine and synaptic plasticity, in the same neurons, has not been described. In this study, we focus on the relationship between the pattern of Ca^{2+} influx into a dendritic spine and the plasticity in unitary connections between L2/3 pyramidal cell pairs. We measured synaptic plasticity under different protocols and pharmacological manipulations aimed to generate different spine Ca^{2+} kinetics. The corresponding spine Ca^{2+} kinetics were simulated and were correlated with the experimental results in order to identify the relation between spine Ca^{2+} kinetics and synaptic plasticity. For the simulation, we followed the model described by Markram *et al.* (1998) (see methods) and used parameters from studies in which Ca^{2+} kinetics had been measured directly in rat cortex (Sabatini *et al.*, 2002) (see table 1 in **paper IV** for details).

We found that neither the peak $[\text{Ca}^{2+}]_s$ nor its full time integral can account for the plasticity observed with the various experimental protocols (Figure 7). We therefore hypothesized that the changes in mean EPSP amplitude following the various STDP induction protocol is determined by spine Ca^{2+} kinetics within a short period following presynaptic activation (Window of Plasticity Induction; WPI). Biochemically, the WPI may represent a short-lived calcium-dependent process that is initiated by synaptic activation, and subsequently generates a long-lasting product that linearly correlates with plasticity.



To test the WPI hypothesis directly against experimental perturbations of spine Ca^{2+} dynamics, we employed different levels of Ca^{2+} chelators, EGTA or BAPTA. Both EGTA and BAPTA have similar affinities for Ca^{2+} but differ in their kinetics, with BAPTA exhibiting a ~ 40 times quicker on-rate (Neher and Augustine, 1992; Nagerl *et al.*, 2000). Consequently, BAPTA and EGTA at similar doses have similar steady-state effects on Ca^{2+} but have different effects on the spatial distribution (spatial-pattern) and temporal dynamics of Ca^{2+} (temporal-pattern) (Markram *et al.*, 1998; Nagerl *et al.*, 2000). The dose-response relation between BAPTA or EGTA and synaptic plasticity in L2/3 pyramidal-pyramidal unitary connections was measured experimentally.

We found that the changes in mean EPSP amplitude following an STDP induction protocol are well correlated with the time integral of $[Ca^{2+}]_s$ over a period of 15-20 ms following presynaptic activation (WPI=15-20 ms).

An advantage of the suggested WPI is its simplicity, compared to previous models describing the relationship between plasticity and postsynaptic Ca^{2+} . In the present study we validated the WPI over a range of pharmacological manipulations of postsynaptic calcium, and with a variety of stimulation protocols.

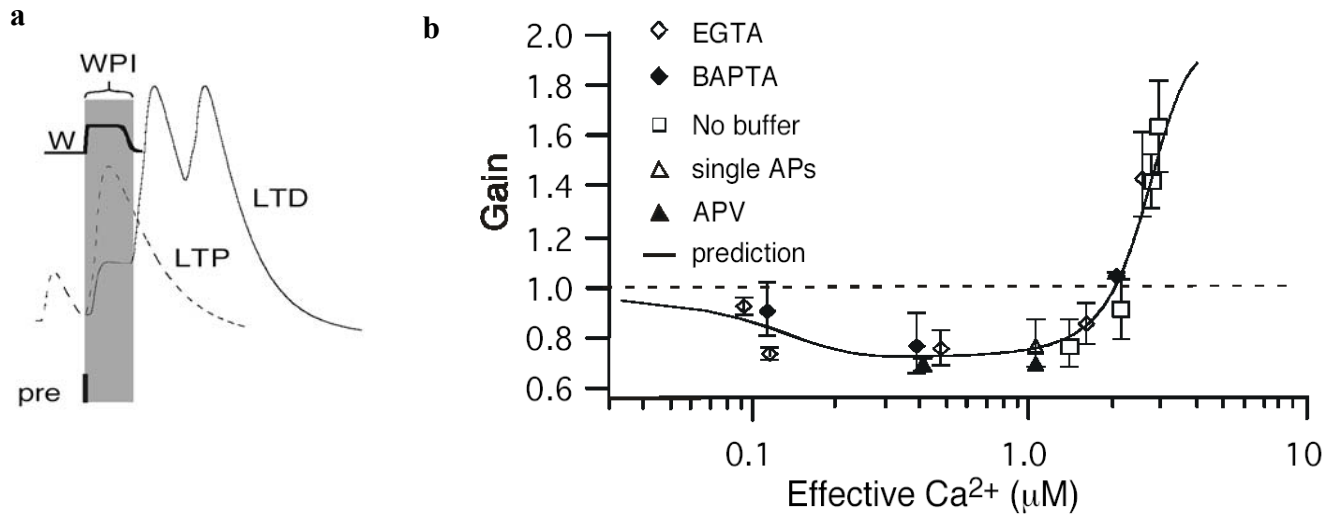


Figure 8: *The window of plasticity induction (WPI)*

(a) WPI (shaded zone) is a period after presynaptic activity, where sensitivity of plasticity to Ca^{+2} (sensitivity-index, W; solid line) is assumed to rise transiently. WPI is superimposed over two traces of $[Ca^{+2}]_s$ simulated for two STDP protocols yielding LTP or LTD (dashed and solid gray lines, respectively).

(b) Synaptic plasticity obtained with different experimental paradigms is plotted against the amount of calcium sensed during the WPI (effective- Ca^{2+}). All experimental data are fitted with a sum of two sigmoid functions (solid line; see paper V for details).

1.6 STDP IN SPINY NEURONS DURING THE EARLY STAGE OF ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is a progressive neurodegenerative disorder in which the vanishing ability to form new memories occurs before the manifestation of cytopathological indices, including amyloid β protein ($A\beta$)-containing neuritic plaques, in cortical regions (Small *et al.*, 2001, Selkoe, 2004).

$A\beta$ has been implicated in AD-related cognitive decline with studies demonstrating that $A\beta$ is preferentially recruited to excitatory synapses (Lacor *et al.*, 2004) and impairs high frequency stimulation (HFS)-induced long-term potentiation (LTP) in the hippocampus (Walsh *et al.*, 2002, Rowan *et al.*, 2004, Wang *et al.*, 2004a).

Continuous structural refinement of cortical synapses is reflected in the formation of LTP, a dynamic enhancement of synaptic efficacy that is generally regarded as a cellular correlate for particular forms of learning and memory (Bliss and Collingridge 1993; Zhou and Poo 2004). In AD, synaptic function becomes compromised prior to the physical disintegration of synapses (Yao *et al.*, 2003), and synapse loss is disproportionate to the extent of neuronal demise (DeKosky and Scheff 1990). A clinical correlate of synaptic dysfunction is the breakdown of cognitive functions in AD.

The strength of excitatory synaptic connections in the neocortex is adjusted by the timing of action potentials in pre- and postsynaptic pyramidal cells (Markram *et al.*, 1997b, Feldman, 2000, Sjostrom *et al.*, 2001). This spike-timing-dependent synaptic plasticity (STDP) can result in a rapid switch from LTP to long-term depression within a narrow transition window (Bi and Poo 1998). Spike-timing-dependent LTP at excitatory synapses is induced when presynaptic spikes precede postsynaptic activity; consistent with Hebb's original postulate of learning that stresses the importance of causality as a condition for synaptic strengthening (Sejnowski, 1999). Although the cellular mechanisms underlying STDP are still ambiguous, its initiation is usually dependent on the activation of postsynaptic N-methyl-D-aspartate receptors (NMDARs) and a subsequent increase in intracellular Ca^{2+} concentrations (Markram *et al.*, 1997b, Yang *et al.*, 1999, Feldman, 2000, Sjostrom *et al.*, 2001).

Recently, soluble $A\beta$ oligomers have been identified as a critical trigger for early synaptic disorganization. However, it remains unknown whether a deficit of Hebbian-related synaptic plasticity occurs during the early phase of AD.

1.6.1 Impaired STDP during the early stage of Alzheimer's disease, one possible mechanism and an *in vitro* rescue of impaired STDP (paper V)

Alzheimer's disease is formally diagnosed by the presence of neuritic plaques and neurofibrillary tangles in the brain. The early stage of Alzheimer's disease is referred to as the stage preceding these characteristic cytopathological indices. To determine functional changes in synaptic plasticity at the early stage of Alzheimer's Disease, we examined STDP induction at the onset of AD-like plaque pathology.

For that purpose we studied whether spike-timing-dependent synaptic potentiation at excitatory input synapses of layer 2/3 (L2/3) pyramidal cells is affected in mice heterozygously carrying the

human APP695swe and PS1dE9 mutations (APPswe/PS1dE9 mice; Jankowsky *et al.*, 2004). In this animal model of AD the onset of AD-like plaque pathology occurs around 3.5 months old of age whereas fully developed AD-like pathology is observed at 7 months old. Our data demonstrate that STDP in pyramidal cell networks is significantly affected even at the onset of A β pathology.

STDP in pyramidal cells involve alteration in the activity of AMPAR and NMDAR at the synapse. The effects of non-fibrillar, soluble A β oligomers (A β_{ol}) on AMPAR and NMDAR-mediated current ratios, amplitudes, and kinetics were assessed with quantitative current recordings in acute slice preparations and nucleated patches excised from neocortical pyramidal cells. We found that a decline in synaptic plasticity is triggered by a selective reduction of AMPA receptor-mediated currents, induced by A β_{ol} .

The fact that synaptic dysfunction in AD occurs before the manifestation of A β pathology (for review see Selkoe, 2004, Walsh and Selkoe 2004), together with the present finding that A β (25-35) $_{ol}$ is sufficient to produce STDP impairment, implies that synaptotoxicity is mediated, at least in part, by naturally-secreted, soluble A β_{ols} (Kim *et al.*, 2001, Walsh *et al.*, 2002, Wang *et al.*, 2004a, Klyubin *et al.*, 2005). The coincidence of a robust loss of synaptic plasticity with minimal cortical A β plaque load at 3.5 months of age (Savonenko *et al.*, 2005) lends further support to the hypothesis that A β_{ols} are detrimental for synaptic plasticity.

The selective A β -induced impairment of AMPAR-mediated currents prompted us to assess whether pharmacological potentiation of AMPAR functions could restore synaptic plasticity. Therefore, we applied cyclothiazide, an inhibitor of AMPAR desensitization, at a concentration that enhances the amplitude, and markedly prolongs the decay time of EPSCs (Trussell *et al.*, 1993). Our data show that cyclothiazide-induced enhancement of AMPAR-mediated currents rescues STDP at excitatory synapses on L2/3 pyramidal cells in APPswe/PS1dE9 mice exhibiting significant cortical A β pathology.

The major novel finding of our study is that STDP impairment is mediated by the A β_{ol} -induced occlusion of postsynaptic AMPAR currents. The possibility of a substantial restoration of synaptic plasticity by enhancing the open channel state of AMPARs defines a prime target for pharmacological intervention. Accordingly, the novel class of AMPAR modulators, ampakines, has been shown to effectively augment synaptic plasticity (Staubli *et al.*, 1994), and enhance learning and memory performance in humans (Ingvar *et al.*, 1997).

1.7 CONCLUDING REMARKS

The work summarized in this thesis is a result of an effort to apply theoretical insights from one organ system (the ventricular myocard of the heart) into another system (the brain cortex). The thesis based on five papers that combine theoretical and experimental studies with implied clinical relevance.

Thus, the scientific contribution of this thesis is a result of a multidisciplinary effort, which represents, in my view, an essential part of scientific work.

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