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UNIVERSITY OF CALIFORNIA, SAN DIEGO

Nonlinear Dendritic Dynamics and their Effect on the Information Processing Capabilities of Neurons

A Dissertation submitted in partial satisfaction of the requirements
for the degree of Doctor of Philosophy

in

Bioengineering

by

Helen G. Saad Khoury

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2013

The Dissertation of Helen G. Saad Khoury is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

Chair

UNIVERSITY OF CALIFORNIA, SAN DIEGO

2013

DEDICATION

*To my love,
I'm so blessed to have you.*

*To my baby,
I can't wait to meet you.*

EPIGRAPH

God is in the details.

Mies van der Rohe

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LIST OF ABBREVIATIONS

SAAD	S patially A ggregated A ctive D endrite (or Author's last name)
CNS	C entral N ervous S ystem
LTP	L ong- T erm P otentiation
LTD	L ong- T erm D epression
STDP	S pike T iming D ependent P lasticity
BTDP	B urst T iming D ependent P lasticity
MI	M utual I nformation
AP	A ction P otential
BAC	B ackpropagation A ctivated C alcium spike
bAP	backpropagating A ction P otential
PSP	P ostsynaptic P otential
EPSP	E xcitatory P ostsynaptic P otential
IPSP	I nhibitory P ostsynaptic P otential
EPSC	E xcitatory P ostsynaptic C urrent
VGC	V oltage G ated C hannels
VDNC	V oltage- D ependent Na ⁺ C hannels
VDCC	V oltage- D ependent Ca ²⁺ C hannels
NMDA	N -methyl- D -aspartate
AMPA	α - A mino-3-hydroxy-5- M ethyl-4-isoxazole P ropionic A cid
GABA	γ - A minobutyric A cid
XOR	E xclusive O R (bit operation)

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Appendix C, in full, is a reprint of the material as it appears in Kim, Soon-Jong; Blumling, James P.; Davidson, Marie C.; Saad, Helen; Eun, Su-Yong; Silva, Gabriel A. Calcium and EDTA induced folding and unfolding of calmodulin on functionalized quantum dot surfaces, *Journal of Nanoneuroscience*, in press. The dissertation author was a co-author of this paper.

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ABSTRACT OF THE DISSERTATION

Nonlinear Dendritic Dynamics and their Effect on the Information Processing Capabilities of Neurons

by

Helen G. Saad Khoury

Doctor of Philosophy in Bioengineering

University of California, San Diego, 2013

Professor Gabriel A. Silva, Chair

A major challenge in neuroscience is to reverse engineer the brain and understand its information processing and learning capabilities. While the pace of discovery and untangling of the brain's staggering dynamics is advancing at unprecedented speed especially with the recently developed tools and imaging techniques, this advancement is not devoid of risk: the arsenal of novel techniques carries a huge mass of data that may complicate further the unraveling of brain function. Is every ion channel, every spine, every dendrite, every neuron and every synaptic connection necessary to achieve the computational capabilities of the central nervous system? Answering this question rises the need for a two-way communication between experiments and mathematical theoretical work. Neural networks composed of point neurons and endowed with biologically inspired synaptic learning rules have been successfully applied to a variety of challenging learning-related tasks, namely in problems of pattern recognition, associative memory, map formation, among others. While these networks are good at tasks they are built for, there still exists a gap separating us from

fully understanding how the brain is good at the large multitude of tasks it can perform. When we reflect upon most efforts in building and simulating neural networks, we ask ourselves about the appropriate scale for modeling: Given the complexity of the nervous system, is it enough to model the neurons as point-like units in which a weighted sum of synaptic inputs is passed through a single spike-generating mechanism? From a modern perspective, the point neuron seems likely to be a poor representation of synaptic integration in neurons with large, profusely branched, active dendrites that populate brain structures associated with advanced cognitive functions and learning. These dendrites are endowed with nonlinear active conductances that modulate synaptic integration and somatic activity. Does the increased nonlinearity at the level of the neuron enhance the computational power of the neuron, and that of the network? In an effort to find answers to these questions, we implemented a simplified mathematical model of a pyramidal neuron endowed with complex dendritic dynamics and quantified its information processing capabilities using Shannon theory of mutual information. We proved that a neuron that holds multiple sites of independent thresholding of synaptic inputs and passive and active forward and backward propagation along with backpropagating action potential activated calcium spike firing and coincidence detection has a higher capacity for information processing than a point neuron and a network of two point neurons. The advantage in information processing, coupled with the simplicity and scalability of the neuron model implemented, constitute a compelling enough reason to promote the usage of such a spatially extended neuron model in networks that undergo plasticity and learning.

Chapter 1

Preface

Everything should be made as simple as possible, but not simpler.

July 1977

Setting the Stage

Physiological evidence and connectionist theory seem to support the notion that learning, in the brain, involves the modification of connection strengths between neurons, termed plasticity. In the physiological realm, various forms of plasticity have been identified, most notably are long-term potentiation (LTP) and long-term depression (LTD) (Mainen, 1999; Luscher et al., 2000). In the theoretical realm, synaptic weights are considered to be the principal modifiable parameters available for learning (Rosenblatt, 1962; Hebb, 1949). In the practical realm, neural networks architectures endowed with biologically inspired synaptic learning rules have been successfully applied to a variety of challenging learning-related tasks, namely in problems of pattern recognition, associative memory, map formation, and others (Arbib, 1995; Eliasmith et al., 2013). Taken together, these physiological, theoretical, and

practical considerations are based on the core principle that in networks of neuron-like units, information and learning are mainly encoded in the fine patterning of synaptic weights.

Upon more careful examination, there exists experimental evidence that weakens the link between the abstract synaptic weights of connectionist theory and the physical substrate for memory in the brain. First, it is now well established that dendrites of pyramidal neurons contain a large number and variety of physiologically active voltage-dependent channels which highly affect dendritic behavior and synaptic integration. These channels include NMDA channels (Thomson et al., 1998; Schiller et al., 2000) and voltage dependent Na^+ , K^+ , Ca^{2+} channels among others (Golding et al., 1999; Margulis and Tang, 1998) that are capable of generating dendritic action potentials both *in vitro* (Larkum et al., 1999a; Schiller et al., 1997; Svoboda et al., 1997; Golding et al., 1999; Kim and Connors, 1993; Urban and Barrionuevo, 1998) and *in vivo* (Kamondi et al., 1998; Hirsch et al., 1995; Svoboda et al., 1997). This being stated, the notion of a connection strength existing between two neurons is challenged by the fact that the efficacy (weight) of a synaptic contact is highly affected by the ongoing activity of other synapses within the dendritic compartment. The usual concept of a synaptic weight requires thus elaboration to take into account nonlinear dendritic dynamics and synaptic interactions.

Second, a growing body of evidence shows that besides changes in efficacy of synapses, structural plasticity at the axo-dendritic interface may contribute to learning-related processes. This structural plasticity involves synaptogenesis and dendritic and axonal growth and remodeling (Segal et al., 2000; Luscher et al., 2000). New spines can emerge within minutes *in vitro* (Dailey and Smith, 1996; Engert and Bonhoeer, 2000) or *in vivo* (Lendvai et al., 2000; O'Rourke and Fraser, 1990). Growth and remodeling of axonal and dendritic arbors and proliferation of new synapses can occur in the adult brain within days (Woolley et al., 1990; Darian-Smith and Gilbert, 1994). In addition to the rapid dynamics of spine appearance and disappearance, structural morphogenesis and spine motility occur frequently (Matus, 1999). Dendrites seem to grow as needed to accommodate increasing numbers of mature synapses on their arbors. The initially formed synapses between neurons are due

to random activity and are in a 'silent' phase as they cannot communicate information to their targets with their only existing NMDA channels. Silent synapses get co-activated with mature synapses and eventually develop AMPA receptors that allow them to communicate with their post-synaptic targets. Those silent synapses that are poorly correlated with their neighbors may get eliminated (Luscher et al., 2000; Cline et al., 1997; Liao et al., 1995; Shatz, 1990; Segal et al., 2000).

In trying to keep with the standard connectionist theory, one can argue that these dynamics are there to regulate the connection strength between two neurons and they can be represented by a number (weight) in models of connected neuron-like units. However, this statement is not faithful to neuronal communication dynamics and learning-related processes as it ignores fundamental elements in neuronal encoding/decoding and information processing such as nonlinear dendritic physiology, formation of new dendrites and changes in the mapping of synaptic contacts on the dendritic tree. These various mechanisms can constitute important forms of neural plasticity and induce forms of information processing that cannot be expressed in terms of changes in synaptic connection strength.

Brief, evidence for (1) active membrane mechanisms and nonlinearity that affect synaptic weights and synapse formation and location and (2) learning-activated remodeling of dendrites and axons and their connections prove that connection strengths between neurons may not be the main nor the exclusive form of flexibility used by the brain to achieve the learning dynamics and store them. Questioning this central tenet of neuroscience is the leading cause for this dissertation.

Chapter 2

Scope and rationale

Throwing a lot of neurons together and hoping something interesting emerges does not seem like a plausible way of understanding something as sophisticated as the brain.

Chris Eliasmith

A large repertoire of spatio-temporal activity patterns in the brain is the basis for adaptive behavior. Understanding the mechanisms by which the brain's 10^{11} neurons and 10^{15} synapses (Drachman, 2005) manage to produce such a range of cortical configurations in a flexible and self-organized manner remains a fundamental problem in neuroscience. While morphology and function are closely intertwined, plasticity and malleability constantly intervene to establish a concert of controlled dynamics that reshape network morphology and function.

The pace of discovery and untangling of these staggering dynamics is advancing at unprecedented speed especially with the recently developed tools and imaging techniques, such as dendritic patch-clamp recordings, two-photon 2D and 3D imaging techniques, genetically encoded sensors and stimulation, two-photon uncaging of glutamate, and others

applied both *in vitro* and *in vivo*. However, this advancement is not devoid of risk: rather than illuminating new principles, this arsenal of novel techniques carries a huge mass of data that may complicate further the unraveling of brain function. Here comes the fundamental importance of Theory, particularly theoretical work that is tightly linked to experiments. It is therefore due to the constant dialogue between experiments and theory that unifying principles underlying brain function, diversity, and plasticity will be revealed. Given the complexity of the nervous system, is it necessary or even desirable to model every neuron, dendrite, and synapse? If not, then what is the appropriate scale for modeling?

In fact, complexity was formerly - and even today in network models - merely credited to the complexity of the neuronal network disregarding that of the single neuron. As a result the neuron unit most often used in models of brain function is the classical ‘point neuron’, in which a weighted sum of synaptic inputs is passed through a single spike-generating mechanism (McCullough and Pitts, 1943; Rumelhart et al., 1986). From a modern perspective, however, the point neuron seems likely to be a poor representation of synaptic integration in neurons with large, profusely branched, active dendrites. There are several structural and functional properties of neural tissue which constitute the major obstacles in understanding the brain, among those are the active conductances embedded in dendritic membrane and backpropagation into the dendritic tree and the processing of inputs. Dendritic processing of synaptic inputs and the resulting modulation of somatic activity has raised several questions, among them those questions tying neuronal processing to network processing: What is the impact of this complex neuronal computation on the level of the network? Does this increased nonlinearity at the level of the neuron imply increased processing capabilities at the level of the network? For example, how do amacrine cells mediate or modulate the information flow from bipolar cells to ganglion cells? Many of these cells communicate via dendo-dendritic synapses, in which the dendrites function as almost separate compartments from the soma, making it very difficult to deduce the function of this network and to model it faithfully without considering dendrites (Balu et al., 2007).

In keeping with the idea that a dendritic arbor might support a moderately large number of independent nonlinear operations (Shepherd and Brayton, 1987; Rall and Segev, 1987; Mel, 1993; Koch et al., 1982) and that this wealth of operations may be the recipe for increased information processing capabilities in the neuron, the idea of this dissertation was born. Starting with a bottom-up approach of a theoretical investigation, we model the individual neurons as spatially extended entities in order to reproduce the rich propagation dynamics - passive and active forward propagation as well as active backward propagation - that occur in the dendrites along with their modulation of neuronal firing activity. We proved that the modeling approach used is comparable to point neuron models, thus is efficient and scalable and that the spatially extended neuron endowed with nonlinear dendritic dynamics outperforms a point neuron - a linear integrate-and-fire neuron in this case - as well as a pair of bidirectionally connected point neurons. We expect that these nonlinear dynamics, complemented by synaptic plasticity mechanisms may enhance the computational power of networks of these neurons.

Chapter 3

Dendritic Excitability

Until now, the race was who could get a human-sized brain simulation running, regardless of what behaviors and functions such simulation exhibits ... From now on, the race is more about who can get the most biological functions and animal-like behaviors.

Eugene Izhikevich

Populated with a staggering plethora of voltage-gated ion channels that are distributed non-uniformly over the dendritic membrane surface, dendrites constitute the siege for rich dynamic behaviors. Their electrical behaviors range from essentially passive responses, to the initiation of dendritic spikes, to passive and active forward propagation towards the soma, to active backpropagation of the action potential from the soma back into the dendrites (Larkum et al., 1999a; Saraga et al., 2003). Such a large repertoire of electrical behaviors does not only modulate dendritic dynamics but it also enriches the neuron's firing behavior and expands the network's information processing capabilities (Mainen and Sejnowski, 1996).

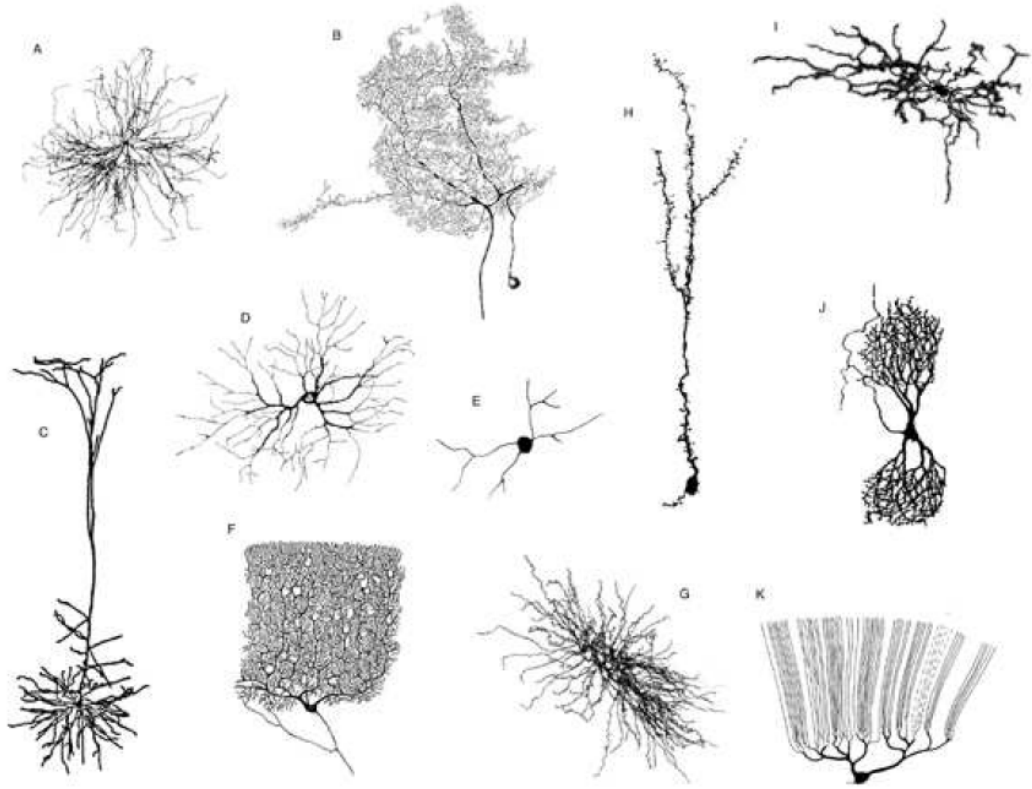


Figure 3.1: **Example of dendritic tree morphologies.** (a) Cat spinal motoneuron. (b) Locust mesothoracic ganglion spiking interneuron. (c) Rat neocortical layer 5 pyramidal neuron. (d) Cat retinal ganglion neuron. (e) Salamander retinal amacrine neuron. (f) Human cerebellar Purkinje neuron. (g) Rat thalamic relay neuron. (h) Mouse olfactory granule neuron. (i) Rat striatal spiny projection neuron. (j) Human nucleus of Burdach neuron. (k) Fish Purkinje neuron. Adapted from (Mel, 1994).

3.1 Introduction and Background

Dendrites are strikingly exquisite and unique structures whose specific morphology is used to classify neurons into classes: pyramidal, Pukinje, amacrine, stellate, etc. Most important is that the majority of the synaptic inputs is conveyed onto the dendritic tree where information is first processed by the neuron (figure 3.2). Dendrites thus constitute the elementary computing device of the brain. One of the most striking observable features of neurons is their extensive dendritic arbor. Not only is the surface area of the dendrites

one or two orders of magnitude larger than that of the soma, but also the shape of the dendritic tree is highly specific and is in fact often used to define neuronal types as shown in figures 3.1 and 3.3. A typical dendritic tree receives approximately ten thousand synaptic inputs distributed over its surface. When activated, each of these inputs produces a local conductance change for specific ions at the postsynaptic site, causing a change in membrane voltage that spreads along the dendritic branches towards the neuron's soma. This spread depends on spine and dendritic morphologies and their electrical properties.

Even though these facts have been known since the 20th century, it is still very natural to wonder “what do dendrites do?”

3.1.1 Dendritic trees: brief anatomy and physiology

Dendrites are thin tubes of nerve membrane. They start with a diameter of a few μm near the soma and become thinner as they successfully branch with their diameter decreasing to below $1 \mu m$. Many types of dendrites are populated by small protrusions, termed spines, which constitute the major postsynaptic target for excitatory inputs and seem to be important loci for plastic processes in the nervous system (Koch and Zador, 1993). Dendritic trees range from very short ($100 - 200 \mu m$) as in the spiny stellate cell of the mammalian cortex to long ($1 - 2 mm$) as in the spinal α -motoneurons. The total dendritic length may reach $1 cm$ if not more. The majority of the brain volume and area is occupied by dendrites. The area of a single dendritic tree is in the range of $2,000 - 750,000 \mu m^2$ and its volume may reach up to $30,000 \mu m^3$.

The dendritic membrane can conduct current via specific transmembrane ionic channels. The resistance to current flow along the dendritic core is smaller than the resistance to current flow across the membrane. The dendritic membrane can store ionic charges, thus behaving like a capacitor. The R-C properties of the membrane imply a time constant $\tau_m = RC$ for charging and discharging the transmembrane voltage. τ_m typically varies between 1 and 100 *msec*. The membrane resistivity implies an input resistance R_{in} at any

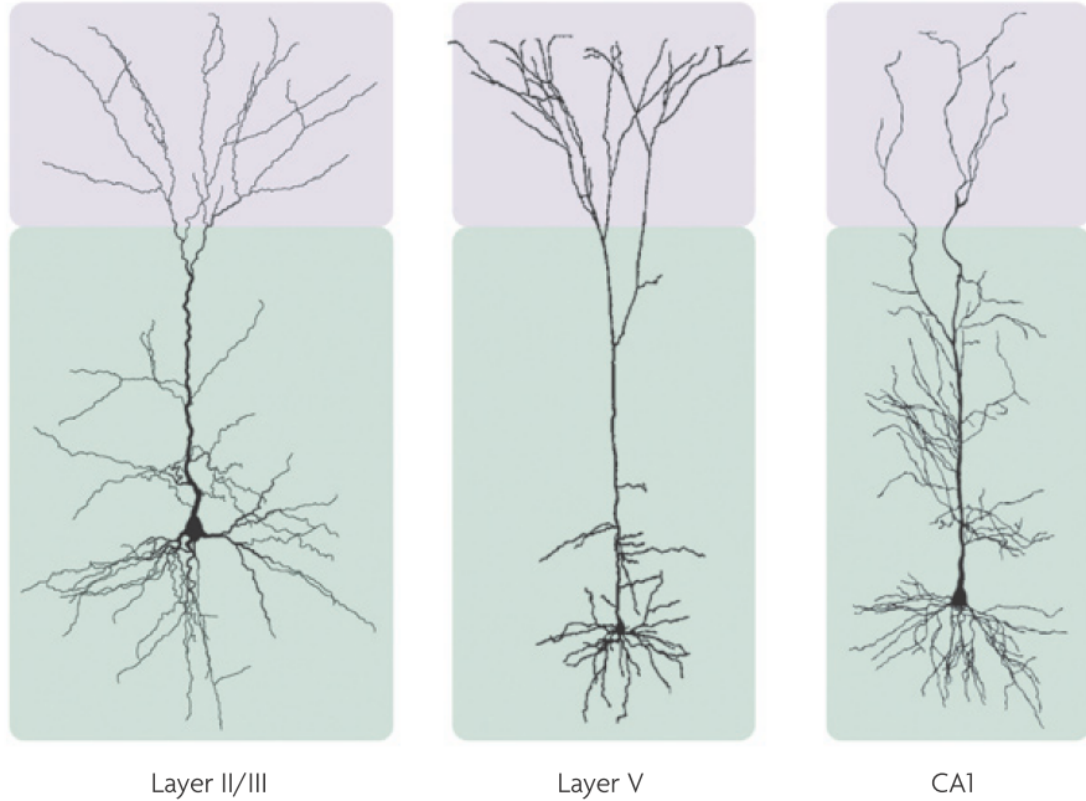


Figure 3.2: **Domains of synaptic input in pyramidal neurons.** The apical tuft (*highlighted with a purple background*) of pyramidal neurons receives excitatory synaptic inputs that have different presynaptic origins than those that form synapses onto more proximal apical dendrites or basal dendrites (*highlighted by a green background*).

point in the dendritic tree. R_{in} values range between $1\text{ M}\Omega$ for thick and leaky dendrites and $1\text{ G}\Omega$ for thin membrane as that of spines.

In classical cable theory, the electrical properties of the membrane are passive and thus voltage-independent. τ_m is thus constant and R_{in} is constant. However, dendrites are active and endowed with voltage-gated ion channels, which implies that τ_m and R_{in} are voltage-dependent.

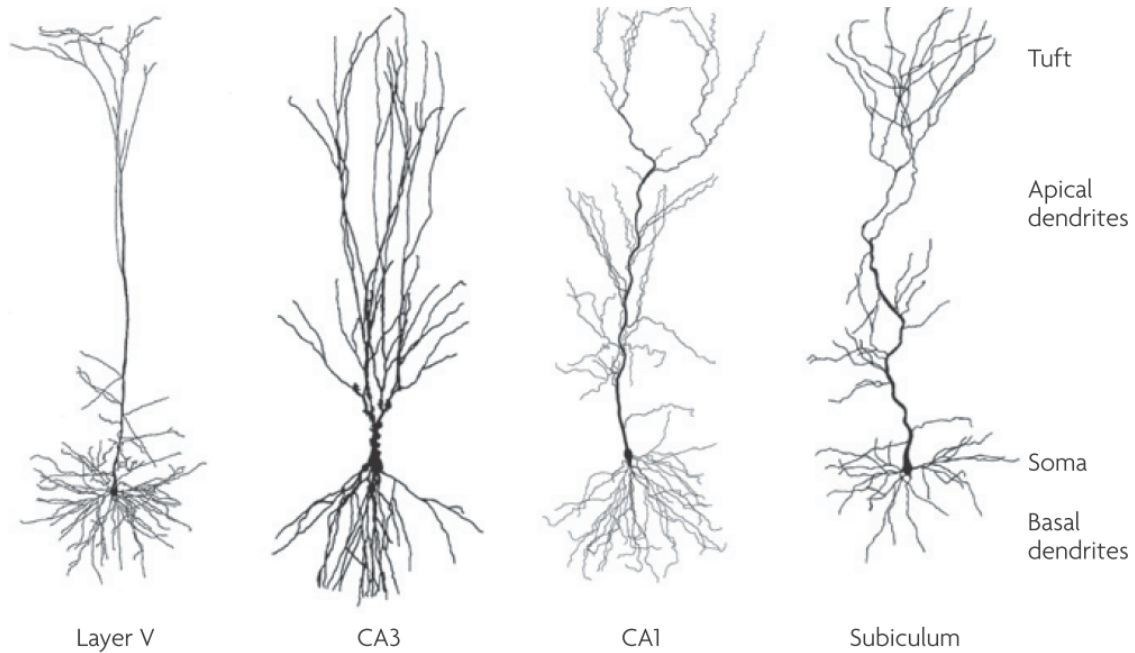


Figure 3.3: **Pyramidal-neuron structure.** The structures of rat pyramidal neurons from different cortical areas highlighting considerable differences between their morphology. The full vertical length of each cell, from left to right, is: $1,180\mu m$; $580\mu m$; $730\mu m$; $790\mu m$. *Courtesy of (Spruston, 2008a)*

3.2 Pyramidal neurons and their connections

Dendritic propagation dynamics and their effect on the neuron's firing activity are studied extensively in pyramidal neurons. Pyramidal neurons are found mainly in brain structures that are associated with advanced cognitive functions and learning, including the cerebral cortex, the hippocampus and the amygdala (y Cajal, 1995). Covered with thousands of dendritic spines, pyramidal neurons' apical and basal dendrites constitute the post-synaptic site for most excitatory glutamatergic synapses. An understanding of these neurons, complemented with an understanding of their function in neuronal networks is thus necessary to elucidate the neural bases of some of the brain's sophisticated functions and information processing abilities.

These neurons are located in layer V of the neocortex, the latter being organized

into horizontal layers parallel to the pial surface and numbered from I to VI. These distinct 6 layers are discriminated by types of neurons and connectivity patterns as shown in figure 3.4. The general notion of information flow between these layered neurons states that most of the extra-cortical input arrives at layer IV, which in turn projects to layer II/III, which then projects to layers V and VI (Mountcastle, 1998). The large pyramidal neurons of layer V receive short- and long-range projections from other cortical areas and represent one of the output levels of the neocortex. With their extensive apical dendritic tree spanning several cortical layers, they receive inputs from a host of different sources, thus representing one of the core integration and relay units in cortical information processing (Binzegger et al., 2004). These neurons project to several sub-cortical regions (thalamic nuclei, pons, superior colliculus, spinal cord and contralateral cortex) as well as to other cortical regions via axon collaterals forming before the axon leaves the cortex. Specific parts of layer V neurons can be differentiated according to their afferents: inhibitory GABA-ergic (γ -aminobutyric acid) input mainly arrives at the soma and axon, while excitatory input terminates mainly on basal/proximal dendrites (origin is local feedforward sources) or distal dendrites (origin is distant thalamic/cortical feedback sources) (Thomson and Lamy, 2007).

In the present work, the non-linear integration mechanisms of layer V pyramidal neurons were studied in a model of these neurons.

3.3 The interplay between passive and active dendritic conductances

The process of integrating postsynaptic potentials constitutes the core of the functional and computational properties of neurons. Synaptic inputs play their music with the dendrites that constitute the major site of integration of a neuron (London and Hausser, 2005). Other factors, among them the spatio-temporal distribution of synaptic inputs, the current and past activity state of the neuron itself, short- and long-term changes in synaptic plasticity, as well as other modulating cellular functions (such as spine growth and the effect

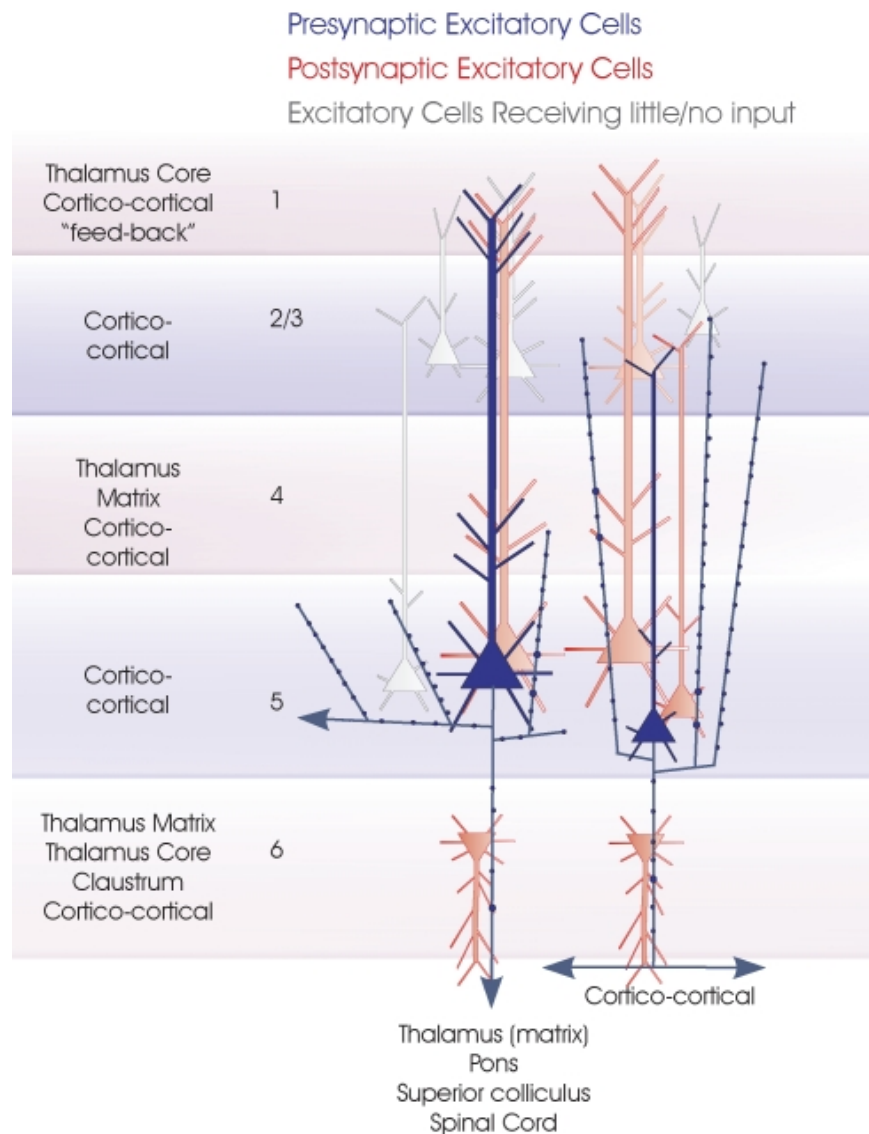


Figure 3.4: **Layer V pyramidal neurons.** The two major subclasses of layer V pyramidal neurons are represented in blue. Large pyramidal neurons with a pronounced apical dendritic tuft in layers I and II project to several subcortical regions. To the left are indicated the major inputs to each cortical layer. Spiny excitatory postsynaptic targets are shown in red, the paler neurons being those that receive sparse and weak inputs. *Courtesy of (Thomson and Lam, 2007)*

of various neurotransmitters) influence the integration behavior of the neuron and modulate its output (Spruston, 2008b).

3.3.1 Passive properties of dendrites

What do passive dendrites do to the transient current inputs that they receive at their synaptic site? The passive cable properties of dendrites filter high temporal frequency postsynaptic potentials (PSPs). A small percentage of synaptic current also leaks out via the dendritic membrane as the intracellular (axial) resistance is substantially smaller than the membrane resistance. The PSPs thus are attenuated, delayed, and their time course (shape) changes as they propagate from the dendrite to the soma. The further from the soma these PSPs are, the slower their rise time and the broader the somatic PSP (Rall, 1967). However, these PSPs reach the soma and thus even in passive dendrites, distal synapses are expected to affect the output of the soma.

The passive cable theory (Rall, 1959) predicted that synaptic inputs are greatly attenuated as they propagate towards the soma with the increase in the distance from the soma, sometimes reaching 100-fold (V_{soma}/V_{dend}) in neocortical layer V pyramidal neurons (Stuart and Spruston, 1998). This attenuation is mainly due to the low membrane resistivity R_m , which causes a current loss along the way to the soma and the low pass filtering effect of the membrane capacitance C_m . The axial resistance in the dendritic membrane, r_i (relates to the dendrite morphology), also contributes to this attenuation since part of the synaptic current does not contribute to the change in membrane potential V_m as it flows into adjacent parts of the dendrite. Dendritic morphology and adjacency also affects the spatio-temporal summation of excitatory and inhibitory postsynaptic potentials (EPSP/IPSP). For example, currents from co-activated proximal synapses undergo sublinear summation due to a reduction in driving force (Gulledge et al., 2005). Shunting inhibition is also a non-linear mechanism of synaptic interactions with the input resistance of the dendrite altered rather than its membrane potential and where excitatory input is vetoed in a precise spatio-temporal manner (Koch et al., 1983; Stuart et al., 1999). As for the shape change

of the local EPSP as it propagates from the dendrite to the soma, its half-width decreases while its amplitude increases due to the geometric differences between the cable-like dendrite and the sphere-like soma (Magee, 2000). The distal dendrite thus displays a much shorter integration time-window if compared to that of the soma (Williams and Stuart, 2002).

3.3.2 Active properties of dendrites

The complex and specific morphology of the dendritic arbor holds a multitude of voltage-gated channels, thus endowing dendrites with the ability to serve as a sieve for an active and fast bidirectional communication between the spine and the soma. Contrary to what was initially believed, forward propagation along the dendrites is not only passive; active voltage-gated conductances play a major role in enabling these compartments to generate fast forward propagating signals, termed dendritic spikes, as well as fast backward propagating signals, referred to as backpropagating action potentials (bAP).

As already stated, distal dendritic inputs are greatly attenuated en route to the soma; however, upon crossing the threshold at the dendritic initiation zone, this passive propagation gets transformed into a fast, active propagation of calcium action potentials. Passive below-threshold dendritic signals can however result in a bursting activity at the soma if they coincide with backpropagating action potentials. The result is a more pronounced effect on the neuron's soma and consequently altered firing dynamics. While passive dendritic propagation speeds lie in the range of $10 - 30 \mu m/ms$, active propagation is more than 10-fold larger with speeds of $150 - 300 \mu m/ms$ (Fromherz and Muller, 1994). This postsynaptic heterogeneous processing of synaptic inputs has a tremendous effect on neuronal dynamics and firing activity as detailed in Larkum et al. 1999a.

In layer V pyramidal neurons, interactions between synaptic events in the dendrite coupled with non-uniformly distributed active conductances have a decisive impact on the integration of synaptic inputs and the somatic voltage. Voltage-dependent ion channels,

found in dendrites of almost every neuron, highly influence and sometimes override the passive properties. As an example, voltage-dependent Na^+ and Ca^{2+} channels (VDNC/VDCC) get activated by sufficiently large postsynaptic currents that can amplify EPSPs and sometimes counter the current loss during the signal propagation to the soma (Williams and Stuart, 2003; Oviedo and Reyes, 2005). The effects of these channels on shaping EPSPs and their propagation towards the soma increase with the increase in the amplitude and number of synaptic inputs since further depolarization activates more voltage-gated channels (Magee, 2000). The interplay of passive and active factors seems to be dynamically regulated to fit the momentary requirements of the neuron, endowing it with the ability to adapt to different stimuli.

3.3.3 Regenerative potentials in dendrites

At first it was believed that the regenerative electrical activity produced by neurons is due to the sodium action potential (AP) at its soma. This AP gets generated near the neuron's soma and transmitted via the axon to postsynaptic targets. Technical advances in imaging and electrophysiological techniques coupled with mathematical models elucidated another form of regenerative activity in neurons: Na^+ - Ca^{2+} -mediated action potentials have been revealed in distal dendrites of major neurons of the CNS, namely in the pyramidal neurons of the hippocampus and neocortex both *in vitro* and *in vivo* (Kim and Connors, 1993; Larkum and Zhu, 2002; Waters et al., 2003). These Na^+ - Ca^{2+} -APs in conjunction with other mechanisms are believed to represent a major tool of non-linear information processing in neurons, considerably expanding the computational capacity of the single unit, the neuron (London and Hausser, 2005). Dendrites are highly plastic, they can grow and diversify even after the proliferation of the neuron has stopped. Thus dendrites constitute the ideal units that increase the complexity of the neuron without increasing the number of units.

3.3.3.1 Regenerative potentials in the apical dendrite

Regenerative potentials in apical distal dendrites of pyramidal neurons are mediated by synaptic co-activation of AMPA ((α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate) and NMDA (N-methyl-D-aspartate) glutamate receptors which causes a transient Ca^{2+} influx. These potentials are a composite of two conductances: Na^+ conductance and Ca^{2+} conductance. The Na^+ conductance produces a sharp all-or-none potential that can be blocked by Tetrodotoxin (TTX) (Schiller et al., 1997). The second conductance, Ca^{2+} is activated by further depolarization and displays a much longer, plateau-like, voltage change that can be blocked by Ca^{2+} and Ni^{2+} (Larkum et al., 2001). In contrast to somatic action potentials which are stereotypic and vary only between different classes of neurons, dendritic spikes show a broad spectrum of shapes ranging from short to complex and long lasting voltage changes (Larkum and Zhu, 2002). These spikes can also be either amplified or attenuated en route to the soma and represent a mechanism of coincidence detection of inputs at different locations in the neuron.

3.3.3.2 Regenerative potentials in the basal dendrite

Thin basal dendrites of layer V pyramidal neurons display another form of regenerative potential whose main conductance is NMDA-receptor mediated (Schiller et al., 1997). The activation of NMDA-receptors associated with these action potentials is due to voltage-dependent relief of a Mg^{2+} block. Na^+ -based APs have been found in basal dendrites but Ca^{2+} -dependence has not been established (Nevian et al., 2007). These basal APs stay localized in the basal dendrite opposite to apical APs which propagate towards the soma.

3.4 Compartmentalization

Morphological properties complemented with the non-uniform distribution of voltage-gated channels and varying spine density (Mountcastle, 1998; Spruston, 2008b) as well as

the different forms of regenerative activity in distinct parts of a pyramidal neuron imply that these neurons possess different functional compartments and have the ability to process information at multiple, independent sites (Hausser and Mel, 2003). For example, dendritic Ca^{2+} spikes boost strong or concurrent input while intrinsic dendritic properties lead to the suppression of weak synaptic inputs, thus providing a mechanism of local non-linear operations at the level of the dendrite. These Ca^{2+} spikes also transmit information from the dendrite to the soma and modulate the latter's output (Larkum et al., 1999b). However, Ca^{2+} spikes do not always reliably propagate to the soma as they get influenced by regulatory instances such as specific inhibition, modulation of voltage-activated channels, activity state of the neuron, among others (Magee, 2000; Larkum and Zhu, 2002; Hausser et al., 2000). This being stated, the integration and computational performance of each compartment could be determined by the synaptic input itself, its conditions and location and the previous and current neuronal activity (such as long-term potentiation/depression (LTP/LTD) or spike history). As a result, the neuron is a highly adaptive and complex information processing device.

3.4.1 Backpropagating APs and coincidence detection

Electrical compartments in the neuron seem to integrate their respective inputs independently but it's the communication between these compartments that influences the somatic neuron output. The dendritic initiation zone is coupled to the neuron's soma via backpropagating action potentials (bAPs) that are initiated at the neuron's soma and effectively invade the dendritic tree thus bridging activity at the distal dendrites to that at the soma (Larkum et al., 1999a,b; Stuart and Sakmann, 1994; Buzsaki and Kandel, 1998). Backpropagating APs are attenuated as they travel more distally due to dendritic geometry and Na^+ channel inactivation with prolonged spiking. This propagation is however rescued by appropriately timed EPSPs that boost the bAP and secure its invasion of distal dendrites (Larkum et al., 1999a; Stuart and Hausser, 2001). The combination of Ca^{2+} spikes and

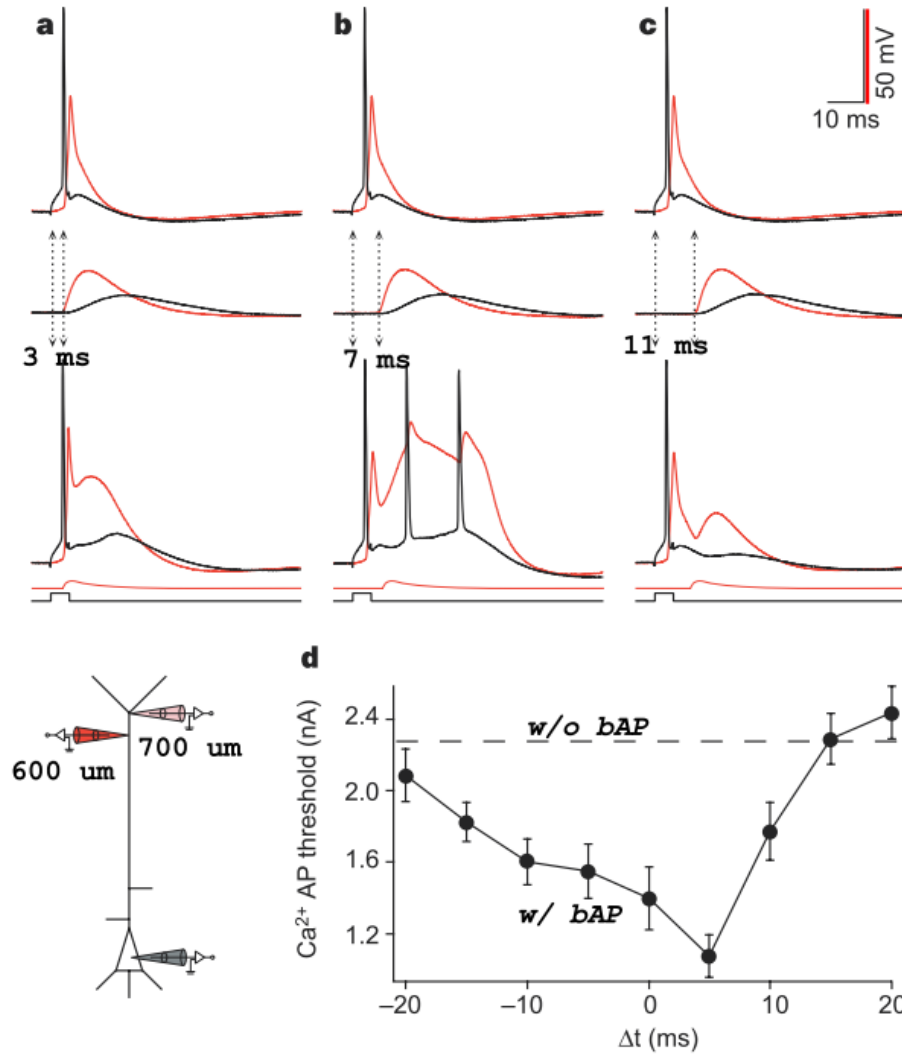


Figure 3.5: **Precision of timing required for BAC firing.** Recordings were made from the dendrite (red; $600\ \mu\text{m}$ from the soma) and the soma (gray). Current was injected at $700\ \mu\text{m}$ from the soma (pink electrode). Δt is the time between the start of the somatic current injection and that of the dendritic injection. Each point in (d) represents the average for 8 neurons and represents the threshold for injected current (in nA) needed to evoke a dendritic Ca^{2+} action potential. *Courtesy of Larkum et al. 1999a*

bAPs could represent a powerful mechanism to link the soma and the dendritic compartment. bAPs travel up into the distal tuft carrying information about the neuron's spiking activity; at the distal dendritic site, they encounter Ca^{2+} spikes and coincidence detection occurs as the spatio-temporal activities of these two compartments coincide. This coupling can be achieved by backpropagating action potential activated Ca^{2+} -spike (BAC) firing as proposed by Larkum and colleagues (Larkum et al., 1999a,b) and as depicted in figure 3.5. Thus, BAC firing occurs when a somatic spike and an EPSP occur within a narrow time window (3-7 ms). This coincidence lowers the threshold to initiate a Ca^{2+} spike and can cause a burst of somatic Na^+ APs (Larkum et al., 1999a). This mechanism also seems to be robust to noise, thus mimicking the conditions that occur *in vivo*, namely the continuous synaptic bombardment (Larkum et al., 2004).

3.5 Synaptic integration in dendrites

Integration of postsynaptic potentials forms the core of the functional and computational properties of neurons. Since dendrites constitute the sieve for the majority of synaptic connections into the neuron, they also represent an fundamental site for integration of incoming inputs (Hausser et al., 2000; London and Hausser, 2005; Spruston, 2008b). Synaptic integration is associated with membrane conductance change. The time course of the synaptic conductance change associated with various input types in a neuron may vary by 1-2 orders of magnitude. The fast excitatory *AMPA* and inhibitory *GABA_A* inputs operate on a time scale of 1 *ms*. The slow excitatory *NMDA* and inhibitory *GABA_B* inputs act on a slower time scale of 10 – 100 *ms* and have a conductance that is about 10 times smaller. Moreover, the spatio-temporal distribution of synapses along with the current and past activity of the neuron constitute various conditions for synaptic integration in different parts of the neuron. Finally short- and long-term changes in synaptic plasticity influence the integration behavior.

3.6 Neural plasticity

One of the major challenges in neuroscience is to gain deeper understanding of learning and memory and how they are represented in the brain. Every day new memories are formed and others erased in humans and animals. These memories are stored in both the network properties of brain areas (Xu et al., 2004) and in the intrinsic excitability of single neurons and their synapses. The quantities associated with these changes are dynamic and all changes are collectively referred to as ‘plasticity’. Plasticity is generally divided into synaptic, intrinsic and structural plasticity. Synaptic plasticity occurs when an existing synapse gets strengthened or weakened. This type of plasticity can be presynaptic (change in the amount of neurotransmitters at the presynaptic terminal or in the probability of their release into the synaptic cleft) or postsynaptic (change in the efficacy of neurotransmitter binding to postsynaptic receptors) (Colicos and Syed, 2006). The conductances of dendrites and soma are intrinsic properties that may get altered with neuron activity and make part of intrinsic plasticity. Structural plasticity is pronounced during the development of the central nervous system and in the repair of injuries (VanOoyen, 2003; Shamy et al., 2007).

Plasticity has been extensively studied in different types of neurons both *in vitro* and *in vivo*. Dendrites are highly involved in plasticity especially when it comes to synaptic as well as structural plasticity (Segal et al., 2000). Active currents in dendrites play a prominent role in plasticity and dendritic spikes constitute a mechanism for cooperative long-term potentiation (LTP) (Stuart et al., 1993). As an example, LTP of synapses on the distal dendrites of hippocampal CA1 pyramidal neurons does require cooperative synaptic inputs, but does not require axonal action potential firing and backpropagation. Rather, locally generated dendritic spikes contribute to the postsynaptic depolarization and calcium entry necessary to trigger potentiation of distal synapses. This mechanism also exists at proximal synapses. Thus dendritic spikes participate in a form of synaptic potentiation that does not require postsynaptic action potential firing in the axon. Backpropagation, on the other hand, is an important feedback mechanism that starts at the soma and invades the dendritic

tree, giving rise to coincidence detection at the level of the dendrite and consequently to plasticity (Markram et al., 1997; Paulsen and Sejnowski, 2000; Xu et al., 2006). Along with this short introduction to neural plasticity, we will briefly provide some background information about the most widely recognized and used forms of synaptic plasticity, Hebbian plasticity and spike-timing-dependent plasticity (STDP).

3.6.1 Hebbian plasticity: the role of firing frequency

Communicated in Donald Hebb’s seminal paper published in 1949, what is referred to as the Hebbian postulate for cellular learning has remained the most widely quoted mechanism of synaptic plasticity (Hebb, 1949). Not only it constitutes a prediction that is experimentally testable, but it also has a simple and appealing phrasing: *“When an axon of cell A is near enough to excite 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 As efficiency, as one of the cells firing B, is increased.”*. While Hebb referred only to long-term potentiation (LTP) of the synaptic connection, Stent suggested the existence of the inverse of LTP, i.e. the weakening or depression of a synapse, referred to as long-term depression (LTD) (Stuart et al., 1973). Stent postulated the following: *“When the presynaptic axon of cell A repeatedly and persistently fails to excite the postsynaptic cell B while cell B is ring under the influence of other presynaptic axons, metabolic change takes place in one or both cells such that As efficiency, as one of the cells ring B, is decreased.”* Intuitively, both LTP and LTD are necessary to optimize information storage in a neural network (Willshaw and Dayan, 1990). However, Hebbian plasticity alone does not determine plasticity; experiments have also revealed the dependence of synaptic plasticity on the firing frequency with potentiation favored at high frequencies, while depression dominates low frequencies (Sjostrom et al., 2001; Froemke et al., 2006).

Inherent to the Hebbian postulate is causality and a need for temporal order, because, by definition, if synaptic strengthening (LTP) occurs when cell A is helping cause activity

in cell B (Hebb’s postulate), this is another way of saying that cell A is firing before cell B. This leads to what is known as Spike-Timing-Dependent Plasticity (STDP).

3.6.2 Spike-timing-dependent plasticity (STDP): the role of timing

In its most basic form, STDP depends on the relative timing of the pre- and post-synaptic spikes: pre-before-post pairings within a narrow timing window result in LTP, whereas the opposite temporal order evokes LTD (Markram et al., 1997; Abbott and Nelson, 2000). To intuitively understand the functional importance of STDP, it is useful to think of the predictive power of the presynaptic spike; if it precedes and predicts the postsynaptic spike, the synaptic connection is rewarded through strengthening, whereas a “postdiction” results in the punishment of the synapse by its weakening. In other words, a synapse with STDP is not only a coincidence detector but also an extractor of temporal order and of causal connections.

STDP has been found in several neocortical layers, such as Layer II/III (Nevian et al., 2006), Layer IV (Egger et al., 1999), and Layer V (Markram et al., 1997); it has also been found in a number of brain regions, including the hippocampus (Debanne et al., 1998), the visual cortex (Sjostrom et al., 2001) and the sensory cortex (Nevian et al., 2006), among others.

However, the rules for STDP are not as simple: beyond the basic asymmetric window, recent studies have revealed several layers of complexity in STDP. Some of the variations of STDP result in the timing window for LTD dramatically widening with increased postsynaptic activity (Sjostrom et al., 2003); even more pronounced is the diametrically opposing plasticity found with distance the gradient from the soma (Sjostrom et al., 2006; Caporale and Dan, 2008); as for spike triplets and quadruplets, the first spike pairing might result in the cancellation of subsequent firing (Froemke and Dan, 2002; Froemke et al., 2006); in the case of an arbitrary firing pattern or bursting dynamics, simply calculating plasticity based on pre-before-post and post-before-pre pairings cannot be robustly applied

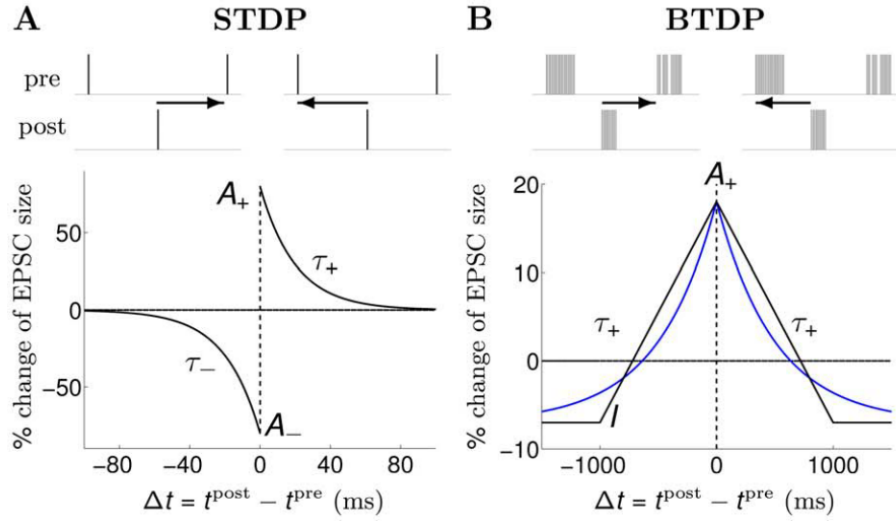


Figure 3.6: **STDP versus BTDP**. STDP modifies synaptic strength based on Δt and the firing order of pairs of pre- and post-synaptic spikes. BTDP governs changes based on the timing, but not the order, of pre- and post-synaptic bursts over a longer time window (1 s). Courtesy of (Gjorgjieva et al., 2009).

(Sjostrom et al., 2001; Gjorgjieva et al., 2009); see figure 3.6 where the window of plasticity for bursting - also referred to as burst-timing-dependent plasticity (BTDP) - is revealed. Thus, neuron type and intrinsic properties (including dendritic morphology), coupled with dependence on the distance between the dendritic site of incoming synaptic input and the neuron's soma, play prominent roles in shaping synaptic plasticity.

3.6.3 The spine as a coincidence detector and the influence of postsynaptic Ca^{2+} concentration and backpropagation

Synaptic plasticity requires that some form of coincidence detection exists between pre- and post-synaptic activity. N-Methyl-D-aspartate (NMDA) receptors positioned on the postsynaptic spine membrane of pyramidal neurons are widely accepted candidates for this mechanism (Schiller et al., 1998; Yuste and Denk, 1995), precisely in the induction of LTP. At resting hyperpolarized membrane potentials, NMDA receptors open only modestly, mainly due to a Mg^{2+} block, even in the presence of glutamate (Mayer et al., 1984). However, in the case of sufficiently strong postsynaptic cell membrane depolarization, blocking

Mg^{2+} ions are expelled from the channel pore, thus unblocking it and allowing for Ca^{2+} influx. Strong postsynaptic depolarization commonly happens in the case of an AP that initiates at the axon hillock and backpropagates to reach the spine and strongly depolarize it. With action potential initiation and backpropagation in the postsynaptic neuron, postsynaptically located NMDA receptors can detect the coincidence of presynaptic glutamate release (due to presynaptic activity) and postsynaptic depolarization (due to postsynaptic spiking). The result is a supralinear rise in postsynaptic Ca^{2+} concentration ($[Ca^{2+}]$) and the ensuing long-term potentiation. While large postsynaptic depolarization-caused NMDA-receptor activation results in LTP, it is generally accepted that the activation of voltage-dependent Ca^{2+} channels before metabotropic glutamate receptors (mGluRs) result in the phospholipase C-dependent (PLC-dependent) synthesis of endocannabinoids, which act as a retrograde messenger to induce LTD (Nevian et al., 2006). Additional work on the importance of coincident postsynaptic backpropagation with dendritic-spine activity and $[Ca^{2+}]$ revealed that the volume-averaged peak elevation of $[Ca^{2+}]$ in dendritic spines of pyramidal neurons (precisely in Layer II/III pyramidal cells) was necessary determinant of the magnitude of long-term potentiation; in particular, sublinear volume-averaged $[Ca^{2+}]$ resulted in LTD whereas supralinear volume-averaged $[Ca^{2+}]$ led to LTP (Nevian et al., 2006).

In this view, the coincidence detector relevant for synaptic plasticity resides in the synaptic spine and gets activated by backpropagation and the rise in dendritic spine $[Ca^{2+}]$. It is worth noting that the existence of a spine coincidence detector and its activation by bAP does not however suggest that it is the only mechanism for postsynaptic depolarization at the spine or the only trigger of plasticity (Hartell, 1996).

In summary, backpropagating action potentials (bAPs) and coincident presynaptic activity trigger long-term synaptic plasticity at the level of the spine and the soma in neurons. The role of dendritic propagation delays is thus extended to include a direct effect on synaptic plasticity mechanisms.

3.7 Conclusion

In summary, postsynaptic heterogeneous processing of synaptic inputs at the level of the dendrites has a major effect on neuronal dynamics, firing activity, and plasticity. The first step in this project was to capture the nonlinear dendritic dynamics in a model of the Layer V pyramidal neuron.

Chapter 4

Modeling the neuron: A balance between detail & abstraction

Towards a fruitful and convenient mixture of simplicity and realism.

Rather than representing neurons as single summing points referred to as ‘point neuron’ models, we decided to proceed with a spatially extended model that allows us to capture the rich dendritic dynamics and their modulation of the neuron’s output as depicted in Larkum et al. 1999a. Often than none in models where dendritic propagation delays are included, they are modeled as being static and passive, which impedes the faith of the model in capturing essential neuronal dynamics. While accounting for the dynamic dendritic propagation delays entails a compartmental model that is naturally more computationally intensive than a point neuron model, we strove in our implementation to keep the model as simple as possible, thus satisfying our aim to incorporate this neuron model as a building block in large neuronal network that can undergo plasticity and learning.

4.1 Experimental findings underlying the neuron model

While there exists an extensive amount of literature detailing experimental findings in cortical pyramidal neurons, we chose to use the findings of Larkum et al. 1999a in his seminal Science paper where he investigated experimentally the coupling of inputs arriving at different cortical layers using electrophysiological measures of a layer V cortical pyramidal neuron *in vitro*. These pyramidal neurons extend their dendrites into all cortical layers and are unusual in having both a dendritic and an axonal zone for the initiating of action potentials, thus they have multiple thresholding units. In addition, the large size of these neurons and their extended dendritic arbors makes them a good candidate for electrophysiological measurements. The main figure from the paper by Larkum et al. 1999a that we based our neuron model on is 4.1. Prior to detailing the neuron model, we will briefly explain the details of this figure.

- a. Triple recordings were made on two sites (depicted in red and blue pipettes) of the apical dendrites and the soma (gray pipette) of a layer V pyramidal neuron. The red pipette is positioned at $770\ \mu m$ from the soma and the blue pipette at $400\ \mu m$ from the soma.
- b. Current injection of EPSP shape (biexponential) and maximum amplitude of $0.3\ nA$ at the distal pipette produced a subthreshold voltage of $1.4\ mV$ at the soma.
- c. Step current injection of $1.1\ nA$ amplitude and $5\ ms$ width at the soma resulted in a somatic action potential that propagated backwards towards the distal dendritic site. The backpropagating action potential decreased in amplitude but increased in width in the dendrite. The afterdepolarization observed at the soma is also pronounced in the backpropagating signal as depicted in the figure.
- d. The combination of somatic and dendritic current injections as in (b) and (c) separated by a time interval of $5\ ms$ resulted in a burst of action potentials and a backpropagation action potential-activated (BAC) Ca^{2+} spike. The backpropagating action potential

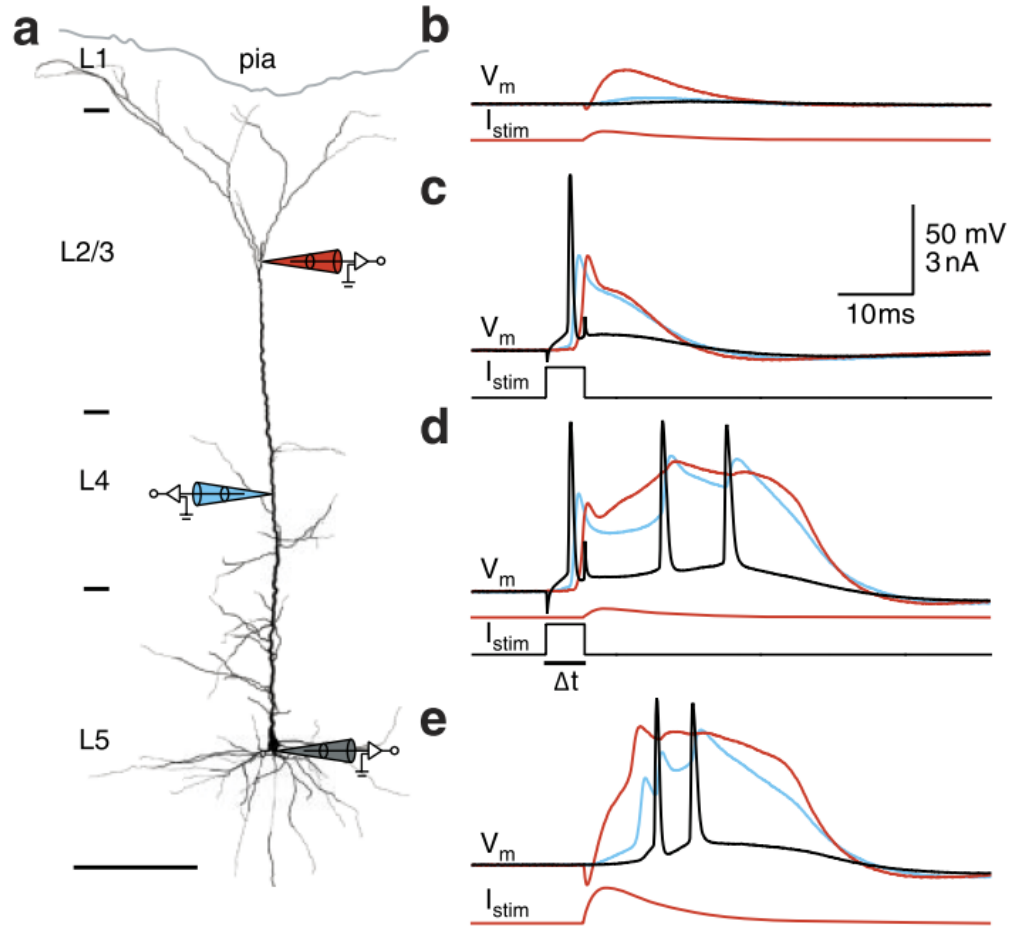


Figure 4.1: **Layer V pyramidal neuron** Active forward propagation and coupling of a backpropagating action potential with subthreshold current injection at the apical dendrite. *Courtesy of Larkum et al. 1999a. Scale bar 200 μ m.*

caused a reduction in the threshold for dendritic Ca^{2+} -spike initiation which resulted in the burst at the level of the soma. This mechanism is better detailed in 3.5.

- e. Current injection in the form of an excitatory postsynaptic potential (EPSP) and maximum amplitude of $1.2nA$ at the distal dendrite evoked a dendritic Ca^{2+} spike that propagated forward and induced a burst of somatic action potentials.

In summary, superthreshold input at the distal dendrite or near-synchronous input to both the distal dendrite and the soma cause a burst of axonal action potentials. Once

threshold is reached at the axonal integration site, the subsequent action potential backpropagating signal enables threshold at the dendritic initiation site to be reached more easily, termed BAC firing. Thus BAC firing constitutes a potential mechanism for binding different cortical regions. This being stated, and owing to its own separate spike-initiation zone, the distal apical dendritic tree can act as a separate synaptic integration unit that facilitates communication to the soma (Larkum et al., 1999a). This mechanism is also likely to exist in other types of pyramidal neurons, such as hippocampal CA1 neurons (Golding et al., 2002).

4.2 Model results and fitting to experimental findings

Modeling the pyramidal neuron involved a compromise between two seemingly mutually exclusive requirements where the model must be (1) computationally simple and efficient to constitute the building block of large neuronal networks, yet (2) capable of producing the rich firing patterns exhibited by real biological neurons that are endowed with non-linear computational units: the dendrites.

The minimal number of components needed to reproduce the results in 4.1 are: the soma, the spine and the dendrite that links the two. For the sake of simplicity and to avoid the need to implement a dendritic tree, we assumed that the pipette located at $700\ \mu m$ from the soma is recording from a spine. Modeling of the spine and soma was inspired by the linear integrate-and-fire (LIF) point neuron model whereby these two separate sites are endowed with thresholding capabilities and give rise to somatic and dendritic spikes. Modeling the dendrite was inspired by the cable model proposed by Rall 1959. Both the LIF model and the Rall cable equation were modified in order to account for the additional properties exhibited by the pyramidal neurons, among these are the active propagation along the dendrite and BAC firing.

The electrical response of the reconstructed model neuron to dendritic and somatic current injection was investigated using the same protocols as in Larkum et al. 1999a. Model results that conform to the rich dynamics of the LayerV pyramidal neuron in 4.1 are

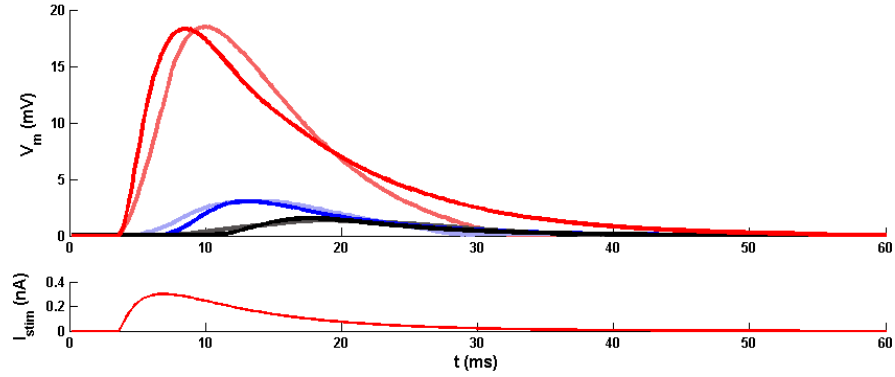


Figure 4.2: **Neuron model: subthreshold current injection at the distal dendrite.** Current injection in the form of EPSP with maximum amplitude of 0.3 nA at the distal dendrite evokes a subthreshold somatic membrane voltage of maximum amplitude 1.4 mV . *Experimental results to which the model was fit are displayed in faded colors that match those of their corresponding model signals*

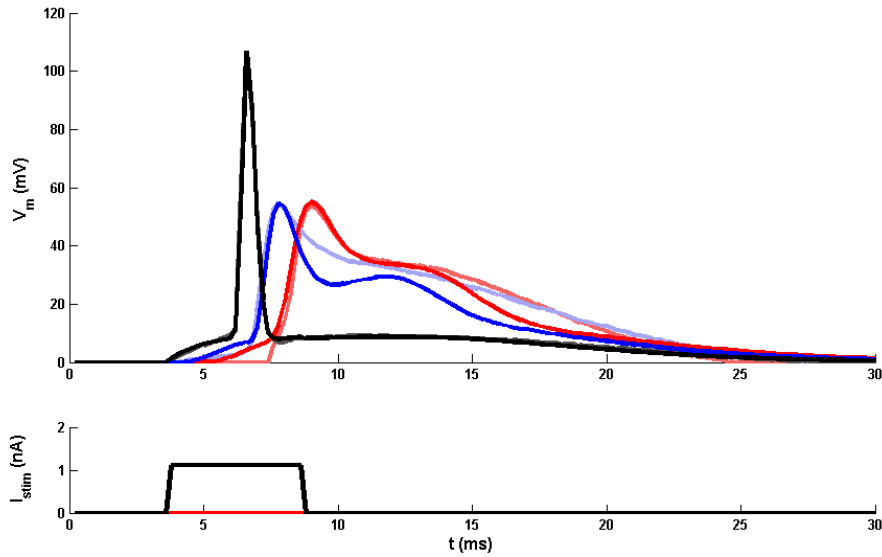


Figure 4.3: **Neuron model: threshold current injection at the soma.** Step current injection at the soma evokes an action potential that backpropagates into the distal dendrite. The backpropagating potential is reduced in amplitude but increased in width in the dendrite. *Experimental results to which the model was fit are displayed in faded colors that match those of their corresponding model signals*

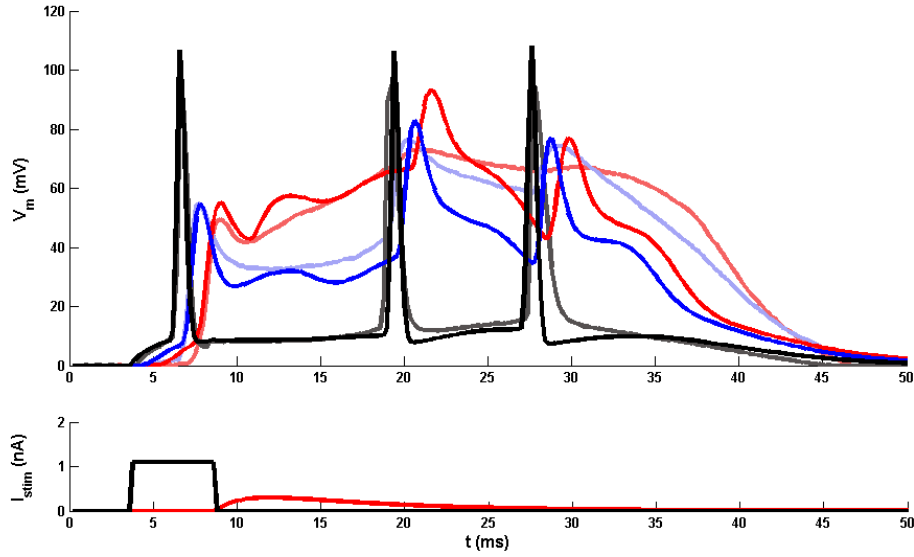


Figure 4.4: **Neuron model: BAC firing.** Threshold step current injection at the soma coupled with subthreshold EPSP current injection at the distal dendrite (0.3 nA) give rise to a burst of action potentials at the soma following the onset of Ca^{2+} -initiated spike at the level of the dendrite. *Experimental results to which the model was fit are displayed in faded colors that match those of their corresponding model signals*

displayed in figures 4.2, 4.3, 4.4 and 4.5. Also in these figures are displayed in faded colors the experimental results to which the model was fit.

4.2.1 Parameter fitting

Parameter fitting was achieved using a modification of the Least Squares method. The Least Squares method is a mathematical procedure for finding the best-fitting curve to a given set of points by minimizing the sum of the squares of the residuals. The residuals vector R is the difference between the observations vector X and the model vector Y .

$$\|r\|^2 = \sum_1^m r_i^2 \quad (4.1)$$

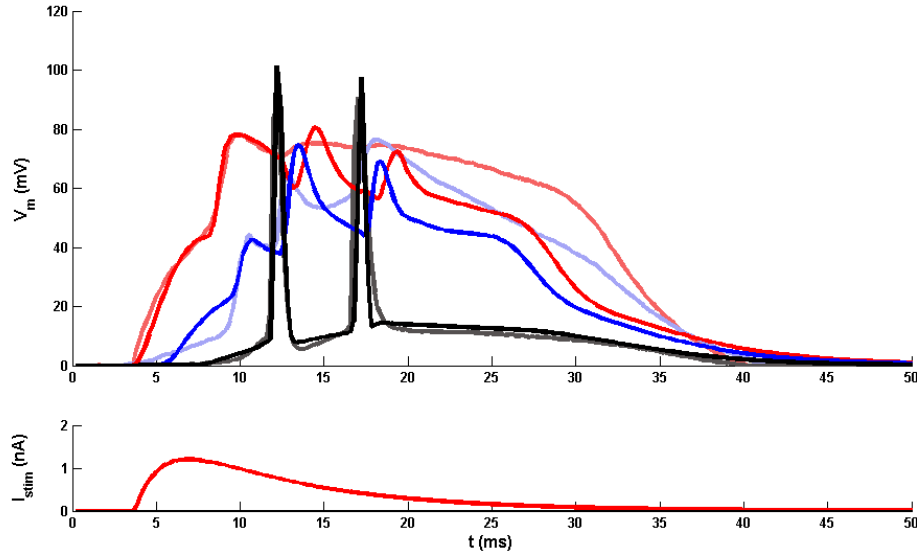


Figure 4.5: **Neuron model: threshold current injection at the distal dendrite.** Current injection in the form of EPSP with maximum amplitude of 1.2 nA at the distal dendrite evoked a Ca^{2+} dendritic spike and consequently a burst of action potentials at the level of the soma. *Experimental results to which the model was fit are displayed in faded colors that match those of their corresponding model signals*

where r refers to elements in the residuals vector and m is the number of observations.

The Least Squares method thus finds coefficients x that solve the problem

$$\min_x \| F(x, xdata) - ydata \|^2 = \min_x \sum_i (F(x, xdata_i) - ydata_i)^2 \quad (4.2)$$

where $xdata$ is the input data, $ydata$ is the observed output data, and $F(x, xdata)$ a vector-valued function of the same size as $ydata$. Lower and upper bounds were used for x in order to limit the search space.

We used a weighted version of the Least Squares method in which we selected important points along the data curve that we need to fit to and assigned them different weights. In other words, we scaled specific observations and model vectors of our choice. As an

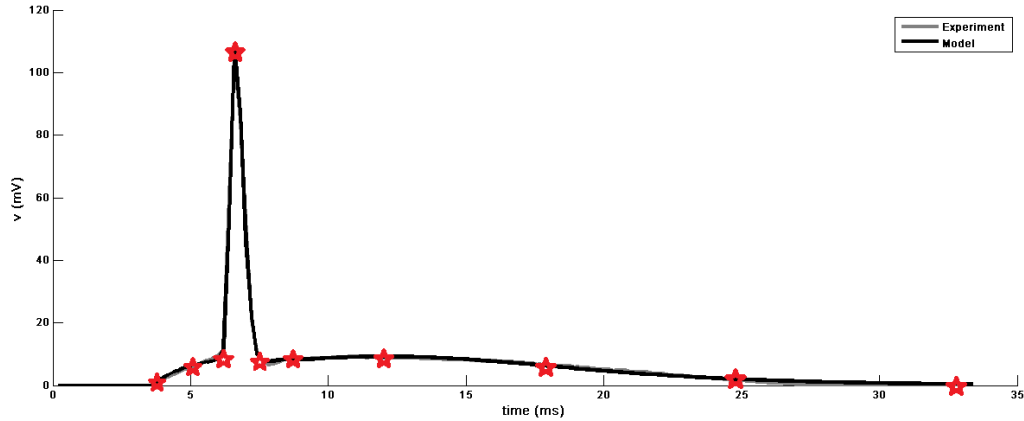


Figure 4.6: Points selected for weighted Least Squares method.

Parameters for the somatic voltage were found using the Least Squares method on the selected points of the model and observations vectors.

example, we refer to the voltage at the level of the soma in figure 4.9, redisplayed here in figure 4.6 with specification of the points selected and weighted.

The weighted method reduces the number of residuals that need to be minimized and thus can result in a much faster and most of the times a more accurate generation of parameters.

4.3 Threshold-initiation units: the soma and the spine

The spatially extended pyramidal neuron model comprises two threshold initiation units: the spine and the soma. We modeled both units as variations of the LIF point neuron model. While the LIF model captures the main nonlinear dynamics of the neuron's soma (action potential), it requires modifications in subthreshold voltage integration, action potential generation and shape as well as afterdepolarization in order to provide the correct fit to the somatic voltage in the layer V cortical pyramidal neuron studied by Larkum et al. 1999a. Thus the need to alter the simple LIF model in a way that ensures the right fit to experimental findings. Prior to detailing the dendritic model and equations, we give a brief overview of the basic integrate-and-fire neuron model.

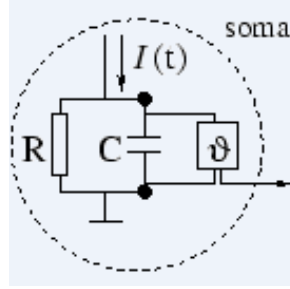


Figure 4.7: **Schematic diagram of the integrate and fire neuron model.** A current $I(t)$ charges the RC circuit. Once the voltage across the capacitances reaches a threshold voltage v , an output pulse $\delta(t - t_{AP})$ is generated. *modified from EPFL Gerstner*

4.3.1 The leaky integrate-and-fire neuron

The properties of the leaky integrate-and-fire (LIF) neuron model have been investigated since 1907. At that time, not much was known about actual spike generation in neurons (Arbib, 1995; Koch, 1999). The LIF model has become a simplified standard approximation to the complex behavior exhibited by real action potential generating neurons (Partridge, 1966). We decided to use a variation of the LIF model because it is a very simple model that offers a good approximation to the behavior of many neuron types, is far more realistic than rate neurons as it includes the non-linearity observed in neurons and it has been shown to be a limiting case of more complex conductance models such as the Hodgkin-Huxley neuron model.

In its standard form, the LIF neuron has two behavioral regimes: sub-threshold and super-threshold. Once the membrane voltage reaches a threshold value, an intrinsic property of the neuron, the super-threshold mode is initiated and the neuron generates an all-or-none action potential that is about $1 - 2\text{ ms}$ in width. As is the case in real neurons, an absolute refractory period forces the LIF model voltage to drop to a reset value a short time after an action potential is generated. The sub-threshold leaky integration of the model is produced by a simple passive resistance-capacitance (RC) circuit with elements having physiological correlates.

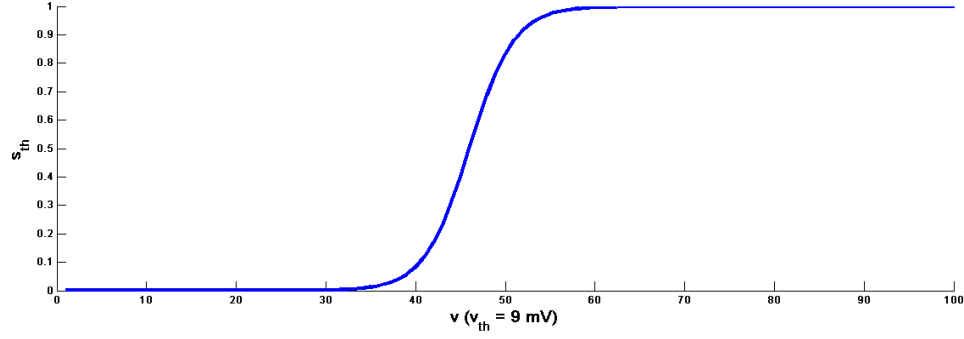


Figure 4.8: **Sigmoid function $s_{th}(v)$ where v_{th} is the inflection point.**

4.3.2 Avoiding discontinuities

Both the spine and the soma are sites for spike generation. Once the membrane potential reaches its threshold value (v_{th} for the somatic AP or u_{th} for the dendritic spike), an all-or-none action potential is initiated. We avoid the discontinuity that accompanies this threshold-caused peak and reset in order to gain a better control over the shape of the action potential initiation and reset. This discontinuity may also complicate the use of some parameter estimation methods that may be needed to estimate and fit parameters to experimental finding. A prominent method here is the Dynamical State and Parameter Estimation (DSPE) method by Abarbanel et al. 2009. There lies the main reason behind the use of the sigmoid function s .

$$s(a, b) \equiv s = \frac{1}{1 + \exp\left(\frac{-(a-b)}{c}\right)};$$

where c denotes the steepness of the ascent of the sigmoid when a is in the vicinity of b . c is set to 0.1 in what follows except when otherwise specified.

As an example $s_{sth} = s(v, v_{th})$ is 0 when the somatic voltage v is much lower than the threshold voltage v_{th} and $s(v, v_{th})$ is 1 when v reaches its apex value in the case of an AP. However s_{sth} takes on values between 0 and 1 in the vicinity of v_{th} as depicted in figure 4.8.

4.3.3 Soma and spine model equations

The soma and the spine are both implemented as threshold-initiating units and thus as variations of the LIF neuron model.

In what follows, we denote by A the initial value of a .

At the level of the soma

In order to achieve the right fit to the experimental findings in figure 4.1, we set the membrane capacitance and resistance to be functions of the membrane voltage. This alteration can reflect the changes that occur in the somatic membrane as a result of the change in membrane potential and ion transfer in and out of the membrane through voltage activated channels. This voltage-dependence entailed the altering of the traditional LIF neuron model by adding 3 state variables α_s , β_s , and γ_s as detailed below.

State variables

The model of the soma is composed of a total of seven state variables (excluding the sigmoid functions that are added only to ensure continuity); these variables are:

$v(t)$ represents the soma membrane voltage (mV),

I_s represents the current at the level of the soma (nA),

$v_{th}(v, t)$ represents the threshold voltage (mV),

$\alpha_s(v, t)$ refers to the voltage-dependent value of the membrane capacitance (nF^{-1}),

$\beta_s(v, t)$ refers to the voltage-dependent value of the membrane time constant (ms^{-1}),

$\gamma_s(v, t)$ represents the voltage-dependent factor that drives the action potential (mV/ms),

τ_{γ_s} is the voltage-dependent time constant of $\gamma_s(v, t)$.

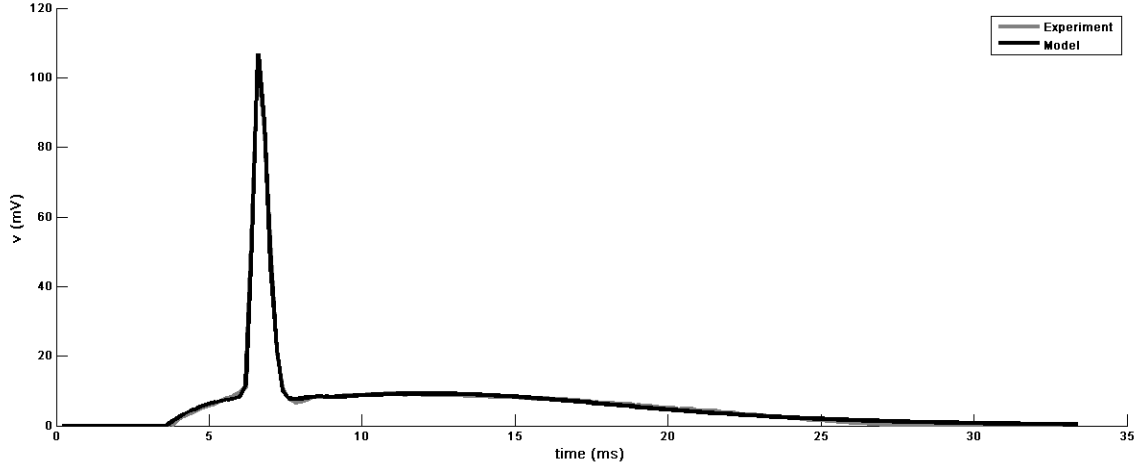


Figure 4.9: **Somatic action potential.** Action potential generated in response to threshold current injection at the soma. Data in gray is extracted from experimental findings in Larkum et al. 1999a and data in black reflect the result of the model.

Parameters

The model parameters are:

- C_s representing the voltage-independent value of the membrane capacitance (nF),
- τ_s representing the voltage-independent value of the somatic membrane time constant (ms),
- V referring to the reset membrane voltage (mV),
- v_{AP} referring to the apex of the action potential (mV),
- τ_{sth} representing the time constant of the threshold voltage (ms),
- p_{γ_s} which is a constant factor that helps ensure the correct shape of the action potential,
- τ_{α_s} referring to the time constant of the α_s (ms),
- τ_{β_s} referring to the time constant of the β_s (ms),
- g representing a factor for the action potential increase.

Equations

The somatic voltage, $v(t)$, is updated at each time step according to equation 4.3

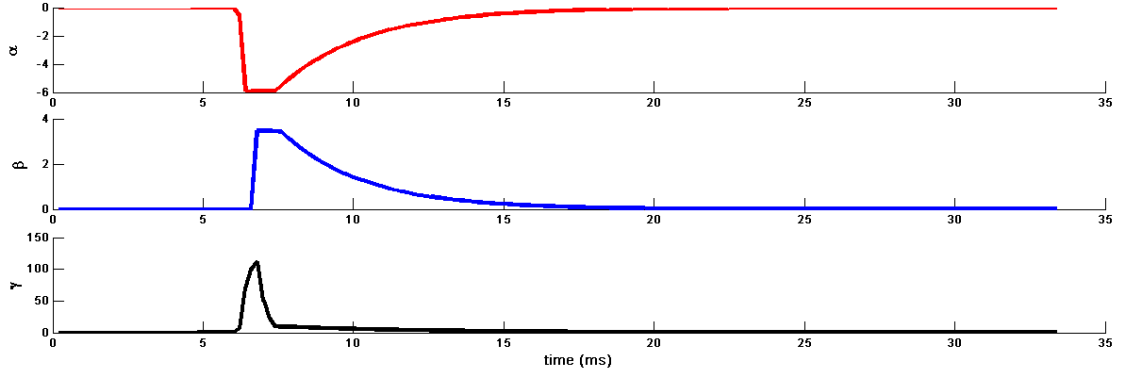


Figure 4.10: **Somatic voltage-dependent variables.** α_s , β_s , and γ_s values corresponding to the somatic action potential in figure 4.9

$$\frac{dv}{dt} = (\alpha_s + C_s^{-1})I_s + (\beta_s + \tau_s^{-1})(V - v) + p_{\gamma_s} \gamma_s; \quad (4.3)$$

Three voltage-dependent state variables are added to the soma model in order to avoid discontinuity.

$$s_{vth}(v) = s(v^{(t)}, v_{th}); \quad s_{ap}(v) = s(v^{(t)}, v_{AP}); \quad s_{\Delta v}(v) = s(v^{(t)}, v^{(t-1)}); \quad (4.4)$$

The voltage-dependent variables α_s , β_s and γ_s are depicted in figure 4.10. These variables, along with the parameter values listed in table 4.1, ensure an almost exact fit to the membrane voltage in 4.1(c) where a step current stimulates the pyramidal neuron soma. The result of the fit is displayed in figure 4.9 with the black curve relating to the model somatic voltage and the gray curve (almost coinciding with the black) relating to the experimental somatic voltage.

$$\alpha_s = -s_{vth} C_s^{-1} + (1 - s_{vth})\left(\alpha_s + dt \frac{-\alpha_s}{\tau_{\alpha_s}}\right); \quad (4.5)$$

α_s is the factor of integration that is added to the inverse of the membrane capacitance to get multiplied by the value of the somatic current I_s . α_s is constant as long as the membrane voltage does not cross its threshold value, the point at which α_s drops to annul the effect of the membrane capacitance and reflect the neuron's absolute refractory period where the factor of the current I is zero and consequently any current applied to the neuron during its all-or-none action potential does not affect the neuron's voltage. α_s recovers with time constant τ_{α_s} in order to reach its initial $pre - v_{threshold}$ value.

$$\frac{d\beta_s}{dt} = s_{ap} \beta_s - \frac{\beta_s}{\tau_{\beta_s}}; \quad (4.6)$$

β_s is the factor of hyperpolarization and behaves similar to α_s except that β_s increases when the membrane voltage reaches its peak (AP maximum value) and resets according to time constant τ_{β_s} .

$$\frac{d\gamma_s}{dt} = s_{vth} s_{\Delta v} g - \frac{\gamma_s}{\tau_{\gamma_s}}; \quad (4.7)$$

where

$$\tau_{\gamma_s} = (1 - s_{vth}) T_{\gamma_s} + s_{vth} T_{\gamma_{vth}}; \quad (4.8)$$

γ_s is the factor for the action potential where γ_s deviates from its initial value once the membrane voltage crosses its threshold. γ_s ensures that afterdepolarization is accounted for as its decay time constant τ_{γ_s} is also voltage-dependent and decays slowly once the decreasing membrane voltage (hyperpolarization) crosses its threshold value.

The neuron's intrinsic mechanism, namely its threshold voltage gets updated as a result of firing an action potential. More precisely, v_{th} is increased by a constant factor f_{vth} once the membrane voltage crosses its value and then decays back to its original value

according to time constant τ_{vth} . This update in the neuron's threshold voltage ensures the control of its firing and bursting.

$$\frac{dv_{th}}{dt} = f_{vth} s_{ap} + \frac{V_{th} - v_{th}}{\tau_{vth}}; \quad (4.9)$$

Table 4.1: Parameter values at the level of the soma

Parameter	Value
C_s^{-1}	5.9055
τ_s^{-1}	0.6467
V	0
p_{γ_s}	3.0374
τ_{α_s}	3
τ_{β_s}	2.8141
τ_{γ_s}	4.5
g	339
T_{γ_s}	4.5
$T_{\gamma_{sth}}$	0.3619
f_{vth}	19
V_{th}	8.4662
τ_{sth}	22

At the level of the spine

Same as with the soma model, we set the spine membrane capacitance and resistance to be functions of the membrane voltage. This voltage-dependence entailed the altering of the traditional LIF neuron model by adding 3 state variables α_d , τ_d , and γ_d and ensured a good fit to the experimental results represented in part (e) of figure 4.1. The fit achieved is reflected in figure 4.5 where the red curve (time 0 to 12 ms preceding the soma action potential as backpropagation of the latter affects the spine membrane voltage) almost coincides with the experimental one that is represented with fading red.

State variables

The model of the spine is composed of a total of seven state variables (excluding the sigmoid functions that are added only to ensure continuity); these variables are:

$u(t)$ represents the soma membrane voltage (mV),

$EPSC$ represents the excitatory postsynaptic current at the level of the spine (nA),

$u_{th}(u)$ represents the threshold voltage (mV),

$\alpha_d(u)$ refers to the voltage-dependent value of the membrane capacitance (nF^{-1}),

$\tau_d(u)$ refers to the voltage-dependent value of the membrane time constant (ms),

$\gamma_d(u)$ represents the voltage-dependent factor that drives the action potential (mV/ms),

τ_{γ_d} is the voltage-dependent time constant of $\gamma_d(u)$.

Parameters

The model parameters are:

C_d representing the voltage-independent value of the membrane capacitance (nF),

U referring to the reset membrane voltage (mV),

u_{DP} referring to the apex of the dendritic spike (mV),

τ_{dth} representing the time constant of the threshold voltage (ms),

b_th representing the threshold value of the backpropagating action potential (mV),

p_{γ_d} which is a constant factor that helps ensure the correct shape of the dendritic spike,

τ_{α_d} referring to the time constant of α_d (ms),

T_d^{-1} referring the initial value of τ_{dth} (ms^{-1}),

τ_{udecay} representing the time constant of τ_{dth} (ms),

$\overline{\gamma_1}$ and $\overline{\gamma_2}$ affecting the rise and decay times of the dendritic spike, respectively,

$\overline{\tau_{\gamma_1}}$ and $\overline{\tau_{\gamma_2}}$ affecting the time constant factor of γ_d ,

$\tau_{\tau_{\gamma_d}}$ representing the time constant of τ_{γ_d} (ms).

Equations

The spine voltage, $u(t)$, is updated at each time step according to equation 4.10

$$\frac{du}{dt} = (\alpha_d + C_d^{-1})EPSC + \tau_d^{-1}(U - u) + p_{\gamma_d} \gamma_d; \quad (4.10)$$

Four voltage-dependent state variables are added to the spine model in order to avoid discontinuity.

$$s_{uth}(u) = s(u^{(t)}, u_{th}); \quad s_{dp}(u) = s(u^{(t)}, u_{DP}); \quad (4.11)$$

$$s_{\Delta u}(u) = s(u^{(t)}, u^{(t-1)}); \quad s_b(b) = s(b^{(t)}, b_{th}); \quad (4.12)$$

$$\alpha_d = -s_{dth} s_{\Delta u} C_d^{-1} + (1 - s_{uth} s_{\Delta u}) \left(\alpha_d + dt \frac{-\alpha_d}{\tau_{\alpha_d}} \right); \quad (4.13)$$

α_d is the factor of integration that is added to the inverse of the membrane capacitance to get multiplied by the value of the postsynaptic current $EPSC$. α_d is constant as long as the membrane voltage does not cross its threshold value, the point at which α_d drops to annul the effect of the membrane capacitance and reflect the neuron's absolute refractory period where the factor of the current is zero and consequently any current applied to the neuron during its all-or-none action potential does not affect the neuron's voltage. α_d recovers with time constant τ_{α_d} in order to reach its initial value.

$$\frac{d\tau_d^{-1}(u)}{dt} = s_{uth} (s_{dp} + s_{\Delta u}) s_b \frac{b}{b_{max}} + \frac{\tau_d^{-1} - \tau_d^{-1}}{\tau_{udecay}}; \quad (4.14)$$

τ_d is the voltage-dependent variable that ensures the leak and hyperpolarization of the spine voltage.

$$\frac{d\gamma_d}{dt} = s_{dth} s_{\Delta} (1 - s_{dp}) \overline{\gamma_1} (1 - (s_b - \frac{v_b}{b_{BAC}})) - s_{dth} s_{\Delta} s_{dp} \overline{\gamma_2} - \frac{\gamma_d}{\tau_{\gamma_d}}; \quad (4.15)$$

γ_d is the factor for the dendritic spike where γ_d deviates from its initial value once the membrane voltage crosses its threshold u_{th} . The dendritic spike rise time is different from its decay time and thus two factors are used to account for this: $\overline{\gamma_1}$ and $\overline{\gamma_2}$. In addition, the dendritic spike rise time and value are affected by the backpropagating action potential.

$$\frac{d\tau_{\gamma_d}}{dt} = -s_{th} s_{\Delta} (1 - s_{dp}) \overline{\tau_{\gamma_1}} + s_{dth} s_{\Delta} s_{dp} \overline{\tau_{\gamma_2}} + \frac{T_{\gamma_d} - \tau_{\gamma_d}}{\tau_{\tau_{\gamma_d}}}; \quad (4.16)$$

τ_{γ_d} is the voltage-dependent time constant of γ_d . By holding different values during the rise and decay of the dendritic spike, this time constant ensures a dendritic spike shape that fits the experimental findings.

The spine's intrinsic mechanism, namely its threshold voltage gets updated upon the occurrence of a dendritic spike. u_{th} is increased by a constant factor f_{uth} once the membrane voltage crosses its threshold value and then decays back to its original value according to time constant τ_{uth} .

$$\frac{du_{th}}{dt} = f_{th} s_{dp} + \frac{U_{th} - u_{th}}{\tau_{uth}}; \quad (4.17)$$

4.4 Neurotransmitter release and synaptic transmission: efficient implementation

While the neuron's soma and spine are modeled phenomenologically, neurotransmitter release and synaptic transmission are modeled using biophysically realistic equations. We

Table 4.2: Parameter values at the level of the spine

Parameter	Value
C_d^{-1}	29.0197
τ_d^{-1}	0.7292
U	0
p_γ	38.8587
τ_{α_d}	5
T_d^{-1}	0.7292
τ_{decay}	1
τ_{γ_d}	2.5
$\overline{\tau_{\gamma_1}}$	2.1
$\overline{\tau_{\gamma_2}}$	6
T_{γ_d}	2.5
$T_{\gamma_{dth}}$	10
$\overline{\gamma_1}$	3.65
$\overline{\gamma_2}$	0.25
b_{BAC}	55
f_{uth}	37
U_{th}	43.46
τ_{uth}	14

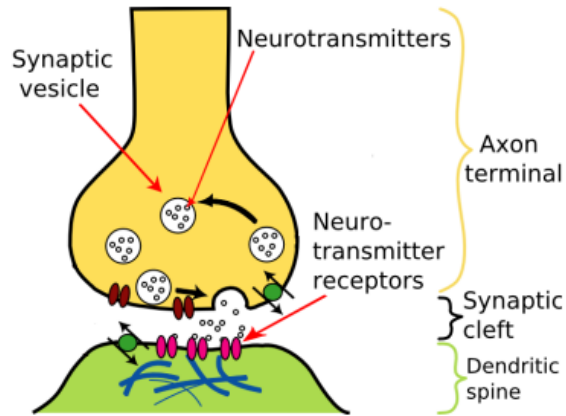


Figure 4.11: **Synaptic Cleft.** Neurotransmitter transmission from the axon terminal to the postsynaptic membrane. *Picture adopted from Julien 1997*

used the Law of Mass Action and receptor binding kinetics to achieve an efficient implementation, thus avoiding the use of time-varying conductance waveforms that are cumbersome in terms of calculation and memory usage.

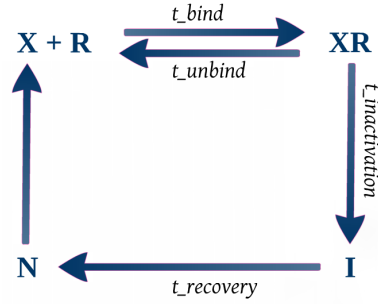


Figure 4.12: **Neurotransmitter release and receptor binding kinetics.**

4.4.1 Modeling receptor binding of neurotransmitters

As illustrated in figure 4.11, the arrival of an action potential at the presynaptic axon terminal triggers the release of neurotransmitter molecules into the synaptic cleft. Some - or all - of the molecules released bind to postsynaptic receptors located on the dendritic spine of the postsynaptic neuron. Receptor-bound neurotransmitters give rise to the postsynaptic current that has the form of an EPSC. The elements of this mechanism are described below according to the schema in figure 4.12.

We denote by:

- N neurotransmitters present in the presynaptic site and ready to be released (assumed to be equal to the recovered neurotransmitters) at any point in time,
- X neurotransmitters present in the synaptic cleft at any point in time and ready to be bound to postsynaptic receptors,
- XR neurotransmitters that are bound to postsynaptic receptors,
- I inactive neurotransmitters (XR upon unbinding from the postsynaptic receptors and while awaiting activation in order to recover and become available for transmission - N - in response to a presynaptic action potential)

The equations of the kinetic model described here are

$$\frac{dN}{dt} = \frac{I}{\tau_{recovery}}; \quad (4.18)$$

$$\frac{dX}{dt} = -\frac{X}{\tau_{bind}} + U_{se} N \delta(t - t_{AP}); \quad (4.19)$$

where U_{se} is a parameter that denotes utilization of synaptic efficacy; Using different values for U_{se} , this model could reflect various possible biophysical mechanisms of synaptic depression - such as receptor desensitization (Destexhe et al., 1994) or depletion of synaptic vesicles - or facilitation (Tsodyks and Markram, 1997). In short, accounting for short term plasticity (STP) or what is referred to as paired-pulse facilitation (PPF) and paired-pulse depression (PPD).

$U_{se} = 1.7$ is used for synaptic depression and $U_{se} = 0.2$ for synaptic facilitation (Mongillo et al., 2008).

$$\frac{dXR}{dt} = \frac{X}{\tau_{bind}} - \frac{XR}{\tau_{unbind}}; \quad (4.20)$$

$$I = 1 - (N + X + XR); \quad (4.21)$$

A total of four state variables are used to model the release of neurotransmitters and their binding to postsynaptic receptors.

Table 4.3: Parameter values for the release and binding of neurotransmitters

Parameter	Value
τ_{bind}	2
τ_{unbind}	8
$\tau_{recovery}$	3
U_{se}	1.7
A_{se}	39

This implementation allows a biophysical representation of postsynaptic receptors binding kinetics that is fast to compute and accounts implicitly for short term plasticity as well as saturation and summation of multiple synaptic events. In addition, the current solution saves memory usage by eliminating the need to store long waveforms in memory arrays and summing them up with the occurrence of every action potential.

4.5 Site for propagation dynamics: the dendrite

Rich dendritic propagation dynamics and variable conduction delays modulate the neuron's firing activity and support a variety of coincidence-detection mechanisms. These mechanisms endow the neuron with a potentially rich repertoire of computational capabilities and are likely to be crucial for synaptic integration and plasticity. We describe in this section the model of the spread of the postsynaptic potential and backpropagation through the dendritic tree.

4.5.1 The passive cable theory

To begin the journey through dendritic modeling we will start with the theory of passive dendrites and modeling that is inspired by the pioneering work of Rall 1959; Wilfrid Rall modeled the dendrites as membrane cylinders connected to each others. The membrane of these cylindrical core conductors was assumed to be passive and current flow in these cylindrical membranes was described by the linear one-dimensional passive cable equation. The compartmental modeling approach is mathematically a finite-difference (discrete) approximation to the cable equation. Compartments are connected to each other via a longitudinal resistivity according to the topology of the tree. Differences in physical properties and potentials occur between compartments since these are assumed to be isopotential. Cable theory complemented by the compartmental modeling approach played an essential role in estimating dendritic parameters and in providing insights about the computational properties of dendrites.

4.5.2 Active forward propagation dynamics modeled using a variation of the passive cable theory

Rather than modeling dendrites as passive transmission cables, we altered the Rall equation to account for both passive and active dendritic propagation. The mathematical challenge was to keep the model of dendritic propagation simple while making a good fit to the rich dynamics depicted in the layer V pyramidal neuron as described in figure 4.1. With the goal of modeling the passive and active propagation dynamics efficiently, we decided to tackle the difference between passive and active forward propagation along the dendrite from an Electrical Engineering perspective. More precisely, we devised a capacitor added in parallel to the intracellular resistance as it figures out in the Rall cable schematic. The reason behind adding this capacitance (labeled C_i) is that in its limit, a capacitor can act as a short circuit with extremely high frequencies and an open circuit with extremely low frequencies. This translates to having the capacitor speed up the propagation of high frequency signals along the dendrite and delay low frequency signals along the same cable. This is indeed the output we wish to get with fast forward propagation of active frequency signals that are larger in amplitude and thinner in width when compared to passive frequency signals. We tested this approach by incorporating the intracellular capacitor into the different dendritic compartments as shown in figure 4.13 and conducting the mathematics to run the model and find the parameters that ensure the right fit to the forward propagation dynamics in the dendrite of the pyramidal neuron being modeled. We describe in what follows the equations and derivations done to achieve the needed results.

We assume that everywhere along the length of the cable, the potential depends only on the length variable and not on radial or angular variables, so that the cable can be viewed as one-dimensional. The cable is divided into a number of short pieces of isopotential membrane of length dx each. Two types of current exist, the transmembrane current and the axial current. The axial current has intracellular and extracellular components (denoted by subscripts i and e , respectively)

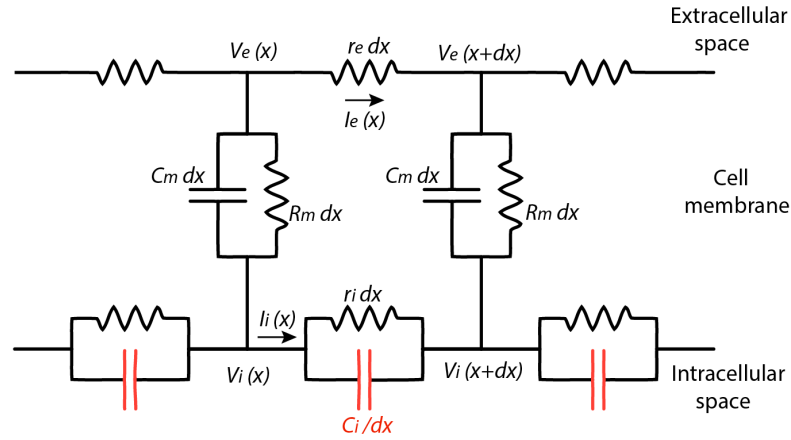


Figure 4.13: **Rall cable schematic modified.** Schematic diagram of a discretized cable, with isopotential circuit elements of length dx .

$$V_m = V_i - V_e; \quad (4.22)$$

V_m refers to the membrane potential (intracellular minus extracellular electric potential)

$$V_e(x + dx) - v_e(x) = -I_e(x) r_e dx; \quad (4.23)$$

$$I_e(x) = \frac{V_e(x + dx) - V_e(x)}{-r_e dx}; \quad (4.24)$$

$$I_i(x) = \frac{-C_i}{dx} \frac{d}{dt} (V_{i+1} - V_i) - \frac{V_{i+1} - V_i}{r_i dx}; \quad (4.25)$$

Kirchhoff's current law:

$$I_t = I_i - I_{i+1} = I_{e+1} - I_e; \quad (4.26)$$

Where I_t is the transmembrane current.

$$I_t = C_m \frac{dV_m}{dt} + \frac{V_m}{r_m}; \quad (4.27)$$

Where C_m and r_m denotes the membrane capacitance and resistance, respectively.

$$I_t = -\frac{\partial I_i}{\partial x} = -\frac{\partial}{\partial x} \left(-\frac{C_i}{\partial x} \frac{d^2 V_i}{dt} - \frac{1}{r_i} \frac{dV_i}{dx} \right); \quad (4.28)$$

$$I_i = \frac{1}{r_i} \frac{\partial^2 V_i}{\partial x^2} + C_i \frac{\partial^3 V_i}{\partial x^2 \partial t} = \frac{\partial^2 V_i}{\partial x^2} \left(\frac{1}{r_i} + C_i \frac{\partial V_i}{\partial t} \right); \quad (4.29)$$

$$C_m \frac{dV_m}{dt} + \frac{V_m}{r_m} = \frac{\partial^2 V_i}{\partial x^2} \left(\frac{1}{r_i} + C_i \frac{\partial V_i}{\partial t} \right); \quad (4.30)$$

Equation characterized by one temporal derivative and two spatial derivatives. It can be shown using Taylor's series that the left hand side of this equation can be expressed in terms of the differences between the value of V_i and the values in the adjacent compartments V_{i-1} and V_{i+1} .

$$\frac{\partial^2 V_i}{\partial x^2} = \frac{V_i - V_{i-1}}{\Delta x^2} + \frac{V_i + V_{i-1}}{\Delta x^2}; \quad (4.31)$$

We explicitly discretized, using Euler method, both space and time in order to reduce the system of equations to a linear algebra form. This discretization is called the finite difference method.

$$C_m \frac{\Delta V_i^{(t)}}{\Delta t} + \frac{V_i^{(t-1)}}{r_m} = \left(\frac{1}{r_i} + C_i \frac{\Delta V_i^{(t-1)}}{\Delta t} \right) \left(\frac{V_i - V_{i-1}}{\Delta x^2} + \frac{V_i + V_{i-1}}{\Delta x^2} \right)^{(t-1)}; \quad (4.32)$$

Here, the subscripts denote the spatial coordinates and the superscripts denote the time coordinates.

We ensured that the implementation of spatial propagation along the dendrite is vectorized in order to avoid looping through the different compartments composing the dendrite, thus increasing the efficiency of the model and reducing the overhead that may result from the inclusion of the spatially extended dendrite in the neuron model.

$$\frac{\Delta V_i^{(t)}}{\Delta t} = -\frac{V_i^{(t)}}{\tau_m} + \frac{1}{C_m} \left(\frac{1}{R_i} + \frac{1}{C_i} \frac{\Delta V_i^{(t-1)}}{\Delta t} \right) \frac{V_{i-1}^{(t-1)} - V_i^{(t-1)}}{\Delta x^2}; \quad (4.33)$$

where

$$\frac{\Delta V_i^{(t)}}{\Delta t} = \frac{V_i^{(t)} - V_i^{(t-1)}}{\Delta t}; \quad (4.34)$$

Rewriting equation 4.33 using the nomenclature defined previously,

$$\frac{du^{(t)}}{dt} = -\frac{u_i^{(t)}}{\tau_{fm}} + \frac{1}{C_{fm}} \left(\frac{1}{R_{fi}} + \frac{1}{C_{fi}} \frac{du_i^{(t-1)}}{dt} \right) \frac{u_{i-1}^{(t-1)} - u_i^{(t-1)}}{dx^2}; \quad (4.35)$$

Table 4.4: Parameter values for the passive and active forward propagation dynamics

Parameter	Value
τ_{fm}^{-1}	0.0914
C_{fm}^{-1}	5.2449
R_{fi}^{-1}	0.6556
C_{fi}	0.004

In conclusion, the addition of the intracellular capacitance C_{fi} enabled us to model active and passive forward propagation dynamics in the dendrite and consequently expose the significant effect of dendrites on the electrical behavior of the neuron.

4.5.3 Modeling backward dendritic propagation

Backward dendritic propagation was modeled using the same cable equation by Rall. This time C_i is set to 0 as signal propagation speeds are constant.

$$\frac{db^{(t)}}{dt} = -\frac{b_i^{(t)}}{(\tau_{bm}^{-1} + T_{bm}^{-1})} + \frac{1}{C_{bm}} \frac{1}{R_{bi}} \frac{b_{i+1}^{(t-1)} - b_i^{(t-1)}}{dx^2}; \quad (4.36)$$

Figures 4.4 and 4.5 reveal a dependence between the backpropagating action potential and the dendritic voltage. We account for this by updating the state variable τ_{bm} as follows:

$$\frac{d\tau_{bm}^{-1}}{dt} = s_{bp}\tau_{bm}^{-1} + \frac{T_{bm}^{-1} - \tau_{bm}^{-1}}{\tau_{\tau_{bm}}}; \quad (4.37)$$

where

$$s_{bp} = (u + b > b_{max}) (sth); \quad (4.38)$$

Table 4.5: Parameter values for the backpropagating action potential along the dendrite

Parameter	Value
C_{bm}^{-1}	2.1643
R_{bi}^{-1}	1.3237
T_{bm}^{-1}	0.001
$\tau_{\tau_{bm}}$	1

4.5.4 At the interface between the soma and dendrite

At the interface between the soma and dendrite lie two important factors: (1) the effect of the dendritic membrane potential on the somatic current as in equation 4.39 and (2) the initiation of the backpropagating action potential modeled using equation 4.40.

$$I_s = r_u^{-1}u; \quad (4.39)$$

Where r_u is the resistance that separates the end of the dendrite from the soma.

$$\frac{db^{(t)}}{dt} = -\frac{b_i^{(t)}}{\tau_{bms}} + \frac{1}{C_{bms}} \frac{1}{r_{bis}} \frac{v^{(t-1)} - b_i^{(t-1)}}{dx^2}; \quad (4.40)$$

The parameter values listed in table 4.6 ensure the widening and decrease in amplitude of the action potential upon reaching the dendrite-soma interface.

Table 4.6: Parameter values for backpropagation at the dendrite-soma interface

Parameter	Value
r_u^{-1}	0.08 (Ω^{-1})
τ_{bms}^{-1}	0.001 (ms^{-1})
C_{bms}^{-1}	2.1831 (nF^{-1})
R_{bis}^{-1}	1.1001 (Ω^{-1})
T_{bis}	3 (ms)
$\tau_{\tau_{bis}}$	0.5 (ms)

As shown in figure 4.3, the decay of the backpropagating action potential gets attenuated. We account for this attenuation by increasing the dendritic resistance (decrease in r_{bis}^{-1}) at the interface between the soma and the dendrite.

$$\frac{d\tau_{bis}}{dt} = f_{\tau_{bis}}\beta(v) + \frac{T_{bis} - \tau_{bis}}{\tau_{\tau_{bis}}}; \quad (4.41)$$

$$r_{bis}^{-1} = -f_{bis} \beta(v) + \frac{R_{bis}^{-1} - r_{bis}^{-1}}{\tau_{bis}}; \quad (4.42)$$

Two state variables are thus used to account for the decaying shape of the backpropagating action potential observed in figure 4.3.

4.5.5 At the interface between the spine and dendrite

The backpropagating action potential reaches the spine level and affects the EPSC according to the following equation:

$$EPSC = (1 - s_{bmax}) I_b; \quad (4.43)$$

where s_{bmax} is used to calculate the factor of $b^{(t)}$ that affects the EPSC.

$$s_{bmax} = s(b^{(t)}, 40); \quad (4.44)$$

Moreover, EPSC is also caused by receptor bound neurotransmitters and thus

$$EPSC = (1 - s_{bmax}) I_b + A_{se} XR; \quad (4.45)$$

where A_{se} is a parameter that refers to the absolute synaptic efficiency i.e. the amplitude of the first EPSC after a long silent period.

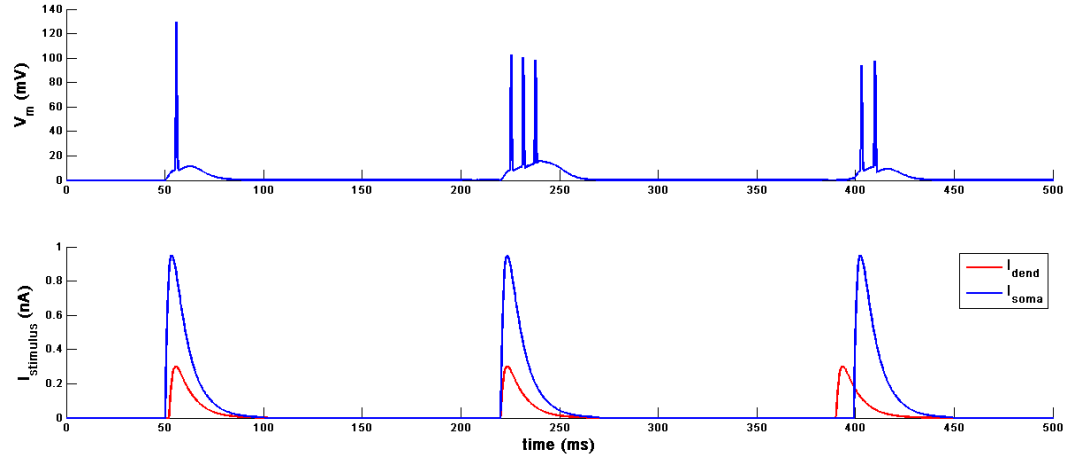


Figure 4.14: **BAC firing: precision of timing required.** Somatic and dendritic current injections separated by a time window that is less than 5 ms give rise to BAC firing which results in bursting at the soma. The somatic EPSC-shaped stimulus is shown in blue and the dendritic EPSC-shaped stimulus in red. $\Delta_t = t(I_{soma}) - t(I_{dend})$ is 8 ms for the first pair of stimuli, 0 ms for the second pair and -9 ms for the third.

4.6 BAC firing

We showed earlier how the combinations of subthreshold dendritic and threshold somatic current injections can give rise to a burst of action potentials at the level of the soma, a mechanism referred to as BAC firing. BAC firing is backpropagation activated Ca^{2+} spike firing. There exists an optimal time window between the EPSC-shaped dendritic stimulus and step-shaped somatic stimulus for BAC firing to occur. For the layer V cortical pyramidal neuron *in vitro* and with a dendritic stimulus that is at $700 \mu m$ from the soma, the window was found to be 3-7 ms (Larkum et al., 1999a). This proves that backpropagating action potentials alter the Ca^{2+} action potential threshold as shown in figure 3.5.

We tested BAC firing and timing precision in the pyramidal neuron model and obtained results that are in agreement with experimental finding whereby there exists a time difference between the dendritic and somatic stimuli that is critical for the initiation of BAC firing. The results are shown in figure 4.14.

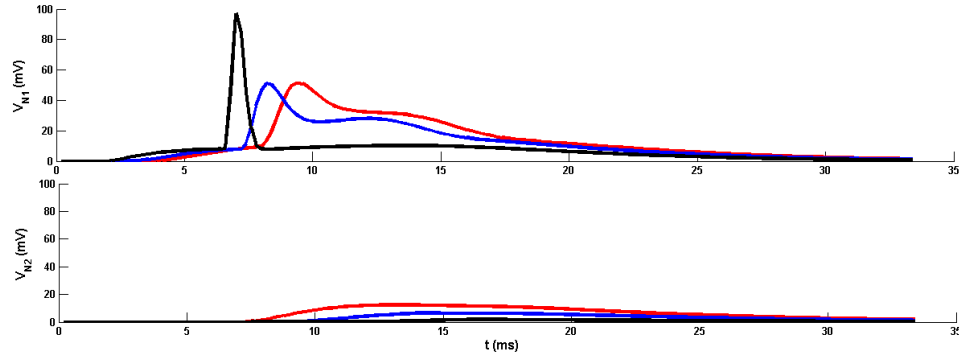


Figure 4.15: **Network of two dendritic neurons.** Neuron 1 is stimulated with a threshold step current and connects to the dendrite of neuron 2 with a weight equivalent to 0.3 nA at the synaptic site.

4.7 Sanity checks using small networks of dendritic and LIF neurons

As a first step towards implementing networks of dendritic and LIF neurons, we performed a sanity check using simple 2- and 3-neuron networks to ensure that all elements of our model are functioning correctly.

4.7.1 Dendritic SAAD neurons

We performed sanity checks on networks composed of two dendritic neurons connected in such a way that neuron 2, postsynaptic to neuron 1 which is in turn stimulated by external inputs, exhibits all different mechanisms depicted in 4.1. Results of these tests are shown in figures 4.15, 4.16 4.17, 4.18 and 4.20.

4.7.2 LIF neurons

We used the exclusive OR (XOR) neuron circuit in order to test the LIF neurons implemented. Results are displayed in figure 4.22;

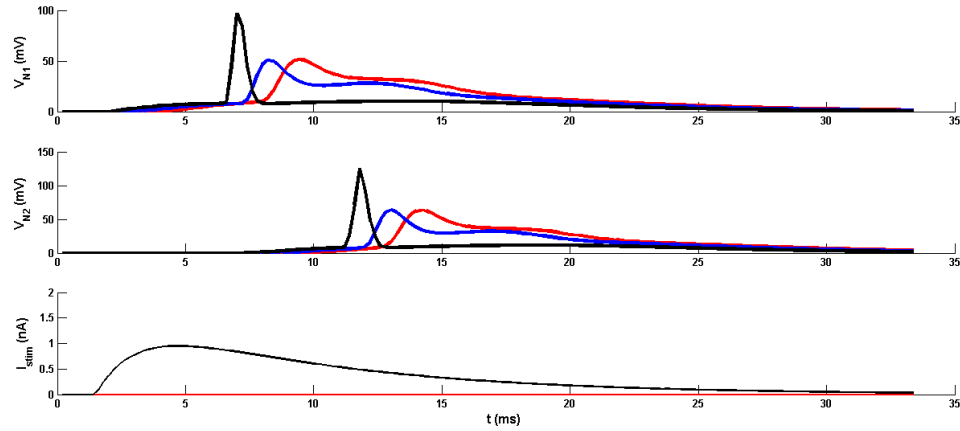


Figure 4.16: **Network of two dendritic neurons.** Neuron 1 is stimulated with a threshold step current and connects to the soma of neuron 2 with a weight equivalent to 1.1 nA at the synaptic site.

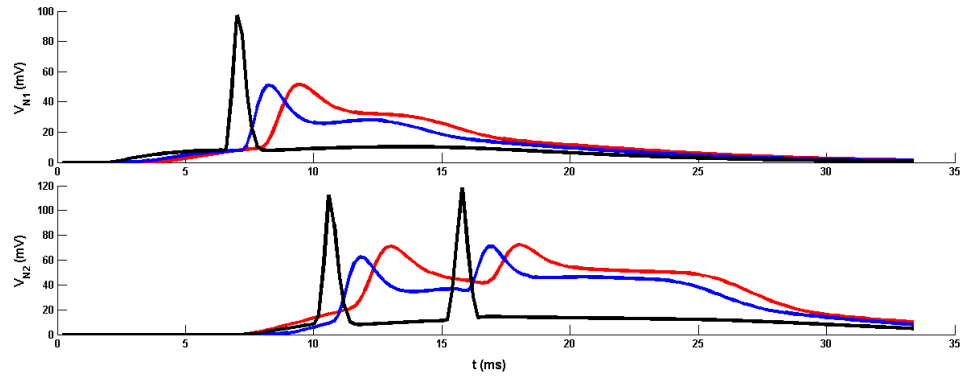


Figure 4.17: **Network of two dendritic neurons.** Neuron 1 is stimulated with a threshold step current and connects to both the dendrite and soma of neuron 2 with weights equivalent to 0.3 nA and 1.1 nA, respectively, at the synaptic sites.

4.8 The neuron model: Conclusion

In an effort to facilitate the study of pyramidal neurons in networks that undergo learning and memory, we devised a phenomenological model for a spatially extended neuron that comprises a spine, a soma and a site for nonlinear two-way propagation dynamics, a dendrite. The spine and soma are modeled as separate thresholding units with point-neuron-like behavior. The modeling of these two compartments is a modification of the LIF

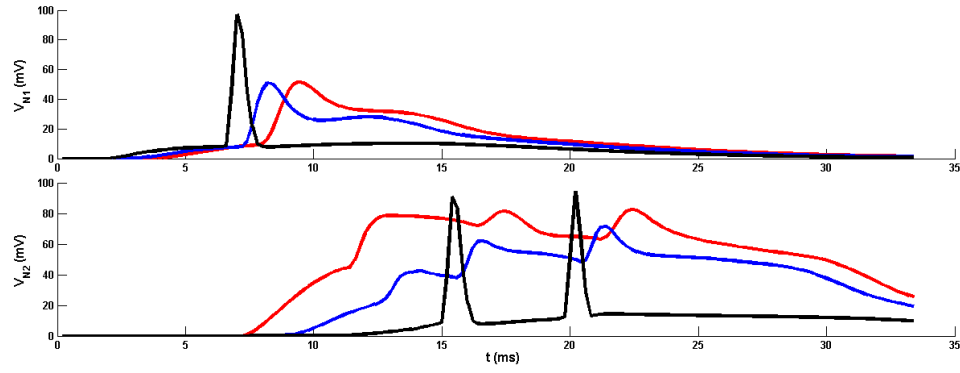


Figure 4.18: **Network of two dendritic neurons.** Neuron 1 is stimulated with a threshold step current and connects to the dendrite of neuron 2 with a weight equivalent to 1.2 nA at the synaptic site.



Figure 4.19: **Schematic for network of two dendritic neurons.** Neuron 1 is stimulated at its dendrite and soma and connects to the soma of neuron 2 with a weight equivalent to 1.1 nA at the synaptic site.

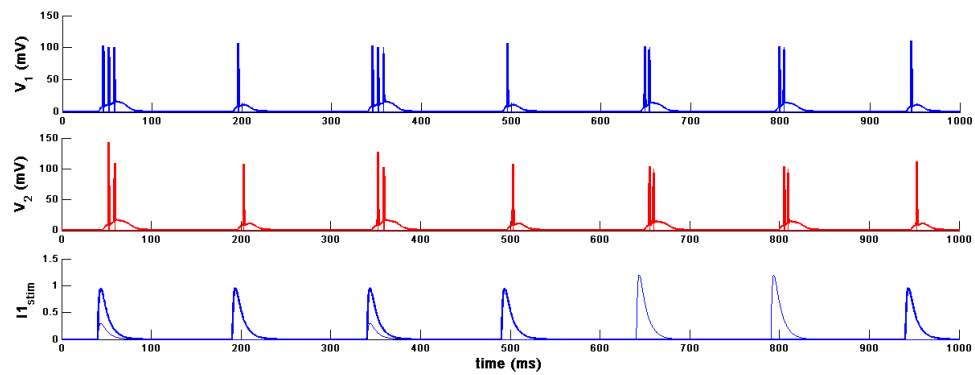


Figure 4.20: **Network of two dendritic neurons.** Neuron 1 is stimulated at its dendrite and soma and connects to the soma of neuron 2 with a weight equivalent to 1.1 nA at the synaptic site.

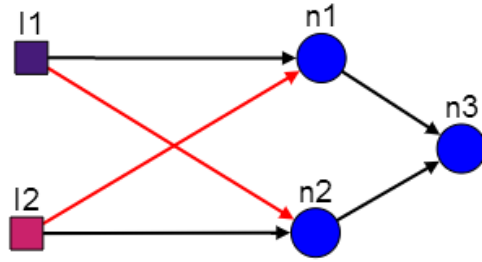


Figure 4.21: **Schematic for the XOR circuit.** Neurons 1 and 2 are stimulated with inputs I1 and I2 with the black connection lines signaling positive weights and the red connection lines signaling their opposites. Both neurons 1 and 2 are presynaptic to neuron 3 which displays the XOR output.

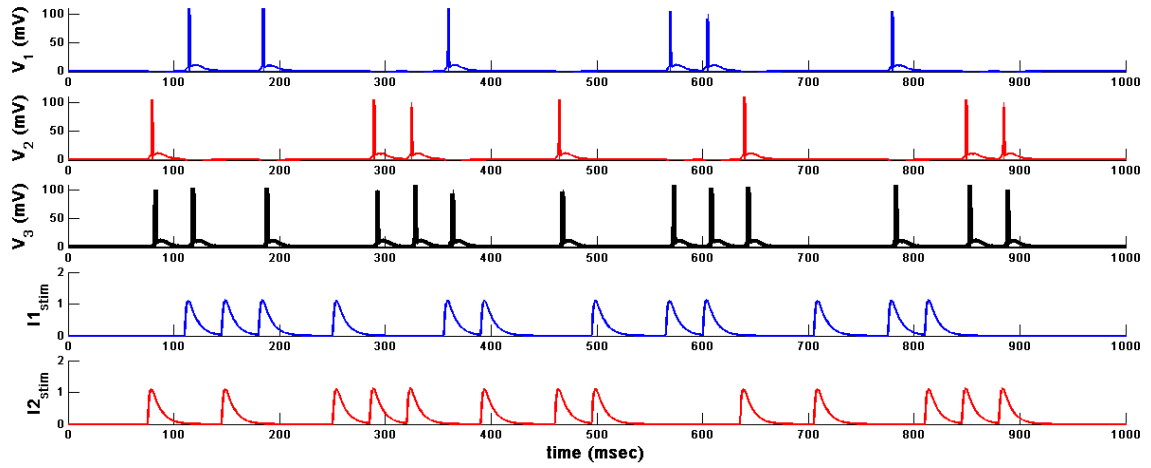


Figure 4.22: **XOR circuit example.** Simulation results for the XOR network.

neuron model whereby the resistive and capacitive components are voltage-dependent. Voltage threshold to initiate an action potential also varies with the voltage, thus the neuron's intrinsic properties are also accounted for in the model. The dendrite is modeled using a variation of Rall's compartmental model in order to capture dendritic passive and active forward propagation as well as backpropagating action potentials en route away from the soma and towards the dendrite. The model captures coincidence detection mechanisms as well as backpropagation-activated dendritic spikes (BAC firing) and exhibits dynamics that are in agreement with the pyramidal Layer V neuron described in Larkum et al. 1999a. Synaptic

transmission is implemented using biophysically realistic equations whereby an action potential reaching the presynaptic axon terminal results in a release of neurotransmitters into the synaptic cleft and the binding of these neurotransmitters to postsynaptic receptors give rise to an excitatory postsynaptic current (EPSC) that invades the postsynaptic neuron's dendrite. While the neuron we implemented is spatially extended and accounts for the multiple properties of pyramidal neurons as described, 29 is the total number of state variables used by the model; we strove to keep this number relatively low for such a detailed neuron model that captures experimental findings with high fidelity.

Chapter 5

Neural coding and Information processing

The question that we seek to answer here is: do dendrites constitute an extra biological detail or are they instead an inherent property for information processing enhancement in neurons?

Having implemented the extended dendritic neuron, also referred to as SAAD neuron, we are now in a position of understanding how these neurons process inputs and whether or not they represent an advantage over point neuron models. Processing inputs involves encoding and decoding assumed to be represented in neural spikes at the level of the neuron's soma or axon. We are at the point of studying the effect of dendritic nonlinear and active processing of synaptic inputs on the coding efficiency and information processing capability of the neuron. The question that we seek to answer is: Do dendrites constitute a biological detail that can be disregarded in studies of neuronal networks or are they instead an inherent and fundamental property for information processing?

We proceed to answer this question quantitatively using measures of information theory applied to the neural code. Information theory has provided a successful means to quantify information encoded and decoded by a communication system. We begin with an

outline of the neural code followed by a discussion of the basic measures of information theory and then we move on to describe the measures used to implement and quantify information in this thesis work.

5.1 Temporal representation in spiking neurons

Neurons in real nervous systems traffic in neural spikes. Neural spikes are fundamental properties of real neurobiological systems and they represent an unavoidable challenge for deciphering neural representation in its 'full-fledged' form. More precisely, neural spikes serve to encode and decode time-varying signals in populations of spiking neurons. Our goal is to describe and compare the representation of time-varying signals by spiking neurons, namely by leaky integrate-and-fire (LIF) neurons and dendritic Saad (SAAD) neurons.

5.2 Temporal codes in neurons

When addressing temporal representation or temporal coding in neurons, it is difficult to avoid the vigorous debate those who take the code to be a rate code (Shadlen and Newsome, 1994; Buracas et al., 1998) and those who take it to be a timing code (Softky and Koch, 1995; Rieke et al., 1997). Both rate and timing codes are clearly time-dependent codes.

5.2.1 Rate coding

A rate code is the one that takes the information about a stimulus to reside in the mean firing rate of a spike train over a relatively long time window (about 100 ms). There are however a wide variety of problems associated with the adoption of rate coding. First, there is experimental evidence that different input spike trains with the same mean firing rate, but different temporal structure, produce significantly different results from the same

neuron (Segundo et al., 1963). Second, most animals are embedded in highly dynamic environments. If these animals needed to integrate information over an extended period of 100 ms, they would have little chance to survive. In fact there is plenty of evidence that many behavioral decisions are made on the basis of one or two neural spikes which can be only a few milliseconds apart (Rieke et al., 1997). Third, rate coding cannot support information transmission rates observed in real neurons (Rieke et al., 1997) while evidence shows that timing codes can (MacKay and McCulloch, 1952). This being stated, there are many reasons to think that the neural code is not a mean rate code.

5.2.2 Time coding

Is the neuron code a timing code? The answer is not necessarily as it depends on what we mean by 'timing code'. There is evidence that the precise timing of spikes is not mandatory for the successful transmission of neural signals (Bialek et al., 1991). The standard timing code is one that takes spike train variability to encode information about the stimulus (MacKay and McCulloch, 1952). Variations in a single signal can be measured by taking the inverse of interspike intervals ($1/ISI$). The same stimulus can elicit different spike trains, thus the measure $1/ISI$ is often averaged over a number of trials. The averaged measure is sometimes called the 'instantaneous' rate code of the neuron (Buracas et al., 1998; Rieke et al., 1997). This measure is equivalent to a rate code with the window size approaching the limit of zero. Other timing codes exist such as the importance of the placement of spikes relative to the stimulus onset in carrying information about the stimulus as is the case of the slowing of firing rates given sustained, super-threshold input. This ubiquity of adaptation in excitatory cortical neurons suggest that this time coding is important.

5.2.3 Code used by the brain

The brain is likely to use different codes for different problems (Zador, 1998; Rieke et al., 1997). Perhaps rapid sensory processing uses a timing code and static sensory processing uses a rate code (Buracas et al., 1998). Given the methodology I adopted, I did not have to 'choose' a code, the 'appropriate' code is instead determined by the signals that are represented and the neuronal properties... The approach is general enough to characterize the kind of coding appropriate to the problem in hand ... This method thus transcends concerns about whether neurobiological systems use rate or timing codes. applies to spike trains with both short and long correlations times, unifying rate and timing codes.

In order to compare the behavior of LIF and SAAD neurons, we decided to use the information metric.

5.3 Information transmission

Because we are interested in characterizing representation, we need to specify a method for decoding the results of this type of encoding and for quantifying this decoding. We use Shannon's theory of mutual information to achieve this goal. There has been a large amount of attention given to the information theoretic properties of neural spike trains (Bialek et al., 1991; Miller et al., 1991; Koch, 1999; Stevens and Zador, 1996; Richmond and Optican, 1990; Bialek and Rieke, 1992). Chief amongst the measures of information processing is Shannon's mutual information theory. Given the success of this measure in providing insights into neural coding, I implemented this method with modest variations.

5.3.1 Mutual information

Information theory was first introduced by Claude Shannon to describe the transmission of information in a communication system as depicted schematically in figure 5.1

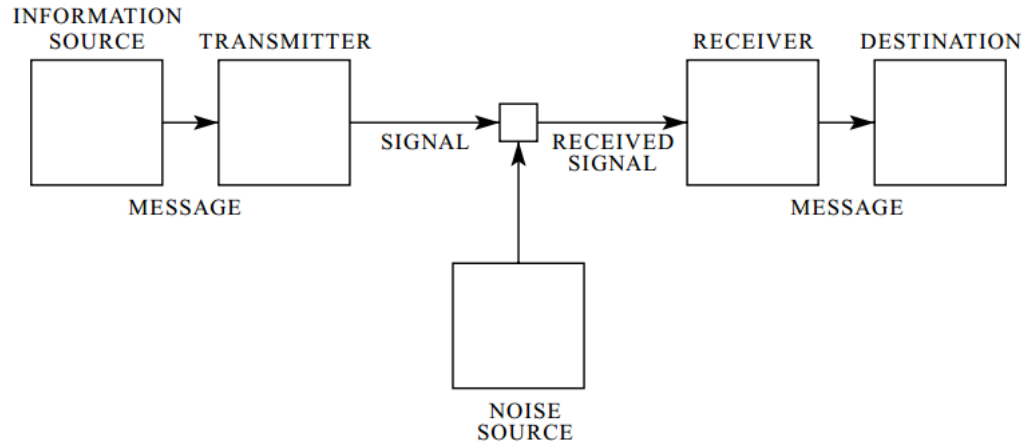


Figure 5.1: **Schematic diagram of a general communication system.**
Courtesy of Shannon 1948

(Shannon, 1948). A communication system consists of essentially five parts:

- (1) An *information source* which produces a message to be transmitted to the receiving terminal.
- (2) A *transmitter* which encodes the message to produce a signal suitable for transmission over the channel.
- (3) The *channel* which constitutes the medium used to transmit information from the transmitter to the receiver and is noisy and thus error prone.
- (4) The *receiver* that decodes and reconstructs the signal by performing the inverse of the operation done by the transmitter.
- (5) The *destination* to whom the message is intended.

Shannon's information theory attaches a number to the amount that can be learned about the world by observing certain signals. Information-theoretic quantities are formed by considering the specific messages x produced by the source as random variables X over $p(x)$, the probability distribution function (PDF) of the set of possible messages. While

information-theoretic quantities can be continuous or discrete, we introduce them in this work in their discrete form.

The fundamental quantity is the Shannon **entropy**, H_X , which represents the average uncertainty associated with any measurement x of a random variable X .

$$H_X = - \sum_x p(x) \log_2 p(x) \quad (5.1)$$

The choice of a logarithmic base corresponds to the choice of a unit for measuring information. We choose here base 2 for all information-theoretic calculations and thus the resulting units are called binary digits, or more briefly bits, a word suggested by J. W. Tukey. H_X quantifies the number of bits needed to encode the random variable X , and can thus be considered the information content of the particular message. This information metric can be interpreted as the level of diversity in the source (Prokopenko et al., 2009).

The **joint entropy** of two (or more) random variables X and Y , $H_{X,Y}$, is a generalization to quantify the uncertainty of the joint distribution of X and Y .

$$H_{X,Y} = - \sum_{x,y} p(x,y) \log_2 p(x,y) \quad (5.2)$$

The conditional entropy of X given Y , $H_{X/Y}$, is the average uncertainty that remains about x when y is known.

$$H_{X/Y} = - \sum_{x,y} p(x,y) \log_2 p(x/y) \quad (5.3)$$

$$p(x/y) = p(x,y)/p(y) \quad (5.4)$$

Thus the conditional entropy can be expressed in terms of the entropy of Y and joint entropy of (X,Y) as

$$H_{X/Y} = H_{X,Y} - H_Y \quad (5.5)$$

The **mutual information** between X and Y measures the average reduction in uncertainty about x that results from learning the value of y , or vice versa.

$$I_{X;Y} = - \sum_{x,y} p(x,y) \log_2 \frac{p(x,y)}{p(x)p(y)} \quad (5.6)$$

$$H_{X/Y} = H_X + H_Y - H_{X,Y} \quad (5.7)$$

$$I_{X;Y} = H_X - H_{X/Y} = H_Y - H_{Y/X} \quad (5.8)$$

The mutual information can be generalized to a set of more than two variables as the **multi-information** or **integration** (Tononi et al., 1994). The multi-information is a measure of the deviation from independence of the G components in the system $\mathbf{X} = X_1, X_2, \dots, X_G$

$$I_{\mathbf{X}} = I_{X_1;X_2;\dots;X_G} = \left(\sum_{g=1}^G H_{X_g} \right) - H_{X_1,X_2,\dots,X_G} \quad (5.9)$$

The **channel capacity** is the maximum amount of information that Y (received signal or output) can contain about X (transmitted signal through the communication channel or input). Thus channel capacity is defined as the maximum mutual information for the channel over all distributions of the transmitted signal

$$C(p(y/x)) = \max_{p(x)} I_{X;Y} \quad (5.10)$$

This definition renders channel capacity as asymmetric and causal (in contrast to mutual information). Channel capacity is a property of the channel itself rather than a property of the dynamics for a specific interaction over the channel. By finding the capacity, we obtain a measure that does characterize the stimulus-response relationship. This approach would seem to provide a measure of information processing capability.

5.3.2 Multivariate mutual information

Typically, mutual information is defined and studied between two variables. This is referred to as bivariate mutual information. However, sometimes intricate dependency exists between more than two variables and their relationship can only be deciphered if they are all considered at once.

As explained earlier, a single-input single-output channel with a discrete input X and output Y having entropies $H(X)$ and $H(Y)$ respectively, has the following amount of information transmitted between X and Y

$$I(X; Y) = H(X) + H(Y) - H(X, Y) \quad (5.11)$$

In the case where two sources U and V transmit information to a single output receiver Y , the mutual information in this two-way channel is written as

$$I(U, V; Y) = H(U, V) + H(Y) - H(U, V, Y) \quad (5.12)$$

One way to reduce the three-dimensional information to two dimensions is to take a weighted sum of the mutual information between U and Y for each value of V .

$$\begin{aligned}
I_v(U; Y) &= I(U; Y/V) = \sum p(v) I(U; Y/V = v) \\
&= I(U, V; Y) - I(V; Y) \\
&= (H(U, V) + H(Y) - H(U, V, Y)) - (H(V) + H(Y) - H(V, Y)) \\
&= H(U, V) - H(U, V, Y) - H(V) + H(V, Y)
\end{aligned} \tag{5.13}$$

The extension of mutual information to a general case of three or more variables was formulated by Fano (Fano, 1961). Fano computes the mutual information between an arbitrary number of events as follows

$$\begin{aligned}
I(X_1; X_2) &= H(X_1) - H(X_1/X_2) \\
&= I(X_1) - I(X_1/X_2)
\end{aligned} \tag{5.14}$$

Extending to a triple product ensemble

$$I(X_1; X_2; X_3) = I(X_1; X_2) - I(X_1; X_2/X_3) \tag{5.15}$$

Generalizing over N variables

$$I(X_1; X_2; \dots; X_N) = I(X_1; X_2; \dots; X_{N-1}) - I(X_1; X_2; \dots; X_{N-1}/X_N) \tag{5.16}$$

Nevertheless, in the case of the dendritic neuron, we decided to use a variation of the bivariate mutual information in order to calculate the mutual information between the neuron's somatic and dendritic inputs and its output. The following chapter details this approach and exposes its results.

Chapter 6

Mutual information

Knowing the neural code can unlock the “secrets” of how neurons work in concert to process and represent information.

6.1 Exploring the neural code

The neuron is the communication system that we focus on in this study. The neuron’s output membrane voltage encodes aspects of the input(s) it receives. In this work we consider the spike train of the neuron as its output for calculating the information that it conveys about its afferents. As can be seen from its definition (equation 5.8) mutual information depends on the input and output probabilities. Mutual information thus measures how different, in a statistical sense, the input and output (also referred to as stimulus and response, respectively) are. Mutual information is equal to zero if the response is uncorrelated or statistically independent of the stimulus.

If we consider the Layer V pyramidal neuron’s output patterns as described in Larkum et al. 1999a, we note the existence of separate all-or-none action potentials as well as output bursts at the level of the neuron’s soma in response to different sets of dendritic

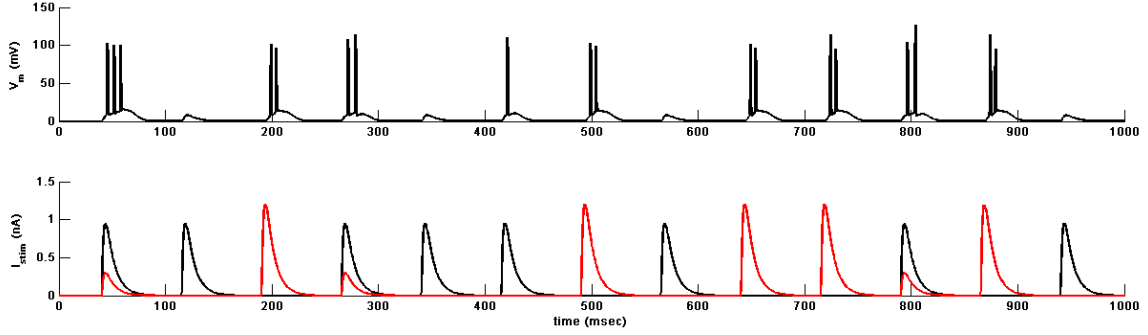


Figure 6.1: **SAAD neuron stimulated by coinciding dendritic and somatic inputs.** The somatic stimulus is represented in black and the dendritic stimulus in red. Modes of operation depicted are: BAC firing, subthreshold somatic activation, firing of a single action potential, and burst firing in response to superthreshold dendritic stimulus

and somatic stimuli. The existence of single spikes and bursts at the level of the neuron's output necessitates knowing when to count bursts and when to count single spikes in the calculation of the output probability distribution. To better clarify this point, we plot in 6.1 the different input/output combinations depicted in 4.1 and observe their correlations. This example is simple as the stimulus presentations are spaced sufficiently far apart in time (inter-stimulus interval = 75 ms) to prevent adaptation and sequential stimulus effects on the neuron's output. Another example would be to stimulate the dendritic neuron at its dendrite and soma by two separate sets of biexponential trains that follow a Poisson distribution of frequency equal to 10 Hz (figure 6.2). While the input/output correlations seemed more obvious in the case of figure 6.1, the same method is used to depict these correlations and calculate input and output probabilities and consequently quantify mutual information in this model of the dendritic neuron.

6.2 Finding correlated patterns of inputs and outputs

Discrete inputs and outputs are used to calculate the entropy and mutual information for the dendritic neuron. As such, the biexponentially shaped input trains are discretized using time steps of $dt = 0.2$ ms and such that the biexponential maximum amplitude is

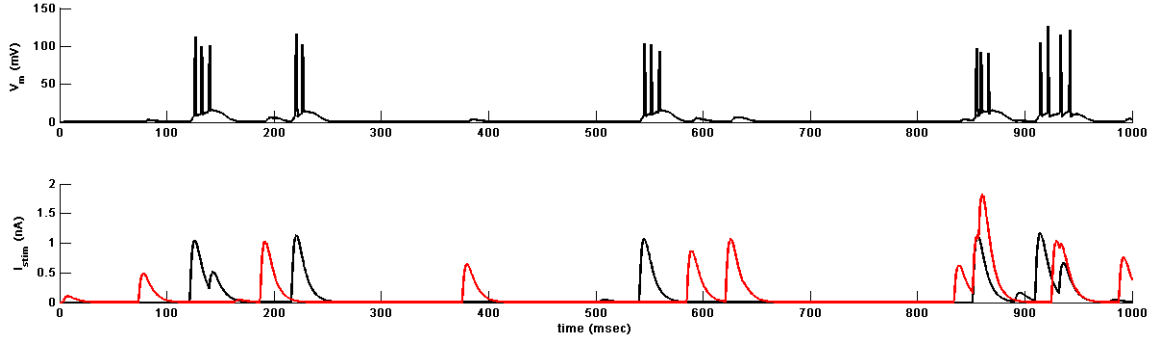


Figure 6.2: **SAAD neuron stimulated by Poisson distributed dendritic and somatic inputs.** Poisson stimuli are of frequency 10 Hz. The somatic stimulus is represented in black and the dendritic stimulus in red. Multiple modes of bursting are observed in response to the naturalistic stimuli.

represented by its value at the time of its occurrence while the rest of the curve is set to zero over the time steps that precede and succeed its peak. This leaves us with an input spike train with non-zero values representing the amplitude and time of occurrence of the spike's corresponding biexponential peak value. The outputs are action potentials as they occur at the neuron's axon terminal(s). We consider the axon terminal rather than the neuron's soma as the locus of output trains in order to account for the axon delays whenever these are positive.

In the case of the dendritic neuron, the input in the calculation of mutual information is the pair of dendritic and somatic stimuli, which leaves us with the case of a multivariate (three variables) mutual information case. The number of variables increases if we consider information calculation in a network of neurons undergoing the effect of multiple stimuli and having multiple output neurons. We devise a method for merging inputs and outputs, thus reducing the multivariate mutual information case to a bivariate case, thus ensuring easier computations.

Input-Output patterns

We devised a method whereby the pair of stimuli (dendritic and somatic) and its corresponding output signal are discretized using a dynamic time resolution that helps

preserve the input/output correlations in order to derive the corresponding entropy and mutual information.

Based on the dendritic neuron outputs obtained for varying input stimuli, we define bursts as sets of consecutive spikes with inter-spike intervals of 10 msec or less. This does not imply however that two consecutive spikes separated by 10 ms or less make necessarily part of a burst. Whether or not an output spike belongs to a burst is determined by the inter-spike interval coupled with its corresponding input. The same reasoning applies to input bursts. This being stated, the merging of inputs and outputs and their characterization as single signals or bursts of signals is done dynamically and gets refined to find the best input/output correlation set. This maximum correlation translates to an increase in the value of mutual information between these inputs and outputs.

Below is a detailed explanation of the merging and patterning method used in this thesis.

- (a) Each input represented by a biexponential curve is binned with a time resolution dt such that the maximum value of the biexponential is represented at the time of its occurrence while the rest of the curve is set to zero. Noise is accounted for by setting a 5% error range for the input amplitude. This ensures that inputs within this range are considered equal in the calculation of input probabilities and consequently of the mutual information.
- (b) Separate binned inputs (dendritic stimulus and somatic stimulus) are then merged into one transmitted signal. This merging is done in a way that preserves the values, time of occurrence and amplitudes of the stimuli. More precisely a dendritic stimulus of amplitude 0.3 nA that coincides with a somatic stimulus of amplitude 0.9 nA are not confused with a dendritic stimulus of amplitude 0.9 nA and a somatic stimulus of amplitude 0.3 nA.

- (c) The output is binned with the same time resolution ($dt = 0.2$ ms) and is envisioned as a spike train such that the presence of a spike is denoted by a 1 and its absence by a zero (at each dt).
- (d) In the case of more than one output signal, merging is done the same way as it is computed for the input sets. Similarly, the order and values of the signals are preserved.
- (e) Output and input bursts are then calculated such that consecutive signals that occur within 10 ms may constitute part of the burst. Based on the correlations between inputs and outputs, a confidence interval (0%-100%) is calculated for each input/output set to ensure that the highest confidence value is reached prior to calculating entropy and mutual information.
- (f) An error of 5% in the burst inter-spike intervals is accepted as it reflects noise that the neuron may be subject to as well as variation in the neuron's intrinsic properties.
- (g) Input and output patterns and confidence intervals are calculated by taking into consideration the patterns of repetition of the following values: input amplitude, inter-stimulus interval, input onset time, output amplitude, inter-spike intervals and number of output spikes within a burst.
- (h) The algorithm is repeated recursively in order to account for the best input/output correlation with confidence values recalculated and bursts reconsidered.

A simple example that illustrates this merging and patterning method is stated in Appendix A.

6.3 Mutual information measures in LIF and SAAD neurons

We calculated mutual information in single SAAD and LIF neurons as well as in networks of these neurons. Results are displayed and explained in what follows.

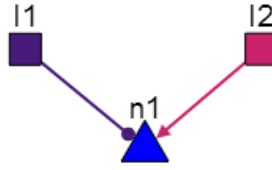


Figure 6.3: **Single SAAD neuron stimulated at the dendrite and soma.** I1 and I2 are dendritic and somatic stimuli, respectively. The pyramidal neuron is represented using a triangle.

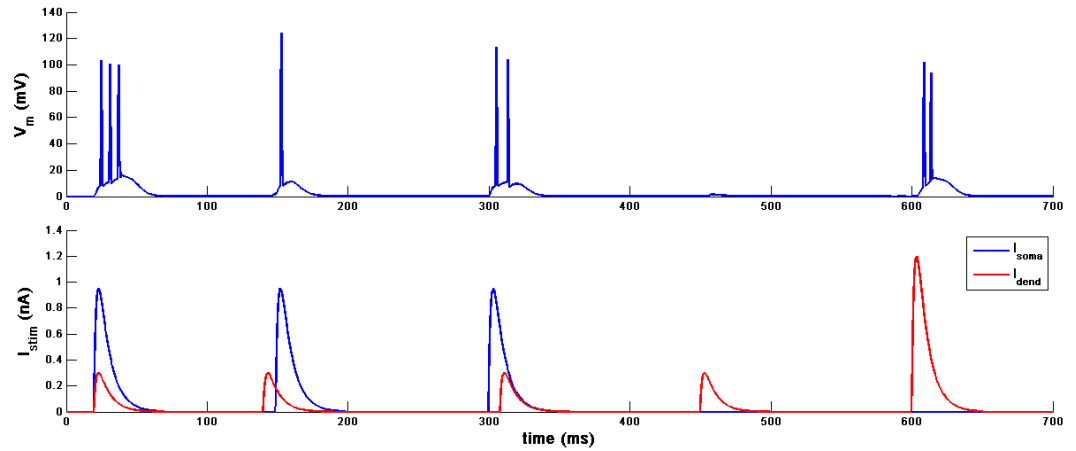


Figure 6.4: **Membrane voltage of SAAD neuron in response to somatic and dendritic stimuli.** Mutual information calculated to be **1.9219 bits** for this stimuli-voltage pair.

6.3.1 Mutual information in a single SAAD neuron and a single LIF neuron

Perhaps the simplest example that can highlight the advantage of the dendritic neuron (SAAD) over the point neuron (LIF) is the one in figure 6.4. The dendritic neuron is stimulated by a somatic and a dendritic stimulus as depicted in the schematic in figure 6.3. The simulation runtime is 700 ms. The pair of stimuli encloses the following five combinations: (1) coinciding somatic and dendritic stimuli which give rise to BAC firing and subsequently a 3-spike burst at the level of the soma. (2) and (3) The time difference between stimuli ($t_{Idend} - t_{Isoma}$) highlights the effect of BAC firing shown in the second and

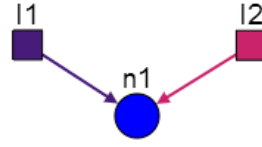


Figure 6.5: **Single LIF neuron stimulated using two external inputs.** I1 and I2 are somatic stimuli. The LIF point neuron is represented using a circle.

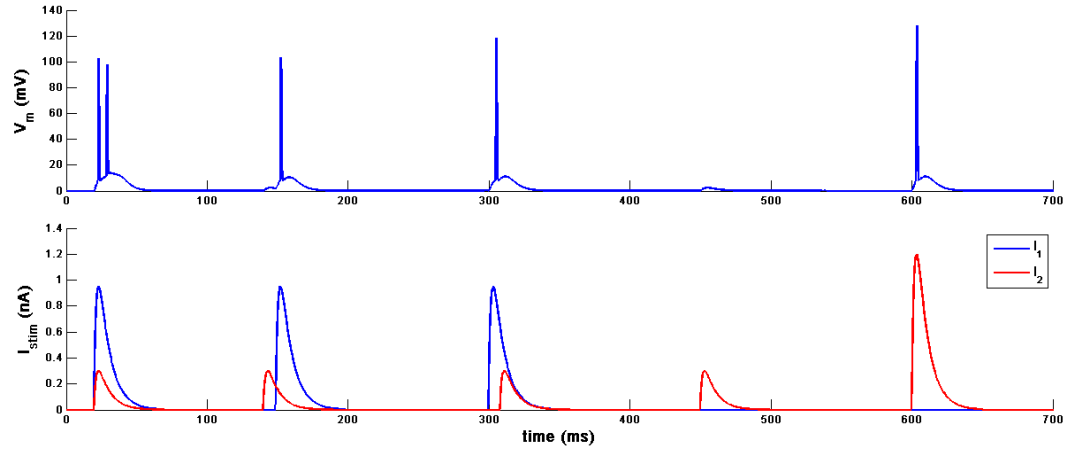


Figure 6.6: **Membrane voltage of LIF neuron in response to two stimuli.** A lower value for mutual information is calculated for the LIF neuron in response to the same stimuli for the SAAD neuron in figure 6.4. Mutual information is calculated to be **0.7219 bits**.

third set of stimuli. (4) Subthreshold dendritic current injection results in subthreshold (0.3 nA maximum amplitude of the EPSC-shaped stimulus) somatic activation. (5) A series of four consecutive (0.2 ms time difference) subthreshold dendritic stimuli (0.3 nA maximum amplitude of the EPSC-shaped stimulus) causes a somatic burst. The same stimulus pair is used as input to the LIF neuron as in figure 6.5. The output of the LIF neuron in response to these inputs is depicted in figure 6.6. It is clear that the LIF neuron cannot differentiate between all five inputs as is the case of the dendritic neuron. Mutual information is calculated to be 1.9219 bits for the SAAD neuron and 0.7219 bits for the LIF neuron.



Figure 6.7: **Network of two bidirectionally connected LIF neurons.**
Neurons n1 and n2 are stimulated by currents I1 and I2, respectively

6.3.2 Comparing mutual information in a network of two LIF neurons and a single dendritic neuron

We proceeded further to calculate mutual information in a network of two LIF neurons bidirectionally connected in order to compare the results with mutual information in one SAAD neuron.

The two-neuron LIF network is shown in figure 6.7 and may be considered ‘comparable’ to a dendritic SAAD neuron stimulated at its dendrite and soma. While we are not comparing similar units, the bidirectional connectivity in the LIF network may be comparable to the forward and backward propagation along the dendrite of the SAAD neuron. With the spine being considered a thresholding unit as the soma, the spine and soma of the SAAD neuron can be compared to two point neuron models. As depicted in figure 6.7, each LIF neuron is stimulated by a separate stimulus: Stimuli 1 and 2 can be considered the equivalents of the dendritic and somatic stimuli onto the dendritic neuron. In calculating the mutual information for the network of two LIF neurons, the input is the pair of stimuli onto neurons 1 and 2 and the output is that of neuron 2. Stimuli in this simulation are of Poisson type and have a frequency of 100 Hz. Amplitudes of the stimuli vary randomly between 0.3 nA and 1.2 nA.

Prior to calculating mutual information in the network of bidirectionally connected LIF neurons, we performed a test on different values of axonal delays (Δ_1 and Δ_2) and different values of synaptic connection weights (w_{12} and w_{21}) in order to determine the

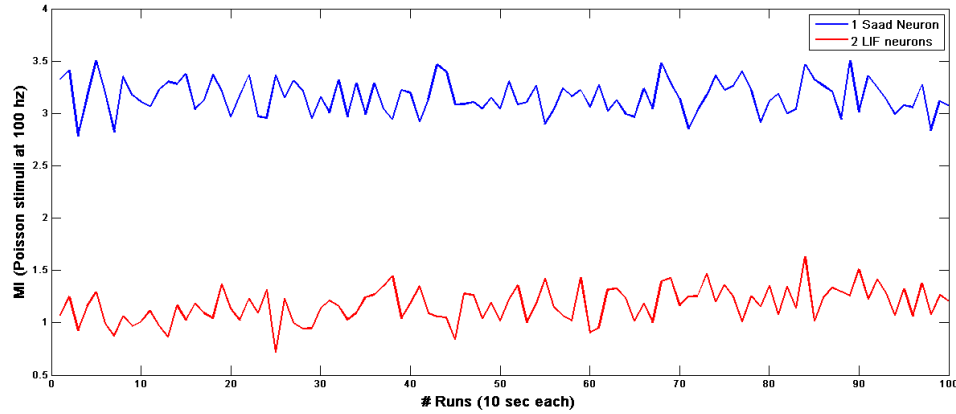


Figure 6.8: **Mutual information comparison in one SAAD neuron and two bidirectionally connected LIF neurons.** The blue data depicts mutual information calculated for the SAAD neuron and the red data refer to the network of two LIF neurons. Multiple runs of 10 sec each are performed and pairs of Poisson stimuli of frequency 100 Hz are presented in each run.

combination of these four variables that endows the network with the maximum capacity to process information given Poisson stimuli of frequency 100 Hz. We test axonal delays that range from 0 to 4 ms (step of 1 ms) and synaptic connection weights between 0.3 and 1 (step of 0.1). The bidirectional neural network exhibited the highest value of mutual information for $\Delta t_1 = \Delta t_2 = 4\text{ ms}$ and $w_{12} = 0.6$, $w_{21} = 1$. These same values are used in what follows except when specified otherwise.

The results are displayed in figure 6.8 and show an advantage of the dendritic neuron's information processing capability over that of the two bidirectionally connected LIF neurons.

6.3.2.1 Poisson stimuli frequency test

In the case of one SAAD neuron and two bidirectionally connected LIF neurons, we performed tests on the value of mutual information upon varying Poisson stimuli frequencies between 5 and 100 Hz. The results, displayed in figure 6.9, show that mutual

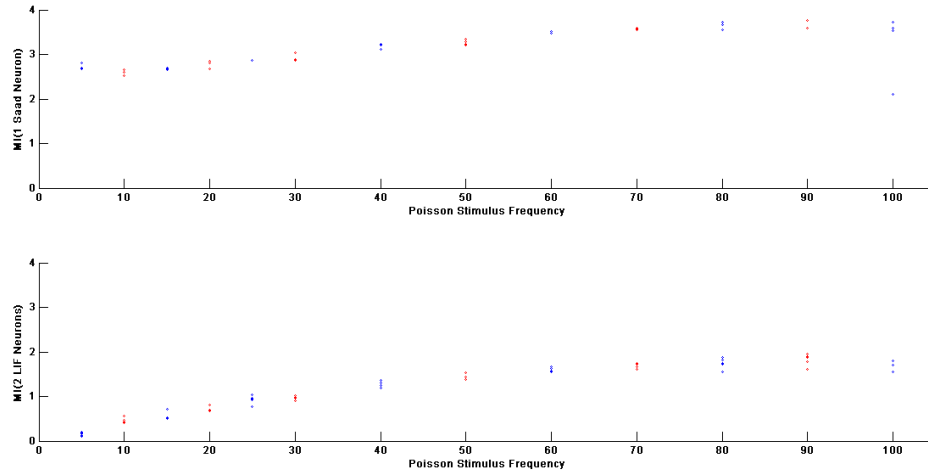


Figure 6.9: **Mutual information in response to Poisson stimulus frequency.** Stimuli frequencies between 5 and 100 Hz were used as inputs to one SAAD neuron and two LIF neurons as described in this section

information measures increase with the increase in stimuli frequency and reaches a ceiling with frequencies in the 100 Hz range.

6.4 Mutual information in large networks

While we are aware of the need of plasticity mechanisms to be implemented in networks of neurons prior to attempting to study information capacity of these networks, we laid the ground by generating different types of neural networks and attempted to ‘sneak-peek’ at mutual information in these networks. More precisely, we generated lattice, small-world, scale-free and random networks with varying connection densities. Networks composed of LIF neurons were set to have 4 input neurons, 2 output neurons, and 24 middle layer neurons, thus totaling 30 LIF neurons. Networks of SAAD neurons were composed of a total of 15 neurons among these 2 input neurons (each having a dendritic and a somatic stimulus) and 1 output neuron. Input neurons in these networks were stimulated with Poisson stimuli of frequency 40 Hz, with the number of stimuli set to be equal to that of input neurons and each stimulus projecting to all input neurons.

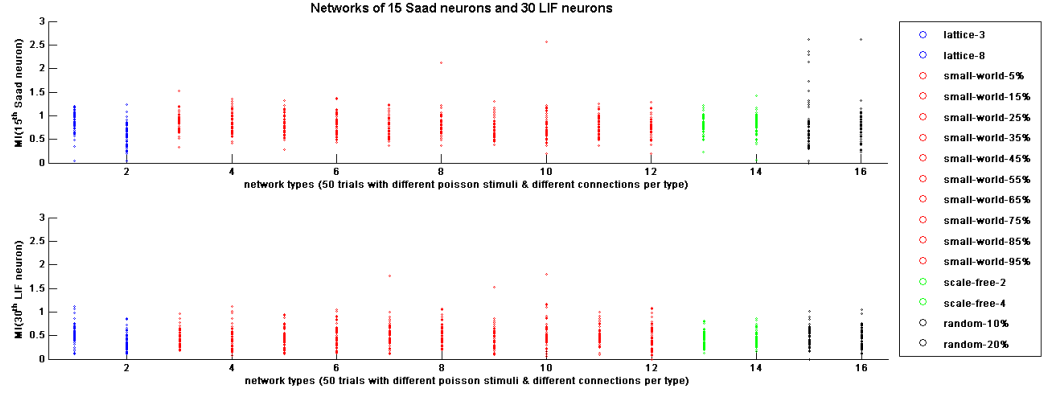


Figure 6.10: **Mutual information in networks of 15 SAAD neurons and 30 LIF neurons.** Mutual information calculation shows that networks of SAAD neurons may be at an advantage in information capacity when compared to networks of 30 LIF neurons. This result is not of great value as plasticity is not implemented in these networks

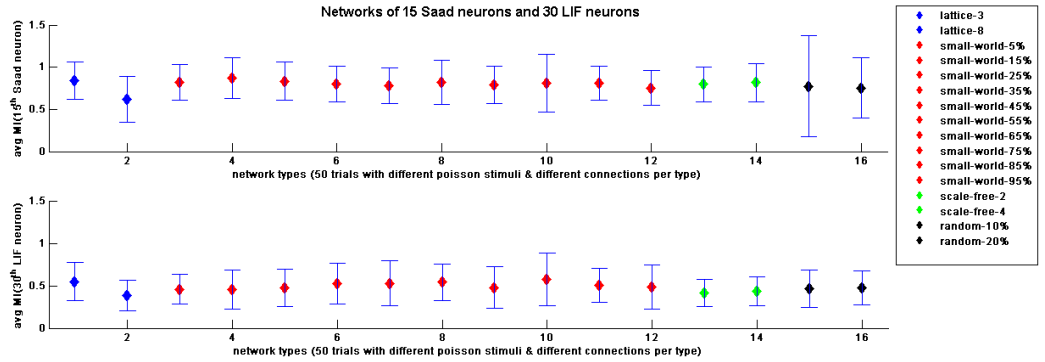


Figure 6.11: **Averaging of mutual information results per network type in networks of 15 SAAD neurons and 30 LIF neurons.** Mutual information is averaged over 50 trials per network type and error bars displayed

Mutual information calculated for each set of networks showed an advantage of networks composed of SAAD neurons over networks composed of LIF neurons. Results in figures 6.10 and 6.11. We will not discuss this advantage as discussion must be in light of plasticity and learning and not based on randomly connected networks.

6.5 Conclusion

The analysis conducted using information theory as the information metric correctly predicts that spatially extended neurons endowed with nonlinear subunits encode substantially more information than simple point neurons such as the linear integrate-and-fire neuron. We quantified information in one SAAD neuron and one LIF neuron, in one SAAD neuron and two bidirectionally connected LIF neurons and in network of 15 SAAD neuron and 30 LIF neurons. Poisson stimuli were used with varying frequencies. While mutual information may not be the best metric out there to calculate the information processing capabilities of neurons, the results of increased mutual information in the dendritic neuron (SAAD) give us an enough incentive to conclude that quantitative measures proved that the spatially extended neuron endowed with active and nonlinear dynamics at the level the dendrite is worthy of being used as the neuron-like unit in neural networks subject to plasticity and learning and where efforts to reverse engineer the brain constitute a main goal.

Chapter 7

Performance and scalability

In complex systems ... the whole is more than the sum of the parts ... given the properties of the parts and the laws of their interaction, it is not a trivial matter to infer the properties of the whole.

Herbert Simon

Using mathematical analysis, we calculated larger information processing capacities in a spatially extended dendritic neuron as compared to a single point neuron and a network of two point neurons bidirectionally connected. Another important consideration is to ensure that the spatially extended neuron is not too bulky but instead suitable to use as the main unit in large neural networks. Answering this question entails tests of performance and scalability, namely of memory usage and CPU time.

7.1 Hardware properties

When we talk about performance and scalability, knowing the hardware specifications is of major importance. A personal computer with 6 free gigabytes of memory and

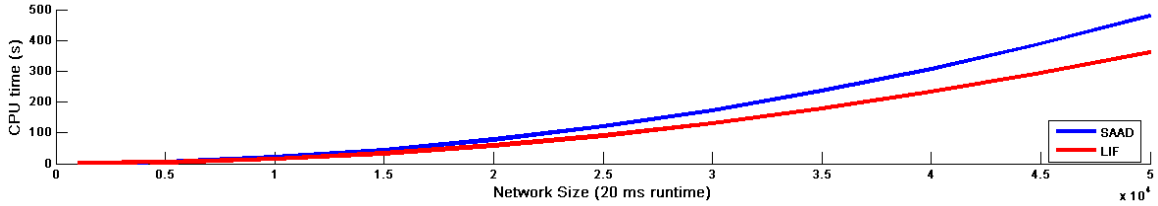


Figure 7.1: **Elapsed CPU time for a 20 ms simulation time of different network sizes.**

four CPU cores (Intel Core i7-20600k) was used to run simulations of the dendritic neuron and networks of this neuron reaching 50,000 neurons. These hardware specifications, although not close to a supercomputer's, did not impede the possibility of running networks of 50,000 dendritic neurons with 25 million synaptic connections. It is also worth mentioning that the code used is written in Matlab and is not compiled which leaves plenty of room for enhancement and holds a promise for the possibility of running much larger networks of these detailed dendritic neurons. GPU availability is another major advantage when present. While we did not run the code on a GPU-based computer, it is implemented in a fully vectorized and parallelizable way in order to take advantage of parallel processing on a multicore CPU, GPU, or supercomputer.

7.2 Performance

Even with the advent of increased computational power and storage space, speed and performance are main issues that highly affect the choice of the neuron model unit used in complex networks. We implemented the dendritic SAAD neuron while keeping in mind the need for a simple, elegant, and fast unit that can behave similarly to a Layer V pyramidal neuron endowed with non-linear processing and rich propagation dynamics.

We calculated the time it takes to achieve 1 sec of runtime for a network of 10,000 dendritic neurons and a network of 10,000 LIF neurons. The results were promising keeping in mind that one dendritic neuron is capable of processing more information than two LIF

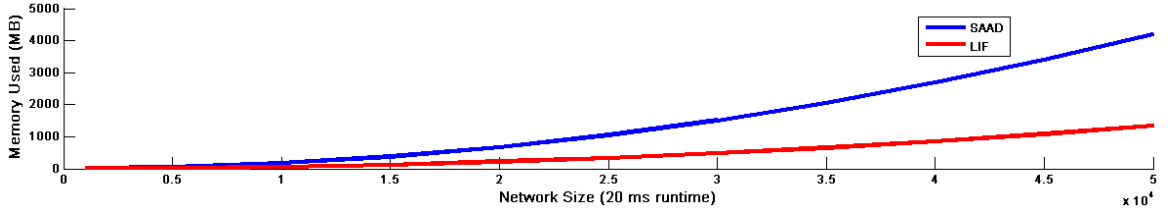


Figure 7.2: **Memory used in networks of SAAD and LIF neurons.**
 Network sizes were varied from 1,000 neurons to 50,000 neurons and memory was measured over a 20 ms runtime

point neurons:

It took 974 s to run a network of 10,000 dendritic neuron for 1 sec and calculate the output voltage of all neurons in response to dendritic and somatic stimuli.

It took 741 s to run a network of 10,000 LIF neurons for 1 sec and calculate their output voltages in response to two stimuli.

The number of connections in networks of 10,000 neurons (of both types) was set to 1,000,000 i.e. an average of 100 connections per neuron. It is worth mentioning that similar LIF-like equations were used to model the soma and spine of the spatially extended neurons, which ensures that the comparison of speed and performance between the LIF model and dendritic model is a fair one.

7.3 Scalability

A system has a finite amount of memory. Thus running neural networks of large sizes is mainly limited by the amount of available memory. For each neuron type (SAAD and LIF), we measured memory usage in small-world networks of sizes ranging from 1,000 neuron to 50,000 neurons (with steps of 5,000 neurons) and corresponding synaptic connections ranging from 10,000 to 25,000,000, respectively. The results are reflected in figure 7.2. Furthermore, figure 7.3 proves that the memory used to run networks of neurons for 10 ms and 20 ms is constant per network size. Increasing the simulation time of the network thus does not

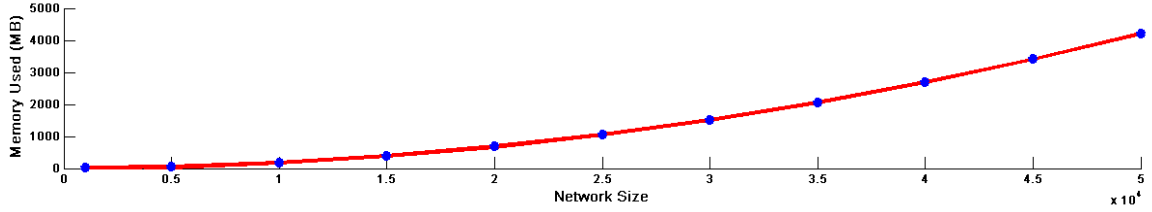


Figure 7.3: **Memory usage independent of simulation times.** 10 ms (red) and 20 ms (blue) of simulation time did not result in increased memory usage for the same number of neurons and synaptic connections

result in memory overload as the latter is kept constant and is only affected by the number of neurons in the network.

On these two bases of increased information capacity and scalability and performance measures as well as the portability of the code implemented to be used on a GPU-enabled supercomputer, it is tempting to conclude that it is worth implementing neural networks with pyramidal neurons as their building blocks.

7.4 Implementation considerations to enhance speed and scalability

Multiple considerations were taken into account while implementing the dendritic neuron model and networks of this neuron. The main ones are listed below.

1. We avoided the use of arrays for storage of state variables over time. Instead, state variables were updated at each time step based on the current factors and its previous time step value. No longer history of these variables was used in the implementation. Thus, no arrays were used to hold state variables over time. This is the reason behind the result displayed in figure 7.3 where the memory is the same for runs of 10 ms and 20 ms of the dendritic neuron.
2. In network implementations, neurons at a certain time step are independent of each others and thus can be vectorized such that their state variables updated in one vector

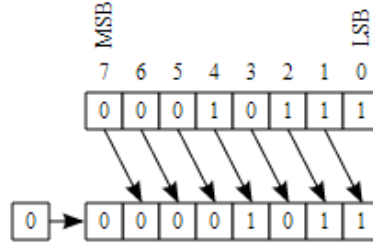


Figure 7.4: **Binary bit shift operation.** LSB and MSB refer to least significant bit and most significant bit, respectively.

operation. Vectorization saves a considerable amount of time compared to looping over the number of neurons in the network.

3. Synaptic transmission is implemented without the need to save previous states of variables over time despite the existence of a delay between the firing of an action potential and the binding of neurotransmitters onto the postsynaptic membrane receptors. Synaptic transmission is described in figure 4.12.
4. Efficient implementation of axon delays whereby one memory address is used to store the delay and only one write is performed to update the propagation along the axon. This implementation is explained in more details in the section that follows.

7.4.1 An efficient implementation of axon delays

Whatever the length of the axon, we ensured in our implementation that only one memory address is used for each axon. In most implementations of axon delay that we are aware of, multiple memory addresses are used per axon i.e. to each axon an array is saved in memory with the number of elements equal to the axon delay in time steps (array length = axon delay (in ms) / dt (in ms)). Once a spike gets initiated at the axon hillock, it propagates down the axon towards its terminal over a time equal to the axon delay. One way to reduce logic operations while achieving this propagation down the axon is to use a circular array, thus avoiding shifting values over the array segment. Another way that is more efficient in terms of memory and computation is to use one memory address to save axonal values. An

action potential is all-or-none, thus it can be represented by a bit (0 signaling its absence and 1 signaling its presence). Once a spike occurs at the axon hillock, the value of the most significant bit in the shift register is set to 1 and a binary shift operation is used at each time step to ensure the propagation of the spike from the site of its initiation to the axon terminal. It takes thus only 1 CPU operation to update the location of the signal for any delay with length 1 to 32 (in units of dt). Since we are using only one memory location to store the axon delay, shifting the signal one step forward towards the axon terminal and away from the soma involves merely a binary shift operation which is equivalent to a division by number 2. It is worth mentioning here that one memory address implies that this space may get used at the level of the CPU which makes it even faster than having it saved in the RAM.

Chapter 8

Conclusion and Future work

Never let the future disturb you. You will meet it, if you have to, with the same weapons of reason which today arm you against the present.

Marcus Aurelius

8.1 Conclusion

We studied the capacity of a spatially extended neuron model endowed with an active dendrite to encode and decode information and compared this capacity to that of simple point neurons. We proved that a dendritic neuron that supports independent thresholding of synaptic inputs and passive and active forward and backpropagation with BAC firing has a higher capacity for information processing. On this basis alone, it is tempting to conclude that pyramidal neurons endowed with highly branched dendritic trees equipped with active channels and voltage-gated conductances that promote thresholding are express design features that greatly enhance the information processing and storage capabilities of these neurons. Nevertheless, this work will be completed when the modeled neuron is used

as the main building block in networks that undergo plasticity and learning. We kept this end goal in mind during the implementation of the neuron model and ensured that the model is lightweight, efficient and fast enough to compete with point neuron models and thus constitute the building block of large networks subject to plasticity mechanisms.

Brief, a renewed interest in the neuron as the basic, but not simple, unit for computation to be used in neural networks that undergo plasticity and constitute the subject of investigations in the conquest to understand learning and information processing in the brain is the main message of this thesis work.

8.2 Future work

How information is processed in complex circuits, with hundreds of thousands of recurrently connected neurons endowed with dendritic nonlinearities, remains a vast mystery. We devised a neuron model that may facilitate future efforts to understand the computing functions of cortical tissue. However, in order to come up with a clear theory of spatiotemporal integration in pyramidal cells and its effect on the network, additional work needs to be completed.

Further steps include:

1. The increase in the number of subunits (spines and dendrites) in the modeled neuron and observing their effect on the neuron's capacity to process information.
2. Testing whether dendritic neurons show a steady increase in capacity with the number of available subunits or instead they reach a limit beyond which adding more subunits does not increase the information and learning capacity of the neuron. Is there an optimal number of dendritic subunits that endow the neuron with a maximal information processing capacity?

3. What about a population of these neurons, keeping in mind that extrapolation from the storage and learning capacity of a single neuron to that of a network of neurons rests on assumptions as to the structure of the network and the learning rule used to train that network.
4. The testing of inhibitory inputs onto the dendritic neuron. This may entail the implementation of basal dendrites and new mechanisms of signal propagation as inhibitory connections mainly signal to basal dendrites that are closer to the soma.
5. The implementation of plasticity mechanisms in the different neuron components that can undergo this change, such as the spine, the dendrite and the soma.
6. Enhance the speed of the neuron model by converting it to a low level compiled code (such as C) and use GPU-enabled computer to allow the implementation of networks of large sizes.

Appendix A

Mutual Information: Merging and Patterning

A.1 Merging and patterning for calculation of mutual information

Input binning and merging

We described in Chapter 6 section 6.2.1 the input-output merging and patterning that we implemented in order to calculate input and output probabilities and entropy values needed for mutual information. In this Appendix, we use a simple example (figure A.1) of input/output sets used to calculate mutual information and explain the results in each step of the calculation in an effort to clarify the method used for merging and finding input-output patterns.

The first step is to bin EPSC-shaped inputs with a time resolution Δt such that the maximum value of the biexponential is represented at the time of its occurrence while the rest of the curve is set to zero. Upon completing the binning, non-zero values are saved into

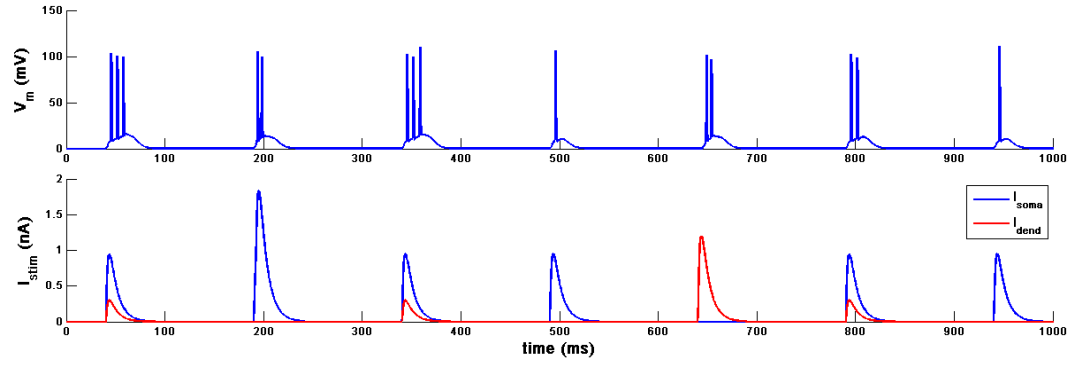


Figure A.1: **Input-output patterning example for calculation of mutual information.**

a separate vector for each stimulus along with the time of occurrence of each value (in units of dt). Each stimulus value is converted to a string with two characters saved for the integer part and 2 characters for the decimal part. Separate binned inputs are then merged into one transmitted signal. This merging is done in a way that preserves the time of occurrence and amplitude of the stimuli. Stimuli that occur at the same time are concatenated. In the case of two stimuli such as the case of this example, 4 characters are saved for each stimulus (see table A.1).

Table A.1: MI Step 1: Merged Stimuli

Amplitude (merged stimuli)	Time of occurrence
00300095	219
00000180	975
00300095	1719
00000095	2469
01300000	3219
00300095	3969
00000095	4719

Output merging

The spike trains at the axon terminal are merged as done for the inputs in the case of multiple outputs. In this example however we are considering one output spike train and thus values represented as in table A.2.

Table A.2: MI Step 2: Merged Responses

Burst ISI	Time of Occurrence	Number of Spikes/burst
31	229	3
22	971	2
31	1729	3
0	2481	1
25	3246	2
31	3979	2
0	4731	1

Input/Output patterning

Input and output patterns are calculated by merging the results obtained in the first two steps and mapping every input to its corresponding output based on the time of occurrence (inputs should precede outputs) and such that the mapping is one-to-one. While it is easy to map inputs to their corresponding outputs in this simple example, the algorithm achieves this mapping for more complicated scenarios by recursively looping over steps a, 2 and 3 until a mapping is found. In addition, the confidence factors are set to 1 (maximum confidence) in this example as it is easy to differentiate bursts from single spikes and find their corresponding inputs due to the low frequency of the stimuli. In more general cases, lower confidence values may be encountered as explained in chapter 5.

Once the input/output patterning is achieved, mutual information is calculated as the difference between the entropy of the input and the conditional entropy of the input given the output (see equation 5.8). The input is composed of the input amplitude and the output is composed of both the ISI and number of spikes as calculated in table A.3

Table A.3: MI Step 3: Mapped Input/Output

In: Amplitude	In: Time	Out: Burst ISI	Out: Time	Out: Num. Spikes/burst
00300095	219	31	229	3
00000180	975	22	971	2
00300095	1719	31	1729	3
00000095	2469	0	2481	1
01300000	3219	25	3246	2
00300095	3969	31	3979	2
00000095	4719	0	4731	1

Appendix B

Source Code

We present in this Appendix the network layout matrices used to represent a certain neuronal network along with the Matlab code used to run the simulations.

B.1 Schematic of a neural network

A sample schematic of a neuron network is used to describe the matrices that we used to denote the network layout and connectivity. As shown in figure B.1, external stimuli are represented using square boxes, pyramidal neurons are represented using triangles, and connections are displayed as arrows. When the postsynaptic neuron's dendrite receives the input, the connection arrow has a rounded tip. The tip is of arrow shape when the postsynaptic neuron's soma receives the input.

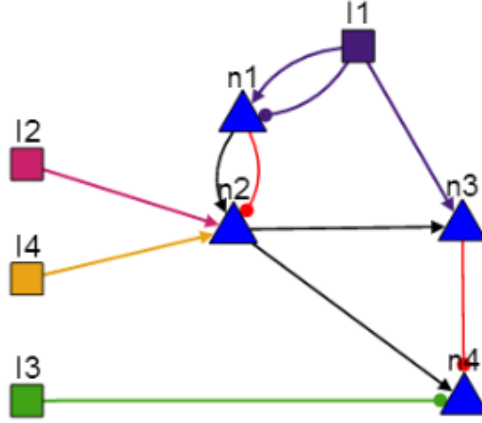


Figure B.1: **Network of 4 SAAD neurons stimulated by 4 external inputs.** SAAD neurons are denoted by triangles. The arrow shape denotes a connection to the neuron's soma, and a round shape denote a connection to the neuron's dendrite.

B.2 Neural network matrix format

Different network layouts are saved in matrix format. As an example, the network whose schematic is presented in figure B.1 is described using the following matrices.

Connectivity matrix

For a network of N neurons, the connectivity matrix is of size $[N \times N]$ with every $[\text{row}, \text{column}]$ pair with the value of 1000 (can occur only along the diagonal) denoting an external stimulus to the neuron $[N_x, N_x]$ and where lower values refer to synaptic connection weights. It is worth mentioning that these connectivity matrices were only used for small networks. In large networks of thousands of neurons and connectivity density less than 60%, the matrices were replaced with arrays corresponding to each neuron whereby the length of the array is equal to the number of outgoing connection from that neuron. This allows us to save in terms of memory usage.

	N1	N2	N3	N4
N1	1000	0.7	0	0
N2	0	1000	1	0.3
N3	0	0	1000	0
N4	0	0	0	0

In the case of the dendritic neuron that can accept incoming connections at its soma and dendrite, a similar connection matrix exists for the dendritic connectivity, assuming that the one described previously relates to the soma. As such the dendritic connectivity matrix for the same network is:

	N1	N2	N3	N4
N1	1000	0.3	0	0
N2	0	0	0	0
N3	0	0	1000	0.6
N4	0	0	0	0

Axon delays

Axon delays are saved in a [1xN] matrix with values denoting the delay in ms.

N1	N2	N3	N4
1	3	0	4

External stimuli

External stimuli are saved in an [mxN] matrix with m denoting the maximum number of stimuli per neuron. In reference to the example represented here, two external stimuli matrices are needed in order to account for somatic and dendritic stimuli.

Somatic stimuli

Dendritic stimuli


```

[dend_synapses_row, dend_synapses_col] = ...
    find((netMatrixDendrite>0)&(netMatrixDendrite<1000));
num_dend_synapses = size(dend_synapses_row,1);

[soma_synapses_row, soma_synapses_col] = ...
    find((netMatrixSoma>0)&(netMatrixSoma<1000));
num_soma_synapses = size(soma_synapses_row,1);
num_synapses = num_soma_synapses+num_dend_synapses;

% Initialize state variables
% N is the neurons structure

% Extract synaptic connections from the network layout matrices
% Synapses connecting to the neuron's somas
S_preNeuron(1:num_soma_synapses) = soma_synapses_row(1:num_soma_synapses);
S_postNeuron(1:num_soma_synapses) = soma_synapses_col(1:num_soma_synapses);
while i <= num_soma_synapses
    % find rows (presynaptic neuron #s)
    preNeuronNum = S_preNeuron(i,1);
    postNeuronNum = S_postNeuron(i,1);
    S_postSomaCurrentAmp(i,1) = netMatrixSoma(preNeuronNum,postNeuronNum);
    N(preNeuronNum).numPostSyns = N(preNeuronNum).numPostSyns + 1;
    N(preNeuronNum).postSyn(N(preNeuronNum).numPostSyns) = i;
    % find cols (postsynaptic neuron #s)
    N(postNeuronNum).numPreSyns = N(postNeuronNum).numPreSyns + 1;
    N(postNeuronNum).preSyn(N(postNeuronNum).numPreSyns) = i;
    i=i+1;
end
% Synapses connecting to the neuron's dendrites
S_preNeuron(num_soma_synapses+1:num_soma_synapses+num_dend_synapses) = ...
    dend_synapses_row(1:num_dend_synapses);
S_postNeuron(num_soma_synapses+1:num_soma_synapses+num_dend_synapses) = ...
    dend_synapses_col(1:num_dend_synapses);
i = num_soma_synapses+1;
while i <= num_soma_synapses+num_dend_synapses
    % find rows (presynaptic neuron #s)
    preNeuronNum = S_preNeuron(i,1);
    postNeuronNum = S_postNeuron(i,1);
    S_postDendCurrentAmp(i,1) = netMatrixDendrite(preNeuronNum,postNeuronNum);
    N(preNeuronNum).numPostSyns = N(preNeuronNum).numPostSyns + 1;

```

```

% synapse linking this neuron to its postsynaptic neuron
N(preNeuronNum).postSyn(N(preNeuronNum).numPostSyns) = i;
% find cols (postsynaptic neuron #s)
N(postNeuronNum).numPreSyns = N(postNeuronNum).numPreSyns + 1;
% synapse linking this neuron to its presynaptic neuron
N(postNeuronNum).preSyn(N(postNeuronNum).numPreSyns) = i;
i = i+1;
end

% Assign stimuli to corresponding neurons' somas and dendrites
for i = 1:num_neurons
    deltaAxon = ceil(delayMatrix(i)/dt);
    % amount to add to axon value in case of a spike (100000000 depends on delay)
    N(i).spikeInc = 2^(deltaAxon-1);
    if deltaAxon == 0
        N(i).spikeInc = 1;
    end
    if ismember(i,1:numStimuli)
        N(i).extSomaStimSpikesIndex = numStimuli;
    else
        N(i).extSomaStimSpikesIndex = 0;
    end
    if netMatrixSoma(i,i)==0
        N(i).extSomaStimSpikes = zeros(1,tsteps(2)-tsteps(1)+1);
        N(i).extSomaStim = zeros(1,tsteps(2)-tsteps(1)+1);
    else
        for s = 1:numStimuli
            N(i).extSomaStimSpikes(1:numStimuli,1:tsteps(2)-tsteps(1)+1) = ...
                stim(s).spikes(1:tsteps(2)-tsteps(1)+1);
            N(i).extSomaStim(s,1:tsteps(2)-tsteps(1)+1) = ...
                stim(s).biexp(1:tsteps(2)-tsteps(1)+1);
        end
    end
    % Same for dendritic stimuli
end

% -----
% MAIN PROGRAM starts here
% -----
for t = tsteps(1):tsteps(2)
    % Update all synapses at the current time step

```

```

for j = 1:num_synapses
    S_X(j,1) = S_X(j,1) + ...
        dt * (-(S_X(j,1)*N(S_postNeuron(j,1)).R)/N(S_postNeuron(j,1)).tauBind ...
            + N(S_postNeuron(j,1)).Use*S_A(j,1)*S_spike(j,1));
    N(S_postNeuron(j,1)).XR = N(S_postNeuron(j,1)).XR + ...
        dt * (S_X(j,1)/N(S_postNeuron(j,1)).tauBind ...
            - N(S_postNeuron(j,1)).XR/N(S_postNeuron(j,1)).tauUnbind);
    S_A(j,1) = S_A(j,1) + dt * (S_I(j,1)/N(S_preNeuron(j,1)).tauRec);
    S_I(j,1) = 1 - (S_A(j,1) + S_X(j,1) + N(S_postNeuron(j,1)).XR);
    % Current caused by activated postsynaptic receptors
    N(S_postNeuron(j,1)).IpostSoma = N(S_postNeuron(j,1)).IpostSoma ...
        + (S_postSomaCurrentAmp(j,1) * N(S_postNeuron(j,1)).Ase ...
            * N(S_postNeuron(j,1)).XR);
    N(S_postNeuron(j,1)).IpostDend = N(S_postNeuron(j,1)).IpostDend ...
        + (S_postDendCurrentAmp(j,1) * N(S_postNeuron(j,1)).Ase ...
            * N(S_postNeuron(j,1)).XR);
end

% Update neurons state variables
for i = 1:num_neurons % for each neuron
    % shift to the right to account for the axon potential propagation
    % along the axon
    N(i).axon = bitshift(N(i).axon,-1);
    N(i).time = double(t)*dt;
    N(i).soma_Istim = sum(N(i).extSomaStim(:,t));
    N(i).soma_I = N(i).soma_Istim;
    N(i).soma_I = N(i).soma_I + N(i).IpostSoma;

    % Forward dendritic propagation dynamics
    for x = 2:maxDelayPassiveDendProp
        fdecay = 1/(1+exp(-(N(i).dend_Vprev(1,x)-N(i).dend_V(1,x))/0.01));
        dVdt = - N(i).dend_invTauf * N(i).dend_V(1,x) ...
            + dend_invCmf * ((N(i).dend_invRif + dend_Cif ...
                * (N(i).dend_V(1,x)-N(i).dend_Vprev(1,x))/dt) ...
                * (N(i).dend_V(1,x-1) - N(i).dend_V(1,x)));
        N(i).dend_V(1,:) = N(i).dend_V(1,:) + dt*dVdt;
    end
    N(i).soma_I = N(i).soma_I + N(i).factorVdend ...
        *(N(i).dend_V(1,maxDelayPassiveDendProp));

    % Backward dendritic propagation dynamics

```

```

% fVbAP = 1 if Vdend+VbAP is close to the max value
fVbAP = 1/(1+exp(-N(i).bAP_V(1,1)-N(i).dend_V(1,1)+VbAPDendMax)/p_fVbAP) ...
    * 1/(1+exp(-N(i).soma_V(1,1)+N(i).soma_Vth)/0.01);

N(i).taufRi = N(i).taufRi + dt * (ftaufRi * N(i).f_Leak + ...
    (N(i).taufRi_init - N(i).taufRi)/0.5);

% Accounting for the slow decrease of bAP (blue & red curves)
% in Larkum 1999a figure 1(c)
N(i).dend_invRibs = N(i).dend_invRibs + dt * (-fRi * (N(i).f_Leak) + ...
    (dend_invRibs_init - N(i).dend_invRibs)/(N(i).taufRi));
dVdt = dend_invCmb * ( N(i).dend_invRibs * ...
    (- N(i).bAP_V(1,maxDelayActiveDendProp) + N(i).soma_V) ) ...
    - N(i).dend_invTaubs * N(i).bAP_V(1,maxDelayActiveDendProp);

N(i).bAP_V(1,maxDelayActiveDendProp) = ...
    N(i).bAP_V(1,maxDelayActiveDendProp) + dt*dVdt;

for x = 1:maxDelayActiveDendProp-1
    dVdt = dend_invCmb * ( N(i).dend_invRib(1,1) ...
        * (- N(i).bAP_V(1,x) + N(i).bAP_V(1,x+1)) ) ...
        - (N(i).dend_invTaub(1,1)+dend_invTaub_init) * N(i).bAP_V(1,x);
end

% update spine
N(i).IbAP = N(i).spine_factorVbAP_target * N(i).bAP_V(1,1);
f = 1/(1+(exp(-(N(i).soma_V-N(i).AP_aboveVT)/0.05)));
N(i).dend_Istim(1,1) = (1+0.8*f)*sum(N(i).extDendStim(:,t));
% Ceiling reached when VbAP > 40 => cannot add dend_Istim
f1 = 1/(1+(exp(-(N(i).bAP_V(1,1)-40)/0.001)));
% if V>V_DP => refractory period (dendritic spike) => cannot add IbAP
f2 = 1/(1+(exp(-(N(i).dend_V(1,1) ...
    -(N(i).spine_Vth_init+N(i).DP_aboveVT))/0.001)));
N(i).EPSC(1,1) = (1-f1)*N(i).dend_Istim(1,1) ...
    + (1-f2)*N(i).IbAP + N(i).IpostDend;
N(i) = SpineVoltage(N(i),dt);

% update soma
N(i) = SomaticVoltage(N(i),dt);

spike = dec2bin(N(i).axon,16);
end

```

```
% % % % % % % % % % % % % % % % % % % % % % % % % % % % % % % % % % %  
%  
% Matlab code for the update of the soma neuron voltage  
%  
% % % % % % % % % % % % % % % % % % % % % % % % % % % % % % % % % % %  
  
function neuron = SomaticVoltage(neuron, dt)  
  
f1 = 1/(1+(exp(-(neuron.soma_V-neuron.soma_Vth)/p_rAP)));  
f2 = 1/(1+(exp(-(neuron.soma_V-neuron.AP_aboveVT)/p_rAP)));  
f3 = 1/(1+(exp(-(neuron.soma_V-neuron.soma_Vprev)/p_rAP)));  
  
neuron.soma_Vth = neuron.soma_Vth + dt * ((fVth*f2) ...  
    + (neuron.soma_Vth_init - neuron.soma_Vth)/neuron.soma_tauVth);  
  
neuron.tauf_AP = (1-f1)*neuron.tauf_AP_init + f1*ftaufAP;  
neuron.f_AP = neuron.f_AP + dt * (f1*f3*AP - neuron.f_AP/neuron.tauf_AP);  
  
neuron.f_Int = -f1*neuron.soma_C_inv + (1-f1) ...  
    * (neuron.f_Int + dt * (- neuron.f_Int/neuron.tauf_Int));  
  
neuron.f_Leak = neuron.f_Leak ...  
    + dt * (f2*Leak - neuron.f_Leak/neuron.tauf_Leak);  
  
neuron.soma_V(1,1) = neuron.soma_V(1,1) ...  
    + dt * ( (neuron.f_Int+neuron.soma_C_inv) * neuron.soma_I ...  
        + (neuron.f_Leak+neuron.soma_tau_inv) ...  
        * (neuron.soma_Vrest - neuron.soma_V(1,1)) ...  
        + neuron.f_AP * p_fAP );  
  
end
```

Function to update the voltage at the level of the spine

```
% absolute refractory period ~ 4.5 ms
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%
% Matlab code for the update of the spine voltage
%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

function neuron = SpineVoltage(neuron, dt)

f1 = 1/(1+exp(-(neuron.dend_V(1,1)-neuron.spine_Vth)/p_rDP));
f2 = 1/(1+exp(-(neuron.dend_V(1,1)+neuron.bAP_V(1,1)...
    -(neuron.spine_Vth_init+neuron.DP_aboveVT))/0.01));
f3 = 1/(1+exp(-(neuron.dend_V(1,1)-neuron.dend_Vprev(1,1))/0.01));
f4 = 1/(1+exp(-(neuron.bAP_V(1,1)-5)/p_rDP));

neuron.spine_Vth = neuron.spine_Vth + dt * ((fVth*f2) ...
    + (neuron.spine_Vth_init - neuron.soma_Vth)/neuron.spine_tauVth);

neuron.tauf_DP = neuron.tauf_DP + dt * (-f1*f3*(1-f2)*fdecay*(1+s2*f4) ...
    + f1*f2*f3*fIncrease*(1+f4*s4) + (neuron.tauf_DP_init - neuron.tauf_DP)/tautau);
neuron.f_DP = neuron.f_DP + dt * (f1*f3*DP - neuron.f_DP/neuron.tauf_DP);

neuron.f_DInt = -f1*f3*neuron.dend_C_inv ...
    + (1-f1*f3)*neuron.f_DInt + dt * (- neuron.f_DInt/neuron.tauf_DInt);

neuron.dend_tau_inv = neuron.dend_tau_inv ...
    + dt * (s3*f1*(f3+f2)*f4*neuron.bAP_V(1,1)/neuron.bAP_max ...
    + ((neuron.dend_tau_inv_init - neuron.dend_tau_inv)/tauLeak));

neuron.dend_V(1,1) = neuron.dend_V(1,1) ...
    + dt * ( (neuron.f_DInt+neuron.dend_C_inv) * neuron.EPSC(1,1) ...
    + neuron.dend_tau_inv * (neuron.dend_Vrest - neuron.dend_V(1,1)) ...
    + neuron.f_DP * p_fDP);

end
```


Appendix C

Calcium and EDTA induced folding and unfolding of calmodulin on functionalized quantum dot surfaces

C.1 Published Work

In this appendix, I include a copy of work published in the Journal of Nanoneuroscience and conducted in the laboratory. This wet lab work has shaped and helped in defining the dissertation work described in the previous chapters.



Calcium and EDTA Induced Folding and Unfolding of Calmodulin on Functionalized Quantum Dot Surfaces

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Calcium is an ubiquitous second messenger signal that is critical to many cellular processes. As such considerable efforts have been made to develop sensitive high resolution calcium sensors. However, organic dye calcium sensors have inherent limitations in signal to noise ratio and spatial resolution. We have developed a novel quantum dot (qdot) based calcium sensor with superior optical properties for biological detection of functional calcium signaling. Here, we discuss the *in vitro* calcium binding properties of our sensor. The sensor was designed as a fluorescence resonance energy transfer (FRET) complex, composed of a dihydrolipoic acid capped quantum (DHQA-qdot) which acts as a fluorescence donor, an organic dye (Alexa Fluor 647) which acts as a fluorescence acceptor, and calmodulin (CaM) which serves as the active calcium sensing element. We confirmed that a significant FRET signal was observed between the donor (610 nm) and acceptor (670 nm) emission wavelengths upon binding with calcium ions with a maximal fractional change in FRET ratio ($\Delta R/R$) of up to 6.6. Unlike this DHQA-qdot system, almost no FRET was observed when qdots were coated with the relatively large surface ligands polyethylene glycol and polyethyleneimine, suggesting negative effects of the bulky ligands on CaM folding. Calcium-induced FRET in our system was reversed by EDTA treatment, suggesting the unfolding of CaM on the nanoparticle surface in the absence of calcium, enabling a regeneration of the sensor system.

Keywords: Quantum Dot, Calmodulin, Calcium, EDTA, Calcium Sensor, FRET, Folding and Unfolding.

INTRODUCTION

Intracellular calcium (Ca^{2+}) signaling is ubiquitous and critical to cellular functions and survival. As such, studies on the regulation of Ca^{2+} dynamics receive significant focus in the life sciences. In order to image and measure these dynamics within cells, numerous calcium indicator dyes have been developed with varying spatial and temporal resolutions. Examples include synthetic dyes with acetoxymethyl (AM) esters (Tsien, 1981) and protein based genetically encoded calcium indicators (GECIs) (Palmer and Tsien, 2006; Heim et al., 2007). The synthetic dyes are easy to use in living cells and have large dynamic ranges, high sensitivity and fast kinetic responses. However, photobleaching, compartmentalization, incomplete AM ester hydrolysis and potential toxicity of hydrolysis products, leakage into the extracellular medium, a short assay window and the inability to target specific intracellular compartments and organelles represent significant limitations of these indicators (Rudolf et al., 2003; Mank and Griesbeck, 2008). The GECIs, including the

Cameleon (Miyawaki et al., 1997) family of sensors, have a number of advantages over the chemical dyes, in particular due to their capacity to target intracellular organelles. The GECIs contain one or two genetically encoded fluorescence proteins for fluorescence detection and a calcium sensing protein component, such as calmodulin (CaM) or troponin, which undergoes conformational transitions upon binding with free calcium ions. The well known Cameleon system consists of a tandem connection of a fluorescence donor protein, CaM, a CaM-binding peptide fragment (such as the M13 peptide derived from skeletal myosin light chain kinase), and a fluorescence acceptor protein. Upon binding with calcium ions, the donor-acceptor distance decreases due to a folding of CaM around the peptide fragment (Miyawaki et al., 1997; Chin and Means, 2000; Truong et al., 2001; Chou et al., 2001). This, in turn, increases fluorescence resonance energy transfer (FRET) (Miyawaki et al., 1997; Truong et al., 2001). The calmodulin based Cameleon was shown to detect free calcium concentrations in the 10^{-8} to 10^{-2} M range (Miyawaki et al., 1997). A detailed review on GECIs can be found in a recent publication (Mank and Griesbeck, 2008; McCombs and Palmer, 2008). CaM (148 amino acids) exists as a monomer in solution and contains two structurally homologous N- and C-terminal domains joined by a flexible linker. Each domain

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binds two calcium ions using two helix-turn-helix motifs (EF-hands) (Chou et al., 2001). The calcium ion binding affinity of CaM (dissociation constant, K_d of 5×10^{-7} M to 5×10^{-6} M) falls into the biologically relevant range of intracellular free Ca^{2+} of 10^{-7} M to 10^{-6} M) (Chin and Means, 2000). Upon binding with calcium ions, CaM's calcium-free apo structure is converted into an extended holo structure with hydrophobic clefts in both domains open for binding to target sequences (Chin and Means, 2000; Chou et al., 2001). Despite the many advantages of GECIs, they suffer from low expression levels in mammalian transgenic animals and experience rather small changes in fluorescence signals, resulting in a poor signal to noise ratio and low sensitivity. Since these sensors are genebased, alteration of target sites requires obtaining different clones or DNA manipulation by end users. Delivery of GECI DNA to cells requires direct or virus assisted transfection followed by a waiting period of one to three days before fluorescence signals can be detected and experimental success can be determined. To address these limitations, we have constructed a calcium sensor composed of a quantum dot fluorescence donor, a mutated *Xenopus laevis* CaM*-M13* pair that acts as an efficient calcium sensing motif that does not interfere with native CaM calcium binding (provided as a generous gift from Dr. Roger Tsien, UC San Diego) (Palmer et al., 2006), and an organic dye that acts as a fluorescence acceptor. We further mutated the CaM* by introducing a cysteine at the 3rd position of the N-terminal to which we attached the organic dye. Our quantum dot based sensor responds to calcium ions and EDTA with very strong and robust FRET changes that produce large signal to noise ratios with fast temporal kinetics. In this paper we describe in detail the physical characterization of CaM* folding and unfolding on quantum dot surfaces.

MATERIALS AND METHODS

Cloning and Purification of CaM Protein

Calmodulin protein with a C-terminal hexahistidine tag was constructed by cloning the CaM*-M13* part of mt-Cameleon in pcDNA3 into pET-28b vector (EMD, Gibbstown, NJ, USA). Two primers (forward: 5'- agcatccatggcggtgcaactgacagaagagcagatt-3'; reverse: 5'-ggctactcgaaggagctggagatcttcttcag- 3') were used to introduce NcoI and XhoI sites (underlined) at the N- and C-termini, respectively. This process also introduced a single cysteine at the 3rd position (underlined and italicized in the forward primer) and alanine at the 2nd position (H2A/D3C). The histidine 2 to alanine 2 mutation introduced by the NcoI site actually corrected the mutation made during the cloning of the mt-Cameleon into the pcDNA3 vector. The PCR product was digested with restriction enzymes and ligated with pET-28b vector restricted with the same enzymes and treated with Antarctic Phosphatase (NEB, Ipswich, MA, USA) to prevent self-ligation. Positive clones were screened by single colony PCR. For the single colony PCR, a small portion of colonies were directly mixed with 25 μ l PCR mixture without a boiling step in water followed by amplification reactions. Using this method, wild-type CaM*-M13* and cysteine mutant CaM*-M13* D3C colonies were identified. The plasmids containing the inserts were purified from DH5 α cells and transformed into BL21 Star (DE3) cells for protein expression. For the protein purification, BL21 Star (DE3) cells were grown at 37 °C to OD_{600 nm} ~ 0.6 and

IPTG was added to give a final concentration of 0.6 mM. After 4 hours of further incubation the cells were collected and purified by PrepEase his-tagged protein purification mini kits (USB, Cleveland, Ohio, USA) by following the protocol provided by the company, except for the lysozyme treatment step which was replaced by sonication. The amount of protein was calculated using an extinction coefficient of $\epsilon_{280 \text{ nm}} = 9,970 \text{ M}^{-1}\text{cm}^{-1}$ calculated from its amino acid composition and molecular mass of 20,652 Da for the CaM* aspartic acid 3 to cysteine 3 mutant protein (CaM*-M13* D3C). Larger scale purifications were made using PrepEase midi kit using a 300 ml culture and saved -80 °C in 20 mM Tris-HCl, pH 8.0 buffer.

Fluorescence Labeling and Spectra Measurements

Fluorescence and absorption spectra were measured using a SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 25 °C. Absorption spectra were measured at 2 nm resolution using a 1 cm path length quartz cuvette. For fluorescence spectra, quartz cuvettes or glass tubes were used depending on the measurements. For quantum dots and sensor complexes, the excitation wavelength was fixed at 400 nm with a photomultiplier voltage set to medium and the emission spectra measured at 2 or 4 nm resolutions at 25 °C.

Fluorescence Labeling of CaM

Before the labeling reaction, CaM*-M13* D3C protein was treated with 10 mM TCEP (*tris*(2-carboxyethyl)phosphine) in 1 $\times$ PBS buffer, pH 7.4 to reduce the single cysteine, followed by removal of the reducing reagent using a 10 kDa Millipore filter (Billerica, MA, USA) in order to improve the labeling efficiency (Shafer et al., 2000). For a typical labeling reaction, CaM*-M13* D3C was mixed with 3-fold molar excess of Alexa Fluor (AF647) maleimide dye (Invitrogen, Carlsbad, CA, USA) at room temperature for 2 hours in PBS, pH 7.4. After the incubation, unreacted dye was removed by gel filtration using a G-25 column followed by buffer exchange with the 10 kDa filter, first with 20 mM Tris-HCl, pH 8.0, containing 10 mM EDTA, followed by the same buffer without EDTA.

Preparation of DHLA and PEI Capped CdSe/ZnS Quantum Dots

Octadecyl amine (ODA) capped hydrophobic CdSe/ZnS quantum dots (ODA-qdot 610, emission wavelength ~610 nm) were purchased from Ocean Nanotech (Springdale, AR, USA). For the DHLA quantum dot (DHLA-qdot), a quantum dot to DHLA molar ratio of 1:40,000 was used for ligand exchange reactions. The molecular weight (MW) of CdSe cores (qdot 610 without ZnS shell) was estimated to be 283,058 with a diameter of 5.09 nm (Yu et al., 2003) and concentrations of qdots were calculated using an extinction coefficient of $436,996 \text{ M}^{-1}\text{cm}^{-1}$ at the first excitation band.¹⁴ For a typical phase transfer reaction, one milligram of ODA-qdot 610 in CHCl_3 was near dried at 70 °C followed by the addition of 25 mg DHLA (Sigma, T8260) dissolved in 0.6 ml of pure ethyl alcohol in a screw-capped eppendorf tube. The ligand exchange reaction was carried out at 70 °C

with occasional mixing during the first two hours and continued overnight with light protection. After the overnight incubation, the reaction mixture was centrifuged at 13,000 rpm using a table top centrifuge for two minutes to remove any unreacted quantum dot particles. The supernatant, which contained qdot-DHLA, unbound DHLA and dissociated ODA, was collected and small grains of potassium tert-butoxide were directly added to the ethanol phase to deprotonate the DHLA for precipitation. Five minutes after the deprotonation, the sample was centrifuged at 13,000 rpm for two minutes and the resulting precipitate washed with 100% ethanol three times to remove free DHLA. After air drying, the DHLA-qdot pellet was resuspended in 20 mM Tris-HCl, pH 8.0. Quantum dots prepared by this method showed both physical and optical stability for more than six months. We found that DHLA (a dithiol) produced a much more stable cap than mercaptododecanoic acid (MDA), which has only one mercapto group (unpublished results). Polyethylene glycol (PEG) capped quantum dots (Qdot 605 ITK amino (PEG)) with amide functional groups were purchased from Life Technologies (Carlsbad, USA) and polyethyleneimine (PEI) capped quantum dots (PEI-qdot) were prepared by a ligand exchange reaction as described by others (Duan and Nie, 2007).

RESULTS AND DISCUSSION

Cloning and Screening of CaM Clones for Bacterial Expression

To produce CaM*-M13* and CaM*-M13* D3C proteins in bacteria, we sub-cloned the CaM*-M13* part of mt-Cameleon/pcDNA3 (Palmer et al., 2006) into NcoI and XhoI sites of pET-28b protein overexpression vector (Fig. 1). The correct colonies containing wild-type and mutant CaM*-M13* D3C inserts were identified by a single colony PCR technique. The results of the single colony PCR screen and the induction

patterns of the calmodulin protein from the selected clones are shown in Figure 2. Though the pET-28b vector is a low copy numbered plasmid, positive clones containing the wild-type CaM*-M13* and CaM*-M13* D3C mutant sequences were easily identified by PCR analysis (Fig. 2, lanes 1, 5 and 7).

Purification and Fluorescence Labeling of Calmodulin

The calmodulin protein was easily purified by a single affinity purification step using NTA-Ni²⁺ columns with the purity in excess of 98% (Fig. 3(A)). Typically, about 7~8 mg of CaM*-M13* D3C was obtained from a 300 ml culture. After the purification, CaM*-M13*D3C was labeled with AF647 maleimide dyes at a cysteine residue introduced at the 3rd position of the N-terminal end of the protein. The fluorescence labeling efficiency of the preparation was estimated to be about 80% by comparing the absorption peaks at 280 nm and 647 nm for free AF647 dye and AF647 labeled protein (Fig. 3(B)). CaM*-M13*-AF647 protein labeled at room temperature showed no indication of degradation (Fig. 3(B) inset). The fluorescence intensity of the protein bands in the gel was rather weak compared to a fluorescence protein band in standard (st) due to the excitation wavelength (340 nm) of the UV box used for the gel image.

Self-Assembly of Fluorescence Labeled CaM*-M13*-AF647 on Quantum Dot Surfaces

Using the hexa-histidine tag at the C-terminal end of the CaM*-M13*-AF647, the protein was easily assembled onto the surface of quantum dots. The histidine tag mediated binding of proteins to DHLA or PEG capped qdots has been previously reported (Medintz et al., 2003; Delehanty et al., 2006 ; Dennis and Bao, 2008), and has a relatively tight K_d of ~1 nM (Sapsford et al., 2007). Histidine tag mediated binding of the protein minimizes the uncertainties of donor to acceptor distances and dye orientations on quantum dots, which are problematic in chemically crosslinked proteins (Mattoussi et al., 2000). Figure 4 shows that the FRET efficiency increases with the number of CaM*-M13*-AF647 positioned near the qdot surface due to enhanced non-radiative energy transfer of excitation energy from the qdot to the acceptor AF647 dye. The experimental FRET efficiency can be determined by following relationship (Lakowicz, 1999):

$$E = 1 - F_{DA}/F_D = nR_o^6/(nR_o^6 + r^6)$$

where F_{DA} and F_D are the fluorescence intensities of quantum dots in the presence and absence of the acceptor AF647 labeled calmodulin, respectively, and n is the number of CaM proteins bound to a quantum dot. The R_o is the Förster radius, which designates a separation distance corresponding to 50% FRET efficiency. Binding of CaM*-M13*-AF647 with DHLA-qdot 610 at different molar ratios (0.99, 2.97, 5.91, 7.90 and 10.9) resulted in a gradual increase in FRET efficiency as judged from the qdot emission peaks (Fig. 4): 12% (1:0.99), 33% (1:2.97), 45% (1:5.91), 52% (1:7.90) and 61% (1:10.9). Since we used an excitation wavelength (400 nm) where there is almost no absorption from the AF647 dye, the decrease of the fluorescence intensities at quantum dot emission peaks and the increased emission of AF647 fluorescence at 670 nm are due to the binding induced

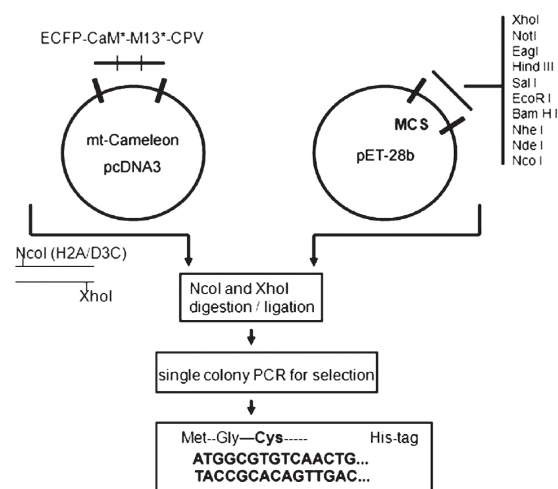


FIG. 1. Summary of sub-cloning stages for CaM*-M13* proteins from mt-Cameleon/pcDNA3 to pET-28b vector. Wild-type (CaM*-M13*/pET-28b) and D3C mutant (CaM*-M13* D3C/pET-28b) were generated by PCR amplification.

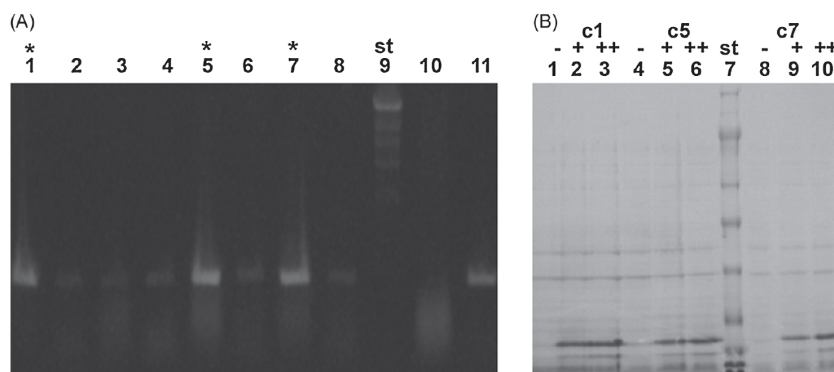


FIG. 2. (A) Single colony PCR screen for CaM*-M13 D3C clones and (B) protein induction of the corresponding positive clones. (A) 1 ~ 8. screened colonies, 9. HindIII DNA standard (st), 10. negative control (pET-28b vector in DH5α cells), 11. positive control (mt-Cameleon/pCDNA3 in DH5α cells). (B) Protein induction patterns of the screened positive clones (*marked 1, 5 and 7 in A and c1, c5 and c7 in B) with (+) and without (–) IPTG. +, 2 hours IPTG induction, ++, 4 hours IPTG induction.

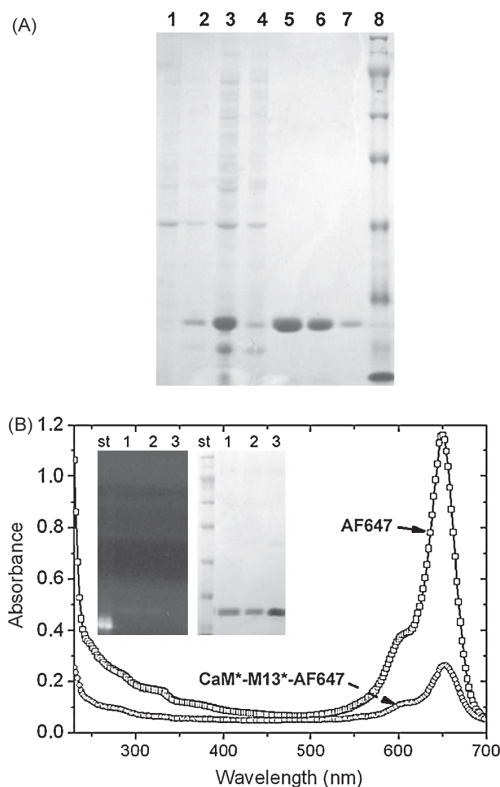


FIG. 3. SDS-PAGE (10%) of the purification steps for CaM*-M13* D3C using a 300 ml culture. (A) 1. – IPTG, 2. + IPTG (0.6 mM, 4 hr), 3. load, 4. flow through, 5~7. eluted fractions, 8. protein standard. (B) Absorption spectra of free dye (AF647) and AF647 labeled CaM*-M13*. (Inset) st. protein standard, 1, 2 CaM*-M13*-AF647 at different concentrations, 3. Unlabeled CaM*-M13* D3C. SDS-PAGE was visualized by UV illumination (left) and coomassie brilliant blue staining (right).

FRET. We also estimated the maximum number of CaM*-M13*-AF647 bound to a single quantum dot to be 10~12 molecules per quantum dot particle, with an optimal number of calmodulin proteins for calcium sensing to be 2 to 5 molecules per quantum dot. The exact stoichiometry of binding and the stoichiometric dependency of calcium sensing parameters are currently under investigation (manuscript in preparation).

Calcium Ion Binding-Induced Folding and EDTA-Induced Unfolding of Calmodulin on Quantum Dot Surfaces

We then tested the calcium sensitivity of the quantum dot-calmodulin complex we constructed. In Figure 5(A), the fluorescence spectra of the qdot sensor at 0, 0.5 and 1 mM calcium ions are shown. Upon binding with 0.5 mM calcium ions, more than 66% of the quantum dot emission was quenched at the donor

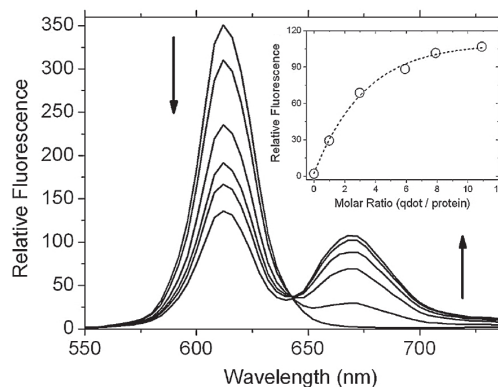


FIG. 4. Titration of DHLA-qdot 610 with CaM*-M13*-AF647. The concentration of the quantum dots was 12.9 nM in 1.5 ml of 20 mM Tris-HCl buffer, pH 8.0. Increasing amounts of 57.2 μM CaM*-M13*-AF647 were added to have quantum dot to protein molar ratios of 0, 0.99, 2.97, 5.91, 7.90 and 10.9 (top to bottom at 610 nm peaks at 25 °C. Excitation wavelength was 400 nm with photomultiplier high voltage set to medium.

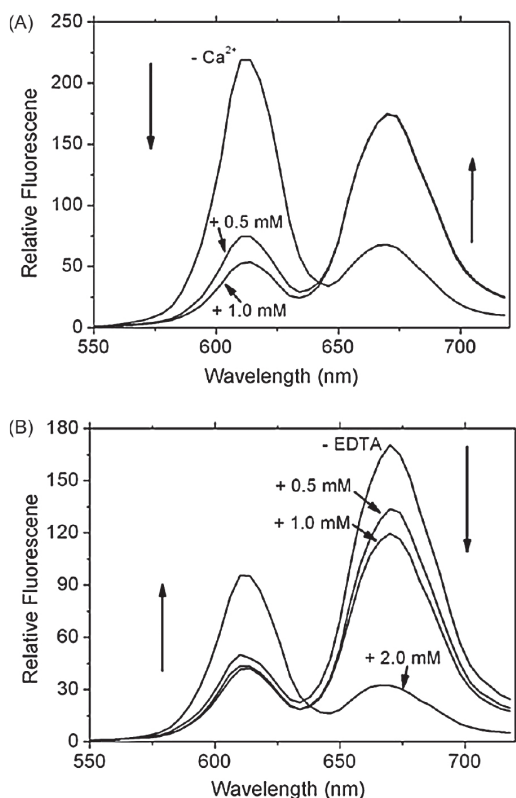


FIG. 5. Calcium and EDTA induced folding and unfolding of calmodulin on the quantum dot surface. (A) Calcium ion binding induced folding of CaM*-M13*-AF647 on the quantum dot surface as indicated by FRET from donor (qdot, 610 nm) to acceptor (AF647, 670 nm). Binding-induced decrease of the donor fluorescence at 610 nm and sensitized emission of acceptor (AF647) at 670 nm is observable. The concentrations of the calcium ions are indicated. (B) EDTA induced unfolding of CaM*-M13*-AF647. Opening of the closed calmodulin structure is evidenced by the reversal of the calcium ion induced FRET. Decrease of acceptor (670 nm) and increase of donor (610 nm) fluorescence are observed. The concentrations of EDTA are indicated. The quantum dot to calmodulin molar ratio was 1 to 5.

fluorescence peaks with about 260% increase in the acceptor emission peak (670 nm). Fractional changes in FRET at 0.5 mM calcium ions were estimated by using a following relationship (Reiff et al., 2005; Heim et al., 2007):

Fractional changes in FRET

$$= \Delta R/R = (R_{\text{Ca}^{2+}\text{-saturated}} - R_{\text{Ca}^{2+}\text{-free}})/R_{\text{Ca}^{2+}\text{-free}}$$

where, R is the acceptor (670 nm) to donor (610 nm) fluorescence intensity ratio. Based on this relationship, the quantum dot sensor gave a $\Delta R/R$ value of about 6.6, which is much greater than the values of 0.5~0.8 for the original Cameleon systems (estimated from figures in Miyawaki et al., 1997) and about 1.7 for troponin C-based calcium sensor (estimated from a supplementary figure in Heim et al., 2007) *in vitro*. Further addition of calcium to 1 mM caused no change in the AF647 emission peak

(670 nm) but resulted in a slight reduction of the quantum dot emission peak (610 nm), probably due to the quenching effects of extra calcium ions on the quantum dot. In the present work we are ignoring any quenching produced by metal or EDTA induced quenching effects on the quantum dots. However, the effects of various metals and ligands on the emission properties of water solubilized CsSe/ZnS quantum dots may not be negligible under certain conditions and may affect the interpretation of experimental data. As such, these effects require further investigation. Since reversible folding and unfolding of the calcium-sensing component (CaM*-M13*) is critical for the system to be used as a calcium ion sensor *in vitro* and *in vivo*, we have investigated whether the folded calmodulin can revert to its calcium deficient apo structure following EDTA treatment (Fig. 5(B)).

The addition of increasing amounts of EDTA up to 1 mM resulted in the folded qdot 610-CaM*-M13*-AF647 complex presenting decreases at both the 610 nm and 670 nm fluorescence peaks, probably due to EDTA induced quenching of the quantum dots. In the presence of 2 mM EDTA, there were noticeable changes in the FRET signals, with a decrease in fluorescence intensity at 670 nm and an increase at 610 nm, implying the reversal of calcium binding-induced calmodulin folding. The reversal of the FRET response strongly suggests that the calmodulin undergoes an unfolding transition upon calcium deprivation resulting in the restoration of its open conformation. The reversibility of calmodulin folding and unfolding on the quantum dot surfaces allows for a wider range of possible applications for this sensor in cell biology.

Effects of Surface Ligands on Ca²⁺ Binding

We also investigated the effects of different surface ligands (PEG and PEI) on the binding of CaM*-M13*-AF647 to quantum dots and on calcium sensing. In Figure 6 the fluorescence spectra for PEG and PEI capped quantum dots upon binding to CaM*-M13*-AF647 and, subsequently, to calcium ions are shown. Binding

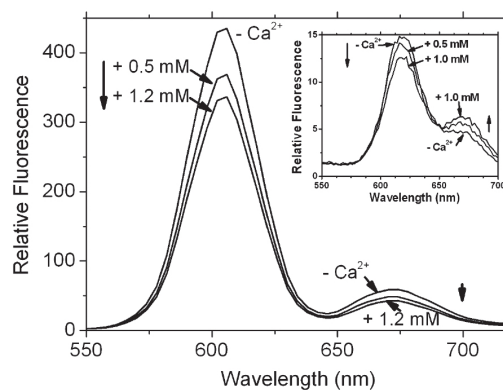


FIG. 6. Effects of surface ligands on the binding and calcium induced folding of calmodulin. Fluorescence spectra PEG capped quantum dot (PEG-qdot) in the presence of calcium ions. Increasing amount of calcium ions decreased fluorescence peaks at both 605 nm and 670 nm. (Inset) Fluorescence spectra of PEI (MW = 2,000) capped quantum dot (PEI-qdot). Calcium ions have a minimal effect on the fluorescence peaks. Concentrations of calcium ions are indicated.

of the protein caused a decrease in fluorescence intensity at 610 nm with a concomitant increase at 670 nm due to FRET from the quantum dot to the AF647 dye labeled on the CaM*-M13* protein. The data indicate that the histidine tagged calmodulin protein can bind to both neutral (PEG, Fig. 6) and positively charged (PEI, Fig. 6 Inset) ligand capped quantum dots, similar to the results of negatively charged DHLA capped quantum dots (Fig. 4). Binding of histidine tagged proteins (Medintz et al., 2003; Dennis and Bao, 2008) and peptides (Delehanty et al., 2006) to PEG or DHLA capped quantum dots have also been reported by others. After we confirmed the binding of the histidine tagged calmodulin protein to all three quantum dots, we tested the calcium sensing capacity of the PEG and PEI capped quantum dots. In contrast to DHLA-qdots, both PEG (Fig. 6) and PEI (Fig. 6 Inset) quantum dots did not show any significant changes in the presence of calcium ions. The minimal changes in the FRET signals suggest that the calmodulins on the PEG and PEI capped quantum dots are unable to fold upon binding with calcium ions. The longer ligands (PEG and PEI) probably cause an unacceptable degree of steric hindrance to optimal CaM*-M13* folding necessary to achieve the strong FRET signals we observed with DHLA-capped quantum dots.

CONCLUSIONS

We have constructed a novel FRET-based calcium sensor composed of a quantum dot acting as a fluorescence donor, a mutated calmodulin-M13 pair (CaM*-M13*) acting as a calcium sensing module, and a fluorescent dye conjugated to the N-terminal end (3rd position) of the protein acting as a fluorescence acceptor. The use of qdots as FRET donors are numerous, due to their robust optical properties, and include ligand binding studies (Medintz et al., 2003), aptamertarget protein binding (Levy et al., 2005), peptide-RNA interactions (Zhang and Johnson, 2006), and measurements of enzyme activity (Xu et al., 2006; Clapp et al., 2008). However, to the best of our knowledge, this is the first quantum dot-based calcium sensor. The FRET sensor we constructed produced a maximal fractional change in FRET ($\Delta R/R$) of about 6.6 upon calcium binding, one of the biggest ratio changes among all known FRET-based quantum dot sensors reported in the literature for any system. Despite the many advantages of genetically encoded calcium indicators (GECIs), they still suffer from small changes in fluorescence signal. Considering the $\Delta R/R$ values of the original Cameleon and troponin-C based GECIs are in the range of 0.5~1.7 (Miyawaki et al., 1997; Heim et al., 2007), the strong FRET response of the quantum dot sensor ($\Delta R/R$ of 6.6, Fig. 5(A)) gives it significant potential to be developed as an intracellular calcium indicator, which our lab is actively pursuing.

Using a hexa-histidine tag (Fig. 1) we were able to assemble calmodulin proteins onto quantum dot surfaces (Fig. 4). The spontaneous complex formation in response to the exposure of different (charged) ligand (PEG, PEI and DHLA)-capped quantum dots facilitate the easy preparation of qdot-protein complexes and the determination of their protein binding stoichiometry. After examining the calcium binding sensitivity of the probes, we examined the unfolding characteristics of the calmodulin (CaM*-M13*). Treatment with EDTA reverted the closed calcium ion-bound conformation to an open structure (Fig. 5(B)), as indicated by the increase of the 610 nm/670 nm intensity

ratios. Our data suggest that in addition to the quantum dot-protein complex capacity as a sensor, it is also an excellent system with which to study the folding-unfolding properties of proteins for structural analysis. We also found that our quantum dot-calmodulin-based calcium sensor was very sensitive to the nature of the surface ligands. When we used longer surface ligands such as PEG (unpublished MW by Life Technologies) or PEI (MW = 2,000), there was almost no FRET response to calcium ion binding (Fig. 6). This is in contrast to the shorter eight-carbon DHLA-capped quantum dots, which showed significant FRET at the same concentration of calcium ions (Fig. 5(A)). The longer ligands probably prevent the folding of calmodulin by steric hindrance causing minimal FRET signals, which should be considered in the development of protein folding-based quantum dot sensors.

Quantum dot-based sensors are also well known for their excellent optical properties (Michalet et al., 2004; Mazumder et al., 2009) including superior photostability, a broad range of excitation wavelengths (ca 350~550 nm), size-tunable narrow emission bands (full-width at half-maximum of about 25~40 nm), minimal crosstalk, long lifetimes, and adjustable optical cross-sections through varying numbers of attached acceptors. These features along with the large quantum dot surface area available for functionalization provide users considerable flexibility in the choice of excitation sources and optical filters, experimental duration, system multiplexing, and intracellular targeting.

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