Subthreshold resonance in central neurons

by

Bruce McCullagh Hutcheon

B.Sc, The University of British Columbia, 1981

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

(Graduate Program in Neuroscience)

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

March 1996

© Bruce McCullagh Hutcheon, 1996
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Pharmacology & Therapeutics

The University of British Columbia
Vancouver, Canada

Date March 25, 96
Abstract

Electrical signals from the mammalian brain often contain large-amplitude components at characteristic frequencies. What properties of central neuronal networks determine these frequencies? One possibility is that individual neurons are electrically tuned to respond better at some frequencies than others. Such tuning has been called electrical membrane resonance and arises from the interaction of the passive properties of the membrane with time- and voltage-dependent membrane currents. In the present work, frequency-domain analyses of neocortical and thalamic neurons were performed to detect resonances at subthreshold potentials. The ionic mechanisms of these resonances and their effect on firing patterns were then examined using a combination of mathematical modeling and experiment. In addition, a novel technique, the reactive current clamp (RCC), was developed for coupling mathematical models of ionic currents to living cells.

In approximately 2/3 of neocortical neurons, the hyperpolarization-activated cation current (I_H) caused a 1 to 2 Hertz resonance near the resting potential. Other currents in these neurons attenuated or amplified the resonance in a voltage-dependent manner. Putative inhibitory neurons of the neocortex did not have an I_H-resonance. The resonance affected neuronal firing patterns. When resonant neocortical neurons were injected with swept-frequency sinusoidal currents, they were most likely to fire action potentials as the input swept through the resonant band; a phenomenon called "frequency-selective firing". In nonresonant neurons, resonance and frequency-selective firing were generated when a model of I_H was coupled to the neuron using the RCC.

A theoretical analysis based on previously published data showed that a different voltage-dependent current, the low-threshold calcium current (I_T), accounts for the 2 to 4 Hz resonance observed in thalamic neurons. In these neurons, spontaneous oscillations of the membrane potential have been observed near the same frequency. It is suggested that spontaneous oscillations arise from resonant mechanisms that are amplified by voltage-dependent currents.
In conclusion, subthreshold resonance is an intrinsic property of neurons that may control the frequencies of coordinated activities in neuronal networks. The resonances described here have frequency ranges that are suitable for stabilizing the spindle and delta brain rhythms that arise during sleep in mammals. In the neocortex, this mechanism seems to be limited to excitatory neurons.
# Table of Contents

Abstract .............................................................................................................. ii

Table of Contents ............................................................................................. iv

List of Tables ...................................................................................................... viii

List of Figures .................................................................................................... ix

Acknowledgments .............................................................................................. xii

Dedication ........................................................................................................... xiii

1. Introduction .................................................................................................... 1
   1.1 Scope of the topic ...................................................................................... 1
   1.2 Resonance and small-signal impedances of neurons .................................. 2
   1.3 The ZAP method ..................................................................................... 7
   1.4 Subthreshold currents .............................................................................. 8
      1.4.1 The hyperpolarization-activated cation current, $I_H$ ....................... 8
      1.4.2 The low-threshold $\text{Ca}^{2+}$ current, $I_T$ ...................................... 9
   1.5 Organization of this thesis ....................................................................... 10

2. The reactive current clamp (RCC) .................................................................. 11
   2.1 Overview .................................................................................................. 11
   2.2 Strengths and limitations of the RCC technique ........................................ 12
   2.3 Theoretical considerations ..................................................................... 13
   2.4 Implementation ....................................................................................... 15
   2.5 Calibration of the RCC system ............................................................... 17
   2.6 Demonstrations of the use of the RCC .................................................... 19

3. Theory of neuronal frequency-response relationships ..................................... 26
   3.1 Introduction ............................................................................................. 26
   3.2 Capacitive-membrane (CM) models ......................................................... 27
List of Tables

Table 4.1 Electrophysiological properties of neocortical neurons ........................................84
Table 4.2 Mean resonant frequencies and Q values for resonant neocortical neurons .............89
Table 5.1 Estimated parameter values for $I_H$ activation at 24 - 26° C .................................118
List of Figures

Figure 2.1 Schematic diagram of the reactive current clamp (RCC) .............................................................. 16
Figure 2.2 Timing scheme for the numerical implementation of Equation (2.6) during the operation of the RCC. ........................................................................................................................................ 18
Figure 2.3 Electronic expression of an artificial I_H in a model cell and an in vitro neuron .................. 20
Figure 2.4 Electronic antagonism of the persistent Na^+ current, I_{Na,p}, and comparison with the effects of TTX .................................................................................................................................................. 22
Figure 2.5 Electronic antagonism and agonism of I_{Na,p} in neocortical neurons ........................................... 23
Figure 2.6 Different levels of electronically expressed I_{Na,p} produce a wide range of firing patterns in a neocortical neuron ........................................................................................................................................ 25
Figure 3.1 Equivalent circuits for three models of membrane electrical activity ........................................ 30
Figure 3.2 Frequency response properties of the CM model ........................................................................ 32
Figure 3.3 Definition of class I and class II currents ..................................................................................... 36
Figure 3.4 Qualitative properties of class I and II currents ............................................................................ 37
Figure 3.5 Frequency-response properties of an attenuated class I model .................................................. 40
Figure 3.6 Frequency-response properties of a resonant class I model ....................................................... 42
Figure 3.7 Frequency-response properties of an amplified class II model .................................................. 44
Figure 3.8 Frequency-response properties of a class II model with attenuated responses ....................... 45
Figure 3.9 Frequency-response properties of a resonant class II model ..................................................... 48
Figure 3.10 Analysis of attenuated and resonant FRCs for class I models ..................................................... 52
Figure 3.11 Low- and high-frequency limits of |Z_{d}(v,w)| for a class I model ................................................. 55
Figure 3.12 Analysis of the FRC of an amplifying class II model ............................................................... 59
Figure 3.13 Low- and high-frequency limits of |Z_{d}(v,w)| for two class II models ......................................... 61
Figure 3.14 Summary of the frequency-response behavior and stability of CM+S models ...................... 64
Figure 3.15 Attenuated and amplified resonance in a CM+SS model ....................................................... 69
Figure 3.16 Stability of a CM+SS model with amplified resonance ............................................................ 71
Figure 3.17 Frequency-response surface for a CM+SS model with an amplified resonance .................... 73
Figure 3.18 Definition of the window conductance for a CM+I model ....................................................... 76
Figure 3.19 Frequency-response surface for a CM+I model with amplified resonance ............................ 78
Figure 4.1 Firing patterns and I-V relations for neocortical neurons .......................................................... 85
Figure 6.6 Changing \( v_{h2} \) has a large effect on resonance but a much smaller effect on the low-threshold spike of the minimal MDT model.

Figure 6.7 The nonlinear responses of the minimal MDT model show frequency selectivity.
Acknowledgments

I wish to express my deepest gratitude to Dr. Ernie Puil and Dr. Robert Miura who jointly supervised this work. They have always been generous with their valuable time and have treated me as a colleague rather than a student. I also wish to thank Lance Corey for writing the computer programs that were crucial to the success of the research.
Dedication

This work is dedicated to my wife, Gitanjali, who has an unfailing supply of support and encouragement when I need it most.
1. Introduction

1.1 Scope of the topic

In this thesis, the subthreshold electrical resonances of neocortical and thalamic neurons are characterized and their ionic mechanisms elucidated through a combination of experiment and modeling. Resonance, in this context, means that a neuron has an intrinsically defined frequency preference that characterizes its responses to inputs. In a resonant neuron, the voltage responses to oscillatory current inputs delivered at the resonant frequency are larger than to inputs of the same size at any other frequency.

Resonance may tune neurons to respond preferentially to oscillatory current inputs at biologically important frequencies. In the mammalian central nervous system, such oscillatory inputs arise during episodes of coordinated rhythmic firing which are associated with well-defined behavioral states such as deep sleep or drowsiness. During these states, convergent synaptic inputs can combine to generate oscillatory currents with characteristic frequencies in the dendrites and somata of neocortical and thalamic neurons. Under these circumstances, the frequency preference of an electrically tuned neuron may favor its participation in one type of coordinated network activity over another. Thus, resonance may be implicated in the control of the behavioral state.

The concept of electrical resonance comes from the theory of electrical circuits. Application of impedance analysis techniques of circuit theory to the search for resonance in neurons is a major theme of this thesis. This includes derivations of the impedances of linearized Hodgkin-Huxley-type models of neurons and direct measurement of the impedances of neurons in vitro slices. Impedance is a linear concept and, therefore, it is most effectively used to examine the small-signal responses of neurons, i.e., responses that do not involve large nonlinearities. For this reason, the work in this thesis focuses on the impedances of neurons at subthreshold membrane potentials where their nonlinear properties are weak enough that they can be well-approximated by a linear system. Nonetheless, it is essential to go beyond the range of linear approximations and attempt to connect the subthreshold frequency-response characteristics of neurons with the highly nonlinear process of action potential generation. This is because subthreshold resonance may be no more than an epiphenomenon if it does not
influence the firing properties of a neuron. To study the connection between resonance and spike firing, the special properties of a swept-frequency, oscillatory current input are used.

Part of this thesis represents a novel form of modeling involving the use of the Reactive Current Clamp (RCC), a system that was developed during the course of this thesis work in the laboratory of Drs. E. Puil and R. M. Miura. The RCC allows a mathematical model of a voltage-dependent ionic current to be electronically added to a living neuron. The RCC also can be used to mask the electrical effects of an endogenous current. Use of the RCC in these ways is called "electronic pharmacology" because of its similarity to the use of drugs for blocking or producing ionic currents. In this thesis, the RCC is used to demonstrate the ability of mathematically defined voltage-dependent currents with specified parameters to create resonance in real neurons.

1.2 Resonance and small-signal impedances of neurons

A great deal of the work in this thesis is concerned with the theoretical and experimental analysis of the impedance of central neurons. The input impedance of a linear electrical system can be thought of as the frequency-dependent relationship between voltage and current measured at the same point. Since a sinusoidal waveform at a particular frequency is described by two independent parameters, the phase and amplitude, the impedance has two corresponding parameters, the phase shift and the amplitude factor. For each frequency, the impedance may therefore be portrayed as a two-dimensional vector or as a complex number. The complex form of the impedance will be considered here. Taking the magnitude of the complex impedance gives the amplitude factor. This constitutes a type of frequency-dependent input resistance. Taking the argument of the complex impedance indicates the phase relationship between the voltage and current. By convention, when the phase of the voltage lags that of the current, the phase is negative. This is the type of response that is normally associated with an electrical circuit containing a capacitor. In contrast, an inductive element in an electrical circuit usually results in the voltage leading the current so that the phase shift is positive.

Impedance analysis has a venerable history in neuroscience and biophysics. It was originally used to study the dielectric and conductance properties of biomembranes in suspension. A series of experimental and theoretical investigations led Fricke (1923, 1925a,b) and then Cole (1928, 1968) to propose that the membrane of eukaryotic cells is a thin, poorly conducting lipid with a capacitance of approximately 1 μF/cm² surrounding a highly conductive
interior. Throughout the 1930s, impedance methods became one of the most accurate and incisive techniques available for studying the structure and function of membranes. By the late 1930s Cole, had ceased working with suspensions and directed his attention towards impedance measurements of the squid giant axon. Using extracellular electrodes to create either longitudinal or transverse alternating electric fields, Cole and his colleagues succeeded in showing that the axon undergoes large conductance changes on excitation (Curtis and Cole 1938; Cole and Baker 1941a, Cole and Curtis 1939). They used these findings to create minimal equivalent electrical circuit models representing the electrical properties of the squid axon. An unexpected finding was the presence of an inductive component in the longitudinal impedance during subthreshold stimulation (Cole and Hodgkin 1939; Cole and Baker 1941b). This inductive component creates a resonance as in a simple RLC electric circuit and suggested to Cole that the conductances in the squid axon had distinctive kinetic as well as voltage-dependent properties (Cole 1968). These findings, however, signaled the end of the dominance of the impedance method for investigating the electrical properties of neurons. There were two reasons for this. First, the development of current- and voltage-clamp recording techniques made possible high-quality, time-domain measurements of membrane conductance and capacitance without the need for impedance measurements. Second, the study of the highly nonlinear conductances underlying action potentials were ill-suited to impedance methods that require the system under study to act in a linear or near-linear fashion.

Impedance methods enjoyed a resurgence as investigators became interested in small-amplitude subthreshold voltage oscillations that occur in the solutions to the Hodgkin-Huxley model of the squid giant axon or were measured experimentally (Hodgkin and Huxley 1952; Cooley and Dodge 1966; Sabah and Leibovic 1969; Mauro et al. 1970). The amplitude of these rhythmic oscillations is voltage dependent and their frequency is close to the firing rate of near-threshold trains of action potentials. Therefore, it was proposed that they act as pacemakers for rhythmic firing at low stimulation intensities (Mauro et al. 1970). Their properties were explained as a manifestation of a linear membrane resonance that arises from the inductance-like behavior of time- and voltage-dependent membrane currents (Mauro et al. 1970). A convenient theoretical framework was provided by linearizing the Hodgkin-Huxley model for the squid giant axon and identifying the resulting system with an electrical circuit containing resistors, capacitors and inductors (Mauro et al. 1970; Koch 1984). Thus, the theoretical impedance analysis of this
circuit provided a simple method for estimating the frequency of the subthreshold oscillations and understanding the conditions that facilitate their appearance.

The study of resonance in the squid axon was pursued by Fishman and colleagues who made direct, frequency-domain measurements of the membrane admittance (the inverse of impedance) in an elegant series of experiments combining voltage-clamp techniques with the pharmacological isolation or blockade of selected ionic conductances (Fishman et al. 1977, 1979; Moore et al. 1981). In accordance with the theoretical calculations of Mauro et al. (1970), they found that there are actually two voltage-dependent resonances in the squid membrane that interact to produce the single resonance usually seen. One resonance is generated by the inactivation of the $Na^+$ conductance system of the squid axon and the other by the $K^+$ system. By fitting the resonant admittances to Mauro's linearized models, they were able to estimate relaxation times for the channels underlying these conductance systems.

Resonances also have been observed in the somata of neurons. Puil et al. (1986, 1988a,b, 1989) have investigated a subthreshold resonance found in the cell bodies of trigeminal root ganglion neurons. In these neurons, most of which prefer to fire a solitary spike when stimulated with current pulses, resonance is associated with a tendency towards repetitive firing. By fitting models to the data they made quantitative estimates of the time- and voltage-dependent properties of the ionic conductances responsible for resonance and of the changes induced in these properties by various drugs (Puil and Gimbarzevsky 1987). They found that the strength of the resonance could be increased or decreased by blockade of different $K^+$ currents (Puil et al. 1989). In the time domain, application of the blockers that increase resonance resulted in a facilitation of repetitive firing and sustained, lightly damped, ringing oscillations of the membrane voltage in response to current pulses. The interpretation of these data is that trigeminal root ganglion neurons possess a $K^+$ current that causes resonance but that the effects of this current are normally masked by another $K^+$ current. In neurons with a relatively weak masking current, the resulting potentiation of oscillatory behavior may lead to the spontaneous discharges implicated in certain types of neuralgia.

The normal physiological functions of the resonance found in trigeminal root ganglion cells are unknown. In other peripheral neurons and excitable cells, however, resonance appears to contribute to the filtering of sensory inputs. For instance, insect and turtle photoreceptors have voltage-dependent resonances that may contribute to a frequency dependence of the receptive
field of these cells (Detwiler et al. 1980; Weckström et al. 1992). Also, many cells of the
auditory and vestibular systems of vertebrates have Ca\(^{2+}\)- and K\(^{+}\)-dependent electrical resonances
with resonant frequencies that approximate the characteristic frequency of the cell’s linear tuning
curve (Crawford and Fettiplace 1981; Fuchs et al. 1988; Hudspeth and Lewis 1988, ). This
suggests that, for these cells, electrical resonance may play a role in determining their
input/output relationships. However, this interpretation is complicated by the existence of
mechanical resonances in these cells (Hudspeth 1983; Crawford and Fettiplace 1985; Eatock et
al. 1993).

Recent work on central neurons using impedance (or admittance) techniques has revealed
a variety of resonant and nonresonant behaviors. Gutfreund et al. (1995) have described a low
frequency (5 - 20 Hz) resonance that occurs at membrane potentials depolarized from rest in
neocortical neurons. The resonance is apparently due to a K\(^{+}\) mechanism since it is abolished by
external application of tetraethylammonium (TEA). A Na\(^{+}\) mechanism also is implicated,
however, since tetrodotoxin (TTX) reduces the resonance. Gutfreund et al. associate the
resonance with spontaneous small-amplitude voltage oscillations observed in the same cells.
Ströhmann et al. (1994, 1995) have performed impedance measurements on neurons at different
levels of the auditory pathway in chicks. They discovered resonance in neurons from several
areas and showed that these neurons could be stimulated to fire most easily by oscillatory
currents near their resonant frequency. Two voltage-dependent currents, active at subthreshold
potentials, were implicated in these resonances, a hyperpolarization-activated cation current, I\(_H\),
and (in rare cases) an inactivating low-threshold Ca\(^{2+}\) current, I\(_T\) (Ströhmann et al. 1994). The
latter current also underlies a low-frequency resonance observed in mammalian thalamic neurons
(Puil et al. 1994). In work that forms part of this thesis, analysis of a realistic model of the
neuron showed that the magnitude of this resonance is crucially affected by small changes in the
relationship between I\(_T\) activation and inactivation (Hutcheon et al. 1994). Jahnsen and Karnup
(1994) have surveyed the power spectra of central mammalian neurons activated by
stochastically fluctuating injected currents. While the measurement of a power spectrum is
different from an impedance, there are enough similarities between these two types of frequency-
domain measurements to identify resonances in their published records from thalamic, CA1
hippocampal, and neocortical neurons. In contrast, cerebellar Purkinje cells were nonresonant.
Many of the neurons and other types of cells mentioned above have complex geometries. The importance of impedance techniques for the study of extended structures can be summarized by the observation that the attenuation of voltage transients in a leaky cable depends on frequency (Jack et al. 1983). In the case of resonance, Koch (1984) has pointed out that transient or periodic synaptic potentials with large frequency components near the resonant frequency will be transmitted with the least attenuation. Moore and colleagues have measured transfer impedances and admittances (the mathematical inverse of the impedance) between two recording sites in cultured neuroblastoma cells and isolated spinal cord neurons from lampreys (Moore et al. 1988, 1993). They found that the same voltage-dependent conductances that contribute to resonant behavior in a space-clamped cell cause resonances in the transfer admittance. However, the resonant frequency often varied with recording location or with the direction of signal transfer emphasizing the role of geometry in signal integration for many neurons.

Like the admittance measurements of the squid axon, determination of these transfer admittances requires that the neuron be voltage clamped to stabilize its membrane voltage. The experiments by Fishman, Moore, and others, follow a style of biophysical analysis that owes more to the work of Cole than to the later analyses of Mauro. The resonances, measured as admittances under voltage clamp, are associated with membrane voltages that are unstable under current clamp. Therefore, their functional relevance is difficult to assess since the membrane normally spends little time near these potentials under physiological conditions. In these investigations, the impedance method reveals more about the physical characteristics of the underlying conductances than about the functional organization of the neuron.

To summarize, some studies treat impedance measurements as probes of the biophysical properties of excitable membranes. Fits of models to impedance data lead to estimates of the kinetic and voltage-dependent properties of ion channels. In this respect, impedance techniques can substitute for the traditional voltage-clamp analysis of ion currents. From another point of view, impedance studies can be used to uncover resonances that may have functional roles such as the electrical tuning of auditory/vestibular cells described above. The approach adopted in this thesis is closer to this second viewpoint. Thus, impedance measurements are used to identify resonances at subthreshold membrane potentials. The frequency, voltage-dependence, and ionic mechanisms of each resonance may then be clues as to its possible function.
1.3 The ZAP method

Impedance analysis is a type of linear systems analysis specialized to electrical circuits. A fundamental property of linear systems is that the response to an input at one frequency is independent of the response at any other frequency. The total response to a input comprised of may frequencies is simply the sum of the responses to the component frequencies in the input. From this, it would seem that it does not matter what waveform is used for the input since its frequency component will never interact. On an abstract level this is true. However, in an experimental situation, some waveforms are more convenient than others. Popular input waveforms for physiological analysis are band limited white noise, sum-of-sines signals, and pseudorandom binary sequences (Marmarelis and Marmarelis 1978; Bendat and Piersol 1986; Sakai 1992).

In this thesis, a swept-frequency sinusoidal ("ZAP") current input is most often used for stimulating neurons. Its use was pioneered by Puil and Miura in the 1980s (Gimbarzevsky et al. 1984; Puil et al. 1986-89). The advantage of this input waveform is that not only can it be used for an impedance analysis when neurons behave in a linear or near linear fashion, it also has advantages when used for the study of nonlinear responses to oscillatory stimuli. This is because the ZAP current is oscillatory, with monotonically increasing (or decreasing) frequency, and so its frequency distribution is evident in the time domain. Thus, the input frequency associated with a feature of interest in the response can be identified by its position. For example, the voltage response of a resonant neuron to a ZAP input is oscillatory with a spindle-shaped envelope. The largest responses within the envelope are generated as the input sweeps past the resonant frequency. Thus the part of the response associated with the resonant frequency is immediately apparent. Other features of interest can now be related to this landmark -- in a later chapter, the input frequencies that most readily evoke action potentials are related to the resonant frequency by visual inspection of the output. This would not be possible using white noise or sum-of-sine-wave inputs where the frequency components are not separated in time.

The ability to combine a quantitative frequency analysis with a simple qualitative assessment of nonlinear frequency sensitivity is thus a distinctive attribute of the ZAP method. Its physiological relevance comes from its use as a simplified model of the oscillatory current inputs that the soma of a neuron receives from the dendrites during coordinated rhythmic activity in the brain. For example, intracellular in vivo recordings from cortical and thalamic neurons of
anesthetized rat or cat, or cerveau isolé preparations show almost sinusoidal, low-frequency oscillations of the voltage caused by rhythmic sequences of synaptic inputs at characteristic frequencies (Steriade et al. 1993b,c; Metherate and Ashe 1993; Cowan and Wilson 1994). These oscillations are the cellular correlates of large-amplitude rhythmic patterns of the EEG reflecting the synchronous activity of large numbers of central neurons. Thus, during some behavioral states, neurons are presented with inputs signaling the presence of synchronized activity at biologically important frequencies in brain circuits. Under these circumstances, the frequency-response characteristics of a neuron can determine whether it will contribute to the propagation and frequency-stabilization of synchronized firing. Therefore, use of the ZAP method to assess the frequency dependence of coupling between oscillatory inputs and action potentials can be viewed as an investigation of how neurons might enter into patterns of coordinated activity in the cortex.

1.4 Subthreshold currents

Neocortical and thalamic neurons possess a number of time- and voltage-dependent currents which are active at subthreshold membrane potentials. Based on theory and on previous experimental work (Ströhmann et al 1994; Puil et al. 1994), two of these currents are particularly well suited to produce resonance -- the hyperpolarization-activated cation current, $I_H$, and the low-threshold $Ca^{2+}$ current $I_T$. Their properties will be reviewed here with emphasis on their contributions to rhythmic behavior and oscillation. Other subthreshold currents occurring in these neurons will be dealt with as they arise in later chapters.

1.4.1 The hyperpolarization-activated cation current, $I_H$. 

$I_H$ (also called $I_Q$, $I_{AR}$ and $I_{f}$) is an inwardly rectifying $Na^+$/K$^+$ current that activates on hyperpolarization and does not inactivate (Spain et al. 1987, 1991; Kamondi and Reiner 1991; Wollmuth and Hille 1992; Maccaferri et al. 1993; Solomon and Nerbonne 1993). Its activation kinetics are slow, with exponential time constants ranging from tens of milliseconds to seconds (Spain et al. 1987; Uchimura et al. 1990; Solomon and Nerbonne 1993a). It is widely distributed in neurons and excitable cells and, in particular, is found in neurons of the neocortex (Spain et al. 1987, 1991; Solomon and Nerbone 1993; Budde et al. 1994) and thalamus (McCormick and Pape 1990b). The voltage dependence of $I_H$ varies from one preparation to another. However, in some neurons the foot of its activation curve is sufficiently depolarized that it contributes to the resting potential (Spain et al. 1987; Tokimasa and Akasu 1990; McCormick and Pape 1990a;
Foehring and Waters 1991). In these neurons, any change in membrane voltage must involve a concomitant change in $I_H$. Thus, $I_H$ is well placed to organize the responses of neurons to time-varying subthreshold inputs (Solomon and Nerbonne 1993a).

In neocortical neurons with $I_H$, a hyperpolarizing current pulse often results in a sagging voltage response followed by an overshooting rebound on termination of the pulse (Foehring and Waters 1991; Spain et al. 1991; Schwindt 1992; Solomon and Nerbonne 1993a). This is reminiscent of the damped oscillatory behavior often associated with resonance. Indeed, as a result of their surveys of central neurons, Jahnsen and Karnup (1994) and Strohmann et al. (1994) both found that neurons with sag and rebound possess resonance. Also, Solomon and Nerbonne (1993b), after an examination of the kinetics of $I_H$ in corticotectal neurons, have specifically suggested that it may “amplify the effects of selected frequencies of depolarizing and hyperpolarizing inputs”. Thus $I_H$ may be implicated in a frequency-selective filtering of inputs that could tune neurons to biologically important frequencies.

In thalamic neurons, $I_H$ is implicated in a different type of rhythmicity where, in concert with $I_T$, it produces spontaneous low-frequency oscillations of the membrane potential (McCormick and Pape 1990; McCormick and Huguenard 1992; Soltesz et al. 1991). Although $I_H$ is apparently small or nonexistent in the region of the thalamus modeled in Chapter 6, in Chapter 4 and Chapter 5, the role of $I_H$ in the resonance of neocortical neurons is thoroughly explored.

1.4.2 The low-threshold Ca$^{2+}$ current, $I_T$

$I_T$ is a transient Ca$^{2+}$ current that activates rapidly at subthreshold potentials in many neurons and inactivates slowly with exponential time constants of hundreds of milliseconds (Carbonne and Lux 1987; Bean 1990; Chen and Hess 1990). It does not seem to occur often in neocortical neurons; however, it is a ubiquitous electrophysiological feature of thalamic neurons. This current often is associated with rhythmic activities in neurons. In neurons of the lateral geniculate and ventrobasal nuclei, it has been implicated in 1 to 4 Hz spontaneous pacemaker oscillations of the membrane potential (Amzica et al. 1992; Curro Dossi et al. 1992; Lerescue et al. 1991; Lerescue 1992; Soltesz and Crunelli 1992), and may support the synchronized δ-wave activity (0.5-4 Hz) recorded during deep sleep (Curro Dossi et al. 1992; Steriade et al. 1991; McCormick et al. 1992).
In a paper that forms the foundation for Chapter 6, Puil et al. (1994) attributed a low-frequency subthreshold resonance in the 2-4 Hz frequency band to $I_T$. In Chapter 6 a model of $I_T$ excitability will be constructed based on the biophysical data of Huguenard and Prince (1992).

1.5 Organization of this thesis

In this thesis, a combination of theoretical and experimental methods are used to investigate subthreshold resonances in neocortical and thalamic neurons. One of these methods involves the use of the reactive current clamp (RCC). Because of its novelty, Chapter 2 will be devoted to describing the theory and implementation of the RCC. In addition, several examples of its use will be given. Chapter 3 contains derivations and analysis of the impedance for several different types of models that will be required in later chapters. A classification of these models on the basis of their frequency-response properties also are given. In Chapter 4, the basic electrical properties of neocortical neurons are described and correlated to the nature of their frequency-responses. A low-frequency subthreshold resonance is identified and its underlying ionic mechanism and effects on firing patterns described. Rectifying conductances that might modify resonance also are investigated. Chapter 5 continues the analysis of resonance in neocortical neurons by developing models of their electrical responsiveness in the subthreshold region. In this chapter, the RCC is used to couple one of these models directly to in vitro neurons. Chapter 6 seeks to account for the findings of Puil et al. (1994) who described a low frequency subthreshold resonance in thalamic neurons. To do this, the impedance of a realistic model of the subthreshold properties of thalamic neurons due to McCormick and Huguenard (1992) is derived. A simplified version of this model is used to investigate the sensitivity of resonance to changes in its parameters. In the final chapter, Chapter 7, the major findings are gathered together and their possible significance discussed.
2. The reactive current clamp (RCC)

2.1 Overview

This thesis combines experiment and modeling to investigate resonance in central neurons. One of the new techniques employed combines these approaches to an unprecedented degree. Because of its novelty, it will be introduced here and several demonstrations of its use will be given.

The reactive current clamp (RCC) is a technique for interactively coupling an arbitrary computer algorithm to a cell. Briefly described, the computer senses the membrane voltage of a cell via a standard electrophysiological recording system. It then performs a calculation using this voltage as the input. The output is used to control the direction and magnitude of a current to be injected back into the cell. The injected current alters the cell’s voltage and, in return, the voltage determines the input to the computer, thus establishing a feedback loop. If the computations are made in real-time and the algorithm is a model of a voltage-dependent conductance, the feedback approximates the relationship between an ionic current through biological ion channels and the cell. Another way to view the RCC is that it is a technique that endows a microelectrode with a time- and voltage-dependent gating.

The RCC was developed during the course of the thesis work reported here. A similar system, called a “dynamic clamp”, was developed independently by a group from Brandeis University in Boston (Sharp et al. 1993a,b). A predecessor of both techniques is the system invented by Yarom (1991) that couples an analog neural model to a neuron. In this case the feedback from the neuron to the computational system is missing since the neuron’s membrane voltage does not affect the behavior of the computational circuit. Nevertheless, the notion of coupling a real-time dynamical model to a neuron is the same.

For electrophysiological experiments, there are two basic ways in which the RCC can be used. First, a current corresponding to a modeled conductance may be electronically added to a cell. This results in the appearance of a new voltage-dependent current amongst its pre-existing repertoire of endogenous currents. This process resembles a technique familiar from molecular
neurobiology — that of expressing a current from a cloned channel against the background of existing currents in a cell. For this reason the addition of a current corresponding to a computationally determined conductance to a cell also will be called the *electronic expression* of the current. The second way the RCC can be used is to enhance or mask the effects of an endogenous conductance. This requires matching the features of the electronically expressed conductance to those of one of the pre-existing conductances within a cell. Once the parameters of the RCC conductance are matched in this way, adding a small amount of RCC current will appear to increase the endogenous conductance. On the other hand, adding a small amount of RCC current with the opposite polarity will mask the electrical effects of the existing conductance. Because of its similarity to the processes of pharmacological agonism and antagonism, these techniques will be called *electronic agonism* and *electronic antagonism* or, collectively, *electronic pharmacology*.

2.2 Strengths and limitations of the RCC technique

The RCC has certain characteristic strengths and limitations that should be mentioned before describing its operation in more detail. Its greatest strength is that it combines the best features of mathematical modeling and experiment. Thus, in the case of electronic pharmacology, since the artificial conductance created by the RCC is computer-controlled, its effects are more selective and more quickly reversed than comparable pharmacological techniques. Also, the mathematical precision with which the model expressed by the RCC is specified means that its parameter space can be systematically explored while its interaction with the endogenous conductances of the cell are assessed. This seems to be a more elegant way of examining the complex dynamics of excitable cells than exploring the parameter space of large models with many conductances which may or may not be biologically realistic. From the point of view of the RCC, the cell is a kind of analogue model of itself.

The RCC also allows observation of variables that cannot be accessed with conventional methods. As an example, the fluctuating levels of activation and inactivation for a modeled current can be recorded as it interacts with the other currents in a cell. This provides a window
into the dynamics of these interactions which was previously only available in large simulation models.

The RCC also has several limitations. The RCC system imitates the electrical but not the chemical nature of the current flowing through voltage-gated channels. For instance, one of the currents that will be a focus of investigation in later chapters (I_H) involves the flow of Na^+ and K^+ ions across the cell membrane (Wollmuth and Hille 1992). The RCC, on the other hand, simply injects a current through an electrode and does not reproduce the changes in internal Na^+ and K^+ concentrations that would normally be associated with a naturally occurring I_H. This could be significant, for example, in studying neurons with Na^+-activated conductances. In the future it may be possible to control this problem by using electrode filled with appropriate solutions, creating pores of known ionic selectivity in the membrane at the tip of a patch electrode in the on-cell configuration, or using caged compounds that can be released in response to light or other stimuli.

A related problem arises for the RCC system because the current it injects is localized to the point attachment of the electrode to the cell. In contrast many biological currents flow through a population of channels with a widespread spatial distribution. This means that the RCC technique cannot replicate the spatial complexity of the interactions between naturally occurring conductances.

As in many other situations, the limitations of the RCC can be turned to the advantage of the investigator. Thus, the recognition that the RCC replicates the electrical but not the chemical nature of a naturally occurring current can be used to show that some phenomena do not rely on the control of intracellular ions.

2.3 Theoretical considerations

This section describes the theory and operation of the RCC. First, consider the process that results in changes in the membrane voltage of a neuron on injection of a current. Assuming that the neuron is isopotential, the relationship between the capacitive, ionic, and injected currents is
\[
    c_m \frac{dv}{dt} = -[I_1 + I_2 + \ldots + I_n] + i_{inj}.
\]  

(2.1)

Here, \(v\) is voltage, \(c_m\) is the total membrane capacitance, \(I_1, \ldots, I_n\) are currents flowing through ion channels, and \(i_{inj}\) is an injected current under the control of the investigator. The use of the RCC technique for electronic pharmacology requires injecting a current that has the time- and voltage-dependent properties of a membrane ionic current into a neuron. This corresponds to adding an extra term, \(-i_{RCC}\), to (2.1). Although this current is part of \(i_{inj}\), we can rewrite (2.1) so that it is expressed in the same way as one of the neuron's endogenous currents,

\[
    c_m \frac{dv}{dt} = -[I_1 + I_2 + \ldots + I_n + i_{RCC}] + \tilde{i}_{inj}(t),
\]  

(2.2)

where \(\tilde{i}_{inj}(t)\) is an explicit function of time \(t\) specified by the investigator (e.g., a current pulse). The total injected current \(i_{inj}\) of (2.1), therefore, is given by

\[
    i_{inj} = -i_{RCC} + \tilde{i}_{inj}(t).
\]  

(2.3)

To control the time- and voltage-dependence of \(i_{RCC}\), a number of different models of ionic currents are available. In this paper, we have used a simple model of a non-inactivating current with HH-type dynamics with the form

\[
    i_{RCC} = \bar{g} m^s (v - v_{rev})
\]  

(2.4)

where \(\bar{g}\) is the maximal conductance, \(v_{rev}\) is an appropriate reversal potential, \(m\) is the activation variable, and the value of the exponent, \(s\), depends on the current to be simulated. The kinetics for \(m\) are given by

\[
    \frac{dm}{dt} = \frac{m_{\infty}(v) - m}{\tau(v)}
\]  

(2.5)

where \(m_{\infty}(v)\) and \(\tau(v)\) describe the voltage dependencies of the steady-state activation and the activation time constant, respectively. The functional forms for \(m_{\infty}(v)\) and \(\tau(v)\) are given in the Results.

The RCC technique instructs the computer to integrate (2.5) numerically. Then, the result of this calculation is used in (2.4) to compute \(i_{RCC}\) for injection into the neuron. Since the
computations are performed in real time, the interaction between \( i_{RCC} \) and the membrane ionic currents of the neuron approximates the relationship between a naturally occurring voltage-dependent current and the other ionic currents. As a result, the neuron behaves as though it possesses a new ionic current.

2.4 Implementation

This section describes the implementation of the RCC as shown schematically in Figure 2.1. A patch-clamp electrode in the whole-cell configuration, connected to the bridge circuit of an Axoclamp 2A amplifier, is used to sense the membrane voltage and inject current. Specially written software running on a 33 MHz 386 computer directs the periodic sampling of the membrane voltage of the neuron and digitizes the voltage values using a 40 kHz analog-to-digital converter (Scientific Solutions). The computer uses the sampled values of the voltage to calculate \( m \) as described below. Then, \( m^s \) is fed through a digital-to-analog converter into a 4-quadrant multiplier where it is multiplied by a driving force. The driving force, calculated by a differential amplifier, is equal to the difference between the membrane voltage and a preset \( v_{rev} \). A voltage divider was used to scale the output from the multiplier and, therefore, played a role corresponding to the maximal conductance of the RCC current (see \( g \) in Figure 2.1). The analog part of the system (differential amplifier, multiplier, and voltage divider) has a bandwidth > 50 kHz; its purpose is to offload calculations involving rapid changes in driving force from the computer.

During the implementation of the RCC, the continuously changing membrane voltage of the neuron is sampled at time intervals of width \( \Delta t \) and delivered to the computer as the input for the numerical integration of (2.5). The output of the computer's calculation, \( m \), also is delivered as a sequence of discrete values at intervals of \( \Delta t \). As shown in Figure 2.2, during the interval from time \( t_n \) to time \( t_{n+1} \), the input to the computer is \( v_n \) and its output is \( m_n \). The problem in finding a suitable procedure for computing \( m_n \) is to predict the change in \( m \) from \( t_n \) to \( t_{n+1} \). For this purpose, an exact solution for the response to a voltage step is available in the special case of (2.5). Referring to Figure 2.2, the value of \( m_n \) is the value of \( m \) predicted for time \( t_{n+1} \), given \( m_{n-1} \) and \( v_n \).
Figure 2.1 Schematic diagram of the reactive current clamp (RCC).

The membrane voltage of a biological neuron (\(v\)) is measured by an Axoclamp 2A amplifier (not shown). The voltage is used as the input to an analog system (gray area) for calculation of a driving force (\(v - v_{\text{rev}}\)) and also, after conversion (A/D), as the digital input (\(v_n\)) to a computer algorithm for the activation variable of a voltage-dependent ionic current. The value of the computed activation variable (\(m_n\)) is converted to an analog signal (D/A) and multiplied by the driving force. The resulting signal is scaled with an attenuator that sets the maximal conductance (\(g\)) of the computed current. The current injected into the neuron (\(I_{RCC}\)) is therefore the same as in (2.4), with \(s = 1\). The relationship between \(I_{RCC}\) and \(v\) simulates the relationship between a voltage-dependent membrane current and the neuron’s membrane potential.
Thus, the formula for updating $m$ is

$$m_n = m_{n-1} + [m_{\infty}(v_n) - m_{n-1}][1 - \exp(-\Delta t/\tau(v_n))]. \quad (2.6)$$

This value then is used to determine $i_{RCC}$ for the interval between $t_n$ and $t_{n+1}$ (see Figure 2.2) according to

$$i_{RCC_n}(t) = \bar{g} m_n^r [v(t) - v_{rev}]. \quad (2.7)$$

The functions $m_{\infty}(v)$ and $e^{-\Delta t/\tau(v)}$ were evaluated at various voltages over a suitable voltage range and stored in arrays for use as lookup tables during on-line calculations. In practice these procedures work well for all values of $\Delta t << \tau$.

The minimum time needed to go once around the RCC feedback loop was 200 µs. We often specified longer cycle times but always insured that $\Delta t/\tau$ was small. It was not necessary to make $\Delta t$ small relative to the fast changes in the membrane voltage because the calculations for the driving force were handled by the analog part of the system.

2.5 Calibration of the RCC system

The RCC depends crucially on the accurate measurement of membrane potential. The use of bridge mode for single-electrode voltage recording and current injection can result in small measurement errors, but were minimized by using in-slice, whole-cell patch recording. Nevertheless, a maximum uncompensated series resistance of 10 MΩ sometimes was present during RCC current injections of ≤ 300 pA. This resulted in a maximum error of 0.3 mV, which was small compared to the reciprocal of the maximum slope of the activation curve of the theoretical conductance. Electrode drift of comparable magnitude sometimes developed. When the uncompensated drift was > 3 mV, the cells were not used for analysis. The junction potential arising from the use of patch electrodes with K⁺-gluconate internal solutions also was compensated by adding -11 mV to all voltage parameters.

After the RCC update equation (2.6) and its lookup tables were installed in the computer, the entire RCC system was calibrated. Three different types of calibration were carried out. First, the RCC circuit was effectively voltage clamped by unhooking the computer feedback to the cell and controlling the voltage input, $v(t)$. Applying voltage steps to the input to the computer produced relaxations in the output resulting from the iteration of (2.6). This output,
Figure 2.2 Timing scheme for the numerical implementation of Equation (2.6) during the operation of the RCC.

Each cycle begins with a discrete measurement \( (v_n, \text{dashed curve}) \) of the continuously varying membrane voltage \( (v, \text{curved solid curve}) \). Immediately after gathering a \( v_n \) level, a new value of the activation variable \( (m_n, \text{solid labeled curve}) \) is calculated for the \( n^{th} \) interval using (2.6) to project the estimated value of \( m \) at \( t_{n+1} \) (filled circle at time \( t_{n+1} \)). The current \( (I_{RCC_n}(t)) \) injected into the neuron throughout the \( n^{th} \) interval, changes the membrane voltage \( (v) \), thus completing the feedback cycle before the next measurement at time \( t_{n+1} \).
normally used to control the current command input to the cell, was proportional to $i_{RCC}$. Its time course was recorded and fitted to a sum of exponential terms just as if it described the voltage clamp current for a real cell. Agreement between the fitted and theoretical time constants indicated that the RCC circuit was working properly. Empirically, it was found that the discrepancy in these time constants was < 5% as long as $\Delta t/\tau(v) < 0.2$. This only caused difficulties in the case of quickly activating currents with time constants that become (theoretically) extremely small at some voltages. To avoid this, a minimal value of 1 ms was assumed for $\tau$. The second type of calibration consisted of setting $m = 1$ and empirically determining the maximal conductance of $i_{RCC}$ for different settings of the potentiometer (labeled "$g$" in Figure 2.1) controlling the gain of the feedback around the RCC loop. During an experiment, the potentiometer readings were recorded and, after the experiment, the corresponding maximal conductances were reconstructed from the empirically determined calibration. Finally, the analog multiplier summing amplifier used in the RCC circuit (see Figure 2.1) was calibrated to remove offsets and assure linearity.

2.6 Demonstrations of the use of the RCC.

This section contains several descriptions of the RCC technique applied to neurons in slices of rat neocortex. These descriptions are included here even though they are not directly relevant to the issue of subthreshold resonance in central neurons because they demonstrate the capabilities of this new technique. The use of the RCC for elucidating the basis of subthreshold resonance will be described in Chapter 5. The methods for preparation of brain slices and physiological recordings used in this section are the same as those given in Chapter 4.

First, Figure 2.3A shows the effects of inserting an artificial $I_H$ current into a model cell (500 MΩ resistor in parallel with a 33 pF capacitor). The artificial $I_H$ produced sags and rebounds in the voltage responses to rectangular square current inputs. This was reflected in a non-ohmic current-voltage (I-V) relationship. Since the model cell is electrically passive, all non-ohmic behaviors are due to the electronic $I_H$ expressed using the RCC. Figure 2.3B shows that the electronic expression of a model $I_H$ in an actual neocortical neuron changes the subthreshold I-V relationship in a similar way.

The use of the RCC for electronic pharmacology, i.e., the electronic agonism and antagonism of an endogenous current, is now demonstrated. In this case the RCC is used to
A. Model cell

The computer-generated $I_H$ causes slow sags and overshoots in the voltage responses of a model cell (500 MΩ resistor in parallel with a 33 pF capacitance) to square current pulses. The current-voltage (I-V) relation of the model-cell/RCC system shows the inward rectification induced by the electronic expression of $I_H$ in the passive model cell. B. Electronic expression of $I_H$ in a neocortical neuron intensifies the subthreshold inward rectification. The quasi-steady-state I-V plots in A and B are generated by injected current ramps (25 pA/s).

Figure 2.3 Electronic expression of an artificial $I_H$ in a model cell and an in vitro neuron.
express or mask the persistent Na$^+$ current, $I_{Na,p}$. This current occurs in many neocortical neurons. Its lack of inactivation, simple kinetics and the fact that it is often the only voltage dependent current expressed in a range of voltages just below threshold make it an ideal candidate for electronic pharmacology. To mask this current, initial parameters for the RCC model of $I_{Na,p}$ were used that were known to be approximately correct according to published data and previous experience. It was then necessary to bring these values into accordance with the actual properties of the endogenous current. This was accomplished by repeatedly subjecting the hybrid neuron-computer system to depolarizing pulses and adjusting the parameters of the model until the inward rectification characteristic of $I_{Na,p}$ was abolished at all voltages between rest and the threshold for Na$^+$ spikes. The results of this process are shown in Figure 2.4 where the antagonism of $I_{Na,p}$ produced by the RCC is compared with the results of its pharmacological antagonism by tetrodotoxin (TTX). At subthreshold potentials, there is little difference between the effects of TTX and the electronic antagonism of $I_{Na,p}$ produced by the RCC as can be seen in Figure 2.4D. The chief difference between the pharmacological and electronic forms of antagonism is that TTX blocks action potentials in addition to blocking $I_{Na,p}$ whereas the electronic antagonism is specifically targeted at $I_{Na,p}$. In fact, there is no known pharmacological blocker of $I_{Na,p}$ which does not also block the transient Na$^+$ current responsible for action potentials. The effects of $I_{Na,p}$ on firing patterns are, therefore, inaccessible to pharmacological analysis.

Figure 2.5 shows how the electronic antagonism and agonism can be used to study the control of firing patterns by $I_{Na,p}$. The middle panel (Figure 2.5A2) shows the voltage responses of a neocortical neuron to depolarizing current pulses before the RCC is engaged. In the left panel (Figure 2.5A1, electronic antagonism), the internal model of the RCC has been tuned to cancel the endogenous $I_{Na,p}$, thus removing the subthreshold rectification and decreasing firing in response to the current inputs. In the right panel, (Figure 2.5A3, electronic agonism) the effective maximal conductance of $I_{Na,p}$ has been doubled by electronic agonism. This results in a lowered current threshold for firing of action potentials, higher firing rates, and the development of a doublet pattern at the beginning of the spike train (arrowhead Figure 2.5A3). On closer inspection, the bursting is associated with the development of a depolarizing afterpotential (DAP) on the falling phase of spikes (Figure 2.5B1). DAPs were often seen in these neurons as described in Chapter 4. Figure 2.6 demonstrates that the expression of an artificial $I_{Na,p}$ at
Figure 2.4 Electronic antagonism of the persistent Na⁺ current, $I_{\text{Na,p}}$, and comparison with the effects of TTX.

A. Voltage responses of a neocortical neuron to depolarizing current pulses of 1s duration. Pulses are in 10 pA increments. B. After $I_{\text{Na,p}}$ has been electronically antagonized, the inward rectification in the depolarizing direction is lost. Action potentials, however, are still present. C. TTX eliminates both the inward rectification due to $I_{\text{Na,p}}$ and action potentials. D. Voltage-current relationships generated by the traces in A, B, and C. Data was gathered 800 ms following initiation of the current pulse.
Figure 2.5 Electronic antagonism and agonism of $I_{Na,p}$ in neocortical neurons.

A. Voltage responses of a neocortical neuron to current pulses having amplitudes between 10 and 70 pA. In A1, the RCC is used to cancel an $I_{Na,p}$ current with a maximal conductance of 0.31 nS. In A2, the RCC circuit is not engaged. In A3, the effective conductance of $I_{Na,p}$ has been doubled with respect to the control conditions in A2 by adding an $I_{Na,p}$ conductance with a maximal conductance of 0.31 nS (producing 0.62 nS in total). The arrowhead in A3 points to a spike doublet. B. The action potentials from the first suprathreshold pulses in A1, A2, and A3 are superimposed by aligning their rising phases. This reveals that the electronic antagonism and agonism of $I_{Na,p}$ modifies the depolarizing afterpotential (DAP) on the repolarizing phase of the action potential.
different conductance levels in a neuron with very little endogenous $I_{\text{Na},p}$ results in the same range of firing patterns as occurs naturally in these neurons. The firing patterns include single spikes (Figure 2.6A-C), long all-or-none bursts (Figure 2.6D), and spike doublets (Figure 2.6E,F). At extreme conductance values, the membrane cannot repolarize after a spike, creating a plateau potential (Figure 2.6G). For comparison, two other neocortical neurons recorded under control conditions are shown (marked by asterisks) and their approximate places within range of firing behaviors indicated by arrows. Thus, the use of the RCC points to the $I_{\text{Na},p}$ current density as a factor controlling firing patterns in neocortical neurons.

In this thesis, the RCC will be used to investigate the influence of an artificial $I_H$ on the frequency-response and firing properties of neocortical neurons (see Chapter 5). However, it is technically difficult to electronically antagonize $I_H$ because it commonly requires two exponential time constants to describe its activation kinetics. For this reason, systematic antagonism/agonism studies were not pursued for $I_H$. 
Figure 2.6 Different levels of electronically expressed $I_{Na,p}$ produce a wide range of firing patterns in a neocortical neuron.

All voltage traces are responses to just-suprathreshold depolarizing current pulses. A. At the lower left is the response of the neuron before expression of $I_{Na,p}$. B - G. The traces from lower left to upper right show the firing patterns evoked in the same neuron when $I_{Na,p}$ is electronically expressed at successively higher maximal conductances (indicated underneath each trace). Asterisks mark just suprathreshold responses of two other neurons. The arrows connect these neurons to the firing behaviors in the neuron with the artificial $I_{Na,p}$ that is most like them.
3. Theory of neuronal frequency-response relationships

3.1 Introduction

In this chapter, expressions for the impedance of various Hodgkin-Huxley-type membrane models are derived and analyzed. This will form a basis for the experimental examination of the frequency responses of neurons in later chapters. Because the experimental results are concerned mainly with the amplitudes of the voltage responses of neurons to currents inputs, this chapter focuses on the analysis of the magnitude of the impedance rather than its phase.

At this point, a brief overview of the models to be considered in this chapter is in order. First, is an elementary model of the passive electrical properties (capacitance and leak conductance) of a neuronal membrane. Although simplistic, it is useful to understand the frequency-domain behavior of this model since it is an essential building block in the construction of other models.

Second, a class of models with a single voltage-dependent current in addition to passive properties is considered. Analysis of the impedance of these models shows that a voltage-dependent current can have one of two possible effects -- it can cause either a low-frequency attenuation of the impedance magnitude or a low-frequency amplification. This forms the basis for the identification of class I (attenuating) and class II (amplifying) currents. This classification is extended to the models containing these currents, i.e., class I models contain attenuating currents and class II models contain amplifying currents. The analysis of the class I and class II models in this chapter is complete in the sense that all qualitatively different frequency response behaviors are given. It is shown that resonance arises both in class I and class II models but, in the latter case, it is associated with an unstable equilibrium potential of the model.

Third, models with two voltage-dependent currents are considered. These are analyzed by treating the individual currents as class I or class II and examining their interaction. In contrast to the models with only one voltage-dependent current, a complete analysis of these models is not offered. Instead, only those cases which will be required in the later chapters or where simplifying assumptions can be made are examined. The major conclusions are that resonance can be attenuated or amplified by class I or class II currents, respectively, and that a sufficiently amplified resonance may lead to spontaneous oscillations of the voltage.
Finally, the impedances of models that possess an inactivating, voltage-dependent current are analyzed by treating the activation and inactivation processes as separate currents. In this way, models with an inactivating current form a subclass of models with two voltage-dependent currents. As in the case of the two-current models, an amplified resonance may form via the interaction of a class I and a class II current. However, the special form of the “window conductance” for models with inactivation restricts the conditions under which this type of resonance is expressed.

Some of these results have been described before. Except for the book by Cole (1968), however, they have been published piecemeal. The distinction between class I and class II currents and their connection with the frequency response is implicit in Cole’s work but was not presented systematically. The concept of amplified and attenuated resonance and the treatment of an inactivating current as if it was composed of two noninactivating currents is new although it was suggested in a paper containing work from this thesis (Hutcheon et al. 1994).

3.2 Capacitive-membrane (CM) models

3.2.1 Definition of CM models

We begin with a model of an isopotential neuron consisting only of the passive electrical properties. This model, called the capacitive membrane (CM) model, consists of a membrane capacitance, a leak current, and a current input. The equation governing the changes in its membrane voltage is

\[ c_m \frac{dv}{dt} = -g_l[v - v_t] + i_{inj}(t). \] (3.1)

Here, \( t \) is time, \( v \) is membrane voltage, \( c_m \) is membrane capacitance, \( i_{inj}(t) \) is an injected current, \( g_l \) is the conductance of the leak current, and \( v_t \) is its reversal potential. Both \( c_m \) and \( g_l \) are positive.

The steady-state voltage-current (V-I) relationship for the CM model is found by setting the derivative term in (3.1) to zero, giving

\[ i_{ss} = g_l[v - v_t]. \] (3.2)

Here, \( i_{ss} \) is the current needed to hold the voltage at \( v \). The slope conductance \( (g_{slope}) \), defined as the slope of the V-I relationship, is equal to \( g_l \). The input resistance \( (r_i) \) is the inverse of the slope conductance and is, therefore, equal to \( 1/g_l \). The chord conductance \( (g_{ch}) \), defined as the sum of
the individual conductances in the model, is also equal to $g_t$. The equality of the slope and chord conductances indicates that the model is voltage-independent. This will not be the case for later models. In fact, the difference between the slope and chord conductances will be an important determinant of the frequency responses of these models.

Equation (3.1) for the time-domain behavior of the CM model is linear in $v$ and, for each constant current input, has a stable equilibrium point $v_o$. If the input is suddenly stepped from one value to another, the voltage relaxes to the new equilibrium exponentially with a membrane time constant of $\tau_m = c_m/g_t$.

### 3.2.2 Impedance of CM models

The procedure for the derivation of the complex impedance, $Z$, of the CM model is given in Appendix A. As described in Chapter 1, the complex impedance for an electrical circuit function of frequency, $\omega$, and contains information about the amplitude and phase relationships between the inputs and outputs for an electrical system. For each frequency, the magnitude of $Z$ (represented by $|Z|$) is the ratio of the amplitudes of the voltage output and current input, and the argument of $Z$ (represented by arg($Z$)) is the phase change between the output and input. For the CM models the expression for $Z(\omega)$ is

$$Z(\omega) = \frac{1}{g_t + j\omega c_m}, \quad (3.3)$$

where $j = \sqrt{-1}$. The equivalent circuit diagram for the CM model with impedance (3.3) is shown in Figure 3.1A. It consists of a circuit with three branches connected in parallel: i) a capacitive branch with impedance given by the expression

$$Z_c(\omega) = \frac{1}{j\omega c_m}, \quad (3.4)$$

ii) a branch representing the impedance due to the leak conductance $g_t$ and having the impedance

$$Z_t = \frac{1}{g_t}, \quad (3.5)$$

and iii) a current source, $i_{inj}$, which models the input. From (3.3), the total impedance of the circuit is
3.2.3 Frequency-response behavior of CM models

The impedance locus for (3.3) is shown in Figure 3.2A. It is plotted in the complex plane with the real and imaginary parts of \(Z(\omega)\) (\(\text{Re}(Z)\) and \(\text{Im}(Z)\) respectively) as the axes. The locus is a semicircle in the complex plane with its low-frequency end at \(1/g\), on the real axis and its high-frequency end at the origin. The frequency associated with the minimum value of the imaginary part of \(Z(\omega)\) is \(\omega = 1/\tau_m\). This is called the membrane frequency of the CM model and is denoted \(\omega_m\). The magnitude of the impedance, \(|Z(\omega)|\), may be plotted against frequency to generate a frequency-response curve (FRC). For each frequency, the magnitude of the impedance is the length of the vector that begins at the origin and extends to the appropriate point on the locus. The FRC may, therefore, be thought of as the length of the complex impedance vector as it sweeps along the impedance locus. The FRC for the CM model is shown in Figure 3.2B. At low frequencies, the value of the FRC approaches \(1/g\), whereas, at high frequencies, it declines monotonically towards zero. The value of \(\omega_m\) controls the rate of this decline since the impedance magnitude falls to \(1/\sqrt{2}\) of its initial value by \(\omega = \omega_m\). The phase change, \(\text{arg}(Z)\) between the output and input at each frequency is the angle between the horizontal (real) axis and the complex impedance vector. This can also be plotted against frequency to give a phase frequency-response curve (pFRC). In Figure 3.2C, the value of the pFRC approaches zero at low frequencies and decreases smoothly towards \(-90^\circ\) at very high frequencies. This means that the current leads the voltage for positive frequencies. The value of the pFRC at \(\omega_m\) is \(-45^\circ\).

Figure 3.2B shows that the CM model acts like a lowpass filter. This means that the responses to high-frequency inputs are attenuated relative to responses to low frequency inputs. This is not standard terminology for electrical circuits because filters normally have both their inputs and outputs in the same units (for example in voltage units) whereas, in the case of impedance magnitudes, the input and output units are different. Nonetheless, the analogy with filters is useful and will be used in the remainder of this thesis. The lowpass filter properties of the CM model, for instance, are described by the “characteristic frequency” \(\omega = \omega_m\). As shown above, this is the frequency where \(|Z|\) is \(1/\sqrt{2}\) of its value at \(\omega = 0\) and \(\text{arg}(Z) = -45^\circ\). This
Figure 3.1 Equivalent circuits for three models of membrane electrical activity.

A. The equivalent circuit for the capacitive membrane (CM) model consists of a current source ($i_{inj}$) in parallel with a capacitive impedance ($Z_c$) and an impedance ($Z_l$) due to a leak conductance. B. The circuit for the capacitive membrane model with a single simple current (CM+S model) has an additional voltage- and frequency-dependent impedance ($Z_x$) due to $I_x$. C. The equivalent circuit for the capacitive membrane model with two simple currents (CM+SS model) has two extra impedances ($Z_b$ and $Z_r$). Details of the models are given in the text.
corresponds to the corner frequency or -3 dB point normally used to describe the behavior of lowpass electrical filters. The fact that the characteristic frequency of the lowpass filter due to the CM model is equal to the membrane frequency, \( \omega_m \), of the CM model simply reflects the fact that the membrane time constant of the model limits the ability of its voltage to follow rapidly oscillating current inputs.

For the more complicated models to be examined below, \( Z \) is a function of frequency and voltage. A plot of the magnitude of \( Z \) against frequency and voltage defines a frequency-response surface or FRS. Since the impedance of the CM model does not depend on voltage, its FRS has the simple structure shown in Figure 3.2D.

3.3 Capacitive membrane models with a simple current (CM+S models)

3.3.1 Definition of CM+S models

We now consider membrane models that contain a time- and voltage-dependent current in addition to the capacitive and leak currents of the CM models. The newly introduced current, "\( I_x \)" , flows in parallel to the capacitive and leak currents. For simplicity, it is assumed that \( I_x \) lacks inactivation or any other gating process that leads to separate, kinetically-defined states. Such a current will be referred to as a *simple* voltage-dependent current.\(^1\) Since these models consist of a CM model together with a simple current, they will be called CM+S (capacitive membrane plus simple current) models.

A dimensionless time- and voltage-dependent variable, \( m_x \), raised to the power \( s_x \), controls the proportion of the total \( I_x \)-conductance, \( g_x \), that is activated. Thus, the \( I_x \) current is given by

\[
I_x = -g_x m_x^{s_x} (v - v_x)
\]  

(3.7)

where \( v_x \) is the Nernst potential, and \( s_x \) can be interpreted as the number of independent activation gates associated with each \( I_x \)-channel.\(^2\) To form the time-domain equations for models of the

\(^1\) In terms of the ion channels underlying membrane currents in neurons, a simple current corresponds to the ensemble activity of a population of voltage-gated ion channels each of which has a single type of gating process governing the transitions between a closed and an open state. This a crude model of channel behavior but has the advantage of simplicity. It will be shown later to yield good predictions of the frequency response behaviors of actual neurons.

\(^2\) When \( s_x > 1 \), \( I_x \) has multiple closed states. It is still considered a simple current, however, as long as it can be described by (3.7).
Figure 3.2 Frequency response properties of the CM model.

A. The impedance locus of the CM model in the complex plane forms a semicircle. As the frequency of the input increases, the impedance travels clockwise around the semicircle beginning at \( Z = 1/g_t \). Large and small solid circles indicate the position of the impedance at \( \omega = 0 \) and \( \omega = \omega_m \), respectively. B. - C. The frequency-response and phase frequency-response curves (FRC and pFRC) portray the magnitude and phase of the impedance as a function of frequency. The values of these curves at \( \omega = \omega_m \) are indicated. D. Frequency-response surface (FRS) for the CM model. In this and other figures in this chapter, the vertical axis of the FRS is normalized taking the values of \( c_m \) and \( g_t \) to be equal to 1. The depolarizing and hyperpolarizing directions along the voltage axis are indicated by arrows labeled “+” and “-”, respectively.
CM+S type, combine equation (3.7) and an equation for the time evolution of $m_x$ with equation (3.1) for the CM model. This gives

$$c_m \frac{dv}{dt} = -g_l (v - v_l) - \bar{g}_x m_x s_x (v - v_x) + i_{ inj} (t), \quad (3.8)$$

$$\frac{dm_x}{dt} = \frac{m_{x, \infty} (v) - m_x}{\tau_x (v)}. \quad (3.9)$$

Here, $\tau_x (v)$ is a voltage-dependent time constant and $m_{x, \infty} (v)$ is a sigmoidal, steady-state activation function given by

$$m_{x, \infty} (v) = \frac{1}{1 + \exp \left( \frac{v - v_{x/2}}{k_x} \right)}, \quad (3.10)$$

where $v_{x/2}$ is the voltage at which $m_{x, \infty} (v) = 1/2$ and $k_x$ characterizes the slope of $m_{x, \infty} (v)$ at $v = v_{x/2}$.

The steady-state V-I relation for $I_x$ is found by substituting (3.10) into (3.7), giving

$$i_{x, ss} = g_x m_{x, \infty} s_x (v) [v - v_x]. \quad (3.11)$$

Differentiating this equation with respect to voltage gives the slope conductance for $I_x$,

$$g_{x, slope} = g_x m_{x, \infty} s_x (v) + m_{x, \infty} s_x^{-1} (v) \frac{d}{dv} m_{x, \infty} (v) [v - v_x], \quad (3.12)$$

where the prime denotes differentiation with respect to $v$.

Equations (3.11) and (3.12) are considerably more complicated than the corresponding expressions for the CM model. To simplify them, we make two definitions. First, define the chord conductance, $g_{x, ch}$, of $I_x$ by

$$g_{x, ch} (v) = g_x m_{x, \infty} s_x (v). \quad (3.13)$$

The chord conductance of $I_x$ at a particular membrane potential, $v$, may be thought of as the summed ohmic conductances of all $I_x$ channels that are open at that potential. While this picture ignores fluctuations in conductance due to the stochastic nature of single channel openings, it is a good approximation for a neuron with a large number of channels. Since the proportion of open
channels changes with potential, the chord conductance is voltage-dependent. Second, define a new, dimensionless parameter, \( \hat{g}_x \), by

\[
\hat{g}_x = s_x \frac{m'_{x,\infty}(v)}{m_{x,\infty}(v)}[v - v_x].
\]

(3.14)

Substituting (3.13) and (3.14) into (3.11) and (3.12) yields simpler expressions for the steady-state current and slope conductance of \( I_x \),

\[
i_{x,ss} = g_{x,\text{ch}}[v - v_x]
\]

(3.15)

and

\[
g_{x,\text{slope}} = g_{x,\text{ch}}[1 + \hat{g}_x].
\]

(3.16)

In these expressions, the various conductances are voltage dependent, although this is not explicitly shown.

The meaning of the term \( \hat{g}_x \) is clarified by comparing expressions (3.16) and (3.13) for the slope and chord conductances of \( I_x \). This shows that, when \( \hat{g}_x = 0 \), the slope conductance equals the chord conductance. Moreover, when \( \hat{g}_x > 0 \), the slope conductance is larger than the chord conductance and, when \( \hat{g}_x < 0 \), the slope conductance is smaller. Finally, when \( \hat{g}_x(v) = 0 \) for all \( v \), by (3.14) \( m_{x,\infty} \) must be independent of voltage (since \( 0 \leq m_{x,\infty} \leq 1 \)). This means that the model behaves ohmically as in the CM model. Thus, \( \hat{g}_x \) describes the departure of \( I_x \) from strictly ohmic behavior. This term will be an important factor in the behavior of the frequency response for CM+S models.

Simple expressions for the steady-state current (\( i_{ss} \)), the chord conductance, and the slope conductance of the complete CM+S model now can be constructed by adding appropriate terms involving the leak current to the expressions above. These are

\[
i_{ss} = g_l(v - v_t) + g_{x,\text{ch}}(v - v_t),
\]

(3.17)

\[
g_{ch} = g_l + g_{x,\text{ch}},
\]

(3.18)

and
3.3.2 Impedance of CM+S models

The differential equations, (3.8) - (3.9), describing the time-domain behavior of the CM+S model are nonlinear. They must be linearized about an equilibrium point before an impedance can be found. As before, the equilibrium points are found by setting the time derivatives in the dynamical equations to zero. The procedure for linearization about an equilibrium point of the system and the derivation of the impedance for a CM+S model is given in Appendix A. The resulting expression for the impedance is

\[ Z(v, \omega) = \frac{1}{g_l + j\omega c_m + g_{x,ch}(v)[1 + X(v, \omega)]} \]  

where

\[ X(v, \omega) = \frac{\hat{g}_x(v)}{1 + j\omega \tau_x(v)}. \]  

The impedance locus, FRC, and pFRC all can be derived from \( Z(v, \omega) \). From (3.20), \( g_{x,ch} \) controls the extent to which \( I_x \) contributes to the overall impedance since, when \( g_{x,ch} = 0 \), (3.20) reduces to the expression (3.3) for the passive impedance of a CM model.

3.3.3 Frequency-response behaviors of CM+S models

An analysis of (3.20) will be given in later sections. First, however, to motivate the analysis, and to develop a feeling for the possible range of frequency-response behaviors for CM+S models, the impedances predicted by (3.20) for several models with different parameter values are presented graphically. These models are distinguished by having either a class I or class II simple current.

For class I currents, \( v_x \) lies in a region of the voltage axis where \( I_x \) is almost completely deactivated, i.e., where \( m_{x,ss}(v) = 0 \). Two examples of possible relationships between the reversal potential and steady-state activation curve for a class I current are shown in Figure 3.3A. Certain properties follow automatically from the definition for a class I current. As shown in Figure 3.4A2, \( i_{x,ss}(v) \) is an s-shaped function of voltage for class I currents. Moreover, \( \hat{g}_x(v) \) is positive for all voltages where \( I_x \) is large enough to contribute significantly to the behavior of the model.
Figure 3.3 Definition of class I and class II currents.

A. For a class I current, the reversal potential ($v_x$, indicated by arrowhead) lies in a voltage region where the current is almost completely deactivated, i.e., where $m_{x,\infty} \approx 0$. Two possibilities are shown. B. For a class II current, the reversal potential lies in a voltage region where $m_{x,\infty} \approx 1$. 
Figure 3.4 Qualitative properties of class I and II currents.

**A. Properties of a class I current.** The relationship between $v_x$ and $m_x$ (A1) shows this is a class I current. As a consequence, the steady-state current, $i_{x,ss}$, has an s-shaped dependence on voltage and $\hat{g}_x$ is positive at voltages where $i_{x,ss}$ is large (A2). Also, for a class I current, the chord and slope conductances are always positive (A3).

**B. For class II currents, $v_x$ lies in the voltage region where $m_x = 1$ (B1).** This means that $i_{x,ss}$ has an n-shaped dependence on voltage and $\hat{g}_x$ is either negative or negligible (B2). When a current is class II, the slope conductance is smaller than the chord conductance and may attain negative values at some points (B3).
According to (3.16), this means that the slope conductance is positive and larger than the chord conductance (Figure 3.4A3).

For class II currents, $v_x$ lies in a voltage region where $I_x$ is almost completely activated, i.e., where $m_{x,\infty}(v) \approx 1$. Two possibilities are shown in Figure 3.3B. As shown in Figure 3.4B1, $i_{x,ss}(v)$ is N-shaped and $g_x(v)$ is either negative, or negligibly small for these currents (Figure 3.4B2). According to (3.16), therefore, the slope conductance of a class II current is smaller than its chord conductance and may be positive, zero, or negative (Figure 3.4B3).

Unfortunately, it is not possible to draw a firm line between class I and class II currents. Theoretically, there are intermediate cases that are neither class I nor class II and where $m_{x,\infty}(v_x)$ is closer to $1/2$ than to either 0 or 1. Few biological currents exist, however, that meet these intermediate conditions. Operationally, if class I currents are defined as those with $m_{x,\infty}(v)_{v_x} < 0.1$, and class II currents as those with $m_{x,\infty}(v)_{v_x} > 0.9$, then most biologically realistic voltage-dependent currents are included in one or the other of these classes. Note that, as shown in Figure 3.3, both depolarization- and hyperpolarization-activated currents can be class I currents and that the same applies to class II currents.

CM+S models containing class I and II currents are referred to as class I and II models, respectively. The following section presents figures showing the impedance locus plots, FRCs, and pFRCs (calculated for $v = V_{1/2}$) for selected class I and class II models. In these figures, the dashed curves show plots of the frequency-response properties of the CM model that results when $I_x$ is eliminated, i.e., when $g_x = 0$. Since only the passive $I_{\text{leak}}$ and the capacitance are left after $I_x$ is eliminated, this will be called the “passive” model corresponding to the larger CM+S model. In the upper part of each figure is a graph of the steady-state activation function and an arrowhead indicating the relative position of $v_{\text{ss}}$. Separate figures of the FRSs for these models

---

3 For a class I current, $g_x(v)$ is negative at voltages that lie on the opposite side of $v_{\text{ss}}$ from $v_x$. However, by definition, $m_{x,\infty}(v) = 0$ at these voltages and so $I_x$ is small there. We shall always refer to class I currents as having $g_x(v) > 0$ with the implicit understanding that, when $g_x(v) < 0$, $I_x$ is negligibly small.

4 For a class II model, $g_x(v)$ is positive at voltages that lie on the opposite side of the reversal potential from $v_{\text{ss}}$. However, in this region, $m_{x,\infty}(v) = 0$ whereas $m_{x,\infty}(v) = 1$; therefore, $g_x(v) = 0$ there. Therefore, $g_x(v)$ will be regarded as being either negative or zero.
are also presented, demonstrating that the frequency responses for class I and class II models are voltage dependent. For all models presented in this chapter, the time constant for the activation of $I_x$ is assumed to be voltage independent, i.e., $\tau_x(v) = \tau_x = \text{constant}$

**Class I models: a class I model with attenuated responses**

First, consider a class I model where $I_x$ activates quickly compared to the membrane time constant ($\tau_x/\tau_m = 0.1$) and where the maximal conductance of $I_x$ is equal to the leak conductance ($\frac{g_x}{g_l} = 1$). The frequency-response properties of this CM+S model are shown by the solid curves in Figure 3.5. For comparison, the frequency response properties of the corresponding passive model are shown as dashed curves. The impedance locus of the model, given in Figure 3.5A, forms a slightly deformed semicircle in the lower half of the complex plane. Note that the radius of the semicircle is smaller than that of the passive model.

Figure 3.5B,C shows the FRC and pFRC for this model. Comparison of these curves with the passive FRC and pFRC shows that $I_x$ induces an attenuation of the impedance magnitude at low frequencies and a positive phase shift at intermediate frequencies. For this reason, this is called the attenuated class I model and the response is called an attenuated response.

In Figure 3.5D, the impedance magnitude of the attenuated class I model is shown as a function of frequency and voltage, thus generating the FRS. The labels indicate various features of the FRS. At depolarized voltages, it has an almost voltage-independent plateau of high impedance ("depolarized plateau") corresponding to a region along the voltage axis where $I_x$ is almost completely deactivated. At hyperpolarized voltages, the FRS has another almost voltage-independent plateau with smaller impedance magnitude ("hyperpolarized plateau") in a voltage region where $I_x$ is almost completely activated. These plateaus, therefore, occur where the voltage dependence of the $I_x$-conductance is minimal. Separating the plateaus is a region of low impedance forming a trough in the FRS. The trough is approximately centered on $v = v_{1/2}$ and extends throughout the region of the voltage axis where the $I_x$-conductance is most steeply voltage dependent. The attenuated FRC of Figure 3.5B corresponds to an isopotential cut through the FRS near the middle of the trough at $v = v_{1/2}$. Finally, throughout the FRS, there is a voltage-independent attenuation of the impedance magnitude at high-frequencies. Comparing Figure 3.5D and Figure 3.2D suggests that this high-frequency attenuation is due to the passive properties of the CM+S model. This is correct, as will be shown in a subsequent section.
Figure 3.5 Frequency-response properties of an attenuated class I model.

For this model $\tau_d/\tau_m = 0.05$ and $g_x/g_t = 1$. The response characteristics of the model are shown as solid curves and the responses of the corresponding passive model as dashed curves. **A.** The impedance locus for the model. The impedance at $\omega = 0$ is indicated by the black dot. **B.** The FRC of the model is attenuated at low frequencies in comparison with its passive counterpart. **C.** The phase of the response is shifted in the positive direction relative to the passive response. **D.** The FRS for the model shows several characteristic features (indicated by labels) that are described in the text. The thick line indicates $v = v_{c2}$. 
A class I model with resonant responses

Figure 3.6 shows the frequency-response characteristics of a class I model with slightly different parameter values. In this model, the activation of $I_x$ is assumed to be a relatively slow process, i.e., $\tau_x/\tau_m = 10$, whereas it was previously assumed to be fast, i.e., $\tau_x/\tau_m = 0.1$. The maximal conductance for $I_x$ is still assumed equal the leak conductance. As seen in Figure 3.6A, the impedance locus for this model is qualitatively different from that of the attenuated class I model. Instead of a semicircle, the impedance locus for $v = v_{x/2}$ forms a broad spiral with its low-frequency end lying in the upper half of the complex plane. This is reflected in the FRC (Figure 3.6B) which has a humped shape with a maximum at a nonzero frequency. This model is, therefore, resonant at the voltage where the FRC has been calculated ($v = v_{x/2}$). The frequency at the peak of the hump will be called the resonant frequency ($\omega_{res}$) and the frequency region surrounding $\omega_{res}$ will be called the resonant band of frequencies. The pFRC of the model also has a peak at a point where the phase is positive (Figure 3.6C). Note, from Figure 3.6C, that the frequency where the phase crosses from positive to negative is not the same as $\omega_{res}$. This property distinguishes the resonance of CM+S models from the resonances of parallel or serial RLC electric circuits which are the usual paradigms of resonant systems. Since the FRC is characterized by a resonant hump, this will be called the resonant class I model.

The FRS for the model is shown in Figure 3.6D. A comparison with the FRS of the attenuated class I model (Figure 3.5D) shows that they share many features including the depolarized and hyperpolarized plateau areas and an intervening trough centered on $v = v_{x/2}$. A new feature is a ridge that runs within a narrow frequency band and spans the trough. Any isopotential cut through the surface that includes this ridge will produce an FRC with a resonant hump. The limited length of the ridge in Figure 3.6D means that the occurrence of resonance in the model is confined to a voltage region between the plateaus (gray area).

The attenuated and resonant class I models above are representative of the entire range of frequency-response behaviors available for CM+S models with class I currents. The value of $\tau_x/\tau_m$ determines whether the response is attenuated or resonant.

Class II models: a class II model with amplified responses

Figure 3.7 shows the frequency-response properties of a class II model. That is, a CM+S model with a class II simple current. For this particular model, the maximal conductance of $I_x$ is
Figure 3.6 Frequency-response properties of a resonant class I model.

For this model, $\tau_\delta/\tau_m = 10$ and $g_x/g_l = 1$. A. For this resonant model, the impedance locus has a spiral shape with its low-frequency end (solid circle) on the real axis. B. The FRC is attenuated with respect to the passive FRC and a resonant hump appears with a peak frequency at $\omega = \omega_{res}$. C. A hump also appears in the pFRC in a frequency region where the phase is positive. The pFRC crosses zero at a frequency near, but not identical, to $\omega_{res}$. D. The FRS for the resonant class I model showing a ridge that spans a region of voltages with resonant FRCs (gray area indicated as "resonant region").
relatively low ($\bar{g}_x/g_l = 0.3$) and the time constant for $I_x$-activation is fast ($\tau_x/\tau_m = 0.1$). The impedance locus (Figure 3.7A) is qualitatively similar to that of the passive model, i.e., it is a slightly deformed semicircle in the lower half of the complex plane. Likewise, the FRC and pFRC (Figure 3.7 B,C) both depend monotonically on frequency as do the corresponding curves for the passive model. However, comparison of the FRC and pFRC of the model with those of the passive system shows that $I_x$ produces an amplification of the impedance magnitude at low frequencies and an additional negative phase shift at intermediate frequencies. Since the FRC of the model is amplified relative to the passive FRC, it will be called an amplified response and the model will be called the amplified class II model.

The FRS for this model is shown in Figure 3.7D. It has a region of enlarged impedance magnitudes at low frequencies that forms a bulge approximately centered on $v = v_{x/2}$. This low-frequency bulge corresponds to the low-frequency amplification of the impedance magnitude seen in the FRC and lies in the same position as the trough of the previous model (see Figure 3.5). At depolarized or hyperpolarized voltages, the impedance magnitude has plateau areas such as those seen in the class I models above. Likewise, the impedance magnitude is attenuated and voltage independent at high frequencies as was the FRS of the class I models.

**A class II model with both attenuated and amplified responses**

Figure 3.8 shows the frequency-response properties of another class II model. The only difference between the parameter values used in the amplified class II model above and in this one is that the maximal conductance of $I_x$ is ten times larger, i.e., $\bar{g}_x/g_l = 3$. In this case, the model has a region of negative slope conductance in its steady state V-I relationship. This change produces a qualitative change in the frequency response of the model. Although the entire impedance locus (at $v = v_{x/2}$ ) still lies in the lower half of the complex plane, the real part of the impedance is negative rather than positive at low frequencies. At higher frequencies, the impedance locus crosses the imaginary axis before turning back towards the origin (inset, Figure 3.8A). The FRC (Figure 3.8B) is similar to the attenuated FRC of the class I model shown in Figure 3.5. Unlike the attenuated class I model, however, the pFRC attains values more negative than -90° and has a maximum at a nonzero frequency (asterisk, Figure 3.8C).
Figure 3.7 Frequency-response properties of an amplified class II model.

For this model $\tau_s/\tau_m = 0.5$ and $g_x/g_t = 0.3$.  

A. The impedance locus for the model forms a slightly distorted semicircle with a larger diameter than the passive impedance locus. 

B. The FRC of the model is amplified at low frequencies in comparison with its passive counterpart. 

C. The phase of the response is shifted in the negative direction relative to the passive response. 

D. The FRS for the amplified class II model showing a bulge at voltages near $v = v_{s/2}$.
Figure 3.8 Frequency-response properties of a class II model with attenuated responses.

For this model $\tau_d/\tau_m = 0.5$ and $g_x/g_t = 0.7$. A. The impedance locus for a class II model with attenuation has a negative real part at low frequencies ($\omega = 0$ indicated by black dots). At higher frequencies it swings around to join the passive impedance locus (magnified in the inset above and to the right). B. The FRC of this model is attenuated relative to the passive FRC. C. The pFRC has phase angles more negative than $-90^\circ$ and has a maximum at high frequencies (indicated by an asterisk). D. The FRS for this class II model has two high ridges bracketing a voltage region of amplified responses where the equilibrium potential of the membrane is unstable.
The FRS (Figure 3.8D) also is qualitatively different from that of the amplifying class II model. Instead of a single bulge in the FRS, there are two narrow ridges, each centered on a particular voltage. Theoretically, the peaks of both these ridges are infinitely high at \( \omega = 0 \). In Figure 3.8D they are finite in amplitude because a log scale is used for the frequency axis and so \( \omega = 0 \) cannot be shown. Both ridges also appear smaller than their actual amplitudes because of the finite size of the mesh used to calculate the surface. For instance, the ridge on the left appears smaller because it is narrower than the peak on the right. The ridges delimit a valley containing the attenuated FRC of Figure 3.8B at \( v = v_{x2} \) (indicated by a heavy line). There is, in fact, an entire voltage region of attenuated FRCs that is centered on \( v = v_{x2} \). Surrounding this region and extending up the inside slopes of the ridges is a region of amplified FRCs. Both the attenuated and amplified FRCs in these regions are associated with impedances having a negative real part (see Figure 3.8A). In contrast, on the outside slopes of the ridges, i.e., on the sides facing away from the valley, there is a region of amplified FRCs with impedances that have positive real parts. The FRCs in these regions, therefore, are similar to FRCs in the amplified class II model above. Finally, flanking the central region containing the ridges, there are depolarized and hyperpolarized plateaus as in the other models.

The existence of impedances with a negative real part at some voltages (and with corresponding phase changes more negative than \(-90^\circ\) ) indicates that the equilibrium membrane potential of the model is unstable. Although the full behavior of the model near an unstable equilibrium point requires a nonlinear analysis, the linear impedance analysis is still useful for three reasons. First, the impedances of neurons at unstable membrane potentials can be measured experimentally in neurons (see Fishman et al. 1977, 1979; Moore et al. 1980, 1988, 1993). This is accomplished by clamping the membrane voltage and measuring the resulting current to find the admittance. Mathematically, the impedance is then found by taking the inverse of the admittance. The approximately linear behavior of the system is assured by limiting the size of voltage transients. Second, some aspects of the nonlinear behavior of neurons near an unstable equilibrium may be predicted from the impedance. In particular, it will be shown in a later section that the frequencies of nonlinear spontaneous oscillations in some models are close to the resonant frequencies of the impedance at the same voltage, or at nearby voltages where the membrane is stable. Finally, the manner in which regions of stability and instability in the models fit together along the voltage axis may be physiologically significant. For these reasons
the impedances of models with voltage regions where the membrane potential is unstable will be examined here.

A class II model with resonant, attenuated and amplified responses

The frequency-response characteristics of a final class II model are shown in Figure 3.9. The parameter values of this model are identical with those of the previous model except that \( \alpha \) is assumed to activate relatively slowly, i.e., \( \tau_i/\tau_m = 10 \). The impedance locus (Figure 3.9A) is an exaggerated version of the impedance locus of the previous model. It is confined to the lower half of the complex plane and has a negative real part at low frequencies. However, the real part of the impedance crosses over to positive values at a lower frequency than in the previous model and attains larger positive values before swinging back towards the origin. The impedance locus thus has a shape like the lower half of a crescent moon rather than a semicircle. These differences are reflected in the FRC (Figure 3.9B) where a resonant hump instead of a simple attenuation is observed, and in the pFRC (Figure 3.9C) where the maximum is more distinct and occurs at a lower frequency than in the previous model. Note that the resonance in this model is distinguished from the resonance in the class I models by the existence of a negative real part in the impedance.

The FRS for this model appears similar to the FRS of the class II model above (Figure 3.9D). There are two ridges bounding a region with amplified and attenuated FRCs as in the previous model. In the middle of this region, however, is a new region containing resonant FRCs. This is shown on the FRS as a gray area. An isopotential cut through the surface in this resonant region (at \( v = v_{r2} \)) corresponds to the FRC of Figure 3.9B. Just as for the resonant class I model studied earlier, resonance appears in the FRC as a ridge spanning a region of attenuated responses. This ridge is indicated in Figure 3.9D but is difficult to see because of the scaling of the figure.

As in the previous model, The FRCs that lie between the peaks in this model are associated with impedances having negative real parts. It will be shown below that the steady state V-I relationship of the model has a negative slope conductance in these same regions and, as a consequence, the membrane potential does not have a stable equilibrium there. In contrast, the class II model possessing only amplified responses as well as the all the class I models are associated with stable equilibrium points.
Figure 3.9 Frequency-response properties of a resonant class II model.

For this model $\tau_d/\tau_m = 10$ and $g_x/g_t = 0.7$.  

**A.** The impedance locus has a crescent shape with its low-frequency end on the real axis ($\omega = 0$ indicated by a solid circle).  

**B.** The FRC of the model is attenuated and has a resonant hump with a maximum at $\omega = \omega_{res}$.  

**C.** The pFRC has a maximum and has phases more negative than $-90^\circ$.  

**D.** The FRS of the model.
Summary of class I and class II responses

To summarize, in this section, five CM+S models have been presented. These models arise from equations (3.8) - (3.9) but the different parameter settings lead to very different frequency-response relationships. Class I models possess resonant or attenuated FRCs depending on whether $\tau_x/\tau_m$ is large or small. Class II models exhibit amplified as well as resonant or attenuated FRCs. For class II models, the resonant and attenuated FRCs are associated with voltages where the equilibrium membrane potential is unstable. These responses may, therefore, only be observed under voltage-clamp conditions.

3.3.4 Qualitative analysis of the frequency response for CM+S models

The section above described the different frequency-response characteristics of class I and class II CM+S models. In this section, equation (3.20) will be analyzed to show how these differences arise. The focus will be on the differences in the FRCs and FRSs.

To proceed, we express $Z(v,\omega)$ as the equivalent impedance for a circuit composed of three component impedances connected in parallel,

$$Z(v,\omega) = \frac{1}{1 + \frac{1}{Z_c(\omega)} + \frac{1}{Z_x(v,\omega)}}$$

(3.22)

where $Z_c(\omega)$ and $Z_t$ are defined as in equations (3.4) and (3.5), and $Z_x(v,\omega)$ is given by

$$Z_x(v,\omega) = \frac{1}{g_{x,\text{ch}} \left[ 1 + \frac{\hat{g}_x(v)}{1 + j\omega \tau_x(v)} \right]}$$

(3.22)

The circuit is shown schematically in Figure 3.1B.

The components of this circuit describe the contributions of the leak current, the capacitance, and $I_x$, respectively, to the total impedance. To understand how they determine the shape of the FRC, consider their relative behavior at low and high frequencies. As $\omega \rightarrow 0$, the contribution of the capacitive component, $1/Z_c$, approaches zero whereas $1/Z_t$ and $1/Z_x(v,\omega)$ approach finite real values. This means that, at low frequencies, the terms involving the ionic currents come to dominate the denominator of (3.22) and control the impedance. At high frequencies, on the other hand, the situation is reversed. Here, as $\omega \rightarrow \infty$, $1/Z_c(\omega)$ becomes
infinitely large and imaginary whereas both $1/Z_l$ and $1/Z_x(v, \omega)$ stay real and finite. Thus, at high frequencies, the capacitive impedance comes to dominate the other terms in the denominator of (3.22), driving the total impedance towards zero. This is the explanation for the high frequency drop-off in the impedance magnitudes of the FRCs and FRSs shown above.

These observations suggest that the total impedance should be analyzed into low- and high-frequency subcircuits. The low-frequency subcircuit will be composed of the $Z_l$ and $Z_x$ impedances in parallel and, therefore, will be called the $\alpha$-subcircuit. From (3.5) and (3.22), its impedance is

$$Z_{lx}(v, \omega) = \frac{1}{g_l + g_{x,ch}(v)\left[1 + \frac{g_x(v)}{1 + j\omega \tau_x(v)}\right]}.$$  

(3.23)

The voltage-dependent characteristic frequency for this subcircuit is $\omega_l(v) = 1/\tau_l(v)$. The high-frequency subcircuit will be composed of the capacitive and leak impedances connected in parallel, together with an extra parallel branch having an impedance equal to the high-frequency limit of $Z_x$. From (3.22), the impedance of this extra branch is due to the chord conductance, $g_{x,ch}(v)$, of $I_x$. It therefore corresponds to the steady-state part of $I_x$ at each voltage, i.e., the current that flows through the proportion of the $I_x$-conductance that is tonically activated at each voltage. At very small times after a change in voltage, the current flowing through $g_{x,ch}(v)$ is nearly ohmic because $I_x$ has a nonzero time constant, $\tau_x$. Thus, if the membrane is initially held at $v_0$ and then instantaneously stepped to $v_1$, the current flowing through the $I_x$-conductance at times that are short compared to $\tau_x(v_0)$ will be well approximated by $i_x = g_{x,ch}(v_0)[v_1 - v_x]$. This means that, at high frequencies, the chord conductance of $I_x$ can be thought of as contributing to the passive properties of the model. The high-frequency subcircuit of the CM+S model will, therefore, be called the passive subcircuit. Its impedance is given by

$$Z_{pass}(v, \omega) = \frac{1}{g_l + j\omega c_m + g_{x,ch}(v)}$$

(3.24)

and its characteristic frequency is $\omega_{pass}(v) = (g_l + g_{x,ch}(v))/c_m = g_{ch}(v)/c_m$. 
**Analysis of class I models: frequency responses of the \( L_x \)- and passive subcircuits.**

Together, the \( L_x \)- and passive subcircuits determine the nature of the frequency response of the CM+S model. Figure 3.10 demonstrates this for the attenuated class I model from Section 3.3.3. Here, the FRCs of the \( L_x \)- (dotted curve) and passive (dashed curve) subcircuits are compared with the FRC of the complete model (solid curve). As deduced from the limiting behaviors of \( Z(v,\omega) \), the FRC of the complete model closely follows the FRC of the \( L_x \)-subcircuit at low frequencies and the FRC of the passive circuit at high frequencies. The FRC of the passive subcircuit is like a high-frequency attenuator or lowpass filter with characteristic frequency \( \omega_{pass} \) and is, therefore, like the FRC for the passive CM model (see Figure 3.2). In contrast the FRC of the \( L_x \)-subcircuit is like a low-frequency attenuator or highpass filter with a characteristic frequency, \( \omega_* \), i.e., its impedance magnitude is smaller at \( \omega = 0 \) than at \( \omega = \infty \).

Therefore, the FRC of the attenuated class I model can be understood as arising from an interaction between two attenuating processes: a low-frequency attenuation due to the \( L_x \)-subcircuit and a high-frequency attenuation due to the passive subcircuit. The approximate frequency regions where these attenuations occur are indicated by gray bars. Whereas the \( L_x \)-subcircuit attenuates mainly at frequencies below \( \omega_* \), the passive circuit attenuates mainly at frequencies above \( \omega_{pass} \). Since \( \omega_* > \omega_{pass} \) for this model, the attenuating regions overlap as indicated by the overlap of the gray bars in Figure 3.10A. The resultant FRC for the complete model is attenuated at all frequencies.

An \( L_x \)-subcircuit with attenuating behavior also accounts for the humped form of the resonant FRC in Figure 3.10B. Once again, the FRC of the complete model closely follows the FRCs of the \( L_x \)- and passive subcircuits. This time though, \( \omega_* \) is small compared to \( \omega_{pass} \) and so the frequency region over which the \( L_x \)-subcircuit attenuates is smaller than in Figure 3.10. This reflects the slow kinetics of \( I_x \)-activation for the resonant model. The resonant hump in the FRC for the complete model arises in the relatively unattenuated region of frequencies that now exists between the attenuating regions of the \( L_x \)- and passive subcircuits. This is indicated by the gap between the gray bars in Figure 3.10B.

Thus, class I currents shape the FRC by creating a region of low-frequency attenuation. When combined with the high-frequency attenuation due to the capacitance, the complete model
Figure 3.10 Analysis of attenuated and resonant FRCs for class I models.

A. The FRC of the attenuated class I model (solid curve) follows the FRC of the $\ell x$-subcircuit (dotted curve) at low frequencies and the FRC of the passive subcircuit (dashed curve) at high frequencies. Limiting values of the FRCs are shown on the left. The FRC of the $\ell x$-subcircuit is like a filter that attenuates at frequencies below $\omega_\ell$ (indicated below the frequency axis) whereas the FRC of the passive subcircuit attenuates at frequencies above $\omega_{pass}$ (also indicated). The regions of attenuation overlap, producing an attenuated FRC for the complete model. The gray bars below the frequency axis show how the attenuations overlap at intermediate frequencies.

B. For the resonant class I model, the regions of attenuation do not overlap. A region of unattenuated responses arises at intermediate frequencies in the gap (indicated below the frequency axis by a dotted line) between the attenuations. Note that the resonant frequency, $\omega_{res}$, lies between $\omega_\ell$ and $\omega_{pass}$. 
may have resonant or attenuated responses, depending on whether or not the low- and high-frequency regions of attenuation overlap. We now show why the \( \ell \)-subcircuits of class I models are attenuating at low frequencies.

**Origin of the low-frequency attenuation in the \( \ell \)-subcircuit**

Why is the \( \ell \)-subcircuit attenuating for class I currents? A mathematical answer comes from examining the expression for the magnitude of its impedance, i.e., its FRC. From (3.23) this is given by

\[
|Z_{\ell}(v, \omega)| = \frac{1}{g_l + g_{x,ch}(v) \left[ 1 + \frac{\hat{g}_x(v)}{1 + i\omega \tau_x(v)} \right]}
\]  

(3.25)

For any \( v \), the form of this function is easy to deduce since the derivative of (3.25) with respect to \( \omega \) is of the same sign over the interval \( 0 \leq \omega \leq \infty \), equal to zero at \( \omega = 0 \) and \( \infty \), and continuous. This means that (3.25) is sigmoidal, with no internal maxima or minima. Furthermore, the sign of its slope at intermediate frequencies is determined by its limiting values at \( \omega = 0 \) or \( \infty \). These limits are

\[
\lim_{\omega \to 0} |Z_{\ell}(v, \omega)| = \frac{1}{|g_l + g_{x,ch}(v)|} = \frac{1}{g_{slope}(v)}
\]  

(3.26)

and

\[
\lim_{\omega \to \infty} |Z_{\ell}(v, \omega)| = \frac{1}{g_l + g_{x,ch}(v)} = \frac{1}{g_{ch}(v)}
\]  

(3.27)

where \( g_{slope} \) and \( g_{ch} \) are the slope and chord conductances of the CM+S model as defined earlier. From (3.26) and (3.27), it is clear that, if \( \hat{g}_x(v) > 0 \), the high-frequency limit must be larger than the low-frequency limit since the slope conductance of the model is larger than its chord conductance. But this is the condition satisfied by class I currents. Therefore, they are attenuating.

A more intuitive explanation of how class I currents result in a low frequency attenuation is that they oppose changes in voltage. Thus, suppose that \( I_x \) is a class I current that activates on

---

[5] Note that the absolute value signs have been dropped from the denominators in (3.27) because \( g_{l} \) and \( g_{x,ch} \) are always positive.
depolarization and has a reversal potential near the foot of its activation curve. Depolarizing the membrane potential can activate $I_x$, resulting in the formation of an outward current that partially counteracts the depolarization. Likewise, a hyperpolarization can deactivate $I_x$ and the subsequent reduction in the flow of outward current will partially reverse the hyperpolarization. The net effect is that $I_x$ attenuates voltage transients -- including those evoked by oscillatory currents. This mechanism is most effective when the changes in voltage are slow enough that the time-dependent activation of $I_x$ can keep up, i.e., at frequencies below $\omega_x$. This argument also works for hyperpolarization-activated currents with a relatively depolarized reversal potential.

**Voltage dependence of the low-frequency attenuation**

The previous section showed that a low-frequency attenuation in the FRC of the $\xi$-subcircuit underlies both resonance and attenuation in class I, CM+S models. However, the characteristics of this attenuation change with voltage. We can plot the low- and high-frequency limits of the $\xi$-subcircuit against voltage to deduce some of the properties of the FRS.

Figure 3.11 plots the low- and high-frequency limits of $|Z_\xi(v, \omega)|$ against $v$ for the attenuated class I model from Section 3.3.3 (note that the limits for the resonant class I model are identical). In the figure, the low- and high-frequency limits of $|Z_\xi(v, \omega)|$ are different from each other only over the band of voltages where the slope of $m'_{\xi, \omega}(v)$ is large. Within this region, the low-frequency limit is smaller than the high frequency limit and so the $\xi$-subcircuit is attenuating. The attenuated responses in this region form the trough that was seen in the FRSs of the class I models examined in Section 3.3.3. Although the attenuated region theoretically extends out to infinitely large positive and negative voltages, the region of measurable attenuation is limited since the low- and high-frequency limits quickly approach each other in the region where $m'_{\xi, \omega}(v)$ is small. Outside the attenuated region, the limits approach the asymptotic values indicated in Figure 3.11. At these voltages, $I_x$ is either completely activated or completely deactivated. These regions correspond to the hyperpolarized and depolarized plateaus in the FRS.
Figure 3.11 Low- and high-frequency limits of $|Z_{pr}(v, \omega)|$ for a class I model.

The asymptotic values of the frequency limits as $v \to \pm \infty$ are indicated. The FRS corresponding to these limits may be attenuated or resonant depending on the relationship between $\omega_r$ and $\omega_x$. 
Resonant frequency

From (3.20) it is possible to derive an expression for the resonant frequency, \( \omega_{res} \), of class I models. For any \( v \), setting \( \partial |Z(v,\omega)| / \partial \omega = 0 \) and solving for \( \omega \), gives an expression for the frequency where \( |Z(v,\omega)| \) is a maximum, i.e., the resonant frequency;

\[
\omega_{res}(v) = \sqrt{\omega_x(v)\left[\xi(v)\omega_{pass} - \omega_x(v)\right]},
\]

where

\[
\xi^2(v) = \frac{g_{x,ch}(v)}{g_{ch}(v)} \left[\frac{g_{slope}(v) + g_{ch}(v)}{g_{ch}(v)} + 2\frac{\omega_x(v)}{\omega_{pass}}\right] > 0.
\]

The inequality in (3.29) is true for class I models because \( \hat{g}_x > 0 \). Since the resonant frequency must be real-valued to be physically realizable, we can also derive a condition for the existence of resonance by requiring the term within the brackets in (3.28) to be real and positive, i.e.,

\[
\xi(v)\omega_{pass}(v) - \omega_x(v) > 0.
\]

Substituting (3.29) into (3.30), the condition for the existence of resonance becomes

\[
\frac{\omega_x(v)}{\omega_{pass}(v)} < a(v) + \sqrt{2a(v)[1 + a(v)]},
\]

where

\[
a(v) = \frac{g_{x,ch}(v)}{g_{ch}(v)} \hat{g}_x(v).
\]

Inequality (3.31) shows that the presence or absence of resonance depends on the parameters of the CM+S model in a complex way. Note, however, if \( \omega_x \) is sufficiently large compared to \( \omega_{pass} \), the inequality is violated and there is no resonance. Further insight into the conditions that CM+S models must meet to sustain resonance can be gained by inserting parameter values in the right hand side of (3.31) that may typically be found in central neurons.

First, suppose that \( g_{x,ch} \) is equal to \( g_t \) at \( v = v_{x/2} \). Since \( g_{ch} = g_t + g_{x,ch} \), this means that

\[
g_{x,ch}(v_{x/2}) / g_{ch}(v_{x/2}) = 0.5.
\]

Next, suppose that \( m_{x,m}(v) \) has the form given by (3.10) and that \( k_{x,l} = 10 \text{ mV}^{-1}, s = 1 \), and \( l v_{x,v_{x/2}} \) has a value between 10 and 100 mV. From (3.14), the value of
\( \hat{g}_x(v_{x/2}) \) is between 1 and 10. Therefore, according to (3.32), the value of \( a(v) \) at \( v = v_{x/2} \) is between 0.5 and 5. Substituting these values of \( a \) into the right hand side of (3.31) shows that, at \( v = v_{x/2} \), \( \omega_x \) can be 1.7 to 12.7 times larger than \( \omega_{\text{pass}} \) and still result in resonance. Thus, the constraint placed on the relative sizes of \( \omega_x \) and \( \omega_{\text{pass}} \) in order to produce resonance is not as strong as appears from the graphical analysis above (see Figure 3.10). In fact, under biologically reasonable conditions, the regions of high- and low-frequency attenuation of the passive and \( \text{x-} \) subcircuits may overlap substantially without abolishing resonance.

It should be borne in mind that inequality (3.31) deals only with the existence of resonance and does not indicate the size of the resonant hump in the FRC. In fact, during many calculations of impedances for CM+S models it was found that while resonances often existed mathematically, they could be almost undetectable in plots of the FRCs. This was particularly true when the attenuating regions of the subsystems were overlapping. Condition (3.31) should, therefore, be used with caution since weak resonances are unlikely to have any functional significance. On the other hand, the above analysis shows that when \( \omega_x/\omega_{\text{pass}} < 1 \), we should expect resonance in models with biologically realistic parameters. This is consistent with the concept, developed above, that resonance arises in the gap between regions of low- and high-frequency attenuation controlled by \( \omega_x \) and \( \omega_{\text{pass}} \), respectively.

**Class II models with positive slope conductance: frequency-responses of the \( \text{x-} \) and passive subcircuits**

For class II models, \( \hat{g}_x(v) \) is negative. The value of the slope conductance may therefore be positive, negative, or zero. The analysis of the frequency response, however, is simple only when the slope conductance is positive since, in a voltage region of negative slope conductance the equilibrium potential is unstable (Hille 1992). From (3.19), the condition that \( \hat{g}_x(v) \) must satisfy to keep the slope conductance positive is

\[
- \frac{g_{ch}(v)}{g_{x,\text{ch}}(v)} < \hat{g}_x(v).
\]  

(3.33)

Note that the left-hand side of this inequality involves terms that change with the size of the individual conductances in the model. In contrast, \( \hat{g}_x(v) \) depends only on parameters concerned with the voltage dependence of \( I_x \) (see equation (3.14))
Figure 3.12 shows FRCs for the $\alpha$- and passive subcircuits (dotted and dashed curves, respectively) of a class II model where inequality (3.33) is satisfied at all voltages. This corresponds to the amplified class II model of Section 3.3.3. Its FRC is shown as the solid curve in Figure 3.12. Since (3.33) is satisfied, the slope conductance of the model is positive. Just as for the class I models, the FRC of the complete model closely follows the FRC of the $\alpha$-subcircuit at low frequencies and the FRC of the passive circuit at high-frequencies. In contrast to the class I models, however, the $\alpha$-subcircuit amplifies rather than attenuates at low frequencies. This low-frequency amplification reinforces the high-frequency attenuation due to the capacitance and the resulting FRC of the complete model is amplified relative to the passive FRC.

*Origin of the low-frequency amplification in the $\alpha$-subcircuit*

Once again, the limiting behavior of $|Z_{\alpha}(v,\omega)|$ at low and high frequencies shows whether the $\alpha$-subcircuit is like a low frequency attenuator or amplifier. From (3.26) and (3.27), the low- and high-frequency limits of $|Z_{\alpha}(v,\omega)|$ are given by $1/g_{\text{slope}}$ and $1/g_{\text{ch}}$, respectively. For a class II model, however, $\hat{g}_\alpha$ is negative so that $g_{\text{slope}} < g_{\text{ch}}$. Since condition (3.33) assures us that $g_{\text{slope}}$ is positive, we also must have $|g_{\text{slope}}| < |g_{\text{ch}}|$. Therefore, the value of $|Z_{\alpha}(v,\omega)|$ is larger at low than at high frequencies and the FRC for this model must be amplifying at low frequencies. This is shown in Figure 3.12 for the amplified class II model of Section 3.3.3.

This can be restated in a more intuitive manner by saying that class II currents produce a low-frequency amplification in the frequency response of the $\alpha$-subcircuit by causing a positive feedback that potentiates voltage transients. For instance, suppose $I_\alpha$ is a current that activates on depolarization and assume that the resulting current is inward, making it a class II current. Under these conditions, a depolarization of the membrane potential away from the equilibrium potential will activate $I_\alpha$, increase the flow of inward current, and depolarize the membrane still further. The positive feedback results in a potentiation of the voltage responses of the membrane but is not strong enough to destabilize the membrane potential as long as the inequality (3.33) is satisfied. On the other hand, a hyperpolarization that turns off $I_\alpha$ will decrease the inward current, leading to further hyperpolarization causing a potentiation of voltage responses similar to that in the depolarizing direction. This mechanism works equally well if $I_\alpha$ is an outward current that activates on hyperpolarization. This parallels the explanation for the attenuating properties...
For the class II model, the \( lx \)-subcircuit amplifies at frequencies below \( \omega_x \). The low-frequency amplification combines with the high-frequency attenuation of the passive subcircuit at frequencies above \( \omega_x \) to produce an amplified FRC for the complete model.
of class I currents. Once again, this mechanism is most effective at low frequencies where the time-dependent activation of $I_x$ can successfully follow the input.

Voltage dependence of the low-frequency attenuation

Figure 3.13A shows the limiting values of $|Z_{\nu}(v,\omega)|$ as a function of $v$ for the amplified class II model. The low-frequency limit has a peak near $v_{\nu/2}$ and is larger than the high-frequency limit (dashed curve). Therefore, this is a region of amplified FRCs. The region of significantly amplified FRCs corresponds to the bulge seen in the FRS of the amplified class II model (see Figure 3.7D).

Class II models with a region of negative slope conductance

Class II models that do not satisfy (3.26) have a negative slope conductance at some voltages. At these voltages $g_{\text{slope}} < g_{ch}$ but $|g_{\text{slope}}| > |g_{ch}|$. This means that the $l\tau$-subcircuit will be a low-frequency attenuator rather than an amplifier.

Figure 3.13B shows the low- and high-frequency limits of $|Z_{\nu}(v,\omega)|$ for a class II model with a voltage region of negative slope conductance (indicated by a gray region). The low-frequency limit (solid curve) has two peaks separated by a valley that dips below the level of the high-frequency limit (dashed curve). The peaks, both of which are infinitely high and are therefore cut off at the top, are associated with regions of low-frequency amplification in the frequency response of the $l\tau$-subcircuit. However, there is a fundamental difference between the amplifying regions on the opposing sides of each peak. On the side of each peak that faces the valley, the slope conductance of the CM+S model is negative and so the equilibrium potential of the membrane is not stable there. In contrast, on the side of each peak that faces outward, away from the valley, the amplifying region is associated with a stable membrane potential. This is like the region of amplified FRCs in the amplified class II model above. In fact the overall structure of the low-frequency limit in Figure 3.13B is produced by interrupting the peak in Figure 3.13A with a region of unstable equilibria accompanied by smaller impedance magnitudes at the points where $g_{\text{slope}}(v) \leq 0$. Mathematically, the smaller impedances arise because, as $g_{\text{slope}}$ becomes large and negative, $1/|g_{\text{slope}}|$ becomes small and positive. The amplified FRCs that arise at the voltages between the peaks where the equilibrium membrane potential is unstable have been observed experimentally (Moore et al. 1993) where they have been attributed to a class II voltage-dependent current.
Figure 3.13 Low- and high-frequency limits of $|Z_{\tau}(v, \omega)|$ for two class II models.

A. Limits for the class II model with amplified responses. For this model, the inequality of (3.33) is satisfied at all voltages and so the slope conductance is always positive. B. Limits for the class II models with amplified, attenuated, and resonant responses. For this model, the slope conductance is negative at the voltages indicated by the gray shading.
In Figure 3.13B the unstable voltage region between the peaks contains a region of attenuated as well as amplified responses. This is not always true of class II models, even when condition (3.26) is violated. In fact, for this attenuating region to exist, \( \hat{g}_x \) must violate the condition,

\[
-2 \frac{g_{ch}}{g_{x,ch}} < \hat{g}. \tag{3.34}
\]

If \( I_x \) is a slowly-activating current, there is the possibility that the low-frequency attenuation will be converted into resonance by a mechanism similar to the resonance of class I models. Comparison of (3.34) with (3.26) shows that, whenever \( \hat{g}_x(v) \) is negative enough to produce a low-frequency attenuation in the \( \alpha \)-subcircuit, it also produces a negative slope conductance in the complete model. This means that attenuated (and resonant) FRCs in class II models are always associated with an instability of the membrane potential. In cases where the activation of the class II current is slow compared to the time constant of the membrane resonance may arise just as in the case of class I currents. Resonances due to class II currents have been observed in the admittance magnitude functions of squid axons where the membrane potential is artificially stabilized by voltage clamping the membrane (Fishman et al. 1977, 1979)

**Summary of the quantitative analysis of CM+S models**

Class I and II currents, and hence class I and II models, are distinguished by the sign of \( \hat{g}_x \). Class I currents cause a low-frequency attenuation of the FRC whereas class II currents cause an amplification. This may be rephrased by saying that class I currents oppose voltage changes due to current inputs whereas class II currents potentiate them. In a CM+S model, a class I current may cause resonance if it attenuates in a band of frequencies that does not overlap the frequency band of the high-frequency attenuation due to the capacitance. This requires the activation kinetics of the current to be slow compared to the membrane time constant. A class II current may also cause resonance by a similar mechanism. However, this requires the equilibrium potential to be unstable so that the resonance may only be measured under a voltage clamp. The functional consequences of this type of resonance for neurons is therefore, doubtful.

Figure 3.14 shows how these properties of CM+S models fit together. The values of \( \hat{g}_x \) and \( g_{ch}/g_{x,ch} \) are usually voltage dependent so that a model may display different behaviors at different voltages. From Figure 3.14, the properties of class I models are particularly simple
since the equilibrium potential is always stable and the \( tx \)-subcircuit is always attenuating -- this means that the FRCs must be either attenuated or resonant. For class II models, on the other hand, there are two different stability regimes and the \( tx \)-subcircuit may be amplifying or attenuating. Thus, there are three types of frequency response behavior -- attenuated, resonant, or amplified -- but for each type of behavior the membrane potential may be stable or unstable.

3.4 Capacitive membrane models with two simple currents (CM+SS models)

We now turn to models that consist of a capacitance, a leak current, and two simple currents. By an obvious extension of the earlier nomenclature, these are called CM+SS models. From the beginning, we will not treat the two voltage-dependent currents in a CM+SS model equally. Instead, one of the currents will be thought of as “basic” and the other as “regulatory”. The basic current, \( I_b \), sets the fundamental frequency response of the model by its interaction with the capacitive and leak currents. The submodel that consists of these three elements is a CM+S model and will be called the “basic submodel”. The regulatory current, \( I_r \), modifies the frequency-response properties of the basic submodel. It may be thought of as a current that can be turned on or off to alter the characteristics of the frequency response.

We saw, in the previous section, that there are three qualitatively different types of FRC exhibited by CM+S models -- resonant, attenuating, and amplifying -- and that these different responses arise from the attenuating and amplifying properties of class I and II currents. It seems reasonable, then, to analyze the currents of the CM+SS models in the same fashion. This is the strategy that will be adopted in this section. Since there are two types of simple currents, there are four types of CM+SS model; those where \( I_b \) is class I and \( I_r \) is class I, those where \( I_b \) is class II and \( I_r \) is class I, etc. The analysis of CM+SS models is, accordingly, more complicated than the analysis of the CM+S models. Therefore, a complete analysis will not be attempted. Instead we will focus on the case where the basic current is class I and leads to a resonance.
Figure 3.14 Summary of the frequency-response behavior and stability of CM+S models.

The horizontal line represents possible values for $\hat{g}_x$. The solid line indicates a region where the equilibrium potential of the model is stable, a dashed line indicates instability. The various types of frequency-response behaviors are indicated below the line. In the attenuated/resonant region, the behavior of the model is further determined by the kinetics of $I_x$. 

--- ---
unstable ($g_{slopes} < 0$)
stable ($g_{slopes} > 0$)
attenuated/resonant
amplified
3.4.1 Definition of CM+SS models

The parameters and functions that describe the voltage dependence and kinetics of \( I_b \) and \( I_r \) will be defined by analogy with corresponding expressions for \( I_x \) of the CM+S model (see Equations 3.7 - 3.10). They will be distinguished with a subscripted “b” or “r”. The system of equations for the CM+SS model is,

\[
c_m \frac{dv}{dt} = -g_t(v - v_t) - \bar{g}_b m_b^{s_b}(v - v_b) - \bar{g}_r m_r^{s_r}(v - v_r) + i_{inj}(t),
\]

(3.35)

\[
\frac{dm_b}{dt} = \frac{m_{b,\infty}(v) - m_b}{\tau_b(v)},
\]

(3.36)

\[
\frac{dm_r}{dt} = \frac{m_{r,\infty}(v) - m_r}{\tau_r(v)}.
\]

(3.37)

The steady state V-I relation for this model is

\[
i_{ss} = g_t(v - v_t) + g_{b,\text{ch}}(v - v_b) + g_{r,\text{ch}}(v - v_r) - i_{inj},
\]

(3.38)

where the chord conductances of \( I_b \) and \( I_r \) are defined by analogy with expression (3.13) for the chord conductance of \( I_x \) in the CM+S model. The slope conductance of the CM+SS model is found by taking the derivative of (3.38) with respect to \( v \), giving

\[
g_{\text{slope}} = g_t + g_{b,\text{ch}}(v)[1 + \hat{g}_b(v)] + g_{r,\text{ch}}(v)[1 + \hat{g}_r(v)]
\]

(3.39)

where \( \hat{g}_b \) and \( \hat{g}_r \) are also defined by analogy with the definitions for the CM+S model. Equation (3.39) can be rewritten in terms of its component slope conductances as

\[
g_{\text{slope}} = g_t + g_{b,\text{slope}} + g_{r,\text{slope}}
\]

(3.40)

where \( g_{b,\text{slope}} \) is the slope conductance of \( I_b \) and \( g_{r,\text{slope}} \) is the slope conductance of \( I_r \). Finally, the chord conductance for the model is

\[
g_{\text{ch}} = g_t + g_{b,\text{ch}} + g_{r,\text{ch}}.
\]

(3.41)

3.4.2 Impedance of CM+SS models

The impedance is derived in a fashion similar to that of the CM+S model (see Appendix A). Its equation can be written as
where the definitions of $X_b(v,w)$ and $X_r(v,w)$ are similar to (3.21).

3.4.3 Qualitative analysis of the frequency response for CM+SS models

To analyze the behavior of $Z(v,\omega)$, rewrite it as the equivalent impedance for a circuit of four component impedances connected in parallel,

$$Z(v,\omega) = \frac{1}{\frac{1}{Z_t} + \frac{1}{Z_c(\omega)} + \frac{1}{Z_b(v,\omega)} + \frac{1}{Z_r(v,\omega)}}$$  \hspace{1cm} (3.43)

At high frequencies, the most significant term in the denominator is, once again, $1/Z_c(\omega)$. At low frequencies, however, this term is negligible compared with the impedances associated with $I_b$ and $I_r$. At high frequencies, therefore, the total impedance follows the impedance of a passive subcircuit. This passive subcircuit is defined similarly to expression (3.24) for the passive subcircuit for the CM+S model except that the chord conductances of both $I_b$ and $I_r$ must now be included. The expression for its impedance is, therefore,

$$Z_{\text{pass}}(v,\omega) = \frac{1}{g_t + j\omega c_m + g_{b,ch}(v) + g_{r,ch}(v)}.$$  \hspace{1cm} (3.44)

At low frequencies, the total impedance follows the impedance of an $\alpha$-subcircuit given by

$$Z_{\alpha}(v,\omega) = \frac{1}{g_t + g_{b,ch}(v)[1 + X_b(v,\omega)] + g_{r,ch}(v)[1 + X_r(v,\omega)]}.$$  \hspace{1cm} (3.45)

At this point in the analysis of the CM+S model in Section 3.3.4, the low- and high-frequency limits of $|Z_{\alpha}(v,\omega)|$ were calculated to determine whether the $\alpha$-subcircuit was a low-frequency attenuator or amplifier. However, in most cases, the low- and high-frequency limits of $|Z_{\alpha}(v,\omega)|$ for the CM+SS model are not as informative as before. This is because $Z_{\alpha}(v,\omega)$ involves two time constants ($\tau_b$ and $\tau_r$) and so $|Z_{\alpha}(v,\omega)|$ does not have a simple sigmoidal form. However, if one of these time constants is either very large or very small, the time dependence of the corresponding current will essentially disappear from $|Z_{\alpha}(v,\omega)|$ and the analysis is made easier. We choose to consider the case where one of the time constants is small. This will be relevant to the experimental investigations on \textit{in vitro} neurons in later chapters.
Models where $I_r$ activates instantaneously

Suppose $I_r$ activates instantaneously so that $\tau_r = 0$. With this condition, the impedance magnitude of the $\ell_x$-subcircuit for the CM+SS model becomes

$$|Z_{\ell_x}(v,\omega)| = \frac{1}{g_l + g_{r,ch}(v)[1 + \hat{\delta}_r(v)] + g_{b,ch}(v)\left[1 + \frac{\hat{g}_b(v)}{1 + j\omega\tau_b(v)}\right]}.$$  \hspace{1cm} (3.46)

For any $v$, this expression depends on $\omega$ in the same way as the impedance magnitude of the $\ell_x$-subcircuit for the CM+S model (see expression (3.25)). It is, therefore, a sigmoidal function of $\omega$. This means that the FRC for the $\ell_x$-subcircuit will have the same range of qualitative behaviors as for the CM+S models, i.e., either attenuating or amplifying at low frequencies. Once again, the low- and high-frequency limits of $|Z_{\ell_x}(v,\omega)|$ will reveal which behavior the FRC adopts. The equations for the limits are

$$\lim_{\omega \to 0} |Z_{\ell_x}(v,\omega)| = \frac{1}{g_l + g_{b,slope}(v) + g_{r,slope}(v)}$$ \hspace{1cm} (3.47)

and

$$\lim_{\omega \to \infty} |Z_{\ell_x}(v,\omega)| = \frac{1}{g_l + g_{b,ch}(v) + g_{r,slope}(v)}.$$ \hspace{1cm} (3.48)

A comparison of these equations with the corresponding expressions (3.26) and (3.27) for the CM+S model reveals that they possess an additional identical term in both denominators. This term, $g_{r,slope}(v)$, describes how $I_r$ modifies the frequency response of the basic submodel.

Attenuated and amplified resonance

We are now in a position to explore how a resonance is affected by the addition of a regulatory current. Suppose that $I_b$ is a class I current that produces resonance in the basic submodel. How is this resonance altered when a class I or class II regulatory current is added? Figure 3.15 shows that the FRC of the model with a class I regulatory current (curve labeled "$I_b + \text{class I}$") is attenuated with respect to the FRC of the basic submodel (curve labeled "$I_b$'"). This is called an attenuated resonance. Likewise, the FRC of the model with a class II regulatory

---

5 This limit is actually indeterminant for $\tau_r = 0$. However, when $\omega$ is large but not infinite, (3.48) is a good approximation.
current (curve labeled “I_b + class II”) is amplified with respect to the FRC of the basic model. This is, therefore, called an amplified resonance. These results seem reasonable since, in the analysis of CM+S models it was found that class I currents are attenuating and class II currents are amplifying.

The conditions for attenuated and amplified resonance are found from equations (3.47) and (3.48) for the low- and high-frequency limits of |Z^G(v,ω)|. If I_r is a class I current, then \( g_{r,\text{slope}} \) is positive and so is \( g_{r,\text{slope}} \). But this increases the denominators of both (3.47) and (3.48) and, therefore, results in an overall attenuation of the resonance as seen in Figure 3.15. On the other hand if I_r is a class II current and \( \hat{g}_r < -1 \), then \( g_{r,\text{slope}} \) is also negative. For moderately negative values of \( g_{r,\text{slope}} \), i.e., values that leave the terms inside the absolute value signs of (3.47) and (3.48) positive, the denominators in (3.47) and (3.48) are reduced in value, thus enlarging the limiting values of |Z^G(v,ω)| and amplifying the resonance as in Figure 3.15. For highly negative values of \( g_{r,\text{slope}} \), there are complex behaviors that will not be analyzed. Finally, when \( \hat{g}_r \) is not negative enough to make \( g_{r,\text{slope}} \) negative (-1 \( \leq \hat{g}_r \leq 0 \), see (3.16)), addition of a class II current causes an attenuation rather than an amplification of resonance. An intuitive explanation of this is that a weakly voltage-dependent regulatory current forms a passive shunting current that attenuates the impedance. The amplifying effects of a class II current must overcome this shunt before it can cause an overall amplification of the FRC. This requires sufficiently strong voltage dependence, i.e., \( \hat{g}_r < -1 \).

Amplified resonance with an unstable equilibrium

Under certain condition, an amplified resonance is associated with an unstable equilibrium potential. This occurs when the slope conductance of the complete model becomes negative, i.e., \( g_{\text{slope}} = g_t + g_{b,\text{slope}} + g_{r,\text{slope}} < 0 \). Under these conditions, small inputs can result in nonlinear behavior of the model. To explore the behavior of such a model further, it is necessary to analyze the original nonlinear system (3.35) - (3.37). Since \( \tau_r = 0 \), the behavior of the model can be assessed in a two-dimensional \((v, m_b)\) phase space by setting \( m_r(t) = m_{r,\infty}(v(t)) \) and \( i_{\text{inf}}(t) = 0 \) in (3.35) and numerically integrating the resulting system. Figure 3.16A shows the phase space diagram and corresponding voltage trajectory for a CM+SS model with an unstable equilibrium. To produce the instability, \( g_r/g_b = 0.25 \) and \( \tau_b = 1.4 \). For these parameter values, the phase space diagram reveals the existence of a stable limit cycle surrounding the
Figure 3.15 Attenuated and amplified resonance in a CM+SS model.

In the absence of the regulatory current, $I_r$, the behavior of the FRC is determined by the basic current, $I_b$, of the model. In this case, the basic FRC is resonant because $I_b$ is assumed to be a class I current (curve labeled $I_b$). When the regulatory current is present, it modifies the resonance. For a class I regulatory current, the resonance is attenuated (line labeled $I_b + \text{class I}$). When the regulatory current is class II, the resonance is amplified (line labeled $I_b + \text{class II}$).
equilibrium point. This signifies the presence of spontaneous oscillations of the membrane potential (see Figure 3.16A2). When the parameters of the model are adjusted slightly (by decreasing the value of $g_r/g_b$ to 0.2), the stability of the equilibrium potential is regained and the model has an amplified resonance. Under these conditions, damped oscillations appear in the voltage trajectory with a frequency close to the frequency of the spontaneous activity in the unadjusted model (Figure 3.16B). Both the spontaneous and damped oscillations are close to the resonant frequency predicted for the model with amplified resonance at the unstable equilibrium (see Figure 3.16C). Thus, an amplified resonance in the frequency-domain behavior of a CM+SS model may indicate the presence of stable or damped oscillations in the time domain. Furthermore, the resonant frequency at an unstable equilibrium may indicate the approximate frequency of stable oscillations.

3.4.3.1.1 Voltage dependence of amplified resonance

In the above analysis of a CM+SS model with an amplified resonance, the half-activation potentials of the simple currents were assumed to be identical, i.e., $v = v_{b/2}$. Figure 3.17 shows how the FRS of such a model changes as the relative positions of $v_{b/2}$ and $v_{r/2}$ are altered. In the FRS at the upper left (Figure 3.17A), the two half-activation potentials are widely separated. Here, the current with the more hyperpolarized half-activation potential is $I_b$ and is assumed to activate with hyperpolarization. The current with the more depolarized half-activation potential is $I_r$ and activates on depolarization. At intermediate voltages, therefore, neither current is activated. Because they are separated in this fashion, the individual characteristics of the currents are visible in the FRS. $I_b$ produces a localized region of resonance, near $v_{b/2}$, which is similar to the resonant region in the FRS of the resonant class I model in Figure 3.6D. In contrast, $I_r$ produces a region of amplification near $v_{r/2}$ which is similar to the bulge in the FRS of Figure 3.7D.

In Figure 3.17B,C, the amplifying and resonant regions of the FRS begin to interact as $v_{r/2}$ shifts closer to $v_{b/2}$. At the interface between these regions, the attenuating properties of $I_b$ begin to reduce the peak magnitude of the FRS in the amplified region. At the same time, the activation curves of $I_b$ and $I_r$ overlap substantially. Here, there is only a single region of amplified resonance visible in the FRS. Thus, the voltage dependence of the individual currents in a CM+SS model determines the form of the frequency response.
Figure 3.16 Stability of a CM+SS model with amplified resonance.

**A.** The two-dimensional \((v,m_b)\) phase plane and voltage trajectory of a nonlinear CM+SS model shows that, when the regulatory (class II) current is large enough to cause the slope conductance to become negative, the equilibrium potential is unstable and surrounded by a stable limit cycle (heavy curve, A1). In (A2), the voltage trajectory shows self-sustained oscillations. **B.** For a smaller regulatory current, the equilibrium is stable (B1) and surrounded by damped oscillations (B2). **C.** A sine wave at the resonant frequency of the amplified resonance in (B). A comparison of the sine wave with self-sustained oscillations in (A) shows that they have nearly equal periods. The small crosses in (A1) and (B1) indicate the initial conditions for the voltage trajectories in (A2) and (B2).
Summary of the qualitative analysis of CM+SS models

The properties of CM+SS models arise from an interaction between their component simple currents. In particular, a pre-existing resonance can be attenuated by a class I current or amplified by a sufficiently voltage-dependent class II current. A strongly-amplified resonance may lead to stable oscillations of the membrane potential that persist in the absence of an oscillatory input and have a frequency near the frequency of resonance. This leads to the view that resonance and spontaneous oscillations are two expressions of the same fundamental frequency preference in neurons.

3.5 Capacitive membrane models with a inactivating current (CM+I models)

Now, consider models that have a single, inactivating, voltage-dependent current. These will be called CM+I models. Although they possess only one voltage-dependent current, they can be analyzed in much the same fashion as the CM+SS models if the processes of activation and inactivation are viewed as two separate currents. Thus we can have a class I activation process paired with a class II inactivation process, or a class II activation process paired with a class I inactivation process. In this section we will only consider the latter possibility since it will be of use in a later chapter.

The voltage-dependent current in the CM+I model will be called $I_x$ by analogy with the current for the CM+S model. It is controlled by the dimensionless activation and inactivation variables $m_x$ and $h_x$, respectively.

3.5.1 Definition of CM+I model

The time domain equations for CM+I model are given by

\[ c_m \frac{dv}{dt} = -g_t(v-v_r) - g_x m_x h_x (v-v_x) + i_{inj}(t), \]  

(3.49)

\[ \frac{dm_x}{dt} = \frac{m_{oxx}(v) - m_x}{\tau_{m,x}(v)}, \]  

(3.50)

and

\[ \frac{dh_x}{dt} = \frac{h_{oxx}(v) - h_x}{\tau_{h,x}(v)} \]  

(3.51)
Figure 3.17 Frequency-response surface for a CM+SS model with an amplified resonance.

A. The FRS of a CM+SS model clearly shows the separate effects of the basic (resonant) and regulatory (amplifying) currents when $v_{b/2}$ and $v_{r/2}$ (indicated by labeled arrowheads) are well separated. B. C. D. As the separation between $v_{b/2}$ and $v_{r/2}$ narrows, the regions of resonance and amplification interact, producing an amplified resonance. Note that the frequency axis is not in log units.
Here, \( m_{\text{ox}}(v) \) and \( h_{\text{ox}}(v) \) are functions that give the steady-state levels of activation and inactivation, respectively, for each voltage. Their form is similar to (3.10) for the steady-state activation curve of the CM+S model. By assumption, they have opposite voltage dependence.

The steady state V-I relationship, slope conductance, and chord conductance for the CM+I models are derived from equations (3.49) - (3.51) in a manner similar to the corresponding relationships for CM+S and CM+SS models. First, define the chord conductance for \( I_x \) by

\[
g_{x,\text{ch}} = m_{\text{ox}} s_m(v) h_{\text{ox}} s_h(v) [v - v_x].
\]

Using this definition, and defining \( \hat{g}_{x,m} \) and \( \hat{g}_{x,h} \) by analogy with \( \hat{g}_x \) for the CM+S model (see equation (3.14)), gives a simple expression for the slope conductance of \( I_x \),

\[
g_{x,\text{slope}} = g_{ch} \left[ 1 + \hat{g}_{x,m} + \hat{g}_{x,h} \right].
\]

The steady-state current, slope conductance and chord conductance of the complete model are, respectively,

\[
i_{\text{ss}} = -g_t (v - v_t) - g_{x,\text{ch}} (v - v_x) + i_{\text{inj}}(t),
\]

\[
g_{\text{slope}} = g_t + g_{x,\text{ch}} \left( 1 + \hat{g}_{x,m} + \hat{g}_{x,h} \right),
\]

and

\[
g_{\text{ch}} = g_t + g_{x,\text{ch}}.
\]

### 3.5.2 Impedance of CM+I models

Linearizing the system (3.49) - (3.51), and finding its impedance as shown in Appendix A gives

\[
Z(v, \omega) = \frac{1}{g_t + j\omega c_m + g_{x,\text{ch}}(v) [1 + X_m(v, \omega) + X_h(v, \omega)]}
\]

where \( X_m(v, \omega) \) and \( X_h(v, \omega) \) are defined in the same way as \( X_t(v, \omega) \) and \( X_c(v, \omega) \) for the CM+SS model or \( X(v, \omega) \) for the CM+S model. They describe the contributions of the activation and inactivation processes, respectively, to the overall impedance. This suggests that activation and inactivation can be treated like the simple currents in the CM+SS model, i.e., they should be analyzed in terms of their ability to produce low-frequency amplification or attenuation in the
FRC. This is the procedure that will be adopted here. An inactivating, voltage-dependent current of the CM+I model will be viewed as an aggregate entity, composed of two simple currents: an activation current and an inactivation current. These currents have opposite voltage dependence but a common reversal potential and chord conductance.

To reinforce this view, (3.57) can be rewritten to look like the impedance for a CM+SS model,

$$Z(v, \omega) = \frac{1}{g_t + j\omega c_m + \frac{g_{x,ch}(v)}{2}[1 + X_m(v, \omega)] + \frac{g_{y,ch}(v)}{2}[1 + X_h(v, \omega)]},$$ (3.58)

With a single exception, this is the same as the impedance (3.42) for a CM+SS model where both currents have a chord conductance of $g_{x,ch}/2$. The exception is that in (3.58), the chord conductance simultaneously depends on both activation and inactivation. In fact, for the CM+I model $g_{x,ch}$ is a “window” conductance for $I_x$. This means that it is only large at voltages where the steady-state activation and inactivation curves overlap to form a window. Outside the window, either $m_x$ or $h_x$ is close to zero and so $g_{x,ch}$ is also near zero. This is shown schematically in Figure 3.18. Referring to (3.57) or (3.58), it can be seen that the voltage region where $I_x$ contributes to the impedance is limited by the width of the window. To stress the difference between the ordinary chord conductance of simple currents and the window conductance of inactivating currents, the window conductance is written “$g_w$“ in Chapter 6 where models of thalamic neurons with an inactivating current are examined.

3.5.3 Voltage dependence of a CM+I model with amplified resonance

The importance of the form of the window conductance is shown for the case of a CM+I model where the activation and inactivation processes of $I_x$ have the properties of class II and class I currents, respectively. This means that activation current is amplifying and the inactivation current is attenuating. Following the example of Section 3.4, we also assume that the class II current activates instantaneously and that the class I current has slow kinetics so that it produces a resonance. Except for the nature of the chord conductance, this model is the same as the CM+SS model with amplified resonance in Section 3.4.
**Figure 3.18 Definition of the window conductance for a CM+I model.**

The window conductance originates from the overlap between the steady state activation and inactivation curves for a CM+I model. Outside of the window region, either $m_{oX} s_m$ or $h_{oX} s_h$ is close to zero and so the window conductance is also near zero.
Figure 3.19 shows the FRS of the model for several different values of $v_{m2}$ and $v_{h2}$. In Figure 3.19A, $v_{m2}$ and $v_{h2}$ are well separated and so the activation and inactivation curves do not overlap. Consequently, the value of the window conductance is near zero at all voltages. Under these conditions, no voltage-dependent features are visible in the FRS. In Figure 3.19B, $v_{m2}$ and $v_{h2}$ begin to approach each other and, as the activation and inactivation curves begin to overlap, a slight bump indicates the voltage region where the window conductance has become large enough to affect the impedance. In Figure 3.19C, the overlap is large enough to generate a large region of amplified resonance in the FRS. As $v_{m2}$ and $v_{h2}$ move still closer to one another the peak magnitude of the amplified resonance grows until, in Figure 3.19D, the membrane potential becomes unstable in a band of voltages in the middle of the amplified resonance and a two-peaked structure is formed as in Figure 3.8.

Thus, the voltage dependence of the activation and inactivation curves controls the location and amplitude of amplified resonance in CM+I models. In addition, $I_x$ only contributes to the impedance at voltages where activation and inactivation interact. In contrast, in the CM+SS model the basic and regulatory currents caused separate regions of amplification and resonance in the FRS even when they did not interact (see Figure 3.17A).

### 3.5.4 Summary of CM+I models

To summarize, the CM+I models are highly similar to the CM+SS models and may be analyzed in terms of component attenuating and amplifying conductances. In contrast to the CM+SS models, however, the effects of the component conductances only appear at voltages where they interact. The relative placement of the activation and inactivation curves determine how extensive this area of interaction will be.
Figure 3.19 Frequency-response surface for a CM+I model with amplified resonance.

A. When $v_{m/2}$ and $v_{h/2}$ are well separated, the overlap between the steady-state activation and inactivation curves (inset) is small. In this case, the window conductance is near zero for all $v$ and $I_x$ does not influence the FRS. B, C. As $v_{m/2}$ approaches $v_{h/2}$ the overlap between the activation and inactivation curves grows (insets). The resulting increase in the window conductance allows an amplified resonance to form. D. As the window conductance continues to grow, the amplification becomes so intense that the membrane potential becomes unstable.
4. **Experiments on neocortical neurons**

4.1 **Introduction**

We now turn to the experimental examination of neocortical neurons. This chapter describes time- and frequency-domain measurements carried out on neurons from neocortical slices maintained in vitro. The time-domain measurements are used to characterize the neurons with respect to their firing patterns and subthreshold rectifying properties. The frequency-domain experiments are intended as probes of the responses of neurons to oscillatory inputs.

As shown in the preceding chapter, voltage-dependent currents can, in theory, confer electrical resonances on neurons. Oscillatory inputs that match the resonant frequencies generated by such currents produce larger responses than those that do not. Thus, a resonant frequency defines an intrinsic, voltage-dependent *frequency selectivity* for a neuron. This suggests the possibility that neurons are tuned by resonance to receive, and respond selectively to, oscillatory inputs at frequencies that hold biological significance. A mechanism of this type can only operate when the neurons function in a nearly, but not quite linear fashion. For neocortical neurons, the most suitable voltage region for such behavior is at subthreshold potentials near rest. There, small current inputs can engage the relatively mild time- and voltage-dependent currents that have been reported at those voltages without stimulating the highly nonlinear currents underlying action potentials.

One goal of this chapter, therefore, is to characterize the voltage dependence and kinetics of subthreshold voltage-dependent currents and relate them to possible subthreshold resonances. Another goal is to explore the relationship between the frequency selectivity generated by subthreshold currents and the production of action potentials. Before proceeding, however, a brief overview of the material to be covered in this chapter is in order. *First*, comes a general survey of the membrane properties and firing patterns observed in neocortical neurons. This will allow a comparison of the neurons studied here with those reported elsewhere. *Second*, is an empirical description of the subthreshold frequency responses of these neurons, their voltage dependence, and how they affect firing patterns. *Third*, is an exploration of the ionic mechanisms underlying the subthreshold rectifying properties of neocortical neurons. This includes, voltage- and current-clamp studies on three noninactivating subthreshold currents that could shape resonant responses: an inwardly rectifying cation current ($I_h$; Spain et al. 1987;
Solomon et al. 1993; Solomon and Nerbonne 1993a, b); an inwardly-rectifying K\(^+\) current (I\(_{\text{IR}}\); Constanti and Galvan 1983; Sutor and Hablitz 1993; Womble and Moises 1993); and a persistent Na\(^+\) current (I\(_{\text{Na},p}\); Alzheimer et al. 1993a, b; Stafstrom et al. 1985). As pointed out in chapter 1, I\(_{\text{H}}\) is of interest because of its involvement in structuring the responses of neocortical neurons to time-varying subthreshold inputs (Schwindt 1992; Solomon and Nerbonne 1993a) and in rhythmic activity of other excitable cells (DiFrancesco et al. 1986; McCormick and Pape 1990). Finally, the ionic mechanisms underlying different features of the frequency response are deduced from their voltage dependence and pharmacology.

4.2 Methods

Sprague-Dawley rats with ages that varied from postnatal day 4 (P4) to P19 were used in these experiments (although most recordings were made in neurons from P6 to P14 rats). After decapitation, the brain was removed and submerged in 5-7 °C artificial cerebrospinal fluid (ACSF). The brain was cut into blocks before being glued to the stage of a Vibroslicer and cut coronally into 350-400 µm slices. Brain slices containing sensorimotor cortex were incubated for 1-2 hours at room temperature before submersion in a Perspex bath (volume 0.3 ml, flow rate 1-1.5 ml/min), which was mounted on the stage of a microscope equipped with Hoffman modulation contrast optics. The slices were thin enough to partially discern the cortical layers which we used as an initial guide for placing the electrode. The recordings were made at room temperature (24-26 °C), and all neurons accepted for analysis had overshooting action potentials and resting potentials more negative than -55 mV. At the end of a session, the distance of the recorded neuron from the cortical edge of the slice was measured using a graticule in the eyepiece of the microscope and the recording site identified using the atlas of Paxinos et al. (1991).

4.2.1 Solutions

The ACSF contained (in mM): NaCl 124, KCl 4, KH\(_2\)PO\(_4\) 1.25, NaHCO\(_3\) 26, dextrose 10, MgCl\(_2\) 2, CaCl\(_2\) 2, which gave a measured osmolarity of 310 mOsmoles. ACSF solutions were maintained at a pH of 7.4 by continuous bubbling with 95% O\(_2\) - 5% CO\(_2\). Patch clamp electrodes were pulled from thin-wall borosilicate glass tubing (outer diameter 1.5 mm) with a Narashige PP-83 puller and were filled with a solution containing (in mM): K-gluconate 140, NaCl 15, ethylene glycol-bis(β-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA) 11, CaCl\(_2\) 1, Na-(N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]) (NaHEPES) 10, Mg adenosine
5'-triphosphate (Mg ATP) 1, Na guanosine 5'-triphosphate (Na GTP) 0.3, and which was balanced to pH 7.2 with KOH and D-gluconic acid. Initial electrode resistance was 5-8 MΩ; seals to the cells were 1 - 11 GΩ before breakthrough (cells with seals <1 GΩ were discarded). To compensate for the junction potential formed between the ACSF and the electrode solution, 11 mV was subtracted from all membrane potentials (e.g., a recorded membrane potential of -60 mV corresponds to an actual potential of -71 mV; see Zhang and Krnjević 1993).

### 4.2.2 Electrical Recordings

An Axoclamp 2A amplifier was used to inject d.c. and oscillatory currents as well as measure voltage responses for frequency-domain analysis. For current clamp and frequency-domain recordings, the amplifier was optimally adjusted to compensate for the electrode resistance. For voltage-clamp studies, however, only 50 to 80% compensation could be achieved in the continuous single-electrode mode. In calibration experiments with dry circuits, it was found that use of the capacitance compensation circuit of the Axoclamp amplifier sometimes had unexpected consequences for frequency-domain recordings. For this reason, capacitance compensation was not used in frequency-domain or current-clamp studies. To minimize the stray capacitance of the electrode, very low fluid levels were used and neurons were recorded within 150 μm of the surface of the slice. This meant that the total immersion of the electrode during whole cell recording was often no more than 200 μm. Under these conditions the benefits derived from capacitance compensation were found to be minimal.

For digital data acquisition, a 40 kHz Labmaster A\D - D\A board was controlled with pClamp software or software written specifically for the purpose of acquisition and analysis of frequency-domain data. Voltage and current traces for time-domain recordings were filtered at 10 kHz and then stored on a computer disk or on video tape (Beta) using a PCM encoder (Sony) and a video recorder (Sony).

### 4.2.3 Frequency-domain analysis

The frequency-domain analysis of neurons using the ZAP method has been previously described (Puil et al. 1986, 1988). Briefly, a d.c. current was first used to hold neurons near a desired membrane potential, then a computer generated current waveform (I) was injected into the neurons, and the resulting voltage response (V) was digitally sampled and recorded in a
computer. For each holding potential, an impedance \((Z)\) was calculated from the ratio of the fast Fourier transforms of the voltage response and the current input using the formula,

\[
Z = \frac{\text{FFT}(V)}{\text{FFT}(I)}.
\]

The magnitude of the complex-valued impedance was plotted against frequency to give a frequency-response curve (FRC). The measurement and analysis systems were calibrated by comparing the measured impedance of a parallel resistor-capacitor circuit with its theoretical impedance.

The ZAP input waveform was of finite duration, \(T\), and was given by the formula

\[
I(t) = a \sin(bt^3), \quad 0 \leq t \leq T.
\]

Here, \(a\) and \(b\) were adjustable parameters controlling, respectively, the amplitude and bandwidth of the input. To avoid aliasing during the sampling procedure, the value of the parameter \(b\) was permanently set so that the highest frequency with significant power in the input signal was \(4/5\) of the Nyquist frequency. In addition, the output filter of the Axoclamp amplifier was used as anti-aliasing filter with an adjustable corner frequency of between 0.3 and 10 kHz. The only aliased noise observed in the experiments was from the 60 Hz line frequency or its harmonics. These components were usually small and did not interfere with the features of interest in the frequency spectra or impedance.

A proper interpretation of the frequency-domain analysis requires an approximately linear relationship between current inputs and voltage responses. Linearity usually was maintained during neuronal recordings as long as voltage responses were less than 20 mV peak-to-peak and action potentials were not evoked. As a confirmation of linear behavior, the amplitude of the ZAP input was often altered with little influence on the calculated impedance. In other cases we used a sine wave input or a sum-of-sine-waves (SSW) input consisting of 128 superimposed sine-waves of equal amplitude and systematically varied frequency and phase. In these cases, significant nonlinearities would have been signaled by the appearance of harmonics in the response. These were not usually observed unless the membrane potential was very close to the threshold for action potential generation.
In the analysis of finite time series, data is often multiplied by a tapering window before decomposition into frequency components by an FFT. This is intended to suppress side-lobes that may artefactually broaden the spectra (Bendat and Piersol 1986). However, for the ZAP input it was found that the use of a Hanning window produced only small changes in the width or placement of peaks in the impedance magnitude. Therefore, tapering windows are not used in this study.

4.3 Results

4.3.1 Electrical properties

The results described in this chapter are based on recordings from 147 neurons in layers II-V of rat sensorimotor cortex. Neurons accepted for analysis had initial spikes that were overshooting by 10 to 20 mV, resting potentials more negative than -55 mV, and did not normally fire action potentials at rest. In cases where electrical recordings were made in control ACSF (103 of 147 neurons), the firing characteristics of neurons were similar to those previously described for juvenile rat neocortex (McCormick and Prince 1987; Kasper et al. 1994a,b). Neurons were identified as regular spiking (RS), intrinsic bursting (IB), and fast spiking (FS) by the properties of their action potentials and the firing patterns evoked by 1 s current pulses (McCormick et al. 1985; Connors and Gutnick 1990, Kawaguchi 1995). The RS neurons were further subdivided into neurons with and without depolarizing afterpotentials (DAPs) on the falling phase of action potentials (RS\textsuperscript{DAP+} and RS\textsubscript{DAP-} neurons as will be described below). Examples of the 4 types of firing pattern are given in Figure 4.1. Some of their basic electrophysiological properties are given in Table 4.1.

Regular spiking neurons

Most neurons (75/103, 73%) were RS neurons as determined from the relatively large ratios of their spike repolarization and depolarization rates (Table 1), ability to respond to just-suprathreshold current inputs with solitary spikes (Figure 4.1A,C), and prominent medium afterhyperpolarizations (mAHPs) lasting from 50 to 200 ms following isolated spikes. At relatively low stimulus amplitudes, action potentials were well-separated with mAHPs occurring between each spike, higher amplitude inputs decreased the separation between spikes. 44 of 75 RS neurons also had distinct slow AHPs (sAHPs) that lasted > 2 s after a train of action potentials.
Table 4.1 Electrophysiological properties of neocortical neurons

<table>
<thead>
<tr>
<th>Firing class</th>
<th>$\text{RS}_{\text{DAP}+}$</th>
<th>$\text{RS}_{\text{DAP}-}$</th>
<th>$\text{FS}$</th>
<th>$\text{IB}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting potential (mV)</td>
<td>-66.3 (5.2)*</td>
<td>-66.5 (6.1)</td>
<td>-66.6 (5.1)</td>
<td>-72.3 (5.5)</td>
</tr>
<tr>
<td>Membrane time constant (ms)</td>
<td>43.2 (17.3)</td>
<td>52.4 (23.0)</td>
<td>50.4 (25.7)</td>
<td>31.6 (10.3)</td>
</tr>
<tr>
<td>Input resistance (MΩ)</td>
<td>303 (189)</td>
<td>446 (331)</td>
<td>450 (255)</td>
<td>218 (107)</td>
</tr>
<tr>
<td>Rise/fall for 1st spike**</td>
<td>3.0 (0.8)</td>
<td>2.8 (1.3)</td>
<td>1.2 (0.4)</td>
<td>2.7 (1.0)</td>
</tr>
<tr>
<td>Rise/fall for 5th spike**</td>
<td>4.1 (1.3)</td>
<td>3.6 (1.4)</td>
<td>1.4 (0.4)</td>
<td>N/A</td>
</tr>
<tr>
<td>Action potential width (ms)†</td>
<td>2.3 (0.4)</td>
<td>2.6 (0.6)</td>
<td>1.3 (0.4)</td>
<td>2.3 (0.7)</td>
</tr>
<tr>
<td>Slow AHP‡</td>
<td>24</td>
<td>20</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>n (total = 103)</td>
<td>39</td>
<td>36</td>
<td>12</td>
<td>16</td>
</tr>
</tbody>
</table>

* data given as mean (standard error)
** maximal rate of rise divided by maximal rate of fall of voltage during first and fifth action potentials.
† measured at 1/2 the height of the action potential
‡ number of neurons exhibiting sAHPs following spike trains with at least 10 action potentials

Regular spiking neurons with depolarizing afterpotentials

In $\text{RS}_{\text{DAP}+}$ neurons, DAPs was evident as a sudden decrease in the rate of repolarization of spikes or, in neurons with large DAPs, as a plateau or depolarizing hump at the bases of spikes (arrow, Figure 4.1A). The DAPs influenced spiking patterns. Normally the largest DAP occurred on the falling phase of the first action potential in a train. Successive DAPs usually progressively diminished in duration. Large current pulses caused the large DAP associated with the first spike to combine with successive spikes, forming a high frequency spike complex (Figure 4.1E1,E2). The high-frequency complex consisted of a spike doublet or triplet with a high, thin, initial spike followed by smaller, broader spikes. Once formed at the beginning of the train, the interspike intervals within a complex were relatively insensitive to further increases in the magnitude of the stimulating current. In contrast, the spike rate in the remainder of the train increased with increasing current.

In 12 of 39 $\text{RS}_{\text{DAP}+}$ neurons, the DAPs first decreased as described above, then increased in magnitude and duration with each action potential. If the magnitude of the injected current pulse was large, these neurons fired secondary spikes on top of the DAPs (Figure 4.1E2).
Figure 4.1 Firing patterns and I-V relations for neocortical neurons.

Voltage responses to 400 ms current pulses are shown for: A. Regular-spiking neurons with a depolarizing afterpotential (RS_{DAP}+); B. Fast-spiking neurons (FS); C. Regular-spiking neurons without depolarizing afterpotential (RS_{DAP}-); and D. Intrinsic bursting neurons (IB). The current-voltage relationships derived from these responses at the times indicated by the filled in circles above the voltage traces are shown below each figure. E. Firing patterns of neurons in response to high-intensity current pulses with amplitudes shown at lower right of each trace. Labels at right indicate the class to which the neuron belongs. The neuron in (E2) is classed as an RS_{DAP}+ rather than an IB neuron because a solitary spike could be evoked in response to small-amplitude current pulses, as in (A).
Regular spiking neurons without depolarizing afterpotentials

RS_{DAP}− neurons (Figure 4.1C) had significantly higher mean input resistances and longer mean time constants than RS_{DAP}+ neurons (Table 1). In addition, 14 of 36 RS_{DAP}− neurons possessed fast AHPs (fAHPs), in contrast to RS_{DAP}+ neurons where 1 of 39 had fAHPs. The spike trains of both RS_{DAP}− and RS_{DAP}+ neurons showed spike rate adaptation during sustained (>500 ms) current pulses.

Intrinsic bursting neurons

IB neurons were similar to RS_{DAP}+ neurons in that they all possessed ADPs. They were further were characterized by relatively low mean input resistances and membrane time constants, and stereotyped spike bursts consisting of 2-3 spikes either at the beginning of a spike train or recurring throughout the train (Figure 4.1D). The bursts in IB neurons were all-or-none. This distinguished them from the high-frequency spike complexes of RS_{DAP}+ neurons that had the appearance of being assembled from separate spikes and DAPs as the magnitude of the injected current was increased.

Fast firing neurons

Fast-spiking (FS) neurons (Figure 4.1B) had high spike rates with little adaptation, thin spikes, prominent fAHPs, small or absent mAHPs, and ratios of <2 for the rate-of-rise to rate-of-repolarization for action potentials (Table 1). These properties are consistent with previous descriptions of cortical FS neurons (McCormick et al. 1985, Kawaguchi 1995). Neurons with this type of firing pattern are regarded as inhibitory interneurons although the evidence for this assertion is not abundant (Kawaguchi 1995). The steady state V-I relations of FS neurons were usually nearly ohmic at potentials more negative than the resting potential. Some RS_{DAP}− neurons had firing rates and nonadapting spike patterns similar to FS neurons. As shown in Table 1, the mean values for the passive electrical properties of RS_{DAP}− and FS neurons were also similar. However, FS neurons lacked the prominent mAHPs of RS_{DAP}− neurons. Perhaps as a consequence, solitary spikes could not be evoked with just-threshold currents as they could in RS_{DAP}− neurons. Instead, multiple spikes appeared when the injected current was larger than spike threshold (Figure 4.1B).

4.3.2 Frequency-response curves (FRCs)

To investigate the frequency preferences of neurons, their frequency response curves (FRCs) were determined. Figure 4.2A,B show the FRCs of and RS and FS neuron near their
respective resting potentials. The responses of the same neurons to current pulses are shown in the upper right of each figure. In these neurons, as in all the neurons we studied, the smallest impedance magnitudes occurred at high frequencies. There were important differences, however, in the low-frequency behavior of the FRCs in different neurons.

**Resonant neurons**

Some neurons were resonant near their resting potentials. For the RS neuron of Figure 4.2A, with a resting potential of -65 mV, resonance appears as a hump in the frequency-response curve (FRC) with a peak at 2.1 Hz\(^1\) and a Q-value (see text) of 1.8. The responses of the same neuron to square current pulses are shown in the inset at upper right: note the sags and depolarizing rebounds which are the manifestations of resonance in the time domain. For the same neuron there were no other resonant peaks in the interval 2 - 500 Hz. Similarly, we could not resolve multiple humps in the FRCs of other neurons. Figure 4.2A2 shows the impedance locus for a different resonant neuron. It forms a broad spiral with impedance values at low frequencies that have positive imaginary parts. The impedance locus is, therefore, similar to that of the resonant class I model in Chapter 3 (see Figure 3.6).

We quantified the resonance of a neuron by measuring its resonant frequency (\(f_{\text{res}}\)) and "Q value". The \(f_{\text{res}}\) of a neuron is defined as the frequency at the peak of the resonant hump in the FRC. The Q value is constructed by dividing the magnitude of the impedance at \(f_{\text{res}}\) by the magnitude at the lowest recorded frequency (Koch 1984). The \(f_{\text{res}}\) of the neuron illustrated in Figure 4.2A is 2.2 Hz. Its Q value, 1.81, was one of the largest we recorded. Table 2 gives the incidence of resonance and the values of \(f_{\text{res}}\) and Q at -70 mV in 59 neurons of different firing types. Approximately 2/3 of RS and IB neurons were resonant at -70 mV. All \(f_{\text{res}}\) values were between 0.7 and 2.5 Hz and most were between 1 and 2 Hz. There were no significant differences between RS and IB neurons with respect to their resonant properties at this membrane potential (two-tail t-tests). The total incidence of resonance among RS and IB neurons is underestimated in Table 2 because some neurons that were nonresonant at -70 mV were resonant at other potentials.

---

\(^1\)Note that, for experimental results, frequencies are given in Hertz (f, cycles/s) rather than as radial frequencies (\(\omega\), radians/s). They are connected by the relation \(\omega = 2\pi f\). The use of cycles/s conforms with the large literature on periodic and oscillatory activities in mammalian brains.
Figure 4.2 Frequency- and time-domain responses of resonant and nonresonant neurons.

A1, 2. FRC and impedance locus for two resonant, RS neurons. B1, 2. FRC and impedance locus for nonresonant neurons. The neuron in (B1) is an FS neuron and the neuron in (B2) is RS. C1-5. ZAP current inputs (lower) and corresponding voltage responses (upper) in a resonant RS neuron, and D1-2 in a nonresonant RS neuron. In this and subsequent figures, action potentials are clipped.
Table 4.2 Mean resonant frequencies and Q values for resonant neocortical neurons

<table>
<thead>
<tr>
<th></th>
<th>$f_{res}$ (Hz)</th>
<th>Q</th>
<th>n†</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSDAP+</td>
<td>1.29 (0.43)</td>
<td>1.33 (0.17)</td>
<td>11/18</td>
</tr>
<tr>
<td>RSDAP-</td>
<td>1.26 (0.35)</td>
<td>1.44 (0.32)</td>
<td>14/23</td>
</tr>
<tr>
<td>IB</td>
<td>1.30 (0.51)</td>
<td>1.28 (0.25)</td>
<td>7/11</td>
</tr>
<tr>
<td>FS</td>
<td>-</td>
<td>-</td>
<td>0/7</td>
</tr>
<tr>
<td>total</td>
<td></td>
<td></td>
<td>32/59</td>
</tr>
</tbody>
</table>

* measured at -70 mV and 24-26 °C, values given as proportion or mean (SD)
† proportion of neurons with resonance

**Resonance does not depend on the input waveform**

The relationship between small amplitude current inputs and voltage responses was almost linear in most neurons. This was evident in the FRCs which were essentially unchanged by increasing or decreasing the amplitude of the ZAP current input when the membrane potential was more negative than -60 mV. In resonant neurons, when this potential was not exceeded, changes in the magnitude of the input affected $f_{res}$ weakly and left the Q value unaltered. Figure 4.3 shows, for a resonant neuron, the FRCs generated near -70 mV by SSW and ZAP inputs with similar frequency bands. The FRCs were nearly identical although the time courses, but not the frequency compositions, of the inputs were very different. A similar result could always be attained for sufficiently small, subthreshold inputs. Thus, subthreshold resonance in these neurons was independent of the form of the input.

**Nonresonant neurons**

All of the FS neurons, and some of the RS and IB neurons, lacked resonant humps in their FRCs at the potentials that were usually investigated (potentials more negative than -55 mV). The neurons that did not have resonant humps at these potentials are called “nonresonant”, although it is recognized that they may have had resonance at other potentials. Figure 4.2B shows a typical FRC for a nonresonant FS neuron at its resting potential (-67 mV). The value of the impedance magnitude decreases monotonically with frequency, resulting in an FRC with the characteristics of a lowpass filter. Thus, the Q-value of this neuron is equal to 1, as is the case for all nonresonant neurons. Note that the voltage responses to square pulses do not show sags or rebounds (inset, upper right). Figure 4.2B2 shows the impedance locus of a nonresonant RS neuron. In contrast to the spiral shape of the locus for resonant neurons, it is a semicircle with no
Figure 4.3 The attributes of resonance are independent of the time course of the input current.

The FRC derived by the ZAP method (gray) is compared to the FRC (black line) derived from a sum-of-sine waves (SSW) input consisting of 128 sine-waves with systematically varied phase and equal amplitude. Although the bandwidth of the SSW input was narrower than that of the ZAP input, the features of the FRC do not change. The time-domain traces for the inputs and their voltage responses also are shown above and to the right of the FRCs (voltage responses shown in upper traces).
positive imaginary part. It is, therefore comparable to the impedance locus of the passive CM model in Chapter 3 (see Figure 3.2)

4.3.3 Coupling of oscillatory inputs to the firing of action potentials

The differences between resonant and nonresonant neurons were reflected in the time courses of their responses to ZAP inputs. Figure 4.2C shows the voltage responses of a resonant RS neuron to constant amplitude ZAP inputs. Because of the systematic order in which the ZAP input sweeps through the frequencies it is easy to see that, for the subthreshold responses, there is a peak at intermediate frequencies. This gives it a spindle shape which is characteristic of resonant neurons. In fact, the largest response is near $f_{res}$. In contrast, the subthreshold voltage responses of the nonresonant neuron in Figure 4.2D is wedge-shaped since the largest response occurred at the lowest frequencies.

In both resonant and nonresonant neurons, large-amplitude ZAP stimuli evoked action potentials. However, there was a clear difference between resonant and nonresonant neurons in the stimulus frequencies that most readily evoked firing. In resonant neurons, action potentials were preferentially generated when the input frequencies were near $f_{res}$ (e.g., Figure 4.2C3). In nonresonant neurons, on the other hand, firing was associated with the lowest frequencies of the input (Figure 4.2D2). This difference between resonant and nonresonant neurons was observed consistently. All resonant neurons tested with large amplitude inputs fired action potentials as the input passed through the resonant band of frequencies ($n = 4$). In 5 of 6 nonresonant neurons the spikes arose at the lowest injected frequencies. In summary, the frequency preference of resonant neurons resulted in a frequency-selective coupling between inputs at frequencies near $f_{res}$ and firing.

This frequency-selective firing does not imply that action potentials will fire at the same frequency as an oscillatory input delivered near the resonant frequency. In trials using single-frequency sine-waves, it was found that action potentials sometimes fired on every other cycle or in some more complicated pattern. Also, the frequency-selective coupling of oscillatory inputs with firing did not occur at all membrane potentials. Firing near the resonant frequency often was observed near -70 mV. When resonant neurons were depolarized above approximately -60 mV by d.c. current, firing occurred at all frequencies below 10 Hz (Figure 4.2C5). The neurons however were still resonant at this potential (Figure 4.2C4). Thus, the ability of resonance to
mediate coupling between oscillatory inputs and the firing of action potentials was voltage dependent.

4.3.4 Voltage dependence of frequency response curves

The FRCs of most neurons were voltage dependent over a 20 mV range near the resting potential. Figure 4.4 shows the FRCs of a resonant RS neuron (treated with 300 nM TTX) at different membrane potentials. Here, d.c. current was used to displace the membrane potential to values between -90 and -50 mV before subjecting it to a ZAP input. The neuron is clearly resonant between -90 and -65 mV. As shown in Figure 4.4B, the $f_{res}$ and Q-values were also voltage dependent. The value of $f_{res}$ increases steadily from a minimum of 1.9 Hz at -65 mV to a maximum of 3.6 Hz at -90 mV. The Q values are near 1 at -60 mV or more depolarized potentials, increase to 1.5 by -77 mV, and remain near 1.5 at more hyperpolarized potentials.

The amplitudes of the FRCs in Figure 4.4A also change with membrane potential. The largest impedance magnitudes are associated with the FRC taken at -72 mV (also see Figure 4.4C). At more hyperpolarized or depolarized potentials, the FRCs have smaller peak values. Above 10 Hz, the FRCs approach asymptotic curves determined by the membrane time constant. Together, the FRCs of this neuron approximate a frequency response surface (FRS). Except for the occurrence of resonance, this FRS looks like the one belonging to the amplified class II model in Section 3.3.3 where a region of amplified responses formed a bulge (see Figure 3.7). It will be shown below that this is an illusion created by the existence of multiple rectifying currents in these neurons with overlapping ranges. In actuality, the FRCs shown in Figure 4.5 come from an FRS more like that of the resonant class I model of Section 3.3.3 flanked by two regions of attenuation. The mechanism of the attenuation at hyperpolarized voltages will be examined in a later section. The mechanism of the attenuation at depolarized levels was not pursued in these investigations.

Figure 4.4 illustrates a pattern commonly seen in resonant neurons treated with TTX, that is, (1) the value of $f_{res}$ increases with hyperpolarization, (2) the Q value is greater at hyperpolarized than at depolarized potentials, (3) the largest impedance values lie between -65 and -75 mV, and (4) the FRCs are independent of voltage at high frequencies.
Figure 4.4 Voltage dependence of the FRCs of a resonant RS neuron (in the presence of 300 μM TTX)

A. A 3-dimensional plot reveals the overall relationship between the FRCs and the membrane voltage. The FRCs shown here and in subsequent 3-dimensional plots are curves fitted by eye to the data. B. The values of Q and the resonant frequency ($f_{res}$) are shown for the same neuron as in A. The increase of $f_{res}$ with hyperpolarization is typical of the subthreshold resonances described in this paper. C. Three of the FRCs from A are superimposed on the same axis. The holding potentials for each FRC are shown. Each curve is the result of a 5 point moving average performed 3 times on the data.
The last two points are also characteristic of the FRCs of nonresonant neurons treated with TTX. For example, as seen in Figure 4.5A, the FRCs at low frequencies are highly dependent on voltage with the largest amplitude FRC occurring between -65 and -75 mV, and smaller amplitude FRCs at more depolarized and hyperpolarized voltages. In contrast, at high frequencies all FRCs have similar impedance magnitudes. Once again, the FRCs of these neurons approximate an FRS with a bulge. This is an illusion created by the existence of hyperpolarized and depolarized regions of attenuation just as in Figure 4.4. In this case, however, there is no intervening region of attenuation.

4.3.5 Subthreshold rectification in RS neurons

As reviewed in Chapter 2 and predicted theoretically in Chapter 3, the voltage dependent features of the impedances above are shaped by voltage-dependent subthreshold currents. Three forms of steady-state, subthreshold rectification were regularly encountered. These were identified tentatively as due to $I_H$, $I_R$, and $I_{Na,p}$ (see Section 4.1). Both $I_H$ and $I_R$ activate with hyperpolarization whereas $I_{Na,p}$ activates on depolarization from rest. In the following sections, we describe the properties of these currents and, in particular, characterize their voltage dependencies and time courses so that they may be compared to the frequency-domain characteristics of RS neurons described above.

Rectifications activated by hyperpolarization: current-clamp studies

Figure 4.1 shows firing patterns and current-voltage (I-V) relations for RS (Figure 4.1A), IB (Figure 4.1B), and FS (Figure 4.1C) neurons. Most I-V relations for RS and IB neurons showed a tendency to curve upwards as the membrane was hyperpolarized from rest. This was not a prominent feature for FS neurons.

Current clamp recordings from RS and IB neurons exhibited two hyperpolarization-activated rectifications which were distinguished on the basis of their voltage dependence, time course, and pharmacology. On hyperpolarization beyond -80 mV, some neurons showed a quickly developing rectification that decreased their input resistances (evident as decreased voltage responses to equally spaced current steps, see Figure 4.6A) and shortened their membrane time constants (dotted line, Figure 4.6A). This can result from the actions of $I_R$. External application of 0.2 to 3 mM Ba$^{2+}$ (n = 4), a blocker of $I_R$ (Sutor and Hablitz 1993; Womble and Moises 1993), increased the membrane time constant and greatly increased the apparent input resistance, especially in the hyperpolarizing direction (Figure 4.7A1,A2; note the
Figure 4.5 Voltage dependence of the FRC of a nonresonant RS neuron

A. A 3-dimensional plot reveals the overall relationship between the FRCs and the membrane voltage. B. The same FRCs as in (A), presented as a 2-dimensional plot of smoothed data. Note that the voltage dependence is small at frequencies above 20 Hz. Note the shallow hump in the FRC at -45 mV. Data gathered in the presence of 300 nM TTX.
different current command steps). These effects of Ba$^{2+}$ were often accompanied by a small depolarization of the resting potential (mean = 1.3 ± 0.8 mV, n = 4) and are consistent with the blockade of I_{IR}.

Some neurons possessed a slowly developing rectification that was evident as sagging voltage responses to hyperpolarizing current pulses (Figure 4.6C1). Overshooting rebounds sometimes crowned by action potentials (Figure 4.6C2) followed the sags on termination of the hyperpolarizing pulses. The sags and rebounds are the time-domain manifestations of resonance. This is seen by putting a waveform consisting of a single square pulse through a digital filter whose properties are determined by the FRC of a resonant neuron. The output waveform from the filter had the same types of sags and rebounds as seen in the neuronal responses to square pulses (Figure 4.8). When the same input was put through a filter based on a nonresonant FRC, the output has no sags or rebounds (not shown).

The amplitudes of the rebounds following a 1 s hyperpolarization were correlated to the magnitudes and durations of the preceding sags (e.g., Figure 4.6C1,2). This is shown quantitatively in Figure 4.6D which displays the relationship between percent sags and rebounds (defined in Figure 4.6B) in 19 consecutively recorded RS neurons. The correlation depended on the holding potential. When neurons were held near -70 mV, the amplitudes of the sags and rebounds were positively correlated ($r^2 = 0.93$, Figure 4.6D1), implying that the sags and rebounds rely on the same current, e.g., I_{H}. When neurons were held near -60 mV, on the other hand, the amplitudes of the rebounds had only a slight dependence on the magnitudes of the preceding sags ($r^2 = 0.47$, Figure 4.6D2). Also, the firing of action potentials during rebounds following the termination of hyperpolarizing pulses was facilitated at -60 mV even though the sags were smaller than those evoked at -70 mV. Thus, at -60 mV, the mechanisms underlying sags and rebounds appear to differ. This dissociation between sags and rebounds at -60 mV is reminiscent of the dissociation between resonance and frequency-preferential firing at the same potentials and could be due to the activation of currents other than I_{H} at potentials near -60 mV.

Application of external 0.5 mM Ba$^{2+}$ did not reduce the sags or rebounds (n = 5, Figure 4.7A). However, application of 3mM external Cs$^+$ (a blocker of both I_{IR} and I_{H}; Constanti and Galvan 1983; Spain et al. 1987; Foehring and Waters 1991) by itself or together with Ba$^{2+}$ eliminated sags and reduced rebounds by > 80% in 7 of 8 neurons (e.g., Figure 4.7A3). This implies that I_{H} is the major current underlying the slow rectification.
Figure 4.6 Consequences of fast and slow rectification in neocortical neurons.

A. Quickly developing hyperpolarization-activated rectification in an RS neuron. The time taken for each voltage response to fall to $1/e$ of its initial value (apparent time constant) is indicated by solid circles connected with a line. B. Quantification of sags and rebounds in resonant neurons. Lowercase letters: percent sag was defined as $100(1 - b/a)$, and percent rebound as $100(1 - c/a)$. C1,2. The sags and rebounds in a resonant RS neuron depend on the magnitude ($C_1$) and duration ($C_2$) of the hyperpolarizing input. Neurons in A, B, and C were initially at their resting potentials. D. Relationship between percent sags and rebounds at -70 (D1) and -60 mV (D2). Linear regression lines are shown with the data.
Figure 4.7 Pharmacological blockade of the voltage sags and overshooting rebounds.

A1,2. Application of Ba$^{2+}$ (0.5 mM, 2 min) in an RS neuron increased input resistance (note changes in current inputs) but did not reduce sags or rebounds. Action potentials were eliminated with 0.3 μM TTX.  A3. Additional application of 3 mM Cs$^+$ to the same neuron eliminated sags and greatly reduced rebounds.  B. Effects of Ba$^{2+}$ and Cs$^+$ on a neuron where substantial rebounds were not preceded by sags.
The possibility of a current other than \( I_{\text{H}} \) contributing to the rebounds was also explored. Rebounds often were incompletely blocked by \( \text{Cs}^+ \) whereas sags were totally eliminated. This may reflect a partial or voltage-dependent blockade of \( I_{\text{H}} \) by \( \text{Cs}^+ \) or may implicate other currents in the formation of rebounds. The latter possibility is highlighted in the case of 4 neurons where large rebounds occurred in the absence of sags during the preceding hyperpolarization. In two of these neurons, external application of 0.5 mM Ni\(^{2+}\) reduced the rebounds by 65\% (Figure 4.7B1-3). In one of the neurons where Ni\(^{2+}\) reduced the rebounds, \( \text{Cs}^+ \) completely blocked the rebounds following small hyperpolarizations, but failed to block rebounds following hyperpolarizations beyond -75 mV (Figure 4.7B3-4). These observations point to the involvement of a low threshold Ca\(^{2+}\) current in some rebounds. We could not determine whether such a current plays a role in rebounds following sags since application of Ni\(^{2+}\) to 4 neurons with sags produced variable results.

Sags in the voltage responses to small hyperpolarizing currents were observed over a wide range of potentials. In some neurons, the sags were evoked by small hyperpolarizations from potentials ranging from threshold to -90 mV. In other neurons, sagging responses were only seen at potentials more negative that -80 mV. The thresholds at which sags could be evoked often appeared to drift to more negative values over the course of an experiment. In contrast, the membrane potential where fast rectification was first detected was consistently between -75 and -85 mV in most neurons and did not change over the course of an experiment. The fast and slow rectifications often coexisted in RS and IB neurons. However, FS neurons seldom had slow sags and rebounds (2 of 12 FS neurons), and, when present, they occurred at very negative potentials (e.g., below -90 mV).

In 6 of 9 neurons tested, \( I_{\text{H}} \) contributed to the resting potential since 3 mM \( \text{Cs}^+ \) reversibly hyperpolarized the membrane potential (mean = -3.1 ± 1.9 mV, range 2 - 12 mV). In 2 of the remaining neurons, \( \text{Cs}^+ \) had no effect on the resting potential and, in a single neuron, evoked a 1.5 mV depolarization.

**Rectifications activated by hyperpolarization: voltage-clamp studies.**

Neurons were voltage clamped to examine the currents underlying their fast and slow hyperpolarization-activated rectifications. When the membrane potential was held near -60 mV and then stepped to more hyperpolarized values in 5 mV increments (Figure 4.9A1), the total evoked inward current could be resolved into a transient component due to the capacitance
Figure 4.8 Measured and reconstructed voltage responses of an RS neuron.

A square current pulse input (bottom, solid line) causes a hyperpolarizing response with a prominent sag and rebound in both an actual neuron (top, dashed line), and the digital filter constructed from its measured impedance (top, solid line). The wavelets appearing at the onset and offset of the reconstructed response arise from the restricted bandwidth of the impedance measurement.
(arrow, Figure 4.9A2), a quickly-activating component, and a slowly activating component identified with \( I_H \) (Figure 4.9A2). The slowly activating inward current was isolated during data analysis by fitting the total current with a sum of exponential terms and ignoring the components in the fit corresponding to the instantaneous and capacitive currents (Scroggs et al. 1994). The resulting fits of \( I_H \) (with a time constant, \( \tau_H \), as long as 4 s at -80 mV) are shown superimposed on the data in Figure 4.9A1. Figure 4.9B (open circles) shows the voltage-current (V-I) relation of the slowly activating component (\( I_H \)) for the same neuron as Figure 4.9A.

The quickly-activating current derived from the fit of the total inward current is composed of a leak current, proportional to the magnitude of the applied voltage step, and an inwardly-rectifying current. The fast activation and voltage dependence (V-I relation in Figure 4.9B, filled symbols) of the rectifying current is consistent with the properties of an inwardly-rectifying \( K^+ \) current that has been described in cortical neurons (Constanti and Galvan 1983; Sutor and Hablitz 1993; Womble and Moises 1993).

Application of 0.2 to 3 mM \( \text{Ba}^{2+} \) greatly reduced the rectifying component of the instantaneous current without blocking the slower components of the total inward current \((n = 2)\). External application of 2 mM \( \text{Cs}^+ \) or its co-application with \( \text{Ba}^{2+} \) resulted in the complete blockade of both hyperpolarization-activated rectifying currents \((n=7)\). These results confirm the identities of \( I_{IR} \) and \( I_H \) as the currents that underlie the fast and slow rectifications, respectively, and show that \( \text{Ba}^{2+} \) can be used in these neurons to block \( I_{IR} \) without blocking \( I_H \).

The activation point of a current was defined as the first membrane potential where the current was > 5 pA. A comparison of the V-I relations of \( I_H \) and \( I_{IR} \) in Figure 4.9B shows that they are simultaneously active at membrane potentials more negative than -85 mV. This appears to be the only region along the voltage axis where they can interact in this neuron. The activation points of \( I_H \) and \( I_{IR} \) in 27 RS neurons were measured and their possible regions of interaction assessed. Figure 4.10 shows the activation points of \( I_H \) and \( I_{IR} \) in these neurons. The values for \( I_H \) (mean = -73.4 ± 10.1 mV) were more depolarized and more variable than those for \( I_{IR} \) (mean = -81.6 ± 3.3 mV). Thus, since both currents are activated by hyperpolarization, the activation point of \( I_{IR} \) determines the range of interaction of \( I_H \) and \( I_{IR} \) in most neurons.
Figure 4.9 Hyperpolarization-evoked inward currents in a voltage-clamped RS neuron.

**A1.** Hyperpolarizing voltage steps from -60 mV in 5 mV increments evoked inward currents. Smooth lines though the data represent $I_H$ as determined by exponential fits to the total current (see text). Short horizontal lines on the left indicate the instantaneous component of the total current. The longer horizontal line on the left indicates the holding current prior to the voltage steps. **A2.** Details of the first second of the total current evoked by the most negative voltage step in A1. Components of the total current are indicated **B.** Steady-state V-I plots of $I_H$ (open circles) and $I_{IR}$ (filled circles) for the neuron in A. The activation points for each current, defined as the membrane potential where the current first becomes larger than 5 pA, are indicated by arrows. **C.** Inwardly rectifying V-I plot (filled circle) of an RS neuron. Application of 300 nM TTX abolished the inward rectification (filled triangle). Subtracting the TTX trace from the control trace gives the voltage dependence of the persistent Na$^+$ current ($I_{Na,p}$, filled square). An arrow indicates the activation point for $I_{Na,p}$. 
Rectification activated by depolarization

Most neurons were outwardly or inwardly rectifying (Figure 4.1) between the resting membrane potential and the threshold for action potentials (generally near -55 mV). We voltage clamped neurons and used slowly ascending (10 mV/s) voltage ramps to acquire quasi-steady-state V-I curves. The same procedure was repeated after application of 10 to 300 nM TTX. Subtracting the trace recorded in TTX from the control trace often revealed a steady-state inward current that activated with depolarization (Figure 4.9C). Because of its voltage dependence, lack of inactivation, and blockade by TTX this inward current is likely the persistent Na$^+$ current, $I_{Na,p}$ (Stafstrom et al. 1985; Alzheimer et al. 1993a, b). In 9 RS neurons, the activation points of $I_{Na,p}$ ranged between -68 and -56 mV. In Figure 4.10, the mean value for the $I_{Na,p}$ activation points (-61.4 ± 4.1 mV) is indicated by a dashed horizontal line and the standard deviation by a gray region. Comparing the $I_{Na,p}$ activation range and the location of the $I_{H}$ activation points shows that $I_{Na,p}$ and $I_{H}$ are likely to interact in some RS neurons. In support of this, we found that TTX reduced the size of rebounds by 12 to 26 % (n = 5). The separation between the distributions for the activation points of $I_{Na,p}$ and $I_{IR}$ (Figure 4.10) indicates that they will not interact.

4.3.6 Effect of ionic blockers on FRCs

Since $I_{IR}$ and $I_{Na,p}$ rarely activate near rest (see Figure 4.10), they are unlikely to account for the resonances we observed there. On the other hand, the voltage range of $I_{H}$ and the slow time course of its activation kinetics are consistent with a possible role in generating low-frequency resonance. Moreover, since the reversal potential of $I_{H}$ is consistently reported to be more depolarized than -45 mV (Spain et al. 1987; Solomon and Nerbonne 1993a), it is a class I current as defined in Chapter 3. Thus, it should produce resonance. In this section, this hypothesis is confirmed through the use of ionic blockers of $I_{H}, I_{K,ir}$, and $I_{Na,p}$ to pharmacologically dissect the FRCs of resonant and nonresonant neurons.

In nonresonant neurons at membrane potentials more negative than -75 mV, Ba$^{2+}$ application increased the amplitudes of FRCs and eliminated their low-frequency voltage dependence (n = 2, Figure 4.11A,B, cf. Figure 4.5A). Most of the Ba$^{2+}$ sensitive increase in the FRCs occurred at frequencies below 20 Hz (Figure 4.11C). At other potentials, Ba$^{2+}$ either slightly increased the amplitudes of the FRCs or left them unaltered (the decreased FRC at -65 mV in Figure 4.11A is anomalous). The effect of Ba$^{2+}$ is therefore to enlarge FRCs over the
Figure 4.10 Activation points for $I_{\text{H}}$, $I_{\text{Na,p}}$, and $I_{\text{IR}}$.

The activation points of $I_{\text{H}}$ and $I_{\text{IR}}$ are plotted for 27 RS neurons. Each solid vertical line indicates, for a single neuron, the gap between the activation point of $I_{\text{H}}$ (filled square) and that of $I_{\text{IR}}$ (short horizontal line). A horizontal dashed line shows the mean activation point for $I_{\text{Na,p}}$, determined in 9 additional neurons and the gray area is ± 1 SD. The arrowhead on the right indicates the mean resting potential for RS neurons.
same voltage range that it blocks $I_{IR}$. On this basis, it is concluded that $I_{IR}$ normally acts like a highpass filter by attenuating the FRCs of neurons at frequencies less than 20 Hz.

This was confirmed in resonant neurons where $\text{Ba}^{2+}$ application increased the amplitudes of the FRCs at low frequencies, enlarged their resonant humps, and shifted $f_{res}$ to lower values ($n = 5$, Figure 4.12). Application of $\text{Cs}^+$, together with $\text{Ba}^{2+}$, further increased the amplitudes of FRCs and either shifted their resonant peaks to very low frequencies or abolished resonance ($n = 3$, Figure 4.12). This was similar to the effect of $\text{Cs}^+$ alone, which abolished resonance and increased the amplitudes of FRCs over a broad range of subthreshold potentials ($n=4$). Like $\text{Ba}^{2+}$, the effects of $\text{Cs}^+$ were most evident at low frequencies ($< 10$ Hz). Unlike $\text{Ba}^{2+}$, $\text{Cs}^+$ increased the impedance magnitudes of FRCs to a greater extent at frequencies below $f_{res}$ than above it. During the application of $\text{Cs}^+$ the FRCs thus adopted the form of lowpass filters. These results point to $I_H$ as the primary mechanism responsible for subthreshold resonance in these neurons. In agreement with the predicted actions of a class I current as discussed in Section 3.3.4 of Chapter 3, the action of $I_H$ is to attenuate the responses of neurons at low frequencies.

At membrane potentials more depolarized than -65 mV, $I_{\text{Na},p}$ also affected the FRCs. In nonresonant and resonant neurons under control conditions, the amplitudes of the FRCs increased two-fold or more as the membrane potential was depolarized from rest to near threshold. This voltage-dependent amplification of the FRCs was confined to frequencies below 10 Hz. In resonant neurons, the amplification included a large increase in the Q value of the resonance which did not have a large effect on $f_{res}$ (Figure 4.13A). In all neurons, application of TTX eliminated the voltage-dependent increase in the FRCs (Figure 4.13B) and also resulted in smoother FRC plots ($n = 10$). In resonant neurons, TTX reduced the size of the hump in the FRC but did not eliminate resonance (Figure 4.13B). These results imply that $I_{\text{Na},p}$ is a low-frequency amplifier of FRCs and resonance.

4.4 Discussion

The main findings in this chapter are that regular spiking (RS) and intrinsic bursting (IB), but not fast spiking (FS), neocortical neurons possess a subthreshold resonance at low frequencies. A hyperpolarization-activated cation current ($I_H$) produces this resonance which mediates a selective coupling of oscillatory current inputs near the resonant frequency to the consequent firing of action potentials. The membrane resonance appears as a hump, with a
Figure 4.11 Effects of external Ba$^{2+}$ application on the FRCs of a nonresonant neuron.

A. Application of Ba$^{2+}$ (200 μM) to the same neuron as in Figure 4.5 blocks attenuation. B. Smoothed FRCs from A replotted in a 2-dimensional format. The membrane potentials for each FRC is shown to the left of each trace. C. Comparison of FRCs at -85 mV (in the presence of 300 nM TTX) before and after Ba$^{2+}$ (control trace redrawn from Figure 4.11B). All FRCs were measured in the presence of 0.3 mM TTX.
Figure 4.12 Effects of ionic blockers on resonance.

Application of Ba^{2+} (200 μM, 3.5 min) to a resonant IB neuron increased the amplitude of the FRC and the Q-value of its resonant hump, and decreased the resonant frequency (middle data trace). Additional application of Cs^{+} (12 min later) greatly increased the amplitude of the FRC and eliminated the resonant hump (top data trace). Washout of the Cs^{+} and Ba^{2+} shows that their effects were completely reversible. Smooth lines through the data are fitted by eye.
nonzero peak frequency, in the frequency-response curve of neurons. When a swept-sine-wave ZAP current of constant amplitude is used as an input, resonance appears as a bulge in the oscillatory voltage response that is largest near the resonant frequency.

4.4.1 Mechanism of resonance

According to the definition in Chapter 3, $I_H$ is a class I current. Since its activation time constant is ordinarily much slower than the membrane time constant, it should result in resonance as discussed in Chapter 2. This was confirmed in this chapter by the many points of similarity between resonant neurons and neurons with $I_H$: (1) resonance and $I_H$ were both evident at subthreshold membrane potentials and had similar voltage dependencies, moreover, when the activation point of $I_H$ was outside its usual range (between -65 and -75 mV), the voltage dependence of resonance was shifted in the same direction; (2) neurons that did not have an $I_H$ were nonresonant; (3) in resonant neurons, $C_{s^+}$, but not $Ba^{2+}$, blocked both $I_H$ and resonance; and (4) the slow time course of $I_H$ activation was consistent with the resonant frequencies observed.

4.4.2 Control and modulation of resonance

The FRCs of resonant and non-resonant neurons were voltage-dependent. For resonant neurons, both the resonant frequencies and the Q-value of the FRCs changed with membrane potential. This suggests that a simple way of controlling the frequency response of resonant neocortical neurons is to change the resting membrane potential -- possibly by the release of modulatory transmitters (McCormick et al. 1993). However, the subthreshold voltage-dependence of resonant and nonresonant FRCs in this study extended only from 0 to about 20 Hz. Above 20 Hz, the FRCs of most neurons were almost voltage-independent even though many neurons had strongly curved I-V relations at these potentials. This reflects the influence of the capacitive, high-frequency attenuation common to all neurons (see Section 3.2.3). The functional implication of this finding is that the processing of low-frequency but not high-frequency rhythmic inputs by neocortical neurons is susceptible to neuromodulation via control of the resting membrane potential.

Two voltage-dependent currents other than $I_H$ affected the FRCs of neurons at potentials below -55 mV. These currents play opposite roles in shaping subthreshold responses to oscillatory stimuli. One of the currents, $I_{Na,P}$, is a class II, simple current as defined in Chapter 3. Therefore, the possibility arises that $I_{Na,P}$ will interact with $I_H$ to produce an amplified resonance
Figure 4.13 Effects of TTX on resonance.

A. In control ACSF, the FRCs of most neurons were larger between -65 and -55 mV than at more hyperpolarized potentials. In the resonant RS neuron shown here, there was a substantial difference in the amplitudes of the FRC and the Q-value of resonance, between -66 and -62 mV.

B. Application of 600 nM TTX (for 3.5 min) reduced the FRC and the Q-value, but did not eliminate resonance. The resonant frequency, $f_{\text{res}}$, is approximately the same before and after TTX.
as described theoretically in Section 3.4. This was confirmed here by using TTX to simultaneously block \( I_{\text{Na,p}} \) and the amplification of resonance.

According to the theoretical understanding of class II currents developed in Chapter 3, \( I_{\text{Na,p}} \) should amplify FRCs whether or not it interacts with \( I_H \) (see Figure 3.17). The voltage region of amplification begins near the foot of the activation curve for \( I_{\text{Na,p}} \) (approximately -65 mV) and extends in the depolarizing direction. An amplified resonance will only form when the foot of the \( I_H \) activation curve overlaps this region. Referring to Figure 4.10, it seems likely that, for many neurons, \( I_H \) activates at potentials that are too hyperpolarized to interact with \( I_H \). This situation may be modified, however, by shifts in the voltage dependence of \( I_H \). In central neurons such shifts are controlled by neurotransmitters (Bobker and Williams 1989; McCormick and Pape 1990b; McCormick and Williamson 1991) and second messengers (\( \text{Ca}^{2+} \), Schwindt et al. 1992; cAMP, Banks et al. 1993; Pape and Mager 1992). By extension, amplified resonance may be a target of neuromodulation in the neocortex.

The other subthreshold current that was found to modify the frequency responses of neurons was \( I_{\text{IR}} \). This current activates at potentials more negative than -80 mV. Its reversal potential is set by the position of the Nernst potential for \( K^+ \) which was calculated at -87 mV under the conditions of the experiments described above. By virtue of these properties, \( I_{\text{IR}} \) is a type I simple current as defined in Chapter 3 and, since it activates quickly, should produce attenuation rather than resonance at all voltages where it is activated. Moreover, at voltages where it is coactivated with \( I_H \) it should produce an attenuated resonance in the FRC. An attenuated resonance is visible at hyperpolarized voltages in Figure 4.4. Comparing the relative activation points for \( I_{\text{IR}} \) and \( I_H \) in Figure 4.10, it seems likely that an attenuated resonance exists at voltages below approximately -80 mV in most neurons with both \( I_{\text{IR}} \) and \( I_H \). It is apparent from the same figure that \( I_{\text{IR}} \) and \( I_{\text{Na,p}} \) will not interact.

The physiological consequences of these interactions are unclear. On the one hand, \( I_{\text{IR}} \) attenuates FRCs at voltages outside the physiological range for the generation of action potentials. On the other hand, the coupling between resonance and firing was weak over much of the activation range of \( I_{\text{Na,p}} \) (i.e., at potentials more depolarized than -60 mV, see Figure 4.2B). Thus, there may be only a narrow range of potentials between -65 and -60 mV where the amplification of resonance due to \( I_{\text{Na,p}} \) might have a functional consequence. It is possible that,
at potentials more positive than -60 mV, $I_{Na}$ has other functions such as the control of firing rate (Stafstrom et al. 1984a,b; Reyes and Fetz 1993).

4.4.3 Resonance mediates a frequency-selective coupling of inputs to firing

Resonant neurons had a selective coupling of frequency components of the current input to the firing of action potentials. This coupling was demonstrated for the case of ZAP current inputs when neurons fired most readily as the input swept past the resonant frequency. A simple mechanism involving resonance near a firing threshold can account for these observations. Figure 4.14 demonstrates this schematically by comparing an idealized voltage response to a ZAP current input to the voltage threshold for the firing of action potentials (horizontal dashed line). Firing is expected where the response exceeds the threshold, as indicated by the filled circles. In this way, the spikes are associated with frequencies of the input near the resonant frequency, i.e., where the subthreshold response was largest. Inputs at other frequencies, in contrast, are attenuated relative to the resonant frequency and so are less likely to result in spiking. Although this mechanism is highly simplified, it accounts for the elements of the coupling as seen from a comparison with the voltage responses of a real neuron to ZAP current input (Figure 4.14B).

It is necessary to emphasize that the occurrence of spikes due to the above mechanism not only depends on the frequency content but also on the amplitude and phase of each frequency component of the input. For instance, it is possible to drive the response corresponding to any frequency component of the input above threshold if the input amplitude is large enough. Thus, as the amplitude of the ZAP current input increases, the spikes appear over a wider region (see Figure 4.14B) because the amplitudes of some of the nonresonant frequencies in the input become large enough to evoke spikes. The notion of a selective coupling of input frequency components to spikes best describes the situation when a resonant neuron responds to sinusoidal inputs with comparable amplitude, each at a different frequency.

A significant finding is that this coupling was voltage-dependent. Resonant neurons held at potentials between -80 and -65 mV could be induced to fire preferentially in response to inputs near their resonant frequency. This was not the case, however, when the same neurons were held near -60 mV -- despite the persistence of resonance at these potentials. The disruption of the frequency-selective coupling between inputs and firing may be due to currents other than $I_{H}$ that activate near -60 mV. This is consistent with our finding that sags and rebounds were poorly
Figure 4.14 Possible mechanism for the selective coupling between firing and near-resonant frequencies in the input.

A. The combination of resonance and a threshold for firing (horizontal dotted line) implies selective coupling. This is shown for idealized responses to ZAP current inputs. In A1, the subthreshold voltage responses are largest near the resonant frequency. When a sufficiently large input is used, spikes appear where the response exceeds threshold, near resonance (A2, filled circles). This mechanism also implies that the frequency band over which the input couples to spikes widens as the amplitude of the ZAP input increases (A3, filled circles). B. The relationship between the firing threshold (horizontal dotted line) and the voltage responses (top traces) to oscillatory ZAP current inputs (bottom traces) in an actual neuron is consistent with the proposed mechanism.
correlated near -60 mV. On the other hand, the simple explanation proposed above to account for the frequency-selective coupling also may be sufficient to account for its voltage dependence. This is because the currents required to drive neurons past threshold from -60 mV may be too small to generate significant differences in the voltage responses to different frequencies (compare Figure 4.2C2 and Figure 4.2C4).

Whatever the mechanism, the voltage-dependence of the coupling suggests the existence of two firing modes in neocortical neurons: (1) a hyperpolarized mode in which the neuron is able to sense and respond selectively to synchronized, convergent inputs with frequencies in the neuron’s resonant band; and (2) a more depolarized mode in which the firing does not couple preferentially to low-frequency inputs. Since many neuromodulators affect conductances that change the resting potential of neocortical neurons (McCormick et al. 1993), these firing modes could form a basis for the voltage-dependent modulation of low-frequency coherent activity by coupling and uncoupling $I_{H}$-generated resonance to spike production in the neocortex.

4.5 Summary

This chapter has identified the existence of a subthreshold, low-frequency resonance in approximately 70% of RS and IB neurons in rat sensorimotor cortex. Resonance was absent in FS neurons. The use of ionic blockers of voltage-dependent currents revealed that $I_{H}$ is a necessary condition for the resonance. This is in agreement with the theoretical investigations of the last chapter. It remains to be shown, however, that $I_{H}$ is the only current responsible for the resonance. This will be the subject of the next chapter.
5. Models of neocortical neurons

5.1 Introduction

In the previous chapter, $I_H$ was identified as prerequisite for the occurrence of a subthreshold resonance found near the resting potential of neocortical neurons. In this chapter it will be shown that $I_H$ is the only voltage-dependent current required to explain the resonance. To accomplish this, the FRCs of resonant neurons are first measured using the ZAP method. The same neurons are then voltage clamped and the parameters characterizing the kinetics and voltage dependence of $I_H$ are determined. Using these parameters, models of the individual neurons can then be constructed and their predicted FRCs compared with the actual FRCs. An agreement between the theoretical and measured FRCs will confirm that $I_H$ is the only voltage-dependent current involved.

In a different vein, the reactive current clamp (RCC) is used to express an artificial $I_H$ in neocortical neurons. The effects of resonance on spike production can then be studied without explicitly modeling the complex dynamics of action potential genesis. This will confirm that the selective coupling between firing and inputs near resonant frequencies described in the last chapter is due to an $I_H$-like current.

Next, simplified models of neocortical neurons are constructed to explore the parameter-dependence of certain features of resonance. This is like the qualitative studies in Chapter 2 except that realistic parameter values derived from voltage clamp experiments are used as the starting point for the investigation. In the final section, the interactions of $I_{IR}$ and $I_{NaP}$ with $I_H$ are modeled using the CM+SS models of Section 3.4 as a framework.

The models used in this chapter include: (1) a model of the process of $I_H$ activation (the basic $I_H$ (BH) model) with parameter values estimated from voltage-clamp experiments; (2) an CM+S model describing the membrane electrical behavior of an isopotential neuron with only two ionic currents -- $I_H$ and a leak current (the reduced membrane (RM) model); (4) a hybrid model, comprised of the BH model and a living neuron, produced with the RCC technique; and (5) a simplified version of the RM model which is used to examine the sensitivity of various aspects of resonance to parameter changes.
5.2 Methods

The methods for dissections, \textit{in vitro} electrical recordings, and small signal impedance techniques are as described in the Methods section of Chapter 4. Procedures and examples of the use of the RCC are given in Chapter 2.

5.2.1 Numerical procedures

The numerical integration program PHASEPLANE (Ermentrout 1990) with the Gear integration option was used to calculate trajectories of the solutions of mathematical models. A simulated 300 ms run takes <4 s on a 33 MHz 486 PC. A tolerance of 0.01 (for the Gear integration method in PHASEPLANE) resulted in accurate computations. This agrees with the observations of Wang et al. (1991) who used the same program to integrate a less complex model involving \( I_H \).

5.3 Results

5.3.1 Construction of the basic \( I_H \) model (BH model)

A model of \( I_H \) was constructed based on experimental observations in neocortical neurons. \( I_H \) often activated with two time constants as has also has been observed in neocortical neurons by others (Spain et al. 1987; Solomon and Nerbonne 1993b). Therefore, to describe \( I_H \) activation, equation (2.7) was adapted by assuming two activation variables, \( m_f \) and \( m_s \), with fast and slow kinetics, respectively. This gives the basic \( I_H \) model,

\[
i_H = -\overline{g}_H \left( p_f m_f + p_s m_s \right) \left[ v - v_H \right],
\]

\[
\frac{dm_f}{dt} = \frac{m_{\infty}(v) - m_f}{\tau_f(v)},
\]

and

\[
\frac{dm_s}{dt} = \frac{m_{\infty}(v) - m_f}{\tau_s(v)}
\]

where

\[
m_{\infty}(v) = \left[ 1 + \exp \left( \frac{v - v_H/2}{k_m} \right) \right]^{-1}.
\]
The notation here is consistent with that of Section 3.3.1 in Chapter 2; $g_H$ is the maximal conductance of $I_H$, $v_H$ is its reversal potential, $m_{\infty}$ is the steady-state activation, $v_{H2}$ is the membrane potential when $m_{\infty} = 1/2$, $k_m$ is a factor that controls the slope of the activation curve. In the BH model, the $m_f$ and $m_s$ share the same voltage dependence but have different time constants given by $\tau_f$ and $\tau_s$, respectively (Solomon and Nerbonne 1993b). The functional forms for $\tau_d(v)$ and $\tau_f(v)$ are not specified; instead, at each membrane potential, experimentally measured values are used. The proportions of the conductance associated with the fast and slow components of $I_H$ are $p_f$ and $p_s$ where $p_f + p_s = 1$.

Activation kinetics

The procedures for estimating the parameter values of $I_H$ are now related. In voltage-clamped neocortical neurons, an inward current that activated slowly in response to hyperpolarizations from holding potentials near threshold was unambiguously identified as $I_H$. Although it is unlikely that a complete space-clamp was achieved in these geometrically complex neurons, $I_H$ activated as a graded function of voltage and its time course of activation did not show signs of an unclamped current.

To extract the time constants of $I_H$ activation, the time courses of currents evoked by hyperpolarizing steps from a holding potential near -60 mV (Figure 5.1A) were fitted with a sum of exponential terms, 

$$I(t) = A_f + A_c e^{-t/\tau_c} + A_f \left(1 - e^{-t/\tau_f}\right) + A_s \left(1 - e^{-t/\tau_s}\right).$$

Equation 5.3, the last 2 terms contain the fast and slow $I_H$ time constants and the asymptotic values of the fast and slow components of $I_H$ ($A_f$ and $A_s$). Both $\tau_f$ and $\tau_s$ were > 0.05 s at membrane potentials more positive than -100 mV. Two other currents, $I_{\text{leak}}$ and $I_{\text{IR}}$, contributed almost instantaneous components to the total current (see Figure 4.9A2). In Equation 5.3, these components are lumped into $A_f$. The term describing the capacitive transient in Equation 5.3 (with parameters $A_c$ and $\tau_c$) was required to produce a reliable fit.

Figure 5.1B shows the voltage-dependence of the $I_H$ time constants in 8 regular-spiking (RS) neurons. These neurons were chosen for analysis because $I_H$ was large. Seven of the neurons required two time constants to describe $I_H$ activation over some part of its range. When the neurons were held near -90 mV, $\tau_f$ ranged from 0.09 to 0.24 s and $\tau_s$ ranged from 0.64 to
Figure 5.1 Extraction of the parameters for the BH model.

**A.** Currents evoked by 2 s hyperpolarizing voltage commands in -8 mV increments from a holding potential of -61 mV. Following a capacitive transient, the evoked inward currents are comprised of a linear leak current ($I_{\text{leak}}$), a rectifying current ($I_{\text{R}}$) that activates rapidly near -80 mV, and a slowly developing current ($I_{\text{H}}$). In this neuron, $I_{\text{H}}$ had 2 time constants. **B1,2.** Time constants for $I_{\text{H}}$ activation in eight neurons with large $I_{\text{H}}$. In B2, the half-open circles indicate values for a neuron that only required only one exponential time constant to fit $I_{\text{H}}$. For the other neurons, the fast and slow time constants for each neuron are shown with matching symbols in B1 and B2. **C.** Determination of the reversal potential, $v_{\text{H}}$. **D.** Steady-state activation curves for $I_{\text{H}}$ determined by fits of tail currents to Equation 5.3. Parameter values from these fits are given in Table 1.
2.40 s (Figure 5.1B1,2). Both time constants tended to decrease with hyperpolarization. In the single neuron where $I_H$ activation was characterized by a single time constant, its value was comparable to the lower range of $\tau_s$ in the other neurons (Figure 5.1B2, half-open circles).

**Reversal potential**

The reversal potential of $I_H$ was estimated by comparing the instantaneous and steady-state V-I relationships of neocortical neurons (Figure 5.1C). Referring to Equation 5.3, the steady-state current associated with hyperpolarization from -60 mV to a given test potential is $I_{ss} = A_i + A_f + A_s$. The instantaneous, noninactivating current, which includes contributions from $I_{\text{leak}}$ and $I_R$, is $I_{\text{inst}} = A_i$. Thus, $I_{ss} - I_{\text{inst}}$ is the total steady-state $I_H$ evoked at the test potential (i.e., $I_{ss} - I_{\text{inst}} = A_f + A_s$). Figure 5.1C gives the V-I relation for $I_{ss} - I_{\text{inst}}$. The reversal potential ($v_H$) of $I_H$ was found by extrapolating the fully-activated portion of the V-I curve for $I_{ss} - I_{\text{inst}}$ (at potentials < -100 mV) back to the point where it intersects the horizontal axis. The values of $v_H$ determined in this way ranged from approximately -35 to -45 mV (Table 1).

<table>
<thead>
<tr>
<th>cell number</th>
<th>$\bar{g}_H$ (nS)</th>
<th>$v_{H/2}$ (mV)</th>
<th>$k_m$ (mV-l)</th>
<th>$v_H$ (mV)</th>
<th>$\tau_f$ (s)</th>
<th>$\tau_s$ (s)</th>
<th>$p_f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.2</td>
<td>-71</td>
<td>8.6</td>
<td>-35</td>
<td>0.13</td>
<td>1.20</td>
<td>0.70</td>
</tr>
<tr>
<td>2</td>
<td>6.0</td>
<td>-81</td>
<td>8.2</td>
<td>-45</td>
<td>0.15</td>
<td>0.84</td>
<td>0.65</td>
</tr>
<tr>
<td>3</td>
<td>3.7</td>
<td>-72</td>
<td>6.6</td>
<td>-40</td>
<td>0.18</td>
<td>2.40</td>
<td>0.49</td>
</tr>
<tr>
<td>4</td>
<td>2.4</td>
<td>-86</td>
<td>9.1</td>
<td>-41</td>
<td>0.17</td>
<td>1.29</td>
<td>0.38</td>
</tr>
<tr>
<td>5</td>
<td>7.2</td>
<td>-74</td>
<td>5.1</td>
<td>-39</td>
<td>0.24</td>
<td>1.73</td>
<td>0.60</td>
</tr>
<tr>
<td>6</td>
<td>13.7</td>
<td>-82</td>
<td>10.3</td>
<td>-40</td>
<td>0.93</td>
<td>0.64</td>
<td>0.69</td>
</tr>
<tr>
<td>7</td>
<td>2.9</td>
<td>-92</td>
<td>4.6</td>
<td>-35</td>
<td>—</td>
<td>1.11</td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td>5.6</td>
<td>-86</td>
<td>10.9</td>
<td>-42</td>
<td>0.91</td>
<td>1.94</td>
<td>0.71</td>
</tr>
</tbody>
</table>

* Fitted time constants for activation of $I_H$ at -90 mV.
† Proportion of $I_H$ associated with $\tau_f$ at -90 mV; $p_f = A_f/(A_f + A_s)$.
‡ Neuron with a single time constant for activation of $I_H$.

As a check on these estimates of $v_H$, we measured $v_H$ in a more direct fashion for two neurons by holding the membrane potential at -110 mV, depolarizing to different test potentials, and observing the potential where the decaying currents reversed polarity. To avoid evoking $K^+$ and $Ca^{2+}$ currents that might interfere with the $I_H$ currents during this procedure (Spain et al. 1987; Solomon and Nerbonne 1993a), 2 mM 4-aminopyridine, 10 mM tetraethylammonium, and
300 nM tetrodotoxin were applied externally to the neurons, and the 2 mM external Ca\(^{2+}\) was replaced by equimolar Mg\(^{2+}\). The values of \(V_H\) measured in this way were -41 mV and -37 mV, consistent with the values estimated by the extrapolation method.

**Steady-state activation**

The parameter values characterizing the voltage dependence of steady-state activation for \(I_H\) were determined by measuring the peak magnitudes of tail currents which were evoked after 2 s prepulses to different test potentials (\(v_{test}\)). After dividing the tail current magnitude by an appropriate driving force to find the conductance, the result was fitted with a Boltzmann equation,

\[
\frac{I_{tail}}{v_{tail} - v_H} = \text{offset} + \bar{g}_H \left[1 + \exp\left(\frac{v_{test} - v_H/2}{k_m}\right)\right]^{-1}.
\] (5.4)

In Equation 5.4, \(I_{tail}\) is the measured peak magnitude of the \(I_H\)-tail current and \(v_{tail}\) is the membrane potential where the tail current was evoked (either -120 or -60 mV). The offset term was necessary in cases where a portion of the total H-current was tonically activated at all test potentials. The results of the fits for 8 RS neurons are summarized in Figure 5.1D and Table 1. Some of the parameter values vary widely and the physiological range of variation may be underestimated because only neurons with the most robust \(I_H\) were chosen for analysis.

### 5.3.2 Construction and analysis of the reduced membrane model (RM model)

The BH model formed the basis for a reduced model of membrane electrical behavior. This was called the reduced membrane (RM) model. Because it contains only \(I_H\), \(I_{leak}\) and a capacitance it is a CM+S model as defined in Chapter 3. This model was most appropriate for describing the electrical behavior of RS neurons between -80 and -65 mV since other subthreshold voltage-dependent currents in these neurons are seldom active over this range of potentials (see Figure 4.10).

The RM model therefore is given by Equations 5.1A-C and 5.2 together with

\[
c_m \frac{dv}{dt} = -g_l [v - v_{leak}] - i_H + i_{inj}
\] (5.5)
where \( g_l \) is the conductance of the leak current and \( v_l \) is its reversal potential. These equations were integrated numerically using parameter values from Table 5.1 above and values of \( g_l \) and \( c_m \) calculated from Table 4.1. Figure 5.2 shows that the responses of the model are similar to those described for resonant neurons in Chapter 4.

After linearization, the frequency response of the RM model is found in the manner described in Appendix A (see also Section 3.3.2 and Puil et al. 1986). The impedance is

\[
Z(f, v) = \frac{1}{g_l + 2\pi jfc_m + g_{H, ch}[1 + H_f + H_s]} \tag{5.6}
\]

where

\[
H_f = p_f \frac{\hat{g}_H(v)}{1 + 2\pi jf\tau_f}, \tag{5.7A}
\]

\[
H_s = p_s \frac{\hat{g}_H(v)}{1 + 2\pi jf\tau_s}, \tag{5.7B}
\]

and

\[
\hat{g}_H(v) = \frac{m'_0(v)}{m_0(v)}[v - v_H]. \tag{5.8}
\]

Here, \( f \) is the stimulation frequency (in Hz), primes denote differentiation by \( v \), and \( g_{H, ch} = \hat{g}_H m_0(v) \) is the chord conductance of \( I_H \) at \( v \). The meaning of the voltage dependent terms \( \hat{g}_H, H_f \) and \( H_s \) in Equations 5.7A,B and 5.8 are the same as for the corresponding terms in Section 3.3.2 of Chapter 3. Briefly, \( \hat{g}_H(v) \) describes the departure of \( I_H \) from an ohmic behavior at any \( v \) whereas \( H_f \) and \( H_s \) are the frequency- and voltage- dependent contributions of the fast and slow components of \( I_H \) to the impedance.

**Predicted and observed frequency-response curves**

For three neurons theoretical FRCs were calculated from Equation 5.6 using the parameter values listed in Table 4.1. These theoretical FRCs were then compared with the measured FRCs of the same neurons near their resting potentials. The results are shown in Figure 5.3 where the theoretical FRCs are shown as dashed curves and the observed FRCs as solid curves. In general, the observed and theoretical FRCs are in good agreement with respect to the resonant frequency.
Parameter values used here are representative of the neurons in this investigation (cf. Table 1): $g_t = 5 \ \text{nS}, \ c_m = 200 \ \text{pF}, \ \bar{g}_H = 8 \ \text{nS}, \ k_m = 8 \ \text{mV}^{-1}, \ v_{H/2} = -80 \ \text{mV}, \ v_H = -40 \ \text{mV}, \ \tau_t = 2 \ \text{s}, \ \tau_f = 0.2 \ \text{s}, \ p_s = 0.5$. A. The responses of the RM model to simulated current pulses contain sags and rebounds similar to those seen in neocortical neurons. B. The response to a ZAP input exhibits a spindle shape indicating resonance.
and the shape and width of the resonant hump. This is demonstrated in the neuron of Figure 5.3A where the observed and theoretical FRCs agree everywhere except at the lowest frequencies.\(^1\) For the neurons of Figure 5.3B,C, however, the detailed agreement between the observed and theoretical FRCs was much improved when certain adjustments were made in the parameters of the RM model. For instance, a voltage offset was added to the membrane potentials of both neurons. Thus, the FRC in Figure 5.3B was measured at -69 mV but the theoretical FRC at -66 mV was the best fit.\(^2\) Such offsets were likely due to small changes in the electrode properties during the course of the recordings. Likewise, the neuron of Figure 5.3C required an offset of +4 mV; also, the relative weights of the fast and slow components of \(I_H\) (i.e., \(p_f\) and \(p_s\)) had to be changed to emphasize the slow component.\(^3\)

When the offset and weight adjustments are made, the resulting theoretical FRCs (dotted curves) fit the measured FRCs very well. This demonstrates that the elements of the RM model (\(I_H\), \(I_{\text{leak}}\), and \(c_m\)) are sufficient to reproduce the major features of the FRCs of neocortical neurons. The predicted FRC when \(g_H\) is set to zero in the model also is shown in Figure 6 (dot-dash curve). Comparison of the FRCs with and without \(I_H\) shows that it produces a low-frequency attenuation as deduced for a class I current in Chapter 3. In view of the demonstration in Chapter 4 that the pharmacological blockade of \(I_H\) abolishes subthreshold resonance in these neurons, it can be concluded that \(I_H\) is necessary and sufficient to produce the observed resonance.

**Predicted frequency response at physiological temperature**

The resonant frequencies predicted for the neurons in the previous section are between 1 and 2 Hz near -70 mV. These values were derived from parameter estimates made at 24-26 °C. However, Spain et al. (1987) have found that the activation time constants of \(I_H\) in cat sensorimotor neurons maintained in vitro at 36 °C are 6 to 11 times faster than those in

---

\(^1\) The parameter values for the neuron in Figure 5.3A are \(g_l = 3\) nS, \(c_m = 210\) pF, \(\overline{g}_H = 6\) nS, \(k_m = 8.2\) mV\(^{-1}\), \(v_{\text{leak}} = -81\) mV, \(v_H = -45\) mV, \(\tau_f = 4.269\) s, \(\tau_s = 0.294\) s, \(p_s = 0.5\).

\(^2\) Adjusted parameter values for the neuron in Figure 5.3B are \(g_l = 3.9\) nS, \(c_m = 290\) pF, \(\overline{g}_H = 3.7\) nS, \(k_m = 3.8\) mV\(^{-1}\), \(v_{\text{leak}} = -72\) mV, \(v_H = -40\) mV, \(\tau_f = 0.945\) s, \(\tau_s = 0.171\) s, \(p_s = 0.4\).

\(^3\) Adjusted parameters for the neuron in Figure 5.3C are \(g_l = 1.7\) nS, \(c_m = 310\) pF, \(\overline{g}_H = 14.2\) nS, \(k_m = 8.6\) mV\(^{-1}\), \(v_{\text{leak}} = -71\) mV, \(v_H = -35\) mV, \(\tau_f = 1.281\) s, \(\tau_s = 0.189\) s, \(p_s = 0.8\).
Figure 5.3 Observed and theoretical FRCs for neocortical neurons.

Solid curves are FRCs measured experimentally with the ZAP method. Dashed curves are theoretical FRCs predicted from the RM model, and dotted curves through the experimental data are the theoretical FRCs after parameter adjustments to produce better fits. Dot-dash curve in each plot is the passive response that remains after the contributions of $I_H$ are removed from the models. A. A neuron that did not require any adjustments to the measured parameter values to produce a good fit between the theoretical and observed FRCs. B. A neuron requiring, for a good fit, a 3 mV offset in membrane potential. C. A neuron requiring a 4 mV offset and a change in $p_s$ from 0.6 to 0.8. D. Theoretical FRC for a cat sensorimotor neuron at 36 °C (dashed curve). Note the large value of $f_{res}$ compared to the values in A, B, C.
Figure 5.3B. Their values for $g_t$ and $\bar{g}_H$ per unit input capacitance also are larger. This suggests that $I_H$ is highly temperature sensitive. In support of this, Solomon and Nerbonne (1993a, b) and Budde et al. (1994) give values for $\tau_s$ and $\tau_f$ in dissociated rat visual cortical neurons at 20 - 22 °C that are comparable to those in Figure 5.3B.

To investigate the possible values of the resonant frequency ($f_{res}$) at physiological temperatures, the parameter values reported by Spain et al. (1987) for their model of a sensorimotor neuron 36°C were used in Equation 5.6. The resulting theoretical FRC for -70 mV, plotted in Figure 5.3D, shows that $f_{res}$ is near 10 Hz under these conditions. This value is voltage dependent and ranges from $f_{res} = 5$ to 15 Hz at potentials from -60 to -80 mV. This indicates that the $I_H$-dependent resonant frequencies measured 24°C in Chapter 4 may be an order of magnitude lower than those at physiological temperatures.

5.3.3 Electronic antagonism and expression of $I_H$

In Chapter 4 it was found that resonant neurons preferred to fire action potentials when stimulated with oscillatory current inputs near $f_{res}$. Stimulation with oscillatory currents at nonresonant frequencies produced smaller subthreshold voltage responses and fewer action potentials. Nonresonant neurons did not have a resonant frequency or exhibit a frequency-selective coupling to the firing of action potentials. Using the reactive current clamp (RCC) we were able to replicate the frequency preference of resonant neurons by coupling nonresonant neurons to a mathematical model of $I_H$.

As explained in Chapter 2, the RCC injects a computer-generated current corresponding to a voltage-dependent conductance into neurons. This current was used either to antagonize an endogenous $I_H$ or to express an artificial $I_H$ in neurons. The interaction of the modeled $I_H$ with the spike firing mechanism of neurons may then be studied. The mathematical model of $I_H$ used for the RCC was similar to the BH model (Equations 5.1A-C) except that, for simplicity, $I_H$ was assumed to activate with a single time constant. Therefore, the model is given by

$$i_H = -\bar{g}_H m_H (v - v_H)$$

(5.9A)

---

4 The parameter values for the model of Spain et al. (1989) are: $g_t = 37$ nS, $c_m = 370$ pF, $\bar{g}_H = 29$ nS, $k_m = 7$ mV$^{-1}$.

$v_{H2} = -78$ mV, $v_H = -35$ mV, $\tau_s = 0.319$ s, $\tau_f = 0.038$ s, $p_s = 0.2$. 
\[
\frac{dm_H}{dt} = \frac{m_{H,\infty}(v) - m_H}{\tau_H(v)} \tag{5.9B}
\]

The functional forms for the steady state activation \(m_{H,\infty}\) and activation time constant \(\tau_H\) are given by an expression analogous to (5.2) and

\[
\tau_H = \frac{2\tau_0}{\exp \left( \frac{V - V_{H/2}}{k_m} \right) + \exp \left( -\frac{V - V_{H/2}}{k_m} \right)}, \tag{5.10}
\]

respectively. Here, \(\tau_0\) is the value of the time constant at \(v_{H/2}\).

**Electronic antagonism of \(I_H\)**

As a check on the fidelity of this model, it was first used to antagonize the endogenous \(I_H\) of a neuron. Figure 5.4 shows the voltage responses for a neuron before and after antagonizing the endogenous \(I_H\) by injection of a negative copy of \(I_H\) generated by a computer. In this case, the electronic antagonism successfully removed the sags and rebounds characteristic of voltage responses to hyperpolarizing current inputs in neurons with \(I_H\). In many neurons, however, antagonizing the sags did not result in the total cancellation of the rebounds. This probably reflected the existence of two activation time constants for \(I_H\) in most neurons (see Figure 5.1).

For each neuron, the electronic antagonism required fine tuning of the model parameters. Since different parameter values were required for each neuron, we initially used parameter values that seemed reasonable, based on prior experience. We then changed the parameter values one by one to produce an effective antagonism. Some of the parameters had a wider range of variation than others. The most variable parameters were adjusted first which produced the following order: \(g_H, \tau_0, v_{H/2}, k_m, \text{ and } v_H\). Often, only \(g_H\) and \(\tau_0\) required adjustment. To show that this procedure results in a well-defined set of parameter values, Figure 5.4 shows the result of intentionally adjusting \(g_H\) and \(\tau_H\) to incorrect values. Although the parameter values used here were within the range of variation found in other neocortical neurons (see Table 5.1), they were clearly unsuitable for the neuron shown here.

**Electronic expression of \(I_H\)**

Electronic expression of an artificial \(I_H\) in a neuron with little or no endogenous \(I_H\) produced sags in the voltage responses to a hyperpolarizing current pulses. The FRC of the
Figure 5.4 Electronic antagonism of $I_H$ using the RCC in an in vitro neuron.

Under control conditions (left), sags in the voltage responses to hyperpolarizing current pulses (arrowhead) indicate the presence of $I_H$. The sags are totally eliminated and the resting potential of the neuron hyperpolarized (middle) by electronically antagonizing $I_H$ in the neuron. To demonstrate the sensitivity of the process, arrows (right) indicate voltage responses to a hyperpolarizing pulse when the values of $\tau_H$ and $g_H$ did not match the properties of the endogenous $I_H$. 
neuron also acquired a resonant hump near 2 Hz (Figure 5.5A). This shows that a current with the voltage dependence and kinetics of $I_H$ can cause resonance in neocortical neurons.

An electronically expressed $I_H$ can be used to demonstrate that the resonance due to $I_H$ leads to frequency-selective firing. This is seen in Figure 5.5A which shows the frequency-coupling of spikes evoked by a ZAP current stimulus in neurons before and after expression of an artificial $I_H$. In the presence of the artificial $I_H$, action potentials fired preferentially as the ZAP input swept through frequencies near $f_{res}$. A similar coupling of resonance to frequency-selective spike firing was seen in neurons with an endogenous $I_H$ in Chapter 4 (see Figure 4.2C). The same phenomenon was observed when current inputs consisting of single-frequency sine waves were used to evoke firing in a neuron with an electronically-expressed $I_H$ (Figure 5.5B). Firing was preferentially associated with the input frequency nearest the resonant frequency, and individual spikes were phase-locked to the sinusoidal voltage response. Note, however, that the spikes and the sinusoidal voltage responses were not frequency-locked.

In Chapter 2 it was pointed out that there are a number of limitations inherent in the use of the RCC technique. However, these limitations can be turned to advantage in contemplating the results above. Thus, since the RCC reproduces only the electrical, and not chemical, nature of the endogenous $I_H$, only the electrical properties are essential for resonance. This implies for instance, that neither resonance nor frequency-selective firing depend on the accumulation of internal $\text{Na}^+$ ions that enter through H-channels. Also, because the conductance created by the RCC technique is restricted to the site of current injection through the electrode, the production of resonance and consequent changes in firing pattern does not require a distributed $I_H$ conductance.

5.3.4 Construction and analysis of the simplified RM model of subthreshold resonance

This section explores the dependence of certain features of resonance on the parameters of the RM model. To facilitate this, a version of the RM model is used which has only a single time constant for $I_H$ activation. This is called the simplified RM model. According to the definitions of Chapter 3, this is a CM+S model with a class I simple current. Its impedance is, therefore, similar to expression (3.20), i.e.,

$$Z(f, v) = \frac{1}{g_l + 2\pi jfc_m + g_{H,ch}[1 + H]}$$  \hspace{1cm} (5.11)
Figure 5.5  **Electronic expression of I_H induces resonance and frequency-selective firing.**

**A.** The FRC of a neocortical neuron is transformed from nonresonant to resonant by the electronic expression of I_H. Insets show the voltage responses to a swept-sine-wave ZAP current input before (right) and after (below) expression of I_H.  

**B.** In the same neuron, sine wave stimulation (frequencies listed below traces) show that the spikes occur at the peaks of the sine wave responses when the frequency is near the resonant frequency (1-2 Hz). Note the differing time scales but identical number of cycles in each case.
where

\[
H = \frac{\hat{g}_H(v)}{1 + 2\pi f \tau_H}.
\]  

(5.12)

Figure 5.6A shows the frequency-response surface (FRS) of the simplified RM model for a set of standard parameter values chosen as representative of the neocortical neurons in these experiments\(^5\) and where \(\tau_H\) is assumed to be voltage independent. The corresponding FRCs are shown in Figure 5.6B. The FRS has the same qualitative features as the FRS of the resonant class I shown in Figure 3.6. At membrane potentials above -50 mV, where \(I_H\) is not activated, the form of the frequency-response surface is solely due to the passive characteristics of the model, i.e., \(I_I\) and \(c_m\). Thus, in Figure 5.6B, the FRC at 0 mV has the form of a lowpass filter with a cutoff frequency (in Hz) of \(f_m = (2\pi \tau_m)^{-1}\) (arrow). At membrane potentials more negative than -50 mV, resonance is apparent as a ridge in the FRS and as a hump with a peak at \(f_{res}\) in the FRC. As in Chapter 3, it is evident from Figure 5.6 that the resonance forms because of a voltage-dependent low-frequency attenuation due to \(I_H\).

Figure 5.6C shows how values of \(Q\) and \(f_{res}\) change with membrane voltage. The Q-value is the ratio of the impedance magnitude at \(f = f_{res}\) to the impedance magnitude at \(f = 0\) Hz. Note that \(f_{res}\) is voltage dependent even though \(\tau_H\) has been made voltage independent. Resonance first appears near -44 mV. At these potentials, however, the Q-value is small (Q-value < 1.05) and \(f_{res}\) is near zero. The Q-value first becomes noticeably different from 1 (i.e. > 1.05) at -60 mV; at this point the value of \(f_{res}\) is near 1.0 Hz. The Q-value then climbs to a peak value of 1.8 at -80 mV whereas \(f_{res}\) peaks at a value of 2.1 Hz near -84 mV. Finally, both the Q-value and \(f_{res}\) decline with further hyperpolarization. Note that, between -60 and -80 mV, the relationship between \(f_{res}\) and voltage is almost linear. These features qualitatively match the typical features of resonance seen experimentally in neocortical neurons (cf. Figure 4.2). Note that all of the voltage-dependent changes in the FRCs and the frequency-response surface occur at frequencies below 20 Hz. This also was observed in neocortical neurons.

---

\(^5\) The standard parameter values for the simplified RM model are \(g_I = 4\) nS, \(c_m = 150\) pF, \(\bar{g}_H = 4\) nS, \(k_m = 8\) mV\(^{-1}\), \(v_{M2} = -80\) mV, \(v_H = -40\) mV, \(\tau_0 = 0.5\) s.
Figure 5.6 Frequency-response properties of the simplified RM model.

A. The frequency-response surface of the simplified RM model with standard parameter values. The half-activation voltage for $I_H$ is indicated by a heavy curve. B. Frequency-response curves at eight membrane potentials (same parameter values as in A). C. Voltage dependence of the Q-value of the resonant hump (dotted curve) and $f_{res}$ (solid curve). The voltage region where the value of Q is greater than 1.05 (i.e., the resonant region) is indicated above the plots of Q and $f_{res}$. The dashed horizontal curves indicate the voltage-independent values of $f_m = (2\pi \tau_m)^{-1}$ and $f_{H} = (2\pi \tau_H)^{-1}$. Note that $f_{res}$ lies between $f_m$ and $f_{H}$ over the entire resonant region.
In Figure 5.6C, the resonant frequency ($f_{\text{res}}$, solid curve) for the simplified RM model with standard parameter values is plotted together with $f_{\text{H}}$ and $f_{\text{pass}}$ (labeled dashed curves) where $f_{\text{pass}} = \omega_{\text{pass}}/2\pi$. Note that $f_{\text{res}}$ lies between $f_{\text{H}}$ and $f_{\text{pass}}$ at most membrane potentials and that it can achieve values more than five times greater than $f_{\text{H}}$. It falls outside the limits established by $f_{\text{H}}$ and $f_{\text{pass}}$ only at the boundaries of the resonant region, where resonance is weak (Q-value < 1.05).

**Effects of parameter variations**

In this section, the effects of parameter variation in the simplified RM model are assessed. The intention here is to discover how variations which might occur from neuron to neuron, or via changes in the properties of a neuron, might affect resonance. For this reason each variation is considered relative to the FRC of the simplified RM model at -70 mV with the standard parameters given in footnote 5 on page 129. Also, all FRCs are calculated for $v = \nu_{H/2}$.

First, consider the properties of $I_H$ that may vary from neuron to neuron. For instance, studies have shown that the $H$-conductance density can differ substantially in cortical neurons (see Table 5.1; also Solomon et al. 1993). In Figure 5.7A, such a difference is replicated via a twofold increase or decrease in the value of $g_H$ (i.e., from 6 to 12 nS or 6 to 3 nS). The increase in $g_H$, while leaving all other parameters unchanged, modified the FRC by decreasing its peak amplitude by a factor of 0.7 and increasing the values of $f_{\text{res}}$ and Q by a factor of 1.3. The decrease in $g_H$ resulted in changes in the opposite direction. These effects may be understood by referring to the analysis of the frequency response in Chapter 3. Thus, changes in $g_H$ produce vertical shifts of the $I$-subcircuit of the simplified RM model without affecting the passive subcircuit. The FRC for the simplified RM model, delineated by these curves, changes accordingly.

The effects of altering the total capacitance ($c_m$) while holding the specific conductances $g_h/c_m$ and $g_l/c_m$ constant (Figure 5.7B) were also examined. Doubling $c_m$ from 150 to 300 pF is equivalent to doubling the size of the modeled neuron without affecting the current densities. This change did not alter the values of $f_{\text{res}}$ and Q; however, the overall amplitude of the FRC

---

6 Recall that $\omega_{\text{pass}}$ was defined in Chapter 3 as $\omega_{\text{pass}}(v) = (g_l + g_{c,m}(v))/c_m = g_{c,m}(v)/c_m$. It characterizes the frequency-dependence of the high-frequency attenuation caused by the combination of the passive properties of the neuron and the steady-state shunting conductance of the voltage-dependent current.
diminished by a factor of 0.5. Decreasing $c_m$ enlarged the FRC by a factor of 2. Thus, differences in size among cortical neurons of the same type would not be expected to alter their resonant characteristics. The resulting changes in input resistance, however, are reflected precisely in the peak values of the FRCs.

The activation time constants for $I_H$ can vary widely among neocortical neurons (see Figure 5.1B1 and Figure 5.1B2; and Solomon and Nerbonne 1993b). Therefore, the effects of altering $f_H$ in the simplified RM model were determined. Changes in $f_H$ affected the bandwidth of the resonant hump and the values of Q and $f_{res}$ as shown in Figure 5.7C. Here, $f_H$ ranges from 32 Hz (corresponding to $\tau_H = 0.005$ s) to 0.032 Hz (corresponding to $\tau_H = 5$ s) whereas the value of $f_{pass}$ at $\nu = \nu_{H/2}$ is 5.8 Hz (corresponding to $\tau_m = 0.027$ s). This is a larger range of $f_H$-variation than actually encountered in our neurons (range: fast activation, $f_{H,f} = 0.4$ to 3.1 Hz; slow activation, $f_{H,s} = 0.05$ to 0.23 Hz). However, consideration of this expanded range clarifies the relationship between $f_H$, $f_{pass}$, and resonance (see below). Decreasing $f_H$ from the standard value of 0.32 Hz ($\tau_H = 0.5$ s) to 0.032 Hz ($\tau_H = 5$ s), broadened the resonant hump and shifted $f_{res}$ to lower values but did not affect the value of Q. This can also be explained by the analysis of Chapter 3. Referring to Figure 3.10, the decrease in $f_H$ corresponds a decrease in $\omega_0$, producing a leftward shift of the FRC of the $\tau$-subcircuit. This widens the frequency gap between the regions of high- and low-frequency attenuation and increases the resonant bandwidth. Conversely, an increase in $f_H$ produces a rightward shift of the FRC for the $\tau$-subcircuit, narrowing the gap, diminishing the bandwidth and amplitude of the resonant hump, and increasing $f_{res}$. Finally, resonance disappears when $f_H$ is large enough to violate inequality (3.31). This situation is demonstrated for $f_H = 32$ Hz in Figure 5.7C and corresponds to a complete overlap between the regions of high- and low-frequency attenuation in the $\tau$- and passive-subcircuits.

Now consider changes in membrane properties that may occur transiently within a neuron. For example, multiple neuromodulatory systems may modify $g_t$ in cortical neurons (McCormick 1992). Compared with the effects of altering $\bar{g}_H$, the effects of changing $g_b$ were complex (Figure 5.7D). Doubling $g_t$ from 4 to 8 nS diminished the peak amplitude of the FRC by a factor of 0.6 and increased $f_{res}$ by a factor of 1.1. These effects were in the same direction as those due to a twofold increase in $\bar{g}_H$. On the other hand, halving $g_t$ raised the Q-value by a factor of 1.3, opposite to the effects of an equivalent change in $\bar{g}_H$. The mixed effects of
Figure 5.7 Effects of changes in the parameter values of the simplified RM model.

A - D. Frequency-response curves at -70 mV for the simplified RM model. Each figure shows the FRCs resulting from changes in one parameter value. Keys (upper right) show adjusted values. The solid curve in each figure shows the FRC for the standard parameter values specified in footnote 5 on page 129 of this chapter.
changes in \( g_t \) may be explained by noting that doubling \( g_t \) doubles the amount of leakage current available to shunt input currents as well as the value of \( f_{\text{pass}} \). The increased shunt accounts for the reduction in the Q-value, whereas the change in \( f_{\text{pass}} \) increases \( f_{\text{res}} \) as seen from Equation (3.28). Graphically, the increase in \( f_{\text{res}} \) is due to a rightward shift in the FRC for the passive-subcircuit of the impedance.

Finally, the effect of a shift in the half-activation point \( (v_{H/2}) \) of the steady-state activation curve for \( I_H \) is to produce a corresponding shift in the voltage dependence of all the properties of the frequency response. Graphically, this corresponds to a shift of the frequency-response surface of Figure 5.6A. This is because the membrane voltage, \( v \), always appears in the combination \( v - v_{H/2} \) in the expression for the impedance of the simplified RM model. Several neurotransmitters result in shifts of the steady-state activation curve for \( I_H \) in central neurons (McCormick and Pape 1990; Banks et al. 1993). One consequence of this for a neuron would be a shift of the voltage dependence of resonance while leaving the other voltage dependent properties of the neuron unchanged.

5.3.5 Amplification and attenuation of resonance by \( I_{IR} \) and \( I_{Na,p} \)

In chapter 3 it was found that the activation range of \( I_H \) in neocortical neurons sometimes overlaps with the activation ranges of two other rectifying, noninactivating currents -- \( I_{IR} \) and \( I_{Na,p} \). With the aid of pharmacological blockers, these currents were shown to interact with \( I_H \) and modify the FRCs of resonant neurons. Coactivation of \( I_{IR} \) and \( I_H \) caused a flattening of the resonant hump and an overall reduction in the peak magnitude of the FRC whereas coactivation of \( I_{Na,p} \) and \( I_H \) sharpened the resonant hump and increased the peak magnitude of the FRC. In this section we show that these actions are consistent with the changes produced by introducing \( I_{IR} \) and \( I_{Na,p} \) into the simplified RM model.

To model \( I_{IR} \) and \( I_{Na,p} \) it was assumed that they both activate instantaneously. This assumption is a good approximation because the activation (\( I_{Na,p} \), Alzheimer 1993; \( I_{IR} \), Sutor and Hablitz 1993) of both currents is much faster than either \( \tau_m \) or the activation time constants of \( I_H \) measured in the neurons in these experiments. The addition of an instantaneously activating simple current to the simplified RM model turns it into a CM+SS model of the type considered in Chapter 3. From the analysis there, we expect that the addition of \( I_{IR} \) (a class I current) should produce an attenuated resonance and that addition of \( I_{Na,p} \) should lead to an amplified resonance.
These expectations are confirmed in Figure 5.8. Addition of $I_{Na,p}$ modified the resonant FRC of the simplified RM model. The curve labeled $I_H + I_{Na,p}$ is the FRC after amplification by $I_{Na,p}$. The resonant frequency and the width of the resonant hump were mostly unaffected by the amplification; the Q-value of the resonance, however, is increased. The addition of $I_{IR}$ resulted in an attenuation of resonance (curve labeled $I_H + I_{IR}$).

5.4 Discussion

The result of primary interest in this chapter is that, together with the capacitive properties and leakage currents of the membrane, the properties of $I_H$ are sufficient to account for a low-frequency subthreshold resonance observed in neocortical neurons. In support of this, a simple model of $I_H$ was able to replicate the FRCs of individual neurons when the parameter values for the model were directly from voltage clamp measurements in the same neurons. Moreover, coupling the model to living neurons using the RCC resulted in resonance and frequency-selective firing.

The present demonstration of the sufficiency of $I_H$ for generating resonance complements the pharmacological demonstration in Chapter 4 that $I_H$ is necessary for resonance. Thus, $I_H$ is the sole major determinant of resonance in a narrow band of potentials between -80 mV and -65 mV in neocortical neurons. However, resonance may be modified by other voltage-dependent currents ($I_{Na,p}$, $I_{IR}$), voltage-independent currents ($g^*$), and changes in the properties of $I_H$. In addition, in neocortical neurons, $I_H$ may be subject to neuromodulation as it is in other central neurons. For instance, an elevation of internal cAMP levels in some neurons shifts the activation curve of $I_{Na,p}$ to more depolarized levels (Banks et al. 1993; McCormick and Pape 1990). As shown in this chapter a shift such as this would produce a corresponding change in the voltage dependence of $I_H$-resonance such that the peak values of Q and $f_{res}$ will be shifted along the voltage axis closer to the firing threshold. According to a simple conceptual model developed in Chapter 4, this would widen the frequency band over which firing couples to oscillatory inputs.

Changes in the maximal H-conductance ($g_H$) also affected the resonant behavior of the simplified RM model. This may be relevant for rat neocortex where $g_H$ depends on age and

---

7 The parameter values for $I_{Na,p}$ are: $g_{Na,p} = 1 \text{nS}, k = -5 \text{mV}^{-1}, v_{Na,p2} = -50 \text{mV}, v_H = 40 \text{mV}$. Parameter values for $I_{IR}$ are:

$g_{IR} = 3 \text{nS}, k = 5 \text{mV}^{-1}, v_{IR2} = -95 \text{mV}, v_{IR} = -85 \text{mV}$
Figure 5.8 $I_{Na,p}$ amplifies the resonance generated by $I_H$.

The dashed curve is the FRC of the simplified RM model (standard parameters) at -60 mV. Addition of $I_{Na,p}$ to the model causes an amplified resonance (solid curve labeled $I_H + I_{Na,p}$). Note that this does not greatly change the value of $f_{res}$. Addition of $I_{IR}$ (solid curve labeled $I_H + I_{IR}$) results in an attenuated resonance.
projection target (Mason and Larkman 1990; Solomon et al. 1993; Kasper et al. 1994) and in cat neocortex where $\bar{g}_H$ may depend on internal [Ca$^{2+}$] (Schwindt et al. 1992).

Changes in $g_t$ also affected resonance. There were two effects of an increase in $g_t$. On the one hand, the increase in the shunting conductance caused an attenuation. On the other hand, the increased $g_t$ speeded up the membrane by decreasing its time constant. This last action may be significant because a class I current that initially produces an attenuation may reveal a resonance when the time constant of the membrane is shortened. This could be a novel mode of neuromodulation in the cortex. Figure 5.9 shows, conceptually, how a resonance may be revealed or hidden by a shift in $f_{pass}$ due to an increase in $g_t$. Since a repertoire of potentially resonant currents with various kinetic properties exist in neocortical neurons (see above), there is the possibility that different resonances are hidden or revealed by changes in $g_t$ that are keyed to different brain states. The range of such a mechanism would be greatly increased by the effective change in $g_t$ that may occur by the summation of synaptic conductances when a neuron is exposed to a sustained random barrage of synaptic (Bernander et al. 1991). Such conditions may occur in the neocortex during EEG-desynchronized brain states. The resulting “effective” time constant can be an order of magnitude smaller than $\tau_m$, thus allowing higher frequency resonances than those described here.
Figure 5.9 An increase in $g_l$ can transform a non-resonant to a resonant neuron.

A. The low- and high-frequency attenuations overlap; therefore, the neuron is nonresonant. B. An increase in $g_l$ shifts the region of high-frequency attenuation to the right, resulting in a resonant hump. In neurons with multiple voltage-dependent currents that produce different low-frequency attenuations, increasing $g_l$ could result in multiple resonant humps.
6. Models of Thalamic Neurons

6.1 Introduction

In a paper on the frequency-response properties of neurons of the nucleus mediodorsalis thalami (MDT) in guinea pigs, Puil et al. (1994) reported the presence of two voltage-dependent, subthreshold resonances. One of these resonances occurred at voltages depolarized from the resting potential in neurons with unusually elevated firing thresholds. Another resonance, with a resonant frequency between 2 and 4 Hz at 34 °C, was evident near the resting potential or at more hyperpolarized voltages. This last resonance could be blocked by antagonists of the low threshold T-type Ca\textsuperscript{2+} current, I\textsubscript{T}, suggesting that I\textsubscript{T}, a ubiquitous current in thalamic neurons, was the underlying cause. On theoretical grounds also, I\textsubscript{T} is suspected of being able to generate resonance (Koch 1984). In the terminology developed in Chapter 3, the process of I\textsubscript{T}-inactivation is like a class I current that can combine with I\textsubscript{T}-activation (a class II current) to produce an amplified resonance. The low-frequency range of the reported resonance also matched the slow kinetics of I\textsubscript{T} inactivation (hundreds of milliseconds).

Other currents, known to occur in thalamic neurons, could also produce or modify resonance. Among these currents are a transient Na\textsuperscript{+} current (I\textsubscript{Na}), a persistent Na\textsuperscript{+} current (I\textsubscript{NaP}), one rapidly inactivating and two slowly inactivating K\textsuperscript{+} currents (I\textsubscript{A}, I\textsubscript{K2}, and I\textsubscript{A}s), the hyperpolarization-activated cation current (I\textsubscript{H}), one or more Ca\textsuperscript{2+} activated K\textsuperscript{+} currents (I\textsubscript{C} or I\textsubscript{K1}), and a high threshold Ca\textsuperscript{2+} current (I\textsubscript{L}) (see review by McCormick 1992; also Huguenard and Prince 1991; McCormick 1991). The kinetics and voltage dependence of I\textsubscript{T} and some of these other currents have been carefully measured by Huguenard and others using voltage-clamp methods in acutely dissociated cells (Huguenard and Prince 1991; Huguenard et al. 1991; Huguenard and McCormick 1992). These efforts resulted in the construction of a realistic model of the electrophysiological properties of thalamocortical neurons, subsequently published by McCormick and Huguenard (1992).

The existence of this model (the MH model) opens the possibility of predicting the impedances of thalamic neurons from a secure biophysical foundation. In this chapter the MH model will first be modified so as to apply to MDT neurons (the MDT model) and then used to calculate impedances for comparison with the data of Puil et al. (1994). The goal of the analysis is to determine if I\textsubscript{T} is sufficient to account for the resonance in MDT neurons. Using a minimal
MDT model containing only $I_T$ and $I_i$ (a CM+I model according to the terminology of Chapter 3), the dependence of resonance on the values of certain parameters will also be examined.

6.2 Methods

6.2.1 Structure of the model

The MH model, developed to describe electrical properties ventrobasal thalamocortical neurons, was modified for the MDT neurons. The MH model uses a formalism based on the theory of Hodgkin and Huxley (1952) and is an extension of the models defined in Chapter 3. The value of the membrane capacitance ($c_m = 400$ pF) for the MDT model was determined from the experimentally measured time courses of responses of MDT neurons to subthreshold current pulses in Pupil et al. (1994).

In the MH model the changes in membrane voltage, $v$, are given by

$$
c_m \frac{dv}{dt} = -(i_t + i_T + i_L + i_{Na} + i_{NaP} + i_H + i_A + i_{K2} + i_C),
$$

(6.1)

where the right hand side of (6.1) contains the contributions from the various ionic currents whose details are specified in Appendix B. The leak current, $I_l$, is the product of a constant, voltage independent conductance and a voltage driving force

$$
i_l = g_l \cdot (v - v_l),
$$

(6.2)

where $v_l$ is the reversal potential of the leak current and $g_l$ is the leak conductance. Other currents in the model have a similar form except that the conductance, $g$, may depend on voltage and the levels of activation, $m^s$, and inactivation, $h$,

$$
g = \bar{g} m^s h,
$$

(6.3)

where $s$ is a positive integer. For most currents $\bar{g}$ is a constant. In the case of Ca$^{2+}$ currents the constant field equation is required (Goldman 1943, Hodgkin and Katz, 1949, Hagiwara and Byerly 1981). This makes the maximal conductance a function of voltage

$$
\bar{g}_{Ca}(v) = -2P_{Ca} F v \xi \frac{[\text{Ca}^{2+}] - [\text{Ca}^{2+}]_0 e^{-\frac{\xi}{v}}}{(1 - e^{-\frac{\xi}{v}})(v - v_{Ca})}, \quad \xi = \frac{zF}{RT},
$$

(6.4)
where $P_{Ca}$ is the maximal permeability (in units of $10^{-6}$ cm$^2$/s), $[Ca^{2+}]_0$ and $[Ca^{2+}]_i$ are external and internal Ca$^{2+}$ concentrations (in mM), $\nu_{Ca}$ is the reversal potential for Ca$^{2+}$, and $F$, $R$, and $T$ are thermodynamic quantities. (Mathematically, the expression for $\bar{g}_{Ca}(v)$ is undefined at $v = \nu_{Ca}$ and $v = 0$; however, the limiting values are finite and positive). The time courses of the activation and inactivation variables for the different currents in the model are given by relaxation equations similar to 3.50 and 3.51.

6.2.2 Parameter values describing ionic currents

The functions describing the currents $I_T$, $I_L$, $I_A$, $I_{K2}$, and $I_C$ in the MH model are given in McCormick and Huguenard (1992) and Huguenard and McCormick (1992). For the most part, these functions are derived from voltage clamp experiments on isolated thalamic neurons (Huguenard et al. 1991, 1992). In thalamic neurons, a Ca$^{2+}$ activated K$^+$ current, and a high threshold Ca$^{2+}$ current have been observed but are not well characterized. Therefore, the MH model uses an $I_C$ from the bullfrog sympathetic ganglion (Yamada et al. 1989), and an $I_L$ from guinea pig CA3 hippocampal pyramidal neurons (Traub et al. 1991). The changes in the parameter values needed to adapt the model to MDT neurons are given in the Results and summarized in Appendix B. The values of the maximal conductance for all currents were scaled by the capacitance to keep the conductance density in the MDT model the same as in the MH model.

6.2.3 Adjustments to $I_T$

In an initial examination of the MH model, it was found that the value of $P_T$ necessary to replicate the modeled current traces in Figure 1 of Huguenard and McCormick (1992), or the voltage clamp data on which the MH model is based (Huguenard and Prince 1992), was 1/1000th of the value reported in Table 1 of McCormick and Huguenard (1992). Other than this change, no alterations were made in the parameters of $I_T$ to accommodate MDT neurons (see Appendix B). In the MDT model, as in the MH model, the maximal permeability ($P_L$) for the high threshold Ca$^{2+}$ current is set equal to twice $P_T$.

6.2.4 Effects of Temperature

The calculations in this chapter are for 34 °C. Following Coulter et al. (1989) and McCormick and Huguenard (1992), a $Q_{10}$ of 3 for the inactivation kinetics and maximal permeability of $I_T$ is used and a $Q_{10}$ of 5 for its activation kinetics. For all other currents the
value of the $Q_{10s}$ are 3 for the kinetic properties and 1.6 for the maximal conductances (McCormick and Huguenard 1992).

6.3 Results

6.3.1 Estimation of model parameters

The MH model includes $I_T$, $I_L$, $I_A$, $I_{K2}$, $I_C$, $I_H$, $I_{Na}$, $I_{NaP}$, and $I_r$. To adapt this model to MDT neurons we can begin by neglecting the contributions of the $Na^+$ currents, $I_{Na}$, and $I_{NaP}$ since the 2 to 4 Hz resonance recorded by Puil et al. (1994) was observed in the presence of TTX. Also, most MDT neurons did not show large voltage sags in response to hyperpolarizing current pulses (Puil et al. 1994) suggesting that the hyperpolarization activated cation current, $I_H$, is often absent in MDT neurons. $I_H$ was therefore excluded from the model but its possible role in the frequency selectivity of thalamic neurons will be dealt with in the Discussion. Excluding $I_{Na}$, $I_{NaP}$, and $I_H$ results in a reduced MH model that contains $I_T$, $I_L$, $I_A$, $I_{K2}$, $I_C$, and $I_r$.

The next step in adapting the MH model was to compare the outputs of the reduced MH model directly to data from MDT neurons. Figure 6.1A compares the voltage output from the reduced MH model with a low-threshold spike evoked in an MDT neuron by a 100 ms current pulse. A $g_r$ of 16 nS was used to match the initial passive phase of the response. Note that the model produces an low-threshold spike of considerably shorter duration than seen experimentally. The rapid repolarization of the low-threshold spike is due to $I_{K2}$ which has much of its activation range at relatively negative potentials. It was found that the reduced MH model cannot duplicate the long-lasting low-threshold spike observed in MDT neurons as long as an appreciable $I_{K2}$ is present. Figure 6.1B shows that the complete removal of $I_{K2}$ considerably improves the fit of the falling phase of the low-threshold spike. However, the lack of a good fit near the peak indicates that a functional equivalent of a delayed rectifier current is still required.

Therefore, $I_{K2}$ was altered by making the fewest parameter changes compatible with a reasonable fit of the data. This altered current is called $I_{Kx}$ in order to distinguish it from the experimentally determined $I_{K2}$. Replacement of $I_{K2}$ by $I_{Kx}$ is the only change made to the voltage dependent properties of the reduced MH model (other than the adjustments noted in the Methods). Appendix B gives the current equations, parameters, and the maximal conductances and permeabilities for the final model (the MDT model) containing $I_T$, $I_L$, $I_A$, $I_{Kx}$, $I_C$, and $I_r$.
Figure 6.1 Alteration of the McCormick and Huguenard (MH) model to fit data from MDT neurons.

Simulated voltage transients for different versions of the MH model are compared to the low threshold spike evoked in MDT neurons stimulated with 100 ms, 0.16 nA current pulses and treated with 0.5 μM TTX.  A. Comparison of the low-threshold spike from an MDT neuron and the reduced version of the MH model (explained in the text).  B. Removal of $I_{K2}$ from the reduced MH model improves the fit of the descending portion of the low threshold spike.  C. Replacing $I_{K2}$ with $I_{RX}$ leads to a good fit of the low threshold spike. The altered model is called the MDT model in the text. Parameter values for the MDT model are given in Appendix B.
The excellent agreement between the modeled and experimental low-threshold spike in Figure 6.1C suggests that the equations and parameter values used for $I_T$ are acceptable approximations for the properties of the low threshold $Ca^{2+}$ current in MDT neurons. Although altering other parameter combinations for $I_T$ and the other ionic currents may have produced a similarly good fit, this was not done so that the model would remain as little changed as possible. It should be stressed that the equations and parameters that describe $I_T$ in the MDT model are completely determined from voltage clamp experiments in thalamic neurons (Huguenard and Prince 1992; Huguenard and McCormick 1992).

6.3.2 Frequency response of the MDT model

The impedance magnitude for the MDT model was calculated using Equation (A2.29) from Appendix A. Figure 6.2A shows a plot of the resulting FRS. An amplified resonance resonant peak in the 2 to 4 Hz range near -70 mV occurs in the FRS. Figure 6.2B shows the calculated FRCs from this surface which can be compared with the corresponding FRCs of an MDT neuron in Figure 6.2C. These plots show qualitative agreement between the predicted and observed responses with regard to the voltage- and frequency-dependence as well as the relative amplitude of the resonant hump.

6.3.3 Contribution of $I_T$ to the impedance

The contributions of $I_T$ to the impedance and the resonant properties of the MDT model were tested in two different ways -- by eliminating $I_T$ from the model (Figure 6.3A) and, following Wang et al. (1991), by creating a minimal MDT model with only two currents, $I_T$ and $I_i$ (Figure 6.3B). A comparison of Figure 6.2A and Figure 6.3A shows that eliminating $I_T$ abolishes the amplified resonance in the FRS of the MDT model. The same effect was observed biologically after application of either $Ni^{2+}$ or octanol, two blockers of $I_T$, to MDT neurons (Puil et al. 1994). In contrast, the FRS of the minimal model has an amplified resonance with approximately the same voltage- and frequency-dependence as for the full MDT model but with an even larger peak magnitude (cf. Figure 6.2A and Figure 6.3B). Since the minimal MDT model is a CM+I model (see Chapter 3), the FRS of Figure 6.3B can be compared to Figure 3.19C showing an amplified resonance in a similar model.

---

1 The impedance data for the MDT neuron was supplied by Drs. Puil, Meiri, and Yarom.
Figure 6.2 Predicted and observed frequency responses for the MDT model and an MDT neuron.

A. Theoretical frequency response as a function of membrane voltage and stimulation frequency showing a large resonant hump with a peak near 4 Hz and -70 mV. B. Frequency response of the MDT model at 4 levels of DC hyperpolarization or depolarization. C. Observed frequency response of an MDT neuron.
Together the above observations imply that $I_T$ determines the qualitative nature of the resonance that appears in the MDT model. By extension, $I_T$ is sufficient to account for the basic properties of the low frequency resonance observed at subthreshold potentials in MDT neurons.

6.3.4 Mechanism of $I_T$ resonance
Figure 6.4A shows how the characteristic features of $I_T$ resonance (the drop-off in the voltage response at low frequencies and the amplification of the response near the peak frequencies) are revealed when a Zap current input is used to stimulate the minimal MDT model. The largest voltage response occurs near the same frequency (3-4Hz) as the peak of the impedance surface in Figure 6.3B (the resting potential in Figure 6.4A is -71 mV with d.c. injection of -0.23 nA). Figure 6.4A also shows how inactivation ($h$) and activation ($m$) contribute to the resonance. At low frequencies the slow $h$ variable is able to track the oscillating input and oppose large voltage changes. At higher frequencies, $h$ cannot follow the voltage and part of its influence is lost. In contrast, $m$ is a fast variable and acts to amplify the voltage response of the model at all frequencies in Figure 6.4A. The $m^2$ trace in Figure 6.4A has a resonant shape not because it is causing the resonance directly, but because it is acting as a follower and amplifier of the voltage. Thus, it is the slow inactivation of $I_T$ that is responsible for the drop-off in response at low frequencies that characterizes resonance whereas the fast activation of $I_T$ is responsible for the amplification near the peak frequencies. This is consistent with the interpretation of $h$ as an "activation" variable for a class I (attenuating/resonant) current and $m$ as the activation variable for a class II (amplifying) current.

To clarify these points further, the effects of $I_T$ activation and inactivation have been separated out in Figure 6.4B. The left trace shows that when the voltage dependence of $I_T$ activation is eliminated, e.g. by setting $m = 1$, a resonance is still present in the response due to inactivation. Conversely, when the voltage dependence of inactivation is eliminated by setting $h = 1$ (Figure 6.4B, middle trace) there is no resonance, but a comparison with the rightmost trace in Figure 6.4B shows that there is an overall enhancement of the voltage response. Thus, the combination of the amplifying effect of $I_T$ activation and the frequency selectivity due to inactivation results in the resonant behavior of the MDT model.
Figure 6.3  $I_T$ is sufficient to explain the resonant in the MDT model.

A. Resonance is lost when $I_T$ is eliminated from the model. This is comparable to the block of $I_T$ by Ni$^{2+}$ or octanol.  B. Resonant frequency response of a minimal model containing only $I_T$ and $I_i$. 

---

B. HUTCHEON 147
Figure 6.4 Responses of the minimal MDT model to simulated ZAP current inputs.

A. Voltage response (top) of the minimal MDT model when the frequency of the input is swept from 0-10 Hz over 5 seconds. The largest voltage response is near 4 Hz. The middle trace shows the time course of the activation variable, $m^2$, during the response. The bottom trace shows the time course of the inactivation variable, $h$.

B. Voltage responses demonstrating that $I_T$ inactivation is responsible for resonance. Peak-to-peak magnitude of 0 - 15 Hz, 7.5 s, ZAP input is 0.05 nA. When only $I_T$ inactivation is operational (left panel, activation held constant at $m^2 = 1$) the response is resonant with a peak near 10 Hz. An $I_T$ with only activation (middle panel, inactivation held constant at $h = 1$) does not produce resonance, but there is an overall amplification of the response compared to the passive case with no $I_T$ (right panel). The maximal permeabilities have been adjusted to produce similar responses at 15 Hz (left, $P_T = 5000 \text{ cm}^3/\text{s}$; middle, $P_T = 300 \text{ cm}^3/\text{s}$; right, $P_T = 0 \text{ cm}^3/\text{s}$).
6.3.5 Significance of the steady state window conductance, $g_w$

Referring to the definitions in Chapter 3, the minimal MDT model is a CM+I model. Therefore, the theoretical results derived there apply to the minimal MDT model. In particular, as in Figure 3.19, the size of the amplified resonance due to $I_T$ in the minimal MDT model should be controlled by the extent of the overlap between the steady-state activation and inactivation activation curves for $I_T$, i.e., by the steady-state window conductance $g_{T,w}$. The resonance in the minimal MDT model is extraordinarily sensitive to the relative positions of the activation and inactivation curves as is shown in Figure 6.5 where a shift of the half-inactivation parameter, $v_{i2}$, in the MDT model from -84 mV (Figure 6.5A) to -87 mV (Figure 6.5B) is enough to reduce the peak amplitude of $g_w$ (panels on the right in Figure 6.5A,B) and almost eliminates the amplified resonance in the FRS (panels on the left in Figure 6.5A,B). Thus a change in $g_w$ effectively transforms the linear frequency response of the model neuron from a bandpass to a lowpass filter.

6.3.6 Dissociation of resonance and the low threshold spike

Although a -3 mV shift in $v_{i2}$ essentially abolishes the resonant properties of the MDT model, it has relatively minor effects on the rebound low-threshold spike after a hyperpolarization. Figure 6.6A shows the linear frequency response of the resonant and nonresonant versions of the MDT model (corresponding to Figure 6.5A and Figure 6.5B respectively) at -73 mV. The response of the nonresonant MDT model is almost the same as the passive response (dotted line Figure 6.6A). In contrast, the rebound low-threshold spike produced by a hyperpolarizing pulse is only slightly attenuated and delayed. Thus, a prediction of the minimal MDT model is that linear resonance and the rebound low-threshold spike can be dissociated even though they both depend on $I_T$.

6.3.7 Spontaneous oscillations in the MDT model

Large (30 mV), spontaneous, 2-3 Hz oscillations develop for the MDT model when the resting potential is set to -75 mV, and the overlap between the activation and inactivation curves is increased by making $v_{a2}$ more positive than -79 mV. These oscillations do not develop if the resting potential is set to -60 mV. A similar phenomenon has been described previously for a minimal model of $I_T$ in a thalamic neuron (Wang et al. 1991). The similarity between the frequency of the spontaneous oscillations and the frequency of the resonant peak in the MDT model suggests that thalamic neurons may have multiple operating modes controlled by small changes in the voltage sensitive properties of $I_T$. 
Figure 6.5 Amplified resonance is extremely sensitive to changes in steady-state activation and inactivation.

A. Frequency response of the resonant MDT model (left) with $V_{h/2} = -84$ mV, and the window conductance, $g_w$ (right). B. Shifting $V_{h/2}$ by -3 mV results in a smaller $g_w$ and almost eliminates the resonance.
Figure 6.6 Changing $v_{h/2}$ has a large effect on resonance but a much smaller effect on the low-threshold spike of the minimal MDT model.

A. Frequency response of the MDT model at -73 mV. Shifting $v_{h/2}$ from -84 mV (solid line) to -87 mV (dashed line) decreases the size of the resonant hump. For comparison the frequency response of the model when $I_T$ is eliminated entirely is also shown (dotted line). B. The rebound low-threshold spike in response to a -0.4 nA current pulse is practically unaffected by the shift in $v_{h/2}$. 
6.3.8 Frequency response of the nonlinear model

The results given above show that I_T is sufficient to explain the frequency preference of MDT neurons stimulated with small periodic currents. The question arises, however, whether large oscillatory inputs that strongly engage the nonlinear properties of neurons can also produce a frequency selectivity as in the neocortical neurons studied in an earlier chapter. This was investigated by stimulating the minimal MDT model with ZAP current inputs of different amplitudes (Figure 6.7). The voltage responses in Figure 6.7 extend from small nearly linear responses (Figure 6.7A,B; bottom panels) to responses that contain large nonlinear components (Figure 6.7A,B; top panels). Figure 6.7A shows that, in the resonant minimal model with \( v_{\text{h2}} = -84 \) mV and hence a large \( g_w \), the frequency selectivity of the neuron is accentuated when a ZAP current input just large enough to evoke nonlinear Ca^{2+} spikes is used (middle panel). When an even larger input is used the low frequency components of the input are able to evoke all-or-none Ca^{2+} spikes. Figure 6.7B shows that the accentuation of the frequency preference is also present in the nonresonant minimal MDT model with \( v_{\text{h2}} = -87 \) mV (and hence a small \( g_w \)). A large enough input to this nonresonant model will produce all-or-none Ca^{2+} as in the top panel of Figure 6.7A (not shown). Thus, there is a region of nonlinear enhancement of the frequency selectivity due to I_T. However, this effect is diminished for inputs that are large enough to allow the full expression of the Ca^{2+} spiking mechanism at low frequencies. Both the input current amplitude and \( g_w \) can control the appearance of frequency selectivity in neurons containing I_T.

6.4 Discussion

6.4.1 I_T underlies low frequency resonance in MDT neurons

The models presented in this chapter show that the properties of the low-threshold Ca^{2+} current, I_T, can account for the voltage-dependent resonance of MDT neurons described in Puil et al. (1994). Different properties of I_T control different aspects of the resonance. The steady state window conductance, \( g_w \), controls the voltage dependence and overall magnitude of the small signal resonance. On the other hand, the interaction of the passive membrane properties of the neuron with the voltage dependence and kinetics of I_T inactivation generates the resonance and determines the resonant frequency. Finally, the fast voltage dependent activation of I_T amplifies voltage responses throughout the resonant frequency range and modulates the location of the peak frequency but does not, in itself, cause resonance. This is similar to the amplified resonance produced in the CM+I model of Chapter 3. According to the discussion there, I_T inactivation can
Figure 6.7 The nonlinear responses of the minimal MDT model show frequency selectivity.

The frequency of the ZAP current input (bottom) is swept from 0-10 Hz in all cases. The peak-to-peak amplitude of the input is given above the individual voltage traces. A. Resonant version of the minimal MDT model ($V_{h/2} = -84$ mV). As the amplitude of the input current is increased, the frequency selectivity due to $I_T$ resonance (almost-linear response, bottom panel) is first enhanced (middle panel) then reduced (top panel) as all-or-none Ca$^{2+}$ spikes are elicited. B. The nonresonant minimal MDT model with $V_{h/2} = -87$ mV and small $g_w$. The model is less sensitive than the resonant model to the oscillatory inputs, however the 0.1 nA input evokes an enhanced frequency selectivity. A 0.25 nA input (not shown) produces all-or-none Ca$^{2+}$ spikes similar to the response to the 0.1 nA input in (A).
be viewed as a class I, simple current capable of producing resonance whereas the activation process of $I_T$ is a class II amplifying current.

6.4.2 Dissociation of resonance and the low threshold spike
In Sections 3.5.2 and 0 it was shown that the amplified resonance of the minimal MDT model depends critically on the size of $g_w$ whereas the low-threshold spike is less sensitive. Thus the small signal responses of thalamic neurons may have different parameter sensitivities than the responses where large nonlinear voltage responses are evoked. This implies that pharmacological agents that selectively alter $g_w$, possibly by shifting the steady state activation and inactivation curves of $I_T$, should change the linear resonant properties of MDT neurons but not their ability to generate a rebound low-threshold spike. Some antagonists of $I_T$ in nonthalamic cells shift $V_{L2}$ in the hyperpolarizing direction as well as reducing $g_T$ (Miyake et al. 1992; Takahashi and Akaike 1991; Chen et al. 1990). In guinea-pig inferior olivary neurons, harmaline shifts the $I_T$ inactivation curve to more depolarized values (Llinás and Yarom 1986). Also, investigations on cloned neuronal Ca$^{2+}$ channels show that the identity of the $\beta_1$ subunit can affect $V_{L2}$ and $V_{T2}$ independent of $g_T$ (Soong et al. 1993). This raises the possibility that pharmacological agents or endogenous substances may have distinct actions depending on whether they alter resonance or the rebound low-threshold spike.

6.5 Significance
In this chapter, it has been shown that $I_T$ is sufficient to account for the low-frequency resonance previously reported in MDT neurons (Puil et al. 1994). This resonance is an intrinsic, voltage dependent property of individual neurons that is expressed under specific conditions, i.e., in the presence of inputs with appropriate frequency components. Therefore, it may promote the development of low-frequency activity in thalamic neurons by acting as a bandpass amplifier to selectively enhance the voltage responses of cells to rhythmic synaptic inputs within the resonant frequency band. Thus, for a neuron with resonance, even weak inputs that arrive within the resonant band of frequencies result in low threshold Ca$^{2+}$ spikes as shown in Figure 6.7. The consequences of this resonance for the nucleus mediodorsalis thalami would be to tune it to detect 2-4 Hz activity in other structures and respond with bursts of activity at the same frequency. In contrast, in neurons with little or no resonance, the frequency-dependent amplification of signals is small or absent so that the connection between small low-frequency
inputs and bursts of spikes due to low threshold Ca$^{2+}$ spikes is weak. Under these conditions the low-threshold spike can still be evoked by sufficiently intense inputs but does not show the sensitive dependence on membrane voltage and input frequency associated with resonant neurons.

These results suggest that low-frequency resonance in thalamic neurons is only one aspect of a range of I_T-dependent rhythmic properties including bandpass filter behavior and spontaneous oscillations that may be useful for tuning their responses to stimuli.
7. Concluding discussion

7.1 Summary of results.

The work of the preceding chapters has uncovered a low-frequency, subthreshold resonance in neocortical neurons and identified the ionic mechanisms underlying resonance in neocortical and thalamic neurons.

In approximately 70% of neocortical neurons, the interaction of their passive properties with the hyperpolarization-activated cation current, $I_H$, created a resonance in the region of the resting potential (between -65 and -70 mV in these experiments) or at more hyperpolarized potentials. At 24 °C, the value of the resonant frequencies was between 1 and 2 Hz at the resting potential and increased to as high as 5 Hz when the membrane was hyperpolarized past -90 mV. An empirical measure of the size of the resonant hump, the Q-value, showed that resonance was often largest when the membrane voltage was slightly hyperpolarized from its resting value. Subthreshold resonance was not detected in fast-spiking neurons -- the putative inhibitory neurons in the sample.

Resonance was modified by the actions of two other subthreshold, voltage-dependent currents in neocortical neurons. The first modifying current -- the persistent Na$^+$ current, $I_{Na,p}$ -- caused an amplification of resonance at potentials where it was coactivated with $I_H$. Based on data from voltage-clamped neurons, the region of amplification begins near -65 mV and may extend up to the threshold for action potentials in some cases. Since the voltage dependence of $I_H$ was found to be much more variable than that of $I_{Na,p}$, the presence or absence of a region of amplified resonance in a neuron depends on the voltage dependence of $I_H$. The second modifying current -- the hyperpolarization-activated, inwardly-rectifying K$^+$ current, $I_{IR}$ -- causes an attenuation of resonance at potentials more negative than approximately -80 mV. Between them, $I_H$, $I_{Na,p}$, and $I_{IR}$ create three regions of distinctive frequency-response behavior at subthreshold potentials in neocortical neurons: a region of amplified resonance that begins near -65 mV and extends to more depolarized potentials; a region of attenuated resonance that begins near -80 mV and extends to more hyperpolarized potentials; and a region of relatively unmodified resonance between -80 and -65 mV.

The frequency-response properties of all of these regions may be understood in the theoretical framework of Chapter 3. There, a voltage-dependent current with no inactivation
process was termed a simple, class I or class II current depending on whether its reversal potential lay at the foot or the head of the activation curve, respectively. Class I currents were shown to be associated with resonance at a stable equilibrium potential when they activate slowly compared to the membrane time constant, and an attenuation of the FRC when they activate quickly. Class II currents cause an amplification of the FRC. Thus, $I_H$ causes a resonance because it is a class I, simple current with slow kinetics. In Chapter 5, a model having a passive resistance and a capacitance in parallel with a class I current (a CM+S model) successfully predicted the resonant FRCs of neocortical neurons near the resting potential when $I_H$ was used as the simple current.

In Chapter 3, examination of models with a passive resistance, a capacitance, and two voltage-dependent simple currents (CM+SS models) showed that the results of interactions between voltage-dependent currents can be simple extensions of their individual behaviors. Thus, when a resonant class I current interacts with an attenuating class I current the result is an attenuated resonance; if the same resonant current interacts with a class II (amplifying) current, the result is an amplified resonance. This was demonstrated in Chapter 5 for a model with realistic parameter values having $I_H$ as the resonant, class I current and $I_{IR}$ or $i_{Na,p}$ as the attenuating or amplifying current.

In thalamic neurons, the analysis of a model due to McCormick and Huguenard (1992) showed that the low-threshold $Ca^{2+}$ current, $I_T$, can account for the features of the hyperpolarized resonance observed by Puil et al. (1994). Theoretically, this resonance is largest near the resting potential and its resonant frequency there is near 2 Hz (at 34 °C). The properties of this resonance also can be understood within the framework introduced in Chapter 3. Thus, with one exception, the minimal MDT model can be regarded as a CM+SS model where the process of $I_T$-inactivation is treated as a class I current capable of producing resonance and the process of $I_T$ activation is treated as a class II amplifying current. The exception is that the conductances of these currents are not independent as they are for true CM+SS models. Instead the effective conductance for both currents is restricted to the voltage region where the steady state activation and inactivation curves of $I_T$ overlap to form a window conductance. This confines the resulting amplified resonance to a narrow band of voltages where the window conductance is large.
7.2 Comparison with other studies on central neurons

There have been relatively few studies of resonance in the central neurons of mammals. In neurons from the auditory thalamus of chicks, Ströhmann et al. (1994) have identified a voltage-dependent resonance that is generated by $I_{H}$ and has resonant frequencies between 6 and 10 Hz (at 30 °C). This resonance is associated with sags and rebounds in the responses of neurons to hyperpolarizing current pulses and has many of the same features as the neocortical resonance explored in Chapters 4 and 5. Resonances dependent on $I_{H}$ were also found in neurons from subthalamic levels of the chick auditory system (Ströhmann et al. 1995). The occurrence of a resonance generated by $I_{H}$ in non-cortical, non-mammalian neurons suggests that the connection between $I_{H}$ and resonance is a general one. Since $I_{H}$ has been reported in many neurons and excitable cells, resonance may also be a widespread phenomenon.

Jahnsen and Karnup (1994) have plotted the power spectra for guinea pig central neurons stimulated with band-limited white noise. They found putative resonances in several areas of the CNS. One such resonance, in hippocampal CA1 neurons, is probably due to $I_{H}$. Consistent with an $I_{H}$ mechanism, the resonance is voltage dependent, blocked by Cs+, and associated with sags and rebounds in the voltage responses to current pulses. The resonant frequency in these neurons was near 12 Hz (see Figure 6F in Jahnsen and Karnup 1994) which is higher than the resonant frequencies measured experimentally (at 24-26 °C) in Chapter 4 but matches the frequency predicted from the voltage-clamp data of Spain et al. (1987). In the same study, Jahnsen and Karnup (1994) failed to find a low-frequency resonance in neocortical neurons. However, the neurons they examined probably did not contain $I_{H}$ since the sagging voltage responses to current steps that usually signal the presence of $I_{H}$ were not observed. It is unclear whether a narrow spike at 24 Hz in the spectra of spontaneously oscillatory neocortical neurons (Figure 10B in Jahnsen and Karnup 1994) is a resonance since it could be due to the spontaneous activity itself rather than a response to stimulation. Finally, Jahnsen and Karnup (1994) describe a low-frequency resonance in thalamic neurons. In agreement with the analysis of the thalamic neuron model in Chapter 6, the resonance is largest at voltages where an $I_{T}$-dependent low-threshold spike can be evoked.

---

1 Although Jahnsen and Karnup investigated power spectra rather than impedances, their data can be taken as comparable to the results of an impedance analysis at frequencies below 50 Hz because the power spectrum of their input signal in this region is approximately flat (see Figure 1, Jahnsen and Karnup 1995).
In neurons of the guinea pig frontal cortex, Gutfreund et al. (1995) have detected a subthreshold resonance with a resonant frequency between 3-15 Hz (34 °C). The resonance is prominent from -65 to -40 mV, has a resonant frequency that increases with depolarization, and is blocked by extracellular application of a combination of TEA and Cs⁺. These properties make the resonance inconsistent with an I_H mechanism and Gutfreund et al. (1995) advocate a slow K⁺ current that activates on depolarization. Based on the analysis of Chapter 3, this would be a simple, class I current and, therefore, capable of producing a resonance. Gutfreund et al. (1995) also demonstrated that a TTX-sensitive mechanism amplifies the resonance. From the voltage dependence and pharmacology of this amplifying mechanism it seems likely that it is due to I_{Na,p} (Gutfreund et al. 1995). Thus, in neocortical neurons of the guinea pig, there is an amplified resonance of the sort modeled here by the CM+SS model of Chapter 3.

A low-frequency resonance at depolarized potentials, similar to that of Gutfreund et al. (1995), was also observed during the experiments described here (see Chapter 4). In Figure 4.4A, for instance, the FRC shows a shallow resonant hump at -45 mV with a peak near 3 Hz. The true identity of this resonance was not ascertained, however, because neurons were not systematically examined for resonance at membrane potentials positive to -50 mV.

7.3 Amplified resonance and spontaneous oscillations

An interesting observation arising from some of the models constructed in this thesis is that spontaneous oscillations of the membrane potential were associated with amplified resonances. Furthermore, the frequencies of these oscillations roughly matched the resonant frequencies. This was demonstrated in the CM+SS model of Chapter 3 and also in the minimal MDT model of Chapter 6 where an amplifying current of sufficiently large conductance destabilized the membrane potential and resulted in the replacement of resonance by spontaneous oscillations at a similar frequency.

Experimentally, spontaneous oscillations were not seen in the neocortical neurons of Chapters 4 and 5, nor were they observed by Puil et al. (1994) in the thalamic neurons that provided the data base for Chapter 6. However, there is evidence that such oscillations exist in resonant neurons and that, in those cases, their frequencies are near the corresponding resonant frequencies. In thalamic neurons, for instance, spontaneous low-frequency oscillations have been described both in vivo (Curro-Dossi et al. 1992; Nunez et al. 1992), and in vitro (Leresche et al. 1992);
In these neurons, the spontaneous oscillations occur near the same frequency as the amplified resonance identified in the thalamic neurons of Chapter 6. In guinea pig neocortical neurons, Gutfreund et al. (1995) proposed that the amplified resonance they saw underlies spontaneous oscillations with a frequency close to the resonant frequency. In those neurons, resonance and spontaneous activity shared the same pharmacological sensitivities except that, whereas TTX completely blocked the amplifying current ($I_{Na,p}$), it only partially blocked resonance. This is consistent with the hypothesis that an amplified resonance is required for spontaneous oscillations since TTX blocks the amplification of the resonance but not the resonance itself. In a model of the neocortical neurons, Gutfreund et al. (1995) showed that resonance and spontaneous oscillations, both generated by the same mechanism and having similar frequency preferences, can coexist at different membrane potentials. Thus, in this model, resonance and spontaneous oscillations are closely linked both mechanistically and functionally.

Alonso and Klink (1993) have described an apparently closely related phenomenon in neurons of the medial entorhinal cortex in vitro. Spontaneous, nearly sinusoidal, oscillations of the membrane potential with a voltage dependent frequency between 5 and 15 Hz, occur in stellate but not pyramidal-like cells. Both of these cell types possess a prominent subthreshold inward rectification due to $I_{Na,p}$, but the stellate neurons possess, in addition, a delayed rectifier-like $K^+$ conductance that the pyramidal-like cells lack. As in the guinea pig neocortical neurons above, the combination of an outwardly rectifying $K^+$ conductance and an inwardly rectifying $Na^+$ conductance should produce an amplified resonance. This leads to the prediction that an amplified resonance underlies the spontaneous oscillations seen by Alonso and Klink and determines their frequency. In support of this, externally applied TTX or Ba$^{2+}$ block the spontaneous oscillations. On the other hand external Cs$^+$ decreases the frequency of the subthreshold oscillations, presumably by blocking a component of $I_{leak}$ (Fig. 6C, D of Klink and Alonso 1993) and thereby lengthening $\tau_m$. An earlier suggestion that the resonance arises from an interaction of $I_H$ and $I_{Na,p}$ (Alonso and Llinás 1989, $I_H$ is referred to as $I_Q$) appears not to be the case.

Thus, except for the single report by Gutfreund et al. (1995), the link between spontaneous subthreshold oscillations and resonance has not been investigated experimentally. Given the probable importance of spontaneous oscillations in controlling the interactions between central neurons (Llinás 1988; Steriade 1993), research targeted at understanding this
link should prove fruitful. Using the RCC, the assertion that amplified resonances result in spontaneous oscillations could be tested by expressing an artificial amplifying current in a neuron with an endogenous resonant current or vice versa.

7.4 Physiological Relevance

What is the function of subthreshold resonance in neocortical and thalamic neurons? By definition, resonance is a filtering mechanism that converts current inputs at the resonant frequency into larger voltage responses than inputs of equal size at higher or lower frequencies. This filtering can shape the post-synaptic potentials arising from synaptic conductances in characteristic ways (Joyner and Westerfield 1982; Puil 1986), establishing a subthreshold processing of synaptic inputs that is keyed to the voltage-dependent resonant frequency. On a larger scale, resonance may be a tuning mechanism that allows central neurons to respond selectively to signals generated at biologically important frequencies in other parts of the brain. From this point of view, resonance extends the notion of the intrinsic neuronal rhythmicity of neurons that has been implicated in the coordination of rhythmic activities in central neural networks (Llinas 1990).

Although the relationship between resonance and synaptic activity was not explored in this thesis, there are two lines of evidence that support the hypothesis that resonance acts as a tuning mechanism: first, the ability of resonance to selectively boost inputs near the resonant frequency past threshold (frequency-selective firing); second, the correspondence between the resonant frequencies of neocortical and thalamic neurons and the frequencies of coherent narrow-band brain activity characteristic of certain behavioral states. These will now be discussed.

In Chapter 4, it was shown that $I_{H}$-resonance leads to an enhanced likelihood for the firing of $Na^+$ action potentials in response to oscillatory current inputs near the resonant frequency. This was confirmed in Chapter 5 where the RCC was used to express an artificial $I_{H}$ in nonresonant neurons and thereby produce frequency-selective firing. A corresponding effect was demonstrated for low-threshold $Ca^{2+}$ spikes in the simplified MDT model where, for a range of input amplitudes, low-threshold spikes were most prominent in response to oscillatory inputs near the resonant frequency (see Figure 6.7). These results demonstrate that subthreshold resonance can affect the way that inputs couple to the highly nonlinear mechanisms of spike generation in neurons. Thus, resonance is like a bandpass filter that rejects or accepts small
inputs arriving at subthreshold potentials. Because of this filtering, off-resonance components of the input will produce relatively small voltage changes and so are unlikely push the membrane past threshold. In contrast, frequency components of the input that are near the resonant frequency will produce relatively large voltage responses that are more likely to result in firing. In this way, the frequency-preference generated by resonance is reflected in the output of the neuron even though resonance is mainly a subthreshold phenomenon.

When resonant neurons are connected together, their individual frequency-preferences may interact to support well-defined states of network behavior. This was shown for electrically connected neurons by Yarom (1992) who coupled resonant electric circuits to inferior olivary neurons of guinea pigs and found that the resulting network showed spontaneous coherent activity near the resonant frequency. In the context of synaptically connected neuronal networks, this suggests that the resonant behavior of individual neurons could support the spread and stabilization of coherent rhythmic activity throughout the brain. Such activity is recognized by large-amplitude, narrow-band frequency components in the EEG and is commonly associated with well-defined behavioral states in vertebrates (Steriade et al. 1990). A hypothesis arising from the work described in this thesis is that the resonant frequencies of neurons are linked to brain rhythms with similar frequencies.

For neocortical neurons at physiological temperatures, the resonant frequencies of either the $I_{H}$-dependent resonance identified in Chapters 4 and 5, or the depolarization-activated resonance identified in guinea pig neurons by Gutfreund et al. (1995), fall within the frequency band of EEG spindle oscillations (8 to 14 Hz). Spindle oscillations in the neocortex are associated with drowsiness and are a response to synchronized, rhythmic synaptic inputs imposed on individual neurons by subcortical afferents and by rhythmic activity in local cortical circuits (Steriade et al. 1990, 1993a). Neocortical neurons with subthreshold resonance may, therefore, be tuned to respond selectively to these distinctive spindle rhythms through the frequency-selective firing discussed above. This proposal is supported by the congruence between the membrane potentials of neurons during spindling and the voltage-dependence of $I_{H}$-resonance. Intracellular in vivo recordings from neocortical neurons show that their mean membrane potentials during and between bouts of spindling are more hyperpolarized than during the brain states accompanying waking (Steriade et al. 1990, 1993b). In agreement with this, the results of Chapters 4 and 5 show that $I_{H}$-resonance is strongest between -70 and -80 mV and that the
frequency-selective firing it engenders is only effective when the resting potentials of neurons are more hyperpolarized than -65 mV. Thus, the general hyperpolarization of neocortical neurons during drowsiness may allow them to engage incoming signals with their resonant filtering mechanisms.

In the thalamus, the $I_T$-resonance may tune neurons to respond to activity within the 0.5 to 4 Hz δ-wave activity seen in the EEG during deep sleep (Curro Dossi et al. 1992; Steriade et al. 1991; McCormick et al. 1992). The frequency band of these oscillations matches the resonant band of frequencies observed by Puil et al. (1994) for MDT neurons and calculated for the MDT model in Chapter 6. Also, as mentioned above, $I_T$ may contribute to spontaneous oscillations within the same frequency band. This leads to a scenario whereby $I_T$ both initiates δ-frequency activity in the thalamus through spontaneous oscillations and also reinforces it via the resonant properties of neurons that do not oscillate spontaneously. Thus, these resonant neurons are tuned to receive δ-frequency inputs either directly from local collaterals (Soltesz and Crunelli 1992) or via indirect connections in thalamocortical or thalamoreticular loops (Steriade 1993). As in the case of neocortical neurons during spindling, thalamic neurons during δ-oscillations are hyperpolarized into a voltage region where their resonant properties are revealed, allowing them to be entrained more easily into the ongoing synchronized activity.

Finally, another aspect of resonance that may be implicated in the synchronization of neurons is the frequency dependence of the effective electrotonic length constant. Dendritic trees equipped with resonant membranes should appear most electrically compact at the resonant frequency (Koch 1984). This means that the spatial integration of synaptic inputs will be most effective if they recur rhythmically near the resonant frequency. In this way, activity in different parts of the neuron are synchronized before the neuron itself becomes entrained into synchronized network oscillations.

7.5 Future research

The first priority for future research is to tie the resonances found in central neurons to the functional aspects of neuronal or network behavior. In this regard, the considerations above suggest that resonance reinforces the participation of neocortical and thalamic neurons in the coordinated rhythmic activities of the brain. This hypothesis is testable, particularly if the RCC is used in vivo to tune or detune neurons to the frequencies of ongoing activity in the brain.
Investigations such as this will shed new light on how individual neurons are recruited into dynamically coherent assemblies. A second direction for future research is to use frequency domain techniques to scan neurons from different parts of functionally defined circuits for resonances at similar frequencies. For example, neurons in various parts of limbic circuits may be assessed for resonances that could facilitate the propagation of θ-band rhythms. Once these resonances are recognized, the study of their modulation by endogenous or manmade agents will provide a new viewpoint on how the elements of the nervous system combine to produce emergent behaviors.
Bibliography


Appendix A

In this Appendix, expressions are derived for the impedance of an isopotential neuron having voltage-dependent and -independent currents. We begin with the case of a CM+S model. The impedance for the CM model is easily deduced from the impedance of the CM+S model by setting the conductance of $I_x$ equal to zero.

Derivation of the impedance for a CM+S model

Assume that the electrical behavior of the neuron is described by the system of equations

$$c_m \frac{dv}{dt} = -g_l (v - v_l) - g_x m_x^\infty (v - v_x) + i^\circ_{\text{inj}},$$  \hfill (A.1)

$$\frac{dm_x}{dt} = \frac{m_x^{\infty}(v) - m_x}{\tau_x(v)},$$  \hfill (A.2)

where the variables and parameters have the same meanings as in Equations (3.8) and (3.9) of Chapter 3 except that the term $i^\circ_{\text{inj}}$ is a constant external current that is under the control of the investigator and used to change the equilibrium voltage of the system.

For each value of $i^\circ_{\text{inj}}$, the system has an equilibrium point $(v_o, m_{x, o})$, determined by setting the time derivatives equal to zero, i.e.,

$$0 = -g_l (v_o - v_l) - g_x m_{x, o}^\infty (v_o - v_x) + i^\circ_{\text{ext}},$$  \hfill (A.3)

$$0 = \frac{m_{x, o}^{\infty}(v_o) - m_{x, o}}{\tau_x(v_o)},$$  \hfill (A.4)

Note that Equations (A.3) and (A.4) yield

$$m_{x, o} = m_{x, o}^{\infty}(v_o).$$  \hfill (A.5)

To make the notation uniform, a subscripted "o" will always indicate the result of evaluating a function at the equilibrium point, i.e.,

$$\tau_{x, o} = \tau_x(v_o).$$  \hfill (A.6)

To proceed further, we assume that $i^\circ_{\text{inj}}$ is replaced by

$$i_{\text{inj}}(t) = i^\circ_{\text{inj}} + \delta I(t),$$  \hfill (A.7)
where $\delta l(t)$ is a small perturbation current. We can then linearize the equations about the point $(v_o, m_o)$ and determine the response of the linearized system to inputs $\delta l(t)$ at different frequencies. Note that some of the nonlinear terms have linear effects which are included in the linearized system.

Introduce the perturbation variables, $V = v - v_o$ and $M = m - m_o$, where $V$ and $M$ are assumed to be small. Then expand Equations (A.1) - (A.2) in a Taylor series in $V$ and $M$ about $(v_o, m_o)$ using Equations (A.5) - (A.7). Neglecting the terms in the expansion that involve products of the perturbation variables yields

$$c_m \frac{dV}{dt} = -g_x V - g_{x,0} \left[ s_x \frac{M}{m_{x,0}} + \frac{v}{(v_o - v_x)} \right] (v_o - v_x) + \delta l(t), \quad (A.8)$$

$$\frac{dM}{dt} = \left( m_{x,0} V - M \right) / \tau_{x,0}, \quad (A.9)$$

where $g_{x,0} = g_x m_{x,0}$, $s_x$ is the steady state chord conductance for $I_x$ at the equilibrium potential, $v_o$, and the prime in (A.9) indicates differentiation by $V$, i.e., $m_{x,0}' = \left( dm_{x,0} / dv \right)(v_o)$.

The linearized system approximates the behavior of Equations (A.1) - (A.2) when the perturbation current, $\delta l(t)$, is small. In experiments with actual neurons, the only variables that are directly measurable are $\delta l(t)$ and the perturbation output voltage $V(t)$. The relationship between these observables is described by the input impedance -- defined as the Fourier transform of the voltage output divided by the Fourier transform of the current input (see Puil et al. 1993). In the case of the linear, homogeneous system Equations (A.8) - (A.9), the use of Fourier transforms can be avoided by assuming that the perturbation input has the form $\delta l(t) = e^{i\omega t}$, i.e., an oscillatory input of unit amplitude at a fixed frequency, $\omega$. Since the input is at a single frequency, we look for solutions of Equations (A.8) - (A.9) at the same frequency in the form

$$V(t) = Z e^{i\omega t}, \quad (A.10)$$

$$M(t) = Y e^{i\omega t}. \quad (A.11)$$

Substituting these expressions into Equations (A.8) - (A.9), and dividing through by $\delta l(t) = e^{i\omega t}$ gives
Finally, eliminating \( Y \) in Equation (A.12) using Equation (A.13) and rearranging yields

\[
Z(\omega, v_0) = \frac{1}{g_l + i\omega c_m + g_{x, ch_0} \left( 1 + \frac{g_{x, o}}{1 + i\omega \tau_{x, o}} \right)}
\]  

(A.14)

where \( \hat{g}_{x, o} \) is the function defined in Equation (3.14) evaluated at \( v = v_0 \). Equation (A.14) gives the linearized impedance of Eqs. (A.1) - (A.2) as a function of frequency for each equilibrium membrane voltage, \( v_0 \). For notational simplicity, the "o" subscripts will not be retained when writing impedance expressions in the remainder of this Appendix or in the body of the thesis.

Models with more than one voltage-dependent current

If there are \( N > 1 \) simple voltage-dependent currents to be considered, each can be expressed in the same form as the terms in (A.1), i.e.,

\[
i_n = g_n m_n^{s_n} (v - v_n), \quad n = 1, 2, ..., N
\]  

(A.15)

where \( s_n \) are integer exponents, \( v_n \) is the reversal potential for current \( I_n \), and \( m_n \) is an appropriate activation variable with a time dependence given by an equation similar to Equation (A.2).

Carrying out the linearization and the small signal analysis, as above, results in an expression that is similar to Equation (A.14) except that there is a separate term in the denominator for each different current, i.e.,

\[
Z(\omega, v) = \frac{1}{g_l + i\omega c_m + \sum_{n=1}^{N} g_{n, ch}(v) F_n(\omega, v)}
\]  

(A.16)

where \( g_{n, ch}(V) \) is the steady state conductance associated with each current and the form of each term, \( F_n(\omega, V) \), is given by
All of the quantities with subscripted "n"s are assumed to be evaluated at the steady state membrane potential. When $N = 2$, (A.16) is the impedance for a CM+SS model. Note from Equation A.17 that a current does not contribute to the impedance in a region where its steady state conductance, $g_{n, ch}$, is near zero.

**Models with an inactivating voltage-dependent current**

For a voltage-dependent current, $I_x$, possessing inactivation the expression for the current flowing through the channels is

$$i_x = g_x m_x s_{m,x} h_x s_{h,x} (v - v_x)$$

where $m_x$ is the activation variable, as before, and $0 < h_x < 1$ is the inactivation variable. The complete system, therefore, requires three state variables and is given by Equations (3.49) - (3.51) in Chapter 3. Linearizing these equations about an equilibrium point $(v_0, m_0, h_0)$ and expressing them in terms of the perturbation variables $V = v - v_0, M = m - m_0, H = h - h_0$ yields

$$c_m \frac{dV}{dt} = -g_t V - g_{x, ch_0} \left[ s_{x, m} m + s_{x, h} H + \frac{V}{(v_0 - v_x)} \right] (v_0 - v_x) + \delta I(t), \quad (A.19)$$

$$\frac{dM}{dt} = \frac{m_{x, o}' V - M}{\tau_{x, m_0}}, \quad (A.20)$$

$$\frac{dH}{dt} = \frac{h_{x, o}' V - H}{\tau_{x, h_0}}. \quad (A.21)$$

Just as in the derivation of the impedance for models with simple currents, assume that the solution of the system (A19) - (A.21) must have the same frequency as the input but with coefficients that need to be determined, i.e.,

$$V(t) = Z e^{j\omega t}, \quad (A.22)$$

$$M(t) = Y e^{j\omega t}, \quad (A.23)$$

and
Substituting (A.22) - (A.24) into the linearized system (A.19) - (A.21) and solving for $Z$ gives

$$j\omega c_m Z = -\bar{g}_t Z - g_{x,ch} \left( s_{x,m} \frac{Y}{m} + s_{x,m} \frac{X}{h_{x,o}} + \frac{Z}{(v_o - v_x)} \right) (v_o - v_x) + 1,$$

(A.25)

$$j \omega Y = \left( m_{x,o} Z - Y \right) / \tau_{x, m_o},$$

(A.26)

and

$$j \omega X = \left( h_{x,o} Z - X \right) / \tau_{x, h_o}.$$

(A.27)

Equations (A.26) and (A.27) can now be used to eliminate the variables $Y$ and $X$ from Equation (A.25) and thus determine the form of $Z$. This gives an expression for the impedance of the CM+I model,

$$Z(\omega, v) = \frac{1}{\bar{g}_t + j \omega c_m + g_{x,ch} \left( 1 + \frac{\hat{g}_{x,m}}{1 + j \omega \tau_{m_o}} + \frac{\hat{g}_{x,h}}{1 + j \omega \tau_{h_o}} \right)},$$

(A.28)

where

$$g_{x,m} = s_{x,m} \frac{m'_{x,o}}{m_{x,o}} (v - v_o)$$

(A.29)

and

$$g_{x,h} = s_{x,h} \frac{h'_{x,o}}{h_{x,o}} (v - v_o) .$$

(A.30)

For models with multiple currents, each inactivating current, $I_n$, contributes the product of its chord conductance and

$$F_n(\omega, v) = \left( 1 + \frac{\hat{g}_{n,m}(v)}{1 + j \omega \tau_{n,m_o}(v)} + \frac{\hat{g}_{n,h}(v)}{1 + j \omega \tau_{n,h_o}(v)} \right)$$

(A.31)

to the sum in the denominator of (A.16).

Models with ion-sensitive currents
The MH and MDT models of Chapter 6 both contain a Ca\(^{2+}\)-activated K\(^+\) current, \(I_C\). This requires an extension of the equations above because \(I_C\) acquires part of its voltage sensitivity indirectly from Ca\(^{2+}\) influx through the high threshold voltage gated Ca\(^{2+}\) channel, \(I_L\). The expressions for the currents due to \(I_C\) and \(I_L\) and the equation for Ca\(^{2+}\) buffering are given in Appendix B.

The derivation of the impedance is similar to the analysis above except that cross terms arise between \(I_L\) and \(I_C\). The contribution of \(I_L\) and \(I_C\) to the sum in the denominator of Equation (A.16) is

\[
g_{L,chn}F_L(\omega, V) + g_{C,chn}F_C(\omega, V) + g_{L,chn}g_{C,chn}\tau_{Ca} s_C \frac{m_C^*}{m_C} \frac{1}{1 + i\omega\tau_{Ca}} F_L(\omega, V) \tag{A.32}
\]

where \(m_C^*\) indicates the derivative of \(m_C\) is with respect to \([Ca^{2+}]_i\) rather than voltage.

**Impedance for the MDT model**

According to the derivations above, the impedance of the MDT model of Chapter 6 is

\[
Z(\omega, v) = \left\{ \bar{g} + j\omega \, c_m + \sum_{n=1}^{N} g_{n,chn}(v)F_n(\omega, v) + g_{L,chn}g_{C,chn}\tau_{Ca} s_C \frac{m_C^*}{m_C} \frac{1}{1 + i\omega\tau_{Ca}} F_L(\omega, v) \right\}^{-1}
\]

\[\text{(A.33)}\]

where \(n\) ranges over the currents in the MDT model as given in Chapter 6.
Appendix B

The MDT model of Chapter 6 uses the voltage-dependent currents \( I_T, I_L, I_A, I_{Ks}, I_C \), and a voltage-independent leak current \( I_L \). This Appendix gives the expressions and parameter values for the currents and for their steady state activation \((m_{\infty})\) and inactivation \((h_{\infty})\) functions and the corresponding time constants \((\tau_m\) and \(\tau_h\)) at 24°C. The total membrane capacitance is 400 pF corresponding to an membrane area of 40,000 \( \mu \text{m}^2 \) if a specific capacitance of 1 \( \mu \text{F/cm}^2 \) is assumed. The maximal permeabilities and conductances given below are followed by their corresponding specific values in parentheses. With the exceptions noted at the end of this Appendix, the parameter values used in the MDT model are the same as those used in the MH model after appropriate scalings for temperature and membrane area (McCormick and Huguenard 1992).

\( I_T \)

\[
I_T = \bar{g}_T(v) m_T^2 h_T \cdot (v - v_{Ca}),
\]

where \( \bar{g}_T(v) \) is given by Equation 6.4 with \( P_T = 0.05 \text{ cm}^2/\text{s} \) (for a specific permeability of 0.0125 cm/s), \([Ca^{2+}]_0 = 2\times10^{-3} \text{ M}, \; \xi = 1/13 \text{ mV}^{-1}, \) and \( F = 9.65\times10^4 \text{ Coul/mole}. \) \( [Ca^{2+}]_i \) is a variable described below and \( V_{Ca} \) is given by the Nernst relation, \( V_{Ca} = \frac{1}{\xi} \ln \left( [Ca^{2+}]_o / [Ca^{2+}]_i \right) \).

\[
m_{\infty_T}(v) = \left(1 + \exp \left( \left( v - v_{mT}/2 \right) / k_{mT} \right) \right)^{-1},
\]

\[
h_{\infty_T}(v) = \left(1 + \exp \left( \left( v - v_{hT}/2 \right) / k_{mT} \right) \right)^{-1},
\]

with \( v_{mT}/2 = -62 \text{ mV}, \; v_{hT}/2 = -84 \text{ mV}, \; k_{mT} = -6.2 \text{ mV}^{-1}, \) and \( k_{hT} = 4 \text{ mV}^{-1} \).

\[
\tau_{mT}(v) = \left( \exp((v + 132)/-16.7) + \exp((v + 16.8)/18.2) \right)^{-1} + 0.612,
\]

\[
\tau_{hT}(v) = \begin{cases} 
\exp((v + 467)/66.6) & \text{for } v < -80 \text{ mV} \\
\exp((v + 22)/-10.5) + 28 & \text{for } v \geq -80 \text{ mV}
\end{cases}
\]

\( I_L \)

\[
I_L = \bar{g}_L(v) m_L^2 \cdot (v - v_L),
\]
where \( \bar{g}_L(v) \) is given by (6.4) with the same parameters as for \( I_T \) except that \( P_L = 2P_T = 0.1 \times 10^{-6} \) cm\(^3\)/s (0.025\(\times\)10\(^{-2}\) cm/s).

\[
m_{\infty L}(v) = \alpha_L(V)/(\alpha_L(v) + \beta_L(v)), \tag{B.7}
\]

\[
\tau_{\infty L}(v) = 1/(\alpha_L(v) + \beta_L(v)), \tag{B.8}
\]

with

\[
\alpha_L(v) = 1.6/(1 + \exp(-0.072(v - 5))), \tag{B.9}
\]

\[
\beta_L(v) = 0.02(v - 1.31)/(\exp((v - 1.3)/5.36) - 1). \tag{B.10}
\]

\[
I_A = \bar{g}_A \cdot (0.6m_{A1}^4 h_{A1} + 0.4m_{A2}^4 h_{A2})(v - v_K), \tag{B.11}
\]

with \( \bar{g}_A = 1.33 \mu S \) (33 pS/\(\mu m^2\) or 300 \(\Omega/cm^2\)) and \( V_K = -105 \) mV.

\[
m_{\infty A_i}(v) = \left(1 + \exp\left((v - v_{m_{A_i}/2})/k_{m_{A_i}}\right)\right)^{-1}, \quad i = 1, 2 ; \tag{B.12}
\]

where \( v_{m_{A1}/2} = -60 \) mV, \( v_{m_{A2}/2} = -36 \) mV, \( k_{m_{A1}} = -8.5 \) mV, and \( k_{m_{A2}} = -20 \) mV.

\[
h_{\infty A}(v) = \left(1 + \exp\left((v - v_{h_{A}/2})/k_{h_A}\right)\right)^{-1}, \tag{B.13}
\]

with \( v_{h_A/2} = -78 \) mV and \( k_{h_A} = 6 \) mV\(^{-1}\).

\[
\tau_{m_{A}}(v) = \left(\exp((v - 35.8)/19.7) + \exp((v - 79.7)/-12.7)\right)^{-1} + 0.37, \tag{B.14}
\]

\[
\tau_{h_{A1}}(v) = \left(\exp((v + 46)/5) + \exp((v + 238)/-37.5)\right)^{-1} \text{ for } v < -63 \text{ mV}, \tag{B.15}
\]

\[
\tau_{h_{A2}}(v) = \left(\exp((v + 46)/5) + \exp((v + 238)/-37.5)\right)^{-1} \text{ for } v < -73 \text{ mV}, \tag{B.16}
\]

otherwise \( \tau_{h_{A1}} = 19 \) ms.

\[
\tau_{h_{A2}} = 60 \text{ ms} \tag{B.17}
\]

\[
I_{Kx} = \bar{g}_{Kx} m_{Kx}(0.4h_{Kx1} + 0.6h_{Kx2})(v - v_K), \tag{B.17}
\]
with $\bar{g}_{K_{x}} = 1.3 \mu S$ (33 pS/\mu m$^2$ or 300 \Omega/cm$^2$).

$$m_{\infty_{K_{x}}}(v) = \left(1 + \exp\left(\frac{(v - v_{m_{K_{x}}}/2)}{k_{m_{K_{x}}}}\right)\right)^{-4},$$  \hspace{1cm} (B.18)

$$\tau_{m_{K_{x}}}(v) = 0.1\left(\exp((v - 81)/25.6) + \exp((v + 132)/-18)\right)^{-1} + 9.9,$$  \hspace{1cm} (B.19)

where $v_{m_{K_{x}}}/2 = -52$ mV and $k_{m_{K_{x}}} = -8$ mV.

$$h_{\infty_{K_{x}}} (v) = \left(1 + \exp\left(\frac{(v - v_{h_{K_{x}}}/2)}{k_{h_{K_{x}}}}\right)\right)^{-1}, \hspace{1cm} i = 1, 2$$  \hspace{1cm} (B.20)

where $v_{h_{K_{x}}}/2 = -58$ mV and $k_{h_{K_{x}}} = 10.6$ mV.

$$\tau_{h_{K_{x}}} (v) = \left(\exp((v - 1329)/200) + \exp((v + 130)/-7.1)\right)^{-1} + 120.$$  \hspace{1cm} (B.21)

The second time constant, $\tau_{h_{K_{x2}}}$, is the same as $\tau_{h_{K_{x1}}}$ for $v < -70$ mV, and

$$\tau_{h_{K_{x2}}} = 8.9\ \text{s for } v \geq -70\ \text{mV}.$$

$I_C$

$$I_C = \bar{g}_C m_C (v - v_K),$$  \hspace{1cm} (B.22)

where $\bar{g}_C = 1.3\ \mu S$ (33 pS/\mu m$^2$ or 300 \Omega/cm$^2$).

$$m_{\infty_{C}}(v) = \frac{\alpha_C(v)}{(\alpha_C(v) + \beta_C(v))},$$  \hspace{1cm} (B.23)

$$\tau_{\infty_{C}}(v) = \frac{1}{(\alpha_C(v) + \beta_C(v))},$$  \hspace{1cm} (B.24)

with

$$\alpha_C(v) = 2.5 \times 10^5 [Ca^{2+}]_i e^{v/24},$$  \hspace{1cm} (B.25)

$$\beta_C(v) = 0.1 e^{-v/24}.$$  \hspace{1cm} (B.26)

$[Ca^{2+}]_i$ buffering

$$d[Ca^{2+}]_i/dt = -\left([Ca^{2+}]_i - \phi\right)/\tau_{Ca} + \zeta I_L,$$  \hspace{1cm} (B.27)

where $\tau_{Ca} = 1$ ms is the Ca$^{2+}$ buffering time constant, $\phi = 5 \times 10^{-8}$ M is the minimal $[Ca^{2+}]_i$ value, and $\zeta = -1.29 \times 10^{-6}$ converts Ca$^{2+}$ currents into changes in $[Ca^{2+}]_i$ in a shell 100 nm below a
membrane of surface area 40,000 μm². Following a report by Jahnsen and Llinas (1984), \(I_T\) is assumed not to contribute to \([Ca^{2+}]_i\).

**\(I_l\)**

The MH model uses separate \(Na^+\)-dependent and \(K^+\)-dependent leak conductances, \(g_{Na\text{leak}}\) and \(g_{K\text{leak}}\), respectively. The MDT model lumps these voltage-independent conductances into a single current,

\[
I_l = \bar{g}_l \cdot (v - v_l), \tag{B.28}
\]

with \(\bar{g}_l = 0.016 \mu S\ (0.4 \text{ pS}/\mu \text{m}^2\ or\ 25000 \text{ \Omega/cm}^2\) and \(v_l = -63\ \text{mV}\).

**Differences between the MDT model and the reduced MH model**

The differences between the MDT model and the reduced MH model lie in the parameters for \(I_{Kx}\) activation (corresponding to \(I_{K2}\) in the reduced MH model), the maximal permeability for \(I_T\), and the maximal conductance for the voltage-independent leak current, \(\bar{g}_l\) (corresponds to \(g_{Na\text{leak}} + g_{K\text{leak}}\) in the reduced MH model).

We altered the activation properties of \(I_{K2}\) to produce \(I_{Kx}\) by decreasing its time constant by a factor of 10, shifting the steady state activation curve by -9 mV, and changing the slope factor, \(k_m\), from -17 to -8 mV.

The \(P_T\) for the MDT model is 1/1000th of the \(P_T\) for the MH model. See Methods section.

The summed maximal conductance of voltage-independent currents in the MH model is 0.028 μS (calculated for 34°C and an input capacitance of 400 pF). The corresponding leak conductance, \(g_l\), for the MDT model is 0.016 μS.