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MODELING OF SINGLE CHANNEL AND WHOLE CELL CURRENT MEASUREMENTS WITH APPLICATION TO Ca CHANNELS

by

DAVID WILSON

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

DOCTOR OF PHILOSOPHY

APPROVED, THESIS COMMITTEE:

Dr. John W. Clark
Professor of Electrical Engineering

Dr. Don H. Johnson
Associate Professor of Electrical Engineering

Dr. Raymon Glantz
Professor of Biology

Houston, Texas
May, 1985
MODELING OF SINGLE CHANNEL AND WHOLE CELL CURRENT MEASUREMENTS
WITH APPLICATION TO Ca CHANNELS
by David L. Wilson

ABSTRACT

Modern measurements of membrane, ionic channel molecules consist
of whole cell current relaxations obtained using various voltage pulse
protocols, current noise power spectra from small numbers of channels,
and single channel intervals in the form of waiting, closed, and open
time histograms. A state-variable description was developed for
predicting these measurements from Markovian state models, and it was
used to analyze the parameter identifiability properties of the models
and measurements. Computer programs were developed for estimating
model parameters; these included maximum likelihood estimation for the
single channel data. In the case of the Ca channel, the parameters
from a variety of models were found to be faster when obtained from
whole cell rather than single channel measurements. The latter
measurements were low pass filtered at 1 kHz in order to reliably
detect the ~1 picoamp openings; the errors that resulted were eluci-
dated by running Monte Carlo simulated channels through the threshold
detection algorithm. Intervals were distorted by the absence of brief
open and closed times as well as falsely prolonged open and closed
times. An analytical technique was developed for computing the
resulting distorted histograms, and after correction there was
reasonable agreement between the measurements. Other errors found
using single channel simulations included the appearance of a bimodal
amplitude distribution from filtered channels having a single
amplitude and the effects of noise and finite record length recording. Of six models considered for describing activation, a 4-state model best fit the whole cell relaxations resulting from simple, short pulse protocols, and it also predicted results from 2-pulse protocols and temperature experiments. Some inactivation models were eliminated because they were unable to simulate recovery from inactivation and single channel failure traces. A family of models was suggested that describes the present experimental results, and possible tests for future model discrimination were evaluated using simulation. Two relatively insensitive tests were the 2-pulse test for "coupled" inactivation and the single channel Hi-Lo sort method for determining Ca-accumulation-dependent processes. It is concluded that the combination of parameter estimation and simulation is a useful tool for interpreting results and helping to plan experiments and analyses.
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Most importantly, I wish to thank my wife, Jane, for her understanding and my son, Stephen, for the joy he gives. I also wish to thank my mother and father for instilling the desire for knowledge that brought me here.
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Chapter 1: Introduction

1.1 Definition of the problem

Membrane Ca channels are large protein molecules which are gated to an open state by depolarization and in the open state conduct ions selectively. As with other ionic channels, the processes of gating and selective conduction may be studied by recording either the macroscopic currents from an entire cell or by recording microscopic currents from a single channel or a small population of channels. These methods and some of the terminology used are given in Section 1.3 and Fig. 1.1. It should be noted that single channel measurements allow one to study the activity of a single macromolecule. Perhaps no other system in chemistry or physics can be studied in such detail. Thus a significant problem concerns how one may use these measurements to understand better the Ca channel. I have used various theoretical analyses to attack this problem and some examples follow below.

The first example has to do with a comparison of the macroscopic and microscopic measurements. On one hand, the signal/noise ratio of the gigaseal patch clamp method (Hamill et al. 1981 and Fig. 1.1) is sufficiently small that recordings must be made at a bandwidth of \( \approx \) 1 kHz or less. On the other hand, whole cell currents, studied at a much higher bandwidth, give very rapid relaxations following voltage-step perturbations; a significant portion of the change in current occurs in the first 200 \( \mu \)sec following a step. As one might anticipate, theoretical analyses in Chapter 4 indicate that the single channel interval measurements suffer from a lack of kinetic resolution. Only after accounting for the effects of filtering did the single channel measurements agree with the macroscopic
Fig. 1.1
Methods and measurements used in studying Ca channels.

A) Shown is the scheme for voltage clamping an isolated small neuron using a suction pipette for passing current and internal perfusion and a microelectrode for voltage measurements. The whole cell current, $I$, is collected from an electrode in the bath.

B and C) Two voltage clamp protocols are shown along with the typical currents obtained. Activation may be studied using short, 5 ms pulses; turn-on results from the positive voltage step, or depolarization, and turn-off from repolarization. Inactivation, or the slow decay of the current, is much slower and can be ignored for short pulses.

D) Single channel currents were recorded using a patch electrode which forms a tight seal to a small patch of membrane. The cell interior was voltage clamped using a conventional 2-microelectrode voltage clamp.

E) Channel openings occur randomly with each depolarization, but by averaging an ensemble of many single channel records together an average current, $\langle I \rangle$, is obtained that has the same characteristics as the whole cell current. Thus the whole cell current is thought to result from a group of $\sim 1.5 \times 10^5$ channels with homogeneous properties.

F) Waiting, closed, and open, time intervals (or WCO intervals) are identified.
measurements. Another example has to do with a particular analysis that has been suggested as a means of discriminating a Ca-accumulation-dependent process for inactivation of the Ca channel (Brown and Lux, 1984). Single channel simulations of a model of this type (Chapter 4) indicated that this test may not be sufficiently sensitive for practical discrimination.

The foregoing examples illustrate how I have tried to compare the information obtained from the macroscopic and microscopic measurements and to use such information to test various models. This work should help identify the measurements and analyses required for future work in understanding Ca channels.

1.2 Why study Ca channels?

First, the importance of studying the macroscopic Ca current, \( I_{Ca} \), will be established then I will turn to the importance of a molecular description. \( I_{Ca} \) has been found in a variety of tissues including skeletal muscle, smooth muscle, cardiac tissue, nerve cells, secretory cells, various receptor cells, and egg cells. In fact, Ca currents may be more universal than Na currents (Hagiwara and Byerly, 1981). Unlike Na currents which appear to be important only in the production of action potentials, \( I_{Ca} \) (or more correctly the accumulation of Ca ions) acts as a trigger or modifier of various cellular functions. These include contraction of muscle, secretion of various substances such as transmitters at nerve terminal endings or hormones, regulation of K conductance (Meech, 1974; Akaike et al., 1983), regulation of \( I_{Ca} \) (Chad, Eckert, and Ewald, 1984; Brown et al., 1981), and perhaps other intracellular functions such as axonal transport as discussed by Kostyuk (1980). Yet another role of Ca may
be the role it plays in "learning" in some simple neuronal systems (Alkon, 1984). In order to understand such processes fully, one must be able to describe $I_{Ca}$ in a quantitative manner.

In addition to a description of macroscopic $I_{Ca}$, it is important to study the molecular nature of the current as argued below. First, the basic physics of the molecule is of interest. In the case of single channel recordings, the conformational history of a single, channel molecule can be studied over an hour time-period. As stated previously, such detailed measurements are probably unique to membrane channel molecules. A second reason is to identify the particular mechanisms of drug action. One study on the β-receptor in heart indicated an increase in the number of channels (Tsien et al., 1983) and another study indicated an increased probability of channel opening (Trautwein et al., 1983). The Ca agonist dihydropyridine drugs have also been studied in this manner (Brown, Kunze and Yatani, 1984; Hess, Lansman and Tsien, 1984). Considering that there are some 7000 dihydropyridine compounds, it is important that a rational approach to understanding their molecular action is developed. A third reason to study the molecular nature of the Ca current is that an electrical, molecular description will complement future studies of the biochemistry of the channel molecule. Several groups are trying to isolate Ca channels and reconstitute them into artificial bilayers. Also the techniques of genetic engineering will come into play as they have for the ACh and Na channels (Kuno et al., 1984). In the future one may be able to build Ca channels with alterations in specific portions of the channel molecule. Such modifications may have specific effects on gating or specificity, etc.
1.3 Measurement techniques

The measurement techniques used by my collaborators and myself for studying Ca currents in snail (Helix Aspersia) neurons are briefly reviewed below. These methods are representative of those presently being used by others. I will first describe the method for measuring whole cell currents and then describe the method for measuring single channels. These methods are found in greater detail in Brown et al. (1983) and Lux and Brown (1984).

Snail neuron cell bodies from the circumoesophageal ganglia of the snail were isolated by mechanical dissection. These cells have a single axo-dendritic process which retracts after it is cut leaving a very spherical cell body with a diameter of \( \sim 100 \mu \). Voltage clamp was achieved with a 2-electrode voltage clamp consisting of a suction pipette for passing current and internal perfusion and a microelectrode for voltage measurement (see Fig. 1.1 and Fig. 1 of Brown et al., 1983). An analog circuit was used to provide a feedback arrangement in which the voltage sensed by the microelectrode was compared to the command potential (Fig. 1.1). Several features of this system were included for fast voltage clamping. Low internal resistance microelectrodes were used, and they were painted with a conductive silver coat which was driven with a capacitance neutralization circuit. The clamp amplifier was constructed from a high frequency op-amp (Teledyne-Philbrick 1030) having a gain-bandwidth product of 100 Mhz; this allowed stable operation at a relatively high bandwidth (Fishman, 1982).

Ca currents were isolated from other ionic currents by substituting non-conducting ions and adding pharmacological blockers, both
intra- and extra-cellularly. The intracellular administration was done by exchanging the solution in the suction pipette. This resulted in a dialysis of the cell interior through the relatively large tip of the suction pipette. In addition to the currents of interest, this procedure left a linear leakage current, an asymmetry, or gating, current, and a small, variable-sized, non-specific current activated at potential >+20mV (Brown et al., 1981 and 1983). The linear leakage current was removed by summing the current from each depolarizing pulse with an equivalent pulse in the hyperpolarizing direction. The asymmetry and non-specific currents were removed by subtracting records obtained after substituting Co for Ca. The remaining current was sensitive to Ca blockers, increased and decreased in amplitude with changes in [Ca]o, and thus was called Ca current. However, in some cells there were indications from tail current measurements that there was yet another contaminating current (see Brown et al., 1983). A recent report suggests that this may be a relatively small H ion current (Byerly and Meech, 1984).

Considerable time was spent studying Ca tail currents which were relatively large and fast and thus stressed the voltage clamp system. Therefore, several possible errors in voltage clamping were examined and are briefly reviewed here. The voltage clamp step was virtually complete in ~30 μsec and the capacitive transient was linear and ~95% complete within 60 μsec. The spatial control was examined with a second, painted microelectrode outside the voltage clamp circuit. It was found that the voltage difference between these two microelectrodes was ~3mV or less within 30 μsec. The series resistance, Rs, of the cell was measured directly using a pseudo-
random noise source as described elsewhere (Tsuda, Wilson and Brown, 1982; Brown et al., 1983; and Brown, Wilson and Tsuda, 1985). $R_s$ was $\leq 5k\Omega$ indicating that the voltage error was $\leq .5mV$ for a peak Ca current of 100 nA. These results indicated that the clamp was quick; the cell was held isopotentially; and the voltage drop across $R_s$ was negligible after 60 $\mu$sec.

Single channel measurements were done using the method developed by D. Lux (Lux and Brown, 1984) which is diagramed in Fig. 1.1. Voltage clamping was done with two low resistance microelectrodes. Single channel currents were measured with a conventional patch clamp system (Hamil et al., 1981). An advantage of this method is that the intracellular voltage is clamped which is not the case in most single channel measurements. Additionally, whole cell currents could be measured from the bath simultaneously. To insure that Ca channels were recorded, Ca or Ba was the only conducting anion in the patch electrode and pharmacological blockers were added as well.

1.4 Review of the pertinent measurements

The kinetic features of $I_{Ca}$ span the time-scale from $\sim 100$ $\mu$sec to $\sim 10$ sec. In order to facilitate discussion, these features are separated into activation and inactivation characteristics (Fig. 1.1). Activation has to do with the rapid turning on or off of inward current with depolarization or repolarization, respectively. Inactivation characteristics have to do with the slow decrease in current during prolonged depolarization and the subsequent slow recovery of the current when it is repolarized. From a practical standpoint the experiments may be labeled as being designed for studying either activation or inactivation. In most cases, a single cell did not
survive long enough to obtain a full complement of both kinds of measurements. Also, the requirements for the two measurement types were different. For example, relatively low resistance electrodes and small cells were used when studying the fast activation properties whereas larger cells and higher resistance electrodes were used when it was desired to maintain a cell for the long period of time required for a complete set of inactivation protocols. Measurements of $I_{Ca}$ activation and inactivation and their interpretation are discussed in turn below.

At least three mechanisms for Ca current inactivation have been suggested. First, inactivation proceeds at a rate dependent only upon voltage, in the same manner as $I_{Na}$ (Kostyuk, Krishtal and Shakhovlov, 1977; Llinas, Steinberg and Walton, 1981). Second, inactivation results from the action of Ca ions at an internal site; thus an accumulation of Ca ions intracellularly reduces the current. Several lines of evidence have suggested this latter theory including changes in inactivation by intracellular administration of EGTA, the amount of inactivation mirroring the peak Ca current-voltage profile, and the amount of inactivation changing as a function of $[Ca]_o$ (representative reports include Brehm, Eckerdt, and Tillotson, 1980; Plant and Standen, 1981). Such results have given rise to what may be called Ca-accumulation-dependent models (Chad et al., 1984; Standen and Stanfield, 1982). Previously we found less evidence for a strict Ca-accumulation-type process and interpreted the results as being due to both a voltage-dependent and a Ca-dependent process (Brown et al., 1981). A third mechanism suggested in skeletal muscle has to do with the accumulation or depletion of Ca ions causing a change in driving
force which results in a decreased $I_{Ca}$ (Almers, Fink and Palade, 1981). Recent single channel measurements have increased our ability to identify the mechanisms involved (Brown, Lux and Wilson, 1984; Brown and Lux, 1984). For example, the third mechanism above may be dismissed for snail neurons on the basis of single channel measurements in which inactivation occurred in the averaged patch records without a change in single channel current. Also Brown and Lux applied some single channel analyses that indicated little involvement of a Ca-accumulation-dependent process (1984).

There have been several recent measurements of macroscopic $I_{Ca}$ activation kinetics (Brown, Tsuda and Wilson, 1983; Marty, Fenwick and Neher, 1982; Byerly and Hagiwara, 1982; Eckardt and Ewald, 1983; Byerly, Chase and Stimers, 1984). In all of these reports, turn-off of the tail currents is shown to occur quite quickly with at least a two-exponential relaxation indicating that at least three states are required. Turn-on exhibits a phasic delay which is also indicative of a multiple-state kinetic scheme. All of the above found discrepancies with a Hodgkin-Huxley type scheme for Ca current activation, i.e., $m^n$ ($n = 1, 2, \ldots$). Marty, Fenwick and Neher (1983) suggested a sequential 3-state model of activation, but Brown, et al. (1983) pointed out certain discrepancies with such a model and suggested a 4-state model as the minimum, linear Markovian model to account for their results. Byerly et al. (1984) supported the latter conclusion.

There have also been several reports of the microscopic characteristics of $I_{Ca}$ from noise and single channel measurements including Fenwick et al. (1982), Hagiwara and Ohmori (1983), Lux and Brown (1984), and Brown, Lux and Wilson (1984). For the most part, these
microscopic measurements reflect the faster "activation" properties of $I_{\text{Ca}}$ rather than the much slower inactivation characteristics.

1.5 My approach to the problem

I have investigated various models for Ca current activation and inactivation using computer simulation and parameter estimation techniques. One objective has been to correlate the macroscopic and single channel measurements and investigate the error involved with the latter method due to bandwidth limitations. Another objective has been to suggest practical tests for model discrimination using the full array of experimental protocols available. The models consider the channel molecule to exist in several different conformations, or states. Transitions between states are random and assumed to be Markovian, thus allowing the use of considerable theoretical analyses previously developed (see next chapter for a historical account).

With regard to the activation properties of $I_{\text{Ca}}$, some six different models were tested including a Hodgkin-Huxley-type $n^2$ model (Hodgkin and Huxley, 1952), various linear 3- and 4-state models, and the non-linear model of Bauman (1981). Identifiability, or the ability to obtain unique rate constants, was analyzed where appropriate. Parameter estimates were obtained from whole cell current relaxations following voltage-step perturbations. These parameters were then compared to single channel measurements using single channel Monte Carlo simulations and analytical techniques. A new method was developed that allowed the computation of the effects of the bandwidth limitations on single channel detection. The latter method assumed a detection system dead time after a recent suggestion (Colquhoun and
Sigworth, 1983). It was found that significant loss of kinetic resolution occurs using the present single channel methodology.

Six different models of $I_{Ca}$ inactivation were also tested. Again the basic approach was to obtain parameter estimates and then to simulate other results to see what may be learned. We found that some techniques previously suggested for model discrimination are not as sensitive as one might hope. This should help eliminate the pursuit of some not so fruitful analyses. A group of models is suggested that will describe most of the inactivation characteristics.
Chapter 2: Theoretical Analyses

2.1 Introduction to state models

To introduce the theoretical work that follows, I will give a brief overview of how channel modeling has evolved in the past few years and point out some of the assumptions involved. The channels are modeled as existing in different conformations, or states, which either conduct or do not conduct ions. Transitions between states are assumed to be Markovian which makes the problem amendable to considerable theoretical analyses. One justification for the Markovian assertion, is the applicability of state transition, or Eyring, theory to many chemical kinetic systems (Espenson, 1981; Colquohoun and Hawkes, 1983). The other justification is that most of the waiting, closed, and open time (WCO) PDFs are well described by sum-of-exponentials-type functions. Fitzhugh was the first to describe the Hodgkin-Huxley currents in terms of a state model in 1965. With the advent of measurements of membrane noise several theoretical analyses appeared including the ones by Stevens (1972), Chen (1973), and Colquohoun and Hawkes (1977). The work of Colquohoun and Hawkes included a description of the WCO PDFs in 1981 at just about the same time that reliable single channel recordings were beginning (Hamil et al., 1981). Several papers concerned with analyzing single channel intervals have followed including Colquohoun and Hawkes (1982) and (1983), and Horn and Lange (1983). In addition to the Markovian assumption is the assertion that the channels behave independently. This seems reasonable on the basis of the channel density \( \sim 3 \) per \( \mu^2 \) for the case of Ca channels (Lux and Brown,
1984). In addition, independence of Na channels was indicated by the binomial analysis of Patlack and Horn (1983).

In this chapter, a state-variable representation of a simple state model is given and used to develop the WCO PDFs of interest, the macroscopic relaxations, and the noise spectra. A diagramatic representation of these equations is also given. To my knowledge no similar development has appeared in the literature. The specific models used in this analysis are then given. This is followed by some other topics including the identifiability properties of the models and an alternative to the above description as given by Colquhoun and Hawkes (1982). The last section concerns how to modify the WCO PDFs to account for a detection system with limited interval resolution.

2.2 The mathematical description of Markovian state models.

A state-variable representation will be developed using, as an example, a three-state model (M$^2$ in Fig. 2.2 on page xx). In this diagram R, A, and O, refer to the "rest", "activated", and "open" states of a channel. State O is the only conducting state; channels initiate from the R state; and state A is an electrically silent intermediate. The following notation is adopted to describe this same model

\[
\begin{align*}
  & \text{(1)} \quad \text{(2)} \quad \text{(3)} \\
  & \begin{array}{c}
    \text{k} \\
    12
  \end{array} \quad \begin{array}{c}
    \text{k} \\
    23
  \end{array} \quad \begin{array}{c}
    \text{k} \\
    21
  \end{array} \quad \begin{array}{c}
    \text{k} \\
    32
  \end{array}
\end{align*}
\]

where R, A, and O have been replaced by states 1, 2, and 3 and the k's are rate constants describing the rates of transitions between states. Since a single channel must be in one of the three states and may
change from one state to another at any instant of time, this is called a Markovian process with discrete states in continuous time (Cox and Miller, 1965).

2.2.1 Development of the state-variable description.

The differential equations describing the probability of a channel being in each of the states may be written in state-variable form which is

\[ \dot{X} = A \dot{X} + B \dot{U} \]
\[ Y = C X. \]

where $X$ is a column vector of $n$ state variables; $A$ is a $n \times n$ matrix and is a function of the rate constants as described later; the product $BU$ describes inputs which drive the system and are not included in the problem at hand; and $Y$ is the output vector which is a linear combination of the state variables. From the outset we should point out that we use "state" to refer to two entirely different things. The term "state" is used in the biophysics and chemical kinetics literature to refer to a particular molecular conformation, whereas system engineers use "state" to refer to the variables in the vector $X$ in equation 2.2.

There are three rules to follow when one writes the equations describing a homogeneous Markovian process:

1. The lifetime in each state is a memoryless, random variable; that is, transitions to states in the future do not depend upon the past history of the channel, only upon the present state of the channel.

2. The probability of more than one transition in an infinitesimal time, $d\tau$, is of higher order and may be neglected.
3. Transition rates are described by constants that have dimensions of time$^{-1}$.

The probability of being in state 1 at \( t+\Delta t \) is denoted \( P_1(t+\Delta t) \), and this may occur in one of two ways:

a) The channel is in state 1 at time \( t \) and remains in this state at time \( t+\Delta t \). This probability is given by
\[
P_1(t) - k_{12} \Delta t P_1(t),
\]
where the latter term describes the probability of leaving state 1 to state 2. The channel may not leave to state 3 because of assertion 2 above.

Assertion 1 is also obeyed; the rate of the probability of leaving depends only upon \( P_1(t) \).

b) The channel enters state 1 from state 2; the probability of this occurring is \( P_2(t)k_{21}\Delta t \).

We thus obtain
\[
P_1(t+\Delta t) = P_1(t)(1-k_{12}\Delta t) + P_2(t)k_{21}\Delta t.
\]
\[\text{Equation 2.3}\]

Similarly, the probabilities of being in states 2 or 3 at \( t+\Delta t \) are expressed as:
\[
P_2(t+\Delta t) = P_2(t)[1 - k_{21}\Delta t - k_{23}\Delta t] + k_{12}\Delta t P_1(t) + k_{32}\Delta t P_3(t)
\]
\[\text{Equation 2.4}\]
\[
P_3(t+\Delta t) = P_3(t)[1 - k_{32}\Delta t] + k_{23}\Delta t P_2(t)
\]
\[\text{Equation 2.5}\]

Equations 2.3 - 2.5 may be rearranged as follows:
\[
\frac{P_1(t+\Delta t) - P_1(t)}{\Delta t} = -k_{12}P_1(t)+k_{21}P_2(t)
\]
\[\text{Equation 2.6}\]
\[
\frac{P_2(t+\Delta t) - P_2(t)}{\Delta t} = k_{21}P_1(t) - (k_{21}+k_{23})P_2(t)+k_{32}P_3(t)
\]
\[\text{Equation 2.7}\]
\[
\frac{P_3(t+\Delta t) - P_3(t)}{\Delta t} = k_{23}P_2(t) - k_{32}P_3(t)
\]
\[\text{Equation 2.8}\]
Taking the limit as $\Delta t$ approaches zero, and writing the system of equations in matrix form one obtains

\[
\begin{bmatrix}
P_1(t) \\
- k_{12} & k_{21} & 0 \\
P_2(t) \\
k_{12} - (k_{21} + k_{23}) & k_{32} \\
P_3(t) \\
0 & k_{23} & -k_{32}
\end{bmatrix}
\begin{bmatrix}
P_1(t) \\
P_2(t) \\
P_3(t)
\end{bmatrix}
= 2.7
\]

The model output probability is

\[
P_3(t) = U_3 P(t) = [0 \ 0 \ 1]
\begin{bmatrix}
P_1(t) \\
P_2(t) \\
P_3(t)
\end{bmatrix}
= 2.8
\]

where $U_3$ is a row vector with all elements zero except a 1 in the 3rd element. In general, the output equation would be formed using $U_j$. Equations 2.7 and 2.8 are in the state-variable form [2.2] with the three state variables being defined as the probability of being in the respective states; the output is scalar and is simply one of the state variables, $P_3(t)$, and there is no driven input. Initial conditions for the system may be written in vector form as $P(0) = [P_1(0) \ P_2(0) \ P_3(0)]^T$. Although equation 2.7 consists of a set of three linear differential equations, it is only a second order system. In general, for such state models, one of the eigenvalues of $A$ must equal zero and the order of the system is the number of states minus one (Cobelli and Distefano, 1980).

Note that equations 2.7 and 2.8 are identical in form to the equations describing a compartmental model which has been connected in the same manner. In the case of a compartmental model, however, the probabilities would be replaced by the amount of material in a
compartment and the transition rate constants would be replaced by rate constants describing flux transfer between compartments. The state variable description has been used in a stochastic analysis of compartmental systems (Eisenfeld, 1977, 1980) and in a description of reliability theory (Shooman, 1968).

2.2.2 **Macroscopic currents.**

Macroscopic current predictions are obtained as follows. We define \( \mathbf{P}_3(t; \mathbf{P}_1(0)=1) \) as the probability of being in state 3 given that the channel initiated in state 1, or the "rest" state; this would be the probability desired for a "turn-on" current measurement. Macroscopic currents are obtained from a collection of many identical channels; thus the probabilistic variable, \( \mathbf{P}_3(t) \), becomes a deterministic variable \( \mathbf{I}(t) \), the current, given by

\[
\mathbf{I}(t) = N \mathbf{i} \mathbf{P}_3(t)
\]

where \( i \) is the single channel current and \( N \) is the number of channels. To find the turn-on current we obtain \( \mathbf{P}_3(t; \mathbf{P}_1(0)=1) \) from equations 2.7 and 2.8 subject to \( \mathbf{P}(0)=[1 \ 0 \ 0] \) and substitute the result into equation 2.9. Other macroscopic current predictions from other initial conditions are obtained using the appropriate \( \mathbf{P}(0) \).

2.2.3 **Fluctuation spectra.**

The kinetic properties of noise or fluctuation measurements may also be predicted. Such measurements are obtained from whole cell current or from large patches and consist of fluctuations due to channel opening and closing superimposed on a mean current. The kinetic properties of the noise are measured by taking the autocovariance of the noise, or more frequently, the power density spectrum. This is normally done under conditions of stationarity (however, see
Sigworth, 1981); thus the measurement is made across a time record and the mean value of the current is a constant given by \( I(\infty) = N_i P_0(\infty) \) where \( P_0(\infty) \) is the probability of being in the open state as time approaches infinity. The autocovariance for channel models such as we describe has been derived in several different places, including Stevens (1972), Chen (1973) and Colquhoun and Hawkes (1978). The result is

\[
C(\tau) = N_i^2 \text{Prob(state O at } t = \tau \mid \text{state O at } t=0) P_O(\infty) - N_i^2 P_O^2(\infty) \tag{2.10}
\]

where O is the open state, \( P_O(\infty) \) is the steady-state occupancy of the open state, N and i were defined earlier, and \( \tau \) represents time in the computed autocovariance function. The conditional probability in the above expression is read "the probability that a channel is in state O at time=\( \tau \) given that it started in state O initially"; in the notation adopted previously this is simply \( P_O(\tau; P_O(0)=1) \). Note that none of the states are made absorbing in this calculation as will be the case for the single channel predictions that follow. For the 3-state model described above one obtains

\[
C(\tau) = N_i^2 P_3(\tau; P_3(0)=1) P_3(\infty) - N_i^2 P_3^2(\infty) \tag{2.11}
\]

The power density spectrum of a time record with the mean subtracted is the Fourier transform of the autocovariance function (Peebles 1976). In general, equation 2.10 will consist of a sum of exponential terms. The Fourier transform of an exponential is a Lorentzian, and the power spectral density will thus be a sum of Lorentzian terms (Stevens, 1972; Chen, 1973; and Colquhoun and Hawkes, 1978). The autocovariance is used to determine the identifiability properties of the fluctuation measurements (Appendix A).
2.2.4 **Single channel WCO PDFs (ideal).**

Now consider the waiting time distribution or the distribution of times that it takes for the first opening of a channel when it initiates from "rest". For the example at hand, this is written as a conditional distribution

\[ P_{13}(t) = \text{Prob}(\text{reach state 3 at time } \leq t | \text{start in 1 at time}=0) \]

which is read "the probability that a channel reaches state 3 at time \( t \) given that it starts in state 1 at time zero". \( P_3(t) \) in equation 2.7 depends on the time required for the channel to first reach state 3 as well as the life-time and reentry-time for state 3. At present, only the former case is of interest and the other possibilities are removed by making state 3 "absorbing"; that is, the rate constant for leaving state 3 is set to zero (i.e. \( k_{32} = 0 \)). In terms of the state variable representation, \( P_{13}(t) \) is obtained by solving for \( P_3(t) \) subject to the initial condition \( P(0) = [1, 0, 0]^T \) with the assertion that state 3 is "absorbing".

Now consider the closed-time distribution. Immediately after a closure the channel resides in state 2. The probability of the channel reopening in time \( t \) is thus given by \( P_{23}(t) \). This conditional probability is found by once again solving for \( P_3(t) \), subject to the new initial condition vector \( P(0) = [0, 1, 0] \), with the constraint that state 3 is absorbing.

The open time distribution is obtained assuming all of the closed states to be absorbing (i.e. \( k_{12} = k_{23} = 0 \)) and solving for \( P_3(t) \) with the initial condition vector \( P(0) = [0, 0, 1] \). In this particular case, where there is only one exit path the open time distribution is given by \( P_{32}(t) \).
In matrix form the conditional probability, $P_{mn}(t)$, is obtained from the solution of

$$ P(t) = A P(t) $$  \hspace{1cm} 2.13

subject to the initial condition column vector $P(0) = U_m^T$ where $U_m$ applies the initial condition to the $m^{th}$ state. The matrix $A$ has elements $[A_{ij}]$, and state $n$ is made absorbng by setting $k_{nq} = 0$, $q = 1, 2, \ldots n$. The output is the probability of being in state $n$ and is obtained by multiplying the column vector $P(t)$ by a row vector $U_n$ thus;

$$ P_{mn}(t) = U_n P(t). $$ \hspace{1cm} 2.14

### 2.2.5 The Laplace-transformed result

One may solve equations 2.7 and 2.8, or 2.13 and 2.14, in many different ways (Swisher, 1976). As shown later, for identifiability analysis one needs not obtain the time domain solution. Instead, the analysis may be done in the Laplace domain using the driving point function. Taking the Laplace transform of equation 2.13 and rearranging gives

$$ P(s) = [sI - A]^{-1} P(0) $$ \hspace{1cm} 2.15

where $I$ is the identity matrix, and "$s$" is the Laplace variable. Substituting this result into the Laplace transform of equation 2.14 yields

$$ P_{mn}(s) = U_n [sI - A]^{-1} U_m^T. $$ \hspace{1cm} 2.16

Letting

$$ [sI - A] = [b_{ij}(s)] $$ \hspace{1cm} 2.17

and using a standard method for inverting matrices one obtains

$$ [sI - A]^{-1} = [\text{cofactor } b_{ij}(s)]^T / \det [sI - A]. $$ \hspace{1cm} 2.18

Substituting [2.18] into [2.16] yields
\[ P_{mn}(s) = \text{cofactor } h_{mn} / \det[sI - A]. \]  

The time domain solution is of course simply obtained from the inverse transform of [2.19].

2.2.6 A diagramatic representation.

In Fig. 2.1 diagrams are shown which represent the equations used to predict each of the measurements described previously. The bold inwardly directed arrows in the diagrams indicate the states to which initial conditions are applied, and bold outwardly directed arrows indicate the states from which measurements are obtained. Where it is appropriate states have been made absorbing by eliminating arrows. These diagrams are a useful shorthand representation, and they help identify certain subsystems in more complex problems.

2.2.7 Other results for single channels.

In the example above, the WCO conditional distributions, indicated by \( P_{ij}(t) \), are cumulative probability distributions and \( P_{ij}(t^{+\infty}) = 1 \). That is, since all of the states are connected to the absorbing j state a channel must eventually reach it. In other cases this may not be true and \( P_{ij}(t^{+\infty}) < 1 \). We thus define a conditional cumulative probability distribution as

\[ F_{ij}(t) = P_{ij}(t) / P_{ij}(t^{+\infty}). \]  

Differentiation of this equation gives a conditional probability density function (or PDF)

\[ f_{ij}(t) = \frac{dF_{ij}(t)}{dt}. \]  

In the identifiability analysis that follows we will use the \( P_{ij} \)'s, or the conditional probability distribution.
Figure 2.1.
Diagramatic representation of the theoretical predictions for various measurements using a 3-state model. Shown are the reductions in the model and the changes in initial conditions that predict the various experimental measurements.

A) State diagram used to obtain $P_O(t; P_R(0)=1)$, or the probability of opening for a turn-on measurement. The bold inwardly directed arrow indicates that an initial condition of unit magnitude is to be applied to the R state. The outwardly directed arrow indicates that state O is the open state, or the observation state.

B) State diagram for the turn-off, or tail current, prediction, i.e., $P_O(t; P_O(0)=1)$. The only difference between A and B is the location of the initial condition.

C&D) State diagram used to represent the waiting time and closed time predictions. Note that state O is absorbing in both cases as indicated by the absence of arrows leaving this state. Also note that the only difference between these two is the location of the initial condition.

E) Open time prediction. Note that state A is absorbing.
A) Turn-on
   1
   R ⇔ A ⇔ 1 →

B) Turn-off
   R ⇔ A ⇔ 1 →

C) Waiting Time
   R ⇔ A → \varnothing →

D) Closed Time
   R ⇔ A → \varnothing →

E) Open Time
   A ← \varnothing →
It is of interest to find the probability of a channel exiting from a state to each of its adjoining states. Consider the following model segment

\[ \begin{align*}
&\text{1} \quad \text{2} \quad \text{3} \\
&\downarrow \\
&\text{4} \quad \text{2.22}
\end{align*} \]

The probability of a channel transitioning from state 2 to any other state in time \( \Delta t \) is called \( P_{2X}(t+\Delta t) \) and is given below.

\[ P_{2X}(t+\Delta t) = k_{21}P_2(t)\Delta t + k_{23}P_2(t)\Delta t + k_{24}P_2(t)\Delta t \quad 2.23 \]

From this equation and a similar expression for \( P_{21}(t+\Delta t) \) the following ratio is obtained.

\[ \frac{P_{21}(t+\Delta t)}{P_{2X}(t+\Delta t)} = \frac{k_{21}}{k_{21} + k_{23} + k_{24}} = \pi_{21} \quad 2.24 \]

Note that this is not a function of time. Thus the ratio \( \pi_{21} \) may be interpreted as the probability that a channel makes a transition from state 2 to 1 given that it left state 2.

2.2.8 The description of Colquhoun and Hawkes.

Colquhoun and Hawkes (1962) analyzed all of the above distributions as well as several distributions associated with burst behavior not described. Their equations are not in the state-variable format; rather their description resembles that used in the probability theory literature (see Cox and Miller, 1965, for example). They have written all the equations in terms of partitioned matrices where the
partitions are open states, short-lived closed states, and long-lived closed states. Their method of solution is the modal expansion, or spectral decomposition, technique. Since they have written their results generally in terms of matrix equations, their results are quite amendable to numerical solution as is described in the next chapter and used in the computer program in Appendix C. We do not develop their results here for the sake of brevity.

2.3 Models used in the analysis of $I_{\text{Ca}}$

Some general properties of the models will be described followed by models for activation and then inactivation. Rewriting [2.9] for the general case of macroscopic relaxations we have

$$I(v,t) = N_i(v) p_o(v,t)$$

where the dependence on voltage, $v$, and time, $t$, is written explicitly.

From tail currents following full activation, the quantity $N_i(v)$ was measured, and the following expression described the result where $v' = 15$ mV, $H = 25.8$ mV and is a constant containing several thermodynamic quantities, $[\text{Ca}_o] = 10$ mV and $C$ is a scaling factor that depends on the number of channels and which was $2.76 \times 10^3$ nA/mV·M for the case of a cell having a maximum peak current of $-55.6$ nA (Brown et al., 1983).

$$N_i(v) = \frac{C(v-v') [\text{Ca}_o] e^{-2v'/H}}{1 - e^{(v-v')/H}}$$

2.3.1 Activation models

The six models denoted $M_1$ through $M_6$ in Fig. 2.2 were candidates for describing $I_{\text{Ca}}$ activation. Under voltage clamp, the input to
Figure 2.2.
Models used for describing $I_{Ca}$ macroscopic activation. $M_1$ is a Hodgkin-Huxley-type model where the probability of channel opening is proportional to $m^2$ and each "m" variable is controlled by a first-order equation as shown. $M_3$ is a non-linear, 3-state aggregation model as suggested by Bauman which is not shown because it would require too much space. The other models are straightforward.

$M_1$: $m^2$, $R \xrightarrow{k_{-1}} A \xrightarrow{k_{+2}} O$, $R \xrightarrow{k_{+1}} O$

$M_2$: $R \xrightarrow{k_{-1}} A \xrightarrow{k_{+2}} O$

$M_3$: Aggregation model of Bauman (1981)

$M_4$: $2a$, $a \xrightarrow{k_{+3}} O$, $b \xrightarrow{2b} k_{-3}$

$M_5$: $a \xrightarrow{2a} k_{+3}$

$M_6$: $3a \xrightarrow{k_{+3}} O$, $b \xrightarrow{3b} k_{-3}$
these models is the voltage which sets the value of the voltage-
dependent rate constants. Model M_5 was selected as best for a variety
of reasons (see later) and it is used in subsequent models of inacti-
vations as shown below. However, the other models could be substi-
tuted and most of the kinetic features would be reasonably described.

2.3.2 Inactivation models

Figure 2.3 shows six models of inactivation which were consi-
dered. Each is discussed separately below. In all cases the activ-
ation parameters were set to those obtained for M_5 from short-pulse
experiments in which inactivation was neglected.

Model A

Model M_A (Fig. 2.3) is a Ca-accumulation-dependent inactivation
model similar to ones proposed by Standen and Stanfield (1983) and
Chad et al. (1984). In the diagram, the R, A's and O have meanings as
described previously, and I indicates the inactivated state. As
indicated on the diagram, transitions to the I state depend on [Ca]_c,
the amount of Ca in a compartment under the membrane; other rate
constants in the diagram depend on voltage only. Below is the equa-
tion describing [Ca]_c in a volume v,

\[
\frac{d [Ca]_c}{dt} = \frac{- (1-P_B) I_{Ca} - k_s [Ca]_c + k_s [Ca]_i}{2Fv} \quad 2.27
\]

The first term on the right-hand side is due to the accumulation of Ca
from I_{Ca}; the negative sign is used because I_{Ca} is a negative quantity
by convention. This term includes a factor for loss due to buffering;
i.e. (1-P_B), where P_B is the probability of a Ca ion being bound. The
second term represents loss due to diffusion to the cell interior.
Figure 2.3.

Inactivation models. $M_A$ is a Ca-accumulation-dependent model while the others are voltage dependent. In the case of $M_C$, activation and inactivation are independent and $P_o$ is given by the product shown. In each case, the activation sequence, $R-A_1-A_2-O$, represents model $M_5$ (Fig. 2.2).
The third term is provided to set a lower limit on \([\text{Ca}]_c\) to be the concentration of Ca in the cell interior, \([\text{Ca}]_i\). Assuming 1-to-1 binding of a buffer, \(P_B\) is given by

\[
P_B = \frac{[B]_{\text{TOTAL}}}{k_B + [B]_{\text{TOTAL}} + [\text{Ca}]_c}
\]

where \([B]_{\text{TOTAL}}\) is the total concentration of buffer and \(k_B\) is the dissociation constant. The fifth order system described in equations 2.29, 2.30 and \(M_A\) (Fig. 2.3) gives the time-course of \(I_{\text{Ca}}\).

Model B

This is a class of voltage-dependent models with two non-independent inactivation states. The three versions, \(M_{B1}'\) and \(M_{B3}'\) are virtually indistinguishable based on macroscopic current measurements. In the case of model \(M_{B3}\), inactivation proceeds in a purely "coupled" fashion, i.e., channels must open before they inactivate. Two features of this model may be inappropriate. First, channels must eventually open in a very long pulse - there can be no failure traces. Second, channels must reopen during recovery from inactivation. The first feature may be a reasonable approximation in the case of snail neurons (Brown et al., 1984) but not in heart cells (unpublished observation). The second feature has not been observed, but it may have been missed. Model \(M_{B2}\) eliminates both of these undesirable features. Model \(M_{B1}\) may be regarded as a superset of both \(M_{B2}\) and \(M_{B3}\). In practice it has too many rate constants to determine uniquely from macroscopic current measurements. However, it was analyzed in the case where \(F_f^*, F_b^*, S_f^*\) and \(S_b^*\) were arbitrarily set to zero.
All of these 5th order models were assumed to have voltage dependent rate constants.

**Model C**

In this model inactivation proceeds independently of activation in a manner analogous to the Hodgkin-Huxley $m^3h$ type formulation. In this case, however, activation and inactivation are more complex and are given by the two diagrams shown. The probability of channel opening, $P_0(t)$, is given by the product $P_{O_A} \times P_{I_A}$ where $P_{O_A}$ and $P_{I_A}$ are probabilities of being in states $O_A$ and $I_A$, respectively. Three independent differential equations describe the activation diagram and 2 describe the inactivation diagram.

**Model D**

Model D is included for completeness because it was considered earlier. It is a model similar to ones recently suggested for Na current (Armstrong and Gilly, 1979, and Horn and Vandenberg, 1984). It may be dismissed as a model for $I_{Ca}$ on the basis of the time course of recovery from inactivation as will be described later.

2.4 **Model identifiability from various measurements**

An identifiable model is one in which all the parameters may be obtained uniquely from the measurements available. The analysis of such problems has mostly been done for the case of compartmental models (Cobelli and DiStefano, 1980; R.F. Brown, 1980). Since the state models may be considered as compartmental models much of this analysis is transferable. Considering the present array of measurements available in electrophysiology, it is of interest to be able to show what combinations of models and experiments yield uniquely identifiable parameters. Certain combinations of measurements and
models can yield either a finite or an infinite number of parameter values which fit the data equally well. Such cases may or may not be obvious in the output from a parameter estimation scheme. Thus, such an analysis should be the first step in a parameter estimation problem.

In Appendix B, a complete analysis of the 3-state model, \( M_2 \), is given in terms of WCO measurements, macroscopic relaxations, and noise measurements. In terms of macroscopic relaxations, it was found that parameters may be uniquely identified from turn-off or tail current measurements but not from turn-on measurements (see Fig. 1.1 for definitions of these protocols). In general this result is obtained for any sequential or catenary structure as can be argued from the work by Bellman and Astrom (1970). This result was used in the formulation of the scheme to estimate parameters from macroscopic relaxations (see later). In Appendix B, it is also shown that all the \( k \)'s can be obtained uniquely from the open and closed-time histograms. Results on other model structures are given elsewhere (Wilson and Clark, 1985).

2.5 Apparent single channel WCO PDF's when there is limited interval resolution.

When a single channel recording is filtered, both "fast" openings and "fast" closures will not be detected. The result is an absence of brief open and closed times as well as falsely prolonged open and closed times in the histograms. In the following, a method is presented for computing these effects. Following a recent suggestion (Colquhoun and Sigworth, 1983), the detection system is modeled as having dead times, namely, \( t_o \), the minimum open time that is
detectable, and \( t_c \), the minimum closed time that is detectable. First, the PDF of the apparent open time, \( p_{ao}(t) \), will be derived; then the waiting and closed time apparent PDF's will be given.

**Apparent open time PDF**

In general, an apparent opening will consist of one of the following sequences of events where an individual "event" is demarcated with parenthesis. A short-hand is used where (open for time > \( t_o \)) stands for the case of an opening that is longer than \( t_o \); the other descriptions follow analogously.

\[
\text{(open for time > } t_o \text{), (closed for time > } t_c \text{)} \\
\text{or} \\
\text{(open for time > } t_o \text{), (closed for time } \leq t_c \text{)} \\
\text{ (open time), (closed for time > } t_c \text{)} \\
\text{or} \\
\text{(open for time > } t_o \text{), (closed for time } \leq t_c \text{), (open time), (closed for } t > t_c \text{)}
\]

The PDF, and its Laplace transform, of each of these events is listed in Table 2.1. A single open state was assumed. Also, in the case of the waiting and closed time PDFs, there are constraints on the A's and \( \tau \)'s such that the integrals of the PDFs are unity and the response is realizable with a state model. In addition to the definitions in Table 2.1, the probability of an opening occurring with duration \( \leq t_o \) is given by

\[
\text{Prob(opening } \leq t_o \text{) } = \int_0^{t_o} p_o(t) dt + t_o/e^{t_o/t_o} 
\]

and a similar expression is used for \( \text{Prob(closure } \leq t_c \) ).

The apparent open time PDF, \( p_{ao}(t) \), is easily derived in the Laplace domain. First, consider the second sequence of events above; i.e.,
Table 2.1
Events associated with the derivation of the apparent WCO PDFs. The PDF of each event and its Laplace transform are also given. The symbol associated with the PDF of the event (open for time $t > t_o$) is $p_o(t|t > t_o)$ and is read "the probability of opening for time, $t$, given that $t$ is greater than $t_o$". Its Laplace transform is indicated by $q_o(s|t > t_o)$. Other nomenclature follows analogously.

<table>
<thead>
<tr>
<th>Event and Symbols</th>
<th>PDF</th>
<th>$L$(PDF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(open time) $p_o(t)$</td>
<td>$\frac{e^{-t/t_o}}{t_o}$</td>
<td>$\frac{1}{t_o}$</td>
</tr>
<tr>
<td>$q_o(s)$</td>
<td>$\frac{1}{s + \frac{1}{t_o}}$</td>
<td></td>
</tr>
<tr>
<td>(closed time) $p_c(t)$</td>
<td>$n \frac{1}{\lambda_i} \frac{e^{-t/t_i}}{t_i}$</td>
<td>$n \frac{1}{s + \frac{1}{t_i}}$</td>
</tr>
<tr>
<td>$q_c(s)$</td>
<td>$\frac{1}{\lambda_i}$</td>
<td>$\frac{1}{s + \frac{1}{t_i}}$</td>
</tr>
<tr>
<td>(waiting time) $q_w(t)$</td>
<td>$\frac{m}{\lambda_i} \frac{e^{-t/v_i}}{v_i}$</td>
<td>$\frac{m}{s + \frac{1}{v_i}}$</td>
</tr>
<tr>
<td>$q_w(s)$</td>
<td>$\frac{1}{\lambda_i}$</td>
<td>$\frac{1}{s + \frac{1}{v_i}}$</td>
</tr>
<tr>
<td>(open for time $t &gt; t_o$) $p_o(t</td>
<td>t &gt; t_o)$</td>
<td>$\frac{e^{-t/t_o} u(t-t_o)}{t_o (1 - Probopening \leq t_o)}$</td>
</tr>
<tr>
<td>$q_o(s)$</td>
<td>$\frac{1}{s}$</td>
<td>$e^{-s} \frac{1}{s + \frac{1}{t_o}}$</td>
</tr>
<tr>
<td>(open for time $t &lt; t_o$) $p_o(t</td>
<td>t &lt; t_o)$</td>
<td>$\frac{e^{-t/t_o} (u(t) - u(t-t_o))}{t_o (1 - Probopening \leq t_o)}$</td>
</tr>
<tr>
<td>$q_o(s)$</td>
<td>$\frac{1}{s}$</td>
<td>$e^{-s} \frac{1}{s + \frac{1}{t_o}}$</td>
</tr>
<tr>
<td>(closed for time $t &gt; t_c$) $p_c(t</td>
<td>t &gt; t_c)$</td>
<td>$\frac{n}{\lambda_i} \frac{e^{-t/c_i} u(t-t_c)}{c_i (1 - Probclosure \leq t_c)}$</td>
</tr>
<tr>
<td>$q_c(s)$</td>
<td>$\frac{1}{\lambda_i}$</td>
<td>$\frac{e^{-s \cdot \frac{t-t_c}{c_i}}}{s + \frac{1}{c_i}}$</td>
</tr>
<tr>
<td>(closed for time $t &lt; t_c$) $p_c(t</td>
<td>t &lt; t_c)$</td>
<td>$\frac{n}{\lambda_i} \frac{e^{-t/c_i} u(t) - u(t-t_c)}{c_i (1 - Probclosure \leq t_c)}$</td>
</tr>
<tr>
<td>$q_c(s)$</td>
<td>$\frac{1}{\lambda_i}$</td>
<td>$\frac{e^{-s \cdot \frac{t-t_c}{c_i}}}{s + \frac{1}{c_i}}$</td>
</tr>
</tbody>
</table>
(open for time > t_o), (closed for time ≤ t_o),
(open time)^O, (closed for time > t_o)^C.

The time interval associated with each individual event is a random variable. Also, the apparent open time for this sequence is given by the sum of these intervals excluding the last closed interval. The PDF of a sum of random variables is obtained from a convolution of the individual PDFs (Papoulis, 1965). Since convolution is equivalent to multiplication in the Laplace domain, the PDF of the apparent opening of this sequence is obtained from the expression below (see Table 2.1 for the definitions of each of these quantities).

\[ g_o(s|t > t_o) g_c(s|t ≤ t_c) g_o(s) \]  

But there are an infinite number of sequences possible. Thus, results from each sequence are weighted with Prob(closure > t_c) and summed to give \( g_{ao}(s) \).

\[ g_{ao}(s) = \sum_{j=0}^{∞} g_o(s|t > t_o) \left[ g_c(s|t ≤ t_c) g_o(s) \right]^j \left[ 1 - \text{Prob}(\text{closure} ≤ t_c) \right] \]  

Rearranging and using the identity

\[ \sum_{j=0}^{∞} h^j = \frac{1}{1 - h}, \quad |h| < 1 \]  

the following result is obtained

\[ g_{ao}(s) = \frac{g_o(s|t > t_o) \left[ 1 - \text{Prob}(\text{closure} ≤ t_c) \right]}{1 - g_c(s|t ≤ t_c) g_o(s)} \]  

Unfortunately [2.33] is too complicated to perform the inverse Laplace transform analytically; thus numerical techniques were employed as described later. However, using some properties of Laplace transforms it is possible to obtain the average open time from this expression (not shown).
It was found that oscillations in the numerical inversion occurred whenever [2.33] was used; however, the following modification improved this situation. Using a well known property of Laplace transforms and making a substitution from Table 2.1, one may write the working equation

$$P_{ao}(t-t_0)u(t-t_0) = L^{-1} \left[ \frac{g_o(s) \left[ 1 - \text{Prob}(\text{closure} \leq t_c) \right]}{1 - g_c(s|t \leq t_c)g_o(s)} \right]. \quad 2.34$$

**Apparent closed time PDF**

In the case of the apparent closed time the possible sequences are:

$$(\text{closed for time} > t_c), \ (\text{open for time} > t_0)$$

or

$$(\text{closed for time} > t_0), \ (\text{open for time} \leq t_0), \ (\text{closed time}), \ (\text{open for time} > t_0)$$

etc.

Combining Laplace transforms as done previously, the expression below is obtained.

$$g_{ac}(s) = g_c(s|t>t_c)\left[ 1 - \text{Prob}(\text{opening} \leq t_0) \right] \prod_{j=0}^{\infty} \left[ g_o(s|t \leq t_0) g_c(s) \right]^{-1} \quad 2.35$$

Using [2.32], some results from Table 2.1, and rearranging for computer implementation, the working equation is obtained.

$$P_{ac}(t-t_c)u(t-t_c) = L^{-1} \left[ \frac{\prod_{i=1}^{n} \frac{A_{ci}}{s + \tau_{ci}} e^{-t_c/\tau_{ci}}}{1 - g_o(s|t \leq t_0) g_c(s)} \right]. \quad 2.36$$

**Apparent waiting time PDF.**

The possible sequences of events for the apparent waiting time are:
(waiting time), (open for \( t > t_o \))

or

(waiting time), (open for \( t \leq t_o \)), (closed time),

(open for \( t > t_o \))

etc.

Combining terms and using the identity in [2.32] one obtains

\[
g_{aw}(s) = \frac{[1 - \text{Prob}(\text{opening} \leq t_o)] g_w(s)}{1 - g_o(s | t \leq t_o) g_c(s)}
\]

2.37

In this case there is no "delay" term in the numerator, and \( p_{aw}(t) \) is computed simply from \( g_{aw}(s) \).

Computational recipe

The computational recipe for obtaining \( p_{ao}(t) \) will be described; other results are obtained analogously. Expressions for \( p_o(t) \) and \( p_c(t) \) are obtained by standard methods, and values for the \( \tau \)'s and \( \lambda \)'s in the respective PDF expressions (Table 2.1) are identified. These values are then substituted into the appropriate Laplace transforms in Table 2.1 and other expressions such as [2.29]. Each of the quantities in [2.34] can now be evaluated, and the result is numerically inverse transformed as described in the next chapter.
Chapter 3: Methods of analysis and computation

In this chapter I have described how the whole cell current and single channel data were analyzed. This includes some practical procedures developed for data preparation (filtering, etc.), the method for detecting and measuring single channels, parameter estimation schemes for obtaining rate constants, etc. In addition, I have described how whole cell currents, single channel PDFs, and single channel Monte Carlo simulations were done. Various computer programs developed for these analyses are highlighted.

3.1 Data acquisition

Data were digitized and stored using either a Nicolet signal averager with a tape drive (Nicolet 1170) or a DEC 11/23 microcomputer system which has included various disk and tape-storage devices. Either device creates files which can be read by the analysis programs. Data acquisition with the computer was done using the program PULSE written by Bill Little. It allows for direct memory access (DMA) acquisition of up to 4096 data points on one or two input channels at a rate of ≥ 10 μsec/point. The A/D board used had a 12-bit resolution. Data acquisition in both cases was triggered from programmable pulse generators used to generate the various voltage-clamp pulse protocols used. In some of the whole-cell current measurements, signal averaging of 10 or 20 pulses was used to improve the S/N ratio. Analog filters were used to precondition the data for digitizing. In the case of noise-spectra recording where particularly significant error due to aliasing might occur, an 8-pole analog filter was used (Rockland #852).
3.2 Whole cell current analysis

Whole-cell currents were analyzed using two computer programs. PULSE, which will be described first, was written to do the types of analyses generally done. The second program, MACEST, was written to obtain parameter estimates for state models.

PULSE and some auxiliary programs include many features for data preparation, data measurement, data plotting and parameter estimation. Data preparation includes removal of linear capacitive artifacts and leakage currents as well as digital filtering. The plotting functions include overlaying test and control plots, plotting of peak current-voltage curves, plotting of isochronal curves, etc. Much of the set-up of the plotting can be done rapidly with a cursor. A practical feature of PULSE is that in addition to terminal output, it allows rapid viewing of data on an oscilloscope screen. This was implemented using a D/A device and ran ~ 1000 times faster than plotting to a terminal via a serial connection. PULSE can also be used to fit data to a library of simple models such as a sum-of-exponentials, $m^2h$, etc. The data fitting is done quite quickly because the Marquardt algorithm is used and analytical, rather than numerical, derivatives are provided (Bevington, 1969; Thomasson and Clark, 1974).

The most interesting feature of MACEST is that it allows one to fit multiple sets of data. This has been used in the analysis of activation data by fitting a set of three measurements simultaneously (see Fig. 4.11 for an example). A model used with MACEST is given Appendix C.2. Sufficient comments are provided that the subroutines could be implemented using another driving program. Various features of MACEST are described below.
The fitting algorithm was a much modified version of the implementation described by Bevington (1969) of the Marquardt method (1963). An unweighted $\chi^2$ error criterion (or objective function) was used as shown below where $n$ is the number of data points, $n_f$ is the number of free parameters, $Y_i$ is the data value, at the $i^{th}$ time interval, and $m_i$ is the corresponding value of the model.

$$\chi^2 = \sum_{i=1}^{n} \frac{(Y_i - m_i)^2}{(n-n_f)}$$  

Inequality constraints on parameters were imposed by adding a penalty function to this objective function. This technique is thought to be superior by some (Bard, 1974) to other techniques in the literature (Box, 1966 and Jennrich and Sampson, 1962). The constraints most often used consisted of requiring rate constants to be $\geq 0$. After reasonable initial guesses were known, the penalty function was eliminated with no difference in the end result.

The model was computed using a 4th-order Runge-Kutta integration scheme primarily because this method requires relatively few lines of computer code as compared to other numerical methods such as the modal expansion technique described earlier. In addition, unlike other methods, this method may be used with nonlinear models. The Marquardt method requires partial derivatives of the model output with respect to each of the parameters at each measurement point. These were calculated in one of two ways: 1) from a 1st-order difference formula, or 2) from sensitivity equations as done by Paulsen et al. (1982). The former method is the easiest to implement and was therefore the method of choice, but the latter method is faster because the derivatives are obtained with a single integration of the equations.
The step size for integration was exponentially varied following a voltage perturbation with smaller integration steps being used immediately following a voltage step when the variables were changing rapidly. When the partial derivatives are calculated using a difference formula this method of varying the step size may be superior to ones where the integration subroutine has control of the step size. As pointed out by Bard (1974) the latter procedure may introduce errors because model values at a given time point may depend on the past history of the step size.

3.2.1 Testing the parameter estimation scheme

The parameter estimation scheme in MACEST was tested by fitting simulated data with appropriate models. Both 3- and 4-state models (Fig. 2.2) were used in the simulations as well as in the fits to the data. Gaussian noise with a known standard derivative, $\sigma_n$, was added to simulate real data. Referring to Table 3.1, the estimated values agreed very well with the true values even in the presence of significant noise. Additionally, the $\chi^2$ values almost exactly equaled the value of $\sigma_n$ indicating no bias in the fit. There were no problems with local minima in any of these fits.

Using these simulations, the F-test for discriminating between models was examined (Munson and Robard, 1980). The value of $F$ was calculated from

$$F = \frac{\chi_1^2 df_1}{\chi_2^2 df_2} \frac{df_2}{df_1}$$

where the $df$'s are degrees of freedom (= $n-n_f$) and subscripts 1 and 2 refer to the simpler model (fewer free parameters) and the more complicated model, respectively. In the case of the 3-state simula-
TABLE 3.1
Results of model-to-model tests on the parameter estimation scheme. Simulations were done with the general 3- and 4-state models using parameters which approximated those obtained from data at -20mv. The usual set of 3 measurements were simulated and the total number of points fit were 3036. Gaussian noise with the $\sigma_n$ shown was added to simulate real data. The simulations were fit with both 3- and 4-state models. The estimated (E) and true values used in the simulation (T) are given in units of ms$^{-1}$, and the $\chi^2$ values are given in units of A$^2$. Note that when the simulation was fit with its respective model excellent agreement was obtained between the T and E values even in the presence of noise. Also, the $\chi$ values almost exactly equaled the value of $\sigma_n$; for example, in the case of the 3-state simulation $\sigma_n = 2.0 \times 10^{-9}$ A and $\chi = 1.997 \times 10^{-9}$ A.

<table>
<thead>
<tr>
<th></th>
<th>$k_{+1}$</th>
<th>$k_{-1}$</th>
<th>$k_{+2}$</th>
<th>$k_{-2}$</th>
<th>$k_{+3}$</th>
<th>$k_{-3}$</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 - State Simulation ($\sigma_n = 0$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-state, T</td>
<td>9.00$\times 10^{-2}$</td>
<td>.600</td>
<td>1.00</td>
<td>5.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3-state, E</td>
<td>8.91$\times 10^{-2}$</td>
<td>.600</td>
<td>.999</td>
<td>5.00</td>
<td>-</td>
<td>-</td>
<td>4.6$\times 10^{-21}$</td>
</tr>
<tr>
<td>3 - State Simulation ($\sigma_n = 2 \times 10^{-9}$ A)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-state, T</td>
<td>9.00$\times 10^{-2}$</td>
<td>.600</td>
<td>1.00</td>
<td>5.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3-state, E</td>
<td>8.90$\times 10^{-2}$</td>
<td>.604</td>
<td>1.01</td>
<td>5.03</td>
<td>-</td>
<td>-</td>
<td>3.990$\times 10^{-18}$</td>
</tr>
<tr>
<td>4-state, E</td>
<td>.585</td>
<td>1.48</td>
<td>.368</td>
<td>.736</td>
<td>1.08</td>
<td>5.08</td>
<td>4.062$\times 10^{-18}$</td>
</tr>
<tr>
<td>4 - State Simulation ($\sigma_n = 1.2 \times 10^{-9}$ A)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-state, T</td>
<td>.500</td>
<td>.450</td>
<td>.270</td>
<td>.840</td>
<td>1.10</td>
<td>5.00</td>
<td>-</td>
</tr>
<tr>
<td>3-state, E</td>
<td>1.10$\times 10^{-2}$</td>
<td>.610</td>
<td>.942</td>
<td>4.88</td>
<td>-</td>
<td>-</td>
<td>1.903$\times 10^{-18}$</td>
</tr>
<tr>
<td>4-state, E</td>
<td>.475</td>
<td>.453</td>
<td>.278</td>
<td>.852</td>
<td>1.11</td>
<td>5.02</td>
<td>1.429$\times 10^{-18}$</td>
</tr>
</tbody>
</table>
tions, the fit of the 4-state model had a larger $\chi^2$ than did that of
the 3-state model; thus the 3-state fit was the better of the two (the
$F$ value was negative). In the case of the 4-state simulation, the
4-state model fit best giving $F=503$ and an extremely high significance
level estimated to be $< 10^{-4}$ from tables.

### 3.3 Single Channel Data Analysis

Single channels were analyzed using computer programs described
elsewhere (Lux and Brown, 1984; Zucker, Wilson, Law, Little, and
Brown, 1985), and only the salient points are described here. The
analysis is divided into the following procedures: 1) data prepara-
tion, including removal of leakage, baseline drift, and capacitive
artifacts; 2) channel idealization, including detection and measure-
ment of channel intervals and amplitudes; and 3) interval analyses,
including WCO histogramming and parameter estimation. Data prepara-
tion techniques are described in the above references. The algorithm
for channel detection and measurement made use of two records - the
original data record and a record which was filtered using a
zero-phase, digital filter as described below. A channel was detected
whenever the filtered trace crossed an opening threshold for a speci-
fied length of time. Closure was determined from the crossing of a
separate closing threshold. Following the initial detection of the
opening and closing transitions, an iterative procedure was used to
measure the opening as follows. The amplitude was estimated from the
mean of the points in the original record between the
transition-times. Next, new opening and closing times were obtained
corresponding to a level at $1/2$ this amplitude. The process was
repeated, resulting in an amplitude and a width at $1/2$ this amplitude.
The latter width was desirable because it is equivalent to the original unfiltered width (see Fig. 3.1). It was found that this method exactly determined the open times of isolated, detected, simulated single channels whenever there was no noise present. Additional procedures were followed for the case of overlapping events. From the assumption of the independence of channels, the number of overlapping openings was expected to follow the binomial distribution. This distribution was measured and a maximum likelihood estimate of the number of channels was obtained using the technique suggested by Patlack and Horn (1982).

Associated with the detection of events are dead times, or minimum resolvable open and closed times (t_o and t_c, respectively). These occur because filtering eliminates threshold crossings of fast openings and fast closures. The dead times depend on the following parameters: the filter characteristics (BW, number of poles, etc.), the threshold levels, and, optionally, the number of points required to exceed threshold. Dead times were evaluated empirically. Using the digital filter described below configured with 4 poles and 1 KHz bandwidth, t_o was ~ .21 ms for a threshold of 60% of the full amplitude.

The input to the interval analysis routine was the idealized data which was in the form of a list that included for each event the elapsed time since the onset of the pulse, the duration, and the amplitude. From such a description, the WCO and amplitude histograms were obtained. Parameter estimates of exponential PDFs were obtained using the maximum-likelihood technique (Colquhoun and Sigworth,
1983). Maximization of the likelihood function was achieved using a modified Marquardt algorithm.

3.3.1 Zero-phase digital filter

A zero-phase digital filter was used to process the single channel data because it eliminated the phasic delay in the pulse response obtained from normal filters (see Fig. 3.1). The use of such a digital filter was important for several reasons. First, this filter allowed the input trace to be directly compared with the filtered and idealized traces in our single channel measurement scheme, and in fact this characteristic was crucial to our analysis method (see above). The second reason is that the zero-phase characteristic allows one to measure the waiting times without error. In the case of data filtered at 1 KHz with analog filters, waiting times could be consistently overestimated by ~ 1-2 msec (Fig. 3.1). In addition to its zero-phase characteristic, the digital filter has other useful attributes. Since the filtering is done off-line, one can digitize the data at a high sampling rate and then choose a filtering scheme that gives a reasonable signal-to-noise ratio yet still allows one to see fast activity in the trace. A by-product of this procedure is that since the anti-aliasing analog filter is set at a relatively high cut-off frequency of 5-10 KHz, the capacitive artifact will decay faster and may therefore reach the dynamic range of the A/D converter more quickly.

A zero-phase digital filter is quite easy to implement for fixed record length data. It consists of taking any available digital filter and passing it through the data in both the forward and
Figure 3.1
Pulse responses of a commercially available analog filter (model 852; Rockland, Inc., Gilbertsville, Pa.) and a zero-phase digital filter.

A) The 2 ms input pulse is shown along with three outputs of the analog filter in the Bessel mode (8 pole). The filter cut-off (3-dB point) was set at 4, 2, and 1 kHz and the "delays" as measured to the time of half-maximal response were .27, .53, and 1.0 ms, respectively.

B) Response of the analog filter in the Butterworth mode (8 pole). Delays were .23, .44, and .84 ms with cut-off frequencies of 4, 2, and 1 kHz. Note the ringing in the response.

C) Pulse response of the zero-phase digital filter described in the text for cut-off frequencies of 4, 2, and 1 kHz (8 pole). Note that there was no time-shifting of the response.

D) Pulse response of the digital filter in the presence of noise. The filtered and unfiltered responses are superimposed to illustrate that no time-shifting occurs and that the original width of the pulse is maintained at half the maximum amplitude.
backward directions. Kormylo and Jain (1974) have given a method for longer lengths of data. We used a single-pole Butterworth filter and applied it repetitively to give any number of poles in the filter response. By using a single-pole Butterworth design, the "ringing" in a multiple-pole Butterworth filter was avoided (Fig. 3.1B). In Figs. 3.1C and 3.1D, the pulse response of the digital filter is shown in the presence and absence of noise. Note that there is absolutely no time shifting of the data.

3.4 Simulations of whole cell currents

In addition to the parameter estimation program for whole cell currents it was found that a companion simulation program was required. The model output once again was calculated numerically using a 4th-order Runge-Kutta method. Several types of plots could be generated, and a number of plots could be placed on one graph in order to determine the effect of varying a parameter. This program could plot the current resulting from a family of voltage clamp pulses; it could produce various complicated voltage clamp pulse protocols; it could plot some measurements versus voltage, such as $h_a(v)$ and $m_a(v)$ curves; etc. A unique feature was that rate constants as a function of voltage could be input to the program from a sketch of an arbitrary curve on a digitizing pad. Thus one need not have a mathematical function for describing a set of rate constants versus voltage, for example. This latter feature was implemented by digitizing points from the pad and then placing a splined curve through them (DeBoor, 1978). The parameters of the spline were saved in a file and used to generate the $Y$ value at any arbitrary $X$ value. Another feature of
this program was the plotting of sensitivity curves (Edelson, 1981 and Paulsen et al., 1982). Also multiple state variables could be plotted as a function of time. These latter features were sometimes helpful in interpreting results as will be demonstrated later.

3.5 Calculation of ideal and apparent WCO PDFs

A computer program for computing all of the single channel PDFs of interest, the macroscopic relaxation curves, and the fluctuation spectra, was developed using the method described in Chapter 2. It used the modal, or spectral decomposition, method and it can be used with any homogeneous, or linear, Markovian system. The computations were implemented quite generally using matrix manipulations. The program input is the rate constants for a general model consisting of 6 states. The output consists of equations describing the desired functions and/or plots. Examples of such calculations are given in Figs. 4.29 and 4.30. A subroutine listing is given in Appendix C.

In addition to the ideal WCO PDFs determined as described above, apparent WCO PDFs were computed that included the effects of limited interval resolution as described in Chapter 2. As described there, the ideal WCO PDFs were obtained and the results were used to calculate $g_{ao}(s)$, $g_{ac}(a)$, and $g_{aw}(s)$. These functions were then numerically inverse transformed to obtain the desired result as described below.

The Graver-Stehfest algorithm as described by Jacquot, Steadman and Rhodine (1983) was chosen for doing the numerical inversions because it is easy to implement and requires relatively few lines of computer code; also it is not restricted to ratios of polynomial functions as some other methods are (Vlach and Singhal, 1983).
Computations were performed on a DEC 11/70 computer with a 32-bit single precision representation of real numbers. As described by Jacquot et al. (1983) when the number of terms in the expansion exceeds a critical number, the inversion becomes unstable due to round-off errors. It was found that the number of terms should not exceed 10 or 20 when computations were done in single or double precision, respectively. Several tests were performed comparing numerical and analytical inverses. Using a decaying exponential test function, the optimal number of terms in the expansion was found to be 8 and 16 for single and double precision, respectively. For an exponential with an initial value of 1, the algorithm gave $\leq 10^{-5}$ deviation from the theoretical inverse in double precision and $\approx 10^{-3}$ deviation in single precision. We have used double precision computations presently. The algorithm tended to oscillate at the discontinuity produced by a delay term in the numerator, i.e., $e^{-st}$. To avoid this problem, the expressions were altered as described previously. This required few changes in the computer code, and gave the same accuracy as described above.

3.6 Single channel simulations

Using the 3-state model as an example (Model $M_2$ of Fig. 2.2), I will demonstrate how the Monte Carlo simulations were done using a method described by Cox and Miller (1965). Equations 2.4-2.6 can be arranged in the form of a matrix equation as given below.

$$F(t + \Delta t) = RF(t)$$  \hspace{1cm} \text{3.3}$$

For this example, $F(t) = [P_1(t), P_2(t), P_3(t)]^T$ and $R$ is the transition probability matrix which may be obtained by inspection or from $R = (\Delta t A + I)$ where $I$ is the identity matrix. The elements, $R_{ij}$, of
R give the probabilities for transitions from state j to state i in time \( \Delta t \). It is informative to label the rows and columns of \( R \) in the following manner.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>1 Final States</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 - ( k_{12} \Delta t )</td>
<td>( k_{21} \Delta t )</td>
<td>0</td>
<td>1 States</td>
</tr>
<tr>
<td>( k_{12} \Delta t )</td>
<td>1 - ( k_{21} \Delta t - k_{23} \Delta t )</td>
<td>( k_{32} \Delta t )</td>
<td>( k_{32} \Delta t )</td>
<td>2 Final States</td>
</tr>
<tr>
<td>0</td>
<td>( k_{23} \Delta t )</td>
<td>1 - ( k_{32} \Delta t )</td>
<td>( k_{32} \Delta t )</td>
<td>3 States</td>
</tr>
</tbody>
</table>

Referring to this diagram, if the channel currently resides in state 1, then the probability of it going to state 2 is \( k_{12} \Delta t \); the probability of it remaining in state 1 is \( 1 - k_{12} \Delta t \); and the probability of it going to state 3 is zero.

To do a Monte Carlo simulation one needs a randomizing device, which in this case was a random number generator subroutine (Version IV Fortran, Digital Equipment Corporation) which gave a uniformly distributed real value between 0 and 1 every time it was called. At each step of the simulation, a properly evaluated \( R \) matrix was obtained from the \( \Delta t \) and rate constant values; this gave numerical values for each of the transition probabilities. Next, each of the possible transitions was assigned an interval between zero and one with a width given by the probability for that transition. For example, if the probability of a transition from state 1 to 2 was .1 and the probability of remaining in state 1 was .9, the intervals (0, .1] and (.1, 1) were assigned to the two transitions, respectively. Transitions occurred depending on the interval in which the value of
the random number fell; i.e., the channel went from 1 to 2 if the random number was .09 in the above example.

The algorithm used by the simulation program follows:

1. Initialize a variable which "points" to the state in which the channel resides.
2. Evaluate \( R \) with appropriate \( \Delta t \) and rate constant values.
3. Obtain a random number, \( R_N \), and increment the elapsed time by \( \Delta t \).
4. Transitions of the pointer, which resides in the \( j \)th state, occurs according to the following table:
   
   \[
   \begin{align*}
   &\text{IF} \ (R_N < R_{1j}), \text{ move to state 1. } \\
   &\text{IF} \ (R_{1j} < R_N < R_{1j} + R_{2j}), \text{ move to state 2. } \\
   &\text{IF} \ (R_{1j} + R_{2j} < R_N < R_{1j} + R_{2j} + R_{3j}), \text{ move to state 3. }
   \end{align*}
   \]

5. Write out an updated state vector which has either a 0 or a 1 in each position depending on the absence or presence of the channel in the state, respectively.
6. Update any time-dependent transition probabilities in \( R \).
7. Unless finished, go back to step 3.

In such a simulation \( \Delta t \) must be much smaller than the average lifetime in any state for the simulation to be accurate. Cox and Miller (1965) suggested \( \Delta t \leq 1/100 \) of the smallest inverse rate constant, but no obvious inaccuracy was found using 1/10, as checked in two different ways. First, single channel WCO histograms obtained from simulated records followed the theoretical PDFs quite will (Fig. 4.25). Also, when many records were averaged, the resulting waveform matched the curve obtained from the same model using a different solution technique (not shown).

The advantages of this method are ease of implementation and ready adaptability to models in which the transition probabilities are not constant. This latter feature proved useful when simulations of
the Ca-accumulation-dependent model of inactivation were done (Fig. 4.23). Note that a more complicated method has been described which also allows for non-constant transition probabilities and may run faster (Clay and deFelice, 1983).

In some of the single channel simulations, Gaussian white noise was added to determine its effect on the detection of events. In order to mimic the real recording situation (a .5 pA channel with RMS noise of ~ 0.7 pA), the RMS value of the noise was adjusted empirically to be .15 of the peak channel opening when the records were filtered at a bandwidth of 1 KHz using the digital filter described earlier.
Chapter 4: RESULTS

In this chapter, the opening section reviews some of the general properties of Ca channels determined from whole cell and single channel measurements and includes results on permeation and kinetics. Next, some properties single-channel waiting, closed, and open (WCO) interval measurements are given. Modeling of activation and inactivation follows in the next two sections. Various tests suggested in the literature for model discrimination are examined. In the last two sections errors involved in single channel measurements are investigated using single channel Monte Carlo simulations and the analytical technique developed for calculating the effect of a detection system having dead times.

4.1 Some general properties of Ca channels

4.1.1 Properties of single channel measurements

The characteristic activity of a single Ca channel when the potential is stepped from -50 to -5 mV is shown in Fig. 4.1. The transitions appear random and there are repetitive openings. Some intervals among a sequence of repetitive openings may be brief compared to others, giving rise to a bursting pattern of discharge (Fenwick et al, 1982; Brown et al, 1982; Hagiwara & Ohmori, 1983). The single channel current is on the order of .4 pA at this potential. The average current from the ensemble of records displays the characteristic features of whole cell current in that it quickly increases in magnitude (activation) and then decreases (inactivation). Note that both of these characteristics occur due to the timecourse of \( p_o(t) \) - there was no change in the single channel current. This is direct evidence for the general model described in equation 2.27.
FIGURE 4.1
Patch recording of a single Ca channel at 28°C following a step depolarization from -50 to -5 mV. The time of the voltage step is indicated by the vertical line. The linear components of capacitive and leakage currents were removed by summation with the current response to a hyperpolarizing pulse of equivalent amplitude. Below are ensemble-averaged patch currents from 68 recordings. Note that this averaged current shows a tendency to "inactivate". No simultaneous openings were obtained; thus it is most likely that there is only one channel in this patch (see Section 3.3). There were no traces without openings, or failures, in this experiment.
Because of the difficulty in obtaining large numbers of records on the same patch, the voltage dependence of the probability of opening was investigated in the following manner. The probability of channel opening was time-averaged across the first 50 ms and across an entire 400-ms pulse; both were then plotted against potential (Fig 4.2). The time-averaged probability of the channel being open was simply the fraction of the time that the channel was open. The average values obtained over 400 ms were smaller than those over 50 ms at potentials above -5 mV. This result is consistent with the inactivation that occurs in whole cell currents at higher potentials at room temperature. The mean open times were similar for the two sets of data, as were the average numbers of openings per burst in agreement with the Markovian assumption.

4.1.2 Properties of the tail currents

Another way of studying the probability of channel opening is by examining the "instantaneous" values of whole cell tail currents using the pulse protocol shown in Fig. 4.3. These tail currents were well described by a sum-of-two-exponentials relaxation. Often, in other cells, a much slower component was recorded (Brown et al, 1983). However this latter Ca-blocker-sensitive current was believed to be a contaminating current on the basis of several kinetic, ion-sensitivity, and temperature sensitivity experiments. The test pulses in Fig. 4.3 were sufficiently long for Ca currents to reach their peaks (3-4 msec), but sufficiently short to be free of inactivation (see later). All of the tail currents were fitted with a sum of two exponentials for an initial estimation of the parameters. The measured time constants were well separated (fast component, $\tau_F =
FIGURE 4.2
The probability of opening, indicated by $P_o$ on the graph, as a function of potential. The values were computed from records that had evidence of only one active channel. The number of records ranged from 4 to 20. On the left-hand side, the $P_o$ values were estimated as the fraction of time that the channel was open over a period of 50 ms, and on the right-hand side, as the same fraction over the total record length of 400 ms.
FIGURE 4.3

Turn-off of $I_{Ca}$. Ca tail currents were measured at the zero-current holding potential of -50 mV following activation to different potentials. As described in the text, from initial fits the time constants did not appear to be a function of the pulse potential ($\tau_F = 0.14 \pm 0.01$ msec and $\tau_S = 1.14 \pm 0.15$ msec); the time constants were thus set equal to the mean values and the resulting curve fits are shown.
0.14±0.01 msec and slow component, $\tau_s = 1.14±0.15$ msec) and had a ratio of approximately 8 instead of the value of 2 required by an $m^2$ model. The distribution of the time constants showed no correlation with the pulse potential, and it was concluded that the $\tau$ values were independent of the activating potential. We thus averaged the $\tau$ values for this experiment and fitted the data again, this time setting the values of $\tau$ to the average values. This procedure resulted in less scatter in the plots of amplitude versus pulse potential and increased our confidence in these parameter estimates. We assumed that $A_F + A_S$ reflected the number of open channels at the end of the pulse; thus by normalizing these values a peak probability of opening curve, or activation curve, was generated (Fig. 4.4B). Note that a large percentage of the channels were closed at +20 mV, the potential for maximum current during the pulse, and the number of open channels did not saturate until about +50 mV or more. The amplitudes of the two components, $A_F$ and $A_S$, showed the normal sigmoidal voltage dependence, and $A_F$ was considerably larger than $A_S$.

The open-channel current-voltage, or instantaneous I-V, relationship may be studied using another type of tail-current voltage-clamp protocol. In one such experiment, the potential was stepped to +46 mV for 3 msec to obtain maximal activation and then returned to different potentials (Fig. 4.4A). The resulting relaxations were fit with the sum of two exponentials and a constant, and $A_F$, $\tau_F$, $A_S$, $\tau_S$ and the steady-state amplitude, $A_{ss}$, were thus obtained as functions of return potential. In Fig. 4.4A these amplitudes and their sum have been plotted, the latter giving the instantaneous I-V curve for the Ca current. Note that $A_S$ is constant from -50 mV to +10 mV, whereas $A_F$
FIGURE 4.4
The activation curve, the instantaneous I-V relationship, and a construction of the peak I-V curve from these two measurements.

A. Instantaneous I-V relationship determined from tail current amplitudes according to the protocol shown in the inset. Tail currents were fitted with the sum of two exponentials, with amplitudes $A_F$ and $A_S'$, and a constant value determined by the value of the current at the end of 5 msec. The instantaneous current is the sum of these three terms. $A_S$ is independent of potential between -50 and 0 mV but $A_F$ and the instantaneous current are linear functions of potential over this range. The peak I-V curve is also shown.

B. The activation curve is obtained by normalizing $A_F + A_S$ as obtained in Fig. 8. The data points are fitted with Eqt. 4.1 of the text and the parameters for the fit are $V_1' = +12.8$ mV and $V_2' = -27.2$ mV.

C. Instantaneous I-V relationship obtained from A. The data points are fitted with Eqt. 2.28 of the text and $V' = 15$ mV, $H = 25.8$ mV and $P = 2.241 \times 10^3$ nA/mV·mM.

D. Constructed peak I-V relationship (continuous curve) and the measured peak I-V (data points). The constructed I-V was obtained by multiplying the function describing the activation curve in B and the function describing the instantaneous I-V in C. Parameters were the same as in B and C with the exception that $P$ was increased by a factor of 1.23 from $2.241 \times 10^3$ to $2.764 \times 10^3$. This value may be expected since between the two runs there was a decrease in the control current by a factor of 1/1.18.
shows a linear dependence on voltage. The instantaneous current, $A_F + A_S + A_{SS}$, is dominated by the $A_F$ components over most of the voltage range studied, and it becomes increasingly negative as the potential is hyperpolarized. This instantaneous I-V curve is very different from measurements published earlier (Kostyuk et al., 1981; Llinas et al., 1981) but agrees with the more recent observations of Fenwick et al. (1982) and Byerly et al. (1984). It appears that the reason for the differences with earlier reports is that the faster component was not resolved in the earlier reports. Measured single channel current-voltage curves resemble this instantaneous I-V (Lux & Brown, 1984).

From an instantaneous I-V curve and an activation curve, both of which were obtained from tail current measurements following 3 msec pulses, an isochronal I-V curve was constructed and compared to a measured one (Fig. 4.4D). In this calculation, it was assumed that equation 2.27 was obeyed. The probability of opening curve, $p_o(t=3\,\text{ms, V})$, is given in Fig. 4.4B and the quantity $N_i(V)$ is the instantaneous I-V shown in Fig. 4.4C. The instantaneous I-V relation must asymptotically approach the zero current axis at depolarized potentials because the peak I-V of the Ca current is never outward over the voltage range studied (Brown et al., 1981). Additionally, such an asymptotic form is obtained from the constant-field equation because the outward going flux is negligible in the case of the highly asymmetric Ca concentrations present ($[\text{Ca}]_i = 10^{-8}$ and $[\text{Ca}]_o = 10\,\text{mM}$). We found the instantaneous I-V relation to be somewhat less steep in form than that given by the constant-field equation, and it was fit with equation 2.28. The activation curve was described by

$$0(V) = \left\{1 + \exp(V_2' - V)\right\}^2 \exp(V_1' - V) + 1.0^{-1}$$

4.1
where the parameters are indicated in the legend. The peak I-V curve was obtained by multiplying these functions. Note that the fit of all three curves to the data points is quite good and recall that the constructed I-V was obtained from tail current measurements alone.

The tail currents obtained with what is called an envelope protocol were also examined. In this protocol, the voltage was stepped to a potential for varying durations before returning to the holding potential. In a cell without the third component, the amplitude, but not the time course, of the tail relaxation appeared to change (Fig 4.5A). This observation was confirmed in Fig. 4.5B where the tail currents obtained at pulse lengths of 10, 40 and 400 msec were fit with constant \( \tau \) values. In Fig. 4.5C, the decay of the tail current amplitudes is found to be similar to the decay of the current during the pulse. This implies that the apparent inactivation observed at +10 mV is due to a true decrease in the Ca current and is not due to contaminating outward currents, in agreement with the earlier single channel result. It would be quite unlikely for a contaminating current to develop without a corresponding change in the time course of the tail current relaxations. Additionally, note in Fig. 10D that \( A_F \) and \( A_S \) decreased equivalently as the pulse was lengthened. These results place constraints on the types of inactivation models to consider (see later).

4.1.3 Surface charge effects on permeation

One particularly interesting result obtained from tail current measurements was that the activation curve shifted to depolarized potentials when the external Ca concentration, \([\text{Ca}]_\text{o}\), was increased (Fig. 4.6). In addition to the activation curve, there was an insert
FIGURE 4.5

The relationship of the envelope of the tails to the current during the pulse. Tail currents were measured on a cell which did not have the slowest component in the tail relaxation.

A. Tail currents measured after the pulse lengths indicated; the spatial arrangement has no relation to the length of the pulse. Data were digitized at 30 μsec/point, and the base line was obtained from separate current measurements.

B. There was no charge in the relaxation time course of the tail currents, and fits are shown at three pulse lengths with the same \( \tau_F \) and \( \tau_S \) values as indicated.

C. Because the tail currents following long pulses were small, we found it necessary to correct for the asymmetry current. After replacing Ca with Co, the asymmetry current obtained did not appear to be a function of the pulse duration. Several of these currents were averaged and the currents were well approximated with a single exponential having an amplitude and time constant of -15.8 nA and 0.52 msec, respectively. We thus subtracted this function from each of the currents shown in A. This procedure was preferable to subtracting current traces because it resulted in less noise in the signal. The resulting currents were fitted with two exponentials, the time constants were averaged, and the currents were fitted once again, this time with the \( \tau_S \) set to their average values (\( \tau_F = 0.18 \) msec, \( \tau_S = 1.6 \) msec). The amplitude, \( A_F + A_S \), is the instantaneous value of the tail current, and it is plotted along with the current at the end of each pulse, designating the pulse current. Note the similarity in the decay of the two curves. After normalization, the time course of the tail envelope almost exactly reflected the decay of the pulse current during the first 200 msec (not shown).

D. The ratio of \( A_S/A_F \) as obtained in C is plotted as a function of the pulse duration. Note that there was little change in the ratio, indicating that inactivation is accompanied by a simple, equivalent scaling of both components of the tail relaxation.
$r_p = 0.2 \text{ msec}$

$\tau_s = 1.3 \text{ msec}$
FIGURE 4.6

Effects of increasing the extracellular Ca concentration, $[\text{Ca}]_o$, on tail current amplitudes obtained at $V_H = -50 \text{ mV}$ and peak currents measured during the activating step. Parameter estimates were obtained as in Fig. 10 (5 mM-Ca, $\tau_F = 0.16 \text{ msec}$ and $\tau_S = 1.58 \text{ msec}$; in 20 mM-Ca, $\tau_F = 0.13 \text{ msec}$ and $\tau_S = 1.24 \text{ msec}$).

A. Increasing $[\text{Ca}]_o$ increased and shifted along the voltage axis the peak current and the amplitudes of both tail current components. Note that the $A_S$ curve is smaller than the peak currents and that $A_S$ is very much smaller than the $A_F$ values.

B. Original traces which show that there was saturation of the tail currents as the pulse potential was increased.

C. Normalizing $A_F + A_S$ one obtains the activation function for $I_{\text{Ca}}$. Increasing $[\text{Ca}]_o$ from 5 to 20 mM results in a shift of the curve by approximately 17 mV. The smooth curves were drawn using the function $(1 + \exp[(V-V')/F])^{-1}$ where $F = 14.5 \text{ mV}$, and $V'$ is 0.5 and 27.5 mV in 5 and 20 mM $[\text{Ca}]_o$, respectively.
equivalent shift to more positive potentials of the peak I-V relationship (Fig. 4.6C). Note also that both $A_p$ and $A_s$ increased at higher [Ca]_o, indicating that both components were due to Ca ion flow. These effects have been examined in detail elsewhere (Wilson, Morimoto, Tsuda & Brown, 1983). The apparent voltage shifts were consistent with the effects of divalent anions on the surface potential resulting from fixed negative surface charge on the membrane, and the results were well-modeled by the Gouy-Chapman, diffuse double-layer theory as modified by Gilbert and Ehrenstein to include specific binding (1969). Selected results are shown in Fig. 4.7A. A ramification of the Gouy-Chapman theory is that the surface concentration of free divalent anions, as calculated from the Boltzmann relation, will be greatly affected by the surface potential. This effect tends to describe the saturating current-concentration relationships of the Ca channel as well as the relative sizes of the currents obtained in Ba, Sr, and Ca (Wilson et al., 1983). Even better agreement was obtained when the theory was modified to include a charge-free disk which represented the channel mouth as done by Apell, Bamberg, and Laüger (1979) for the case of an artificial channel. This latter prediction of the free divalent concentration is compared to the current-concentration relationships in Fig. 4.7B (see also Wilson et al., 1983).

Having established some of the basic properties of the Ca channel and shown how they agree with the types of models described earlier, I will continue by next examining some specific single-channel interval kinetics.

4.2 Single channel WCO histograms
FIGURE 4.7

Modeling of surface charge effects on the Ca channel.

A. Relationship between $V_p$ and bulk divalent cation concentration.
   The lines are theoretical expectations from the Guoy-Chapman equation for the diffuse double layer as modified by Gilbert & Ehrenstein (1969). The upward displacement in the case of Ca is due to the binding of Ca ions, assuming zero binding for Sr, Ba, and Mg. The theoretical curves were obtained from the following parameters where the equations are given in Wilson et al (1983): surface charge density, $\sigma$, of $1e/80 \ \AA^2$, external surface potential, $\Psi$, -116 mV, and $K_A$ which is zero for Mg, Sr and Ba and 0.1 M$^{-1}$ for Ca. Similar values for Na channels (Hille, Woodhull and Shapiro, 1975); K channels (Gilbert and Ehrenstein, 1969), and Ca channel (Ohmori and Yoshi, 1977) have been reported.

B. Relationship between surface concentration of the permeable species and peak current on the ordinates and bulk concentration on the abscissa. The surface concentration was calculated from the surface potential as determined from results in part A, the pore model of Appell et al (1979) assuming a pore of 6.9Å in diameter, and the Boltzmann relationship. Note that Mg reduces the surface concentration.
In Fig. 4.8 are plotted the CO histograms from single channel activity measured at -20 and 0 mV. The distributions of the open times were described by a single exponential PDF indicating a single open state. The mean open times were 1.6 and 1.4 ms at -20 and 0 mV, respectively. Over the range of -20 to 0 mV, at 5 mV increments, there was little voltage dependence of the mean open time. The single channel activity shows very brief closed times and some much longer closed times. Consistent with this observation are the two phases in the decay of the closed time histograms that were fit by a PDF consisting of the sum of two exponentials. A 3-state model of activation was the minimum model consistent with these results, and model M_2 of Fig. 2.2 (page 27) was used to fit the data. In Appendix A, it is shown that all the rate constants are uniquely identifiable from these measurements. Parameter estimates were obtained by fitting model M_2 directly to the data, and the estimates are given in the legend.

A burst length was defined as the length of a group of openings separated from other openings by periods three or more times the fast time constant of the closed time histogram. This definition included single openings. The distribution of burst durations is included in Fig. 4.8. As given in equation A9 of Brown et al. (1984), the apparent burst length is expected to be biexponentially distributed, and the parameters should be uniquely identifiable from the data. However, because of the somewhat arbitrary nature of defining a "burst" and because the bursts were truncated at 6 ms, parameters were not identified from this distribution. Instead, parameters already identified from the CO histograms were used to predict a burst length
FIGURE 4.8
Histograms of open time (left), closed time (middle), and burst length (right) obtained at -20 and 0 mV at 18°C. Data points are plotted in the center of the bins. In the case of the open time and burst duration plots the bin widths are 0.3 ms. The closed time histogram is plotted with bin widths of 0.3 and 3 ms to try to illuminate the two phases in the decay of the data, and the change in bin width causes the discontinuity in these plots. The first bin, combining openings of < 300 μs, is truncated in each plot because of the finite response time of the detection system. In the case of the open and closed time histograms that last data point is a "rest" bin, which includes the rest of the data with longer intervals as described in Brown et al (1984). Smooth curves in the plots are obtained from the appropriate PDFs for model $M_2$. From these two histograms all parameters in the model were identified as shown in Appendix A, and the prediction for the burst length data is plotted using Eqn. A9 of Brown et al (1984). Parameters in units of 1/ms at -20 mV are $k_+1 = 0.17 \pm 0.04$, $k_-1 = 0.37 \pm 0.09$, $k_+2 = 0.19 \pm 0.03$, and $k_-2 = 0.60 \pm 0.02$; the numbers of events measured were 89 and 96 for the closed time and open times, respectively; and the corresponding total numbers of events after extrapolation to time zero were 94 and 115. Parameters at 0 mV are $k_+1 = 0.13 \pm 0.02$, $k_-1 = 0.35 \pm 0.05$, $k_+2 = 0.40 \pm 0.03$, and $k_-2 = 0.70 \pm 0.05$; the number of events measured were 563 and 592 for the closed and open times, respectively; and the corresponding total numbers of events after extrapolation to time zero were 630 and 728. The time constants in milliseconds, computed from the k's above given in the order of the open $\tau$, the faster closed $\tau$, the slower closed $\tau$, the faster burst duration $\tau$, the slower burst duration $\tau$, the faster macroscopic relaxation $\tau$, and the slower macroscopic relaxation $\tau$ are 1.67, 1.5, 21, 1.1, 4.0, 1.0 and 2.7 at -20 mV and 1.43, 1.23, 16, 0.8, 1.6, 0.7 and 3.1 at 0 mV. These calculations were carried out using the expressions for the various time constants as they appeared in Brown et al (1984).
distribution, and the predicted curves are shown in Fig. 4.8. The fit to the measured data was fairly good considering that there were no free parameters.

Distributions of waiting times from patches containing one channel are shown in Fig. 4.9. Two distributions of waiting times taken from the same patch at -20 and 0 mV are shown in A and B, and C shows additional measurements at -20 mV from another patch. The presence of both a rising phase and a falling phase in the data is evidence for at least three states. Adjoining states would require the absence of a preceding rise.

The smooth curve plotted on these histograms is the waiting time predicted from parameters estimated in Fig. 4.8. Note that in each case the peak is larger than the predicted response and occurs later. Thus, the measurements are somewhat "slower" than the predictions. Next, we tried to fit these data with the equation describing the waiting-times for a 3-state model, equation B.1. We found that the fits failed near the peaks, and that the two \( \rho \) values tended to be almost equal. The \( \rho \) values are the eigenvalues for the system of differential equations describing the three-state model with an absorbing open state, and this system of equations is identical for both the closed and waiting-time predictions (Fig. 2.1). However, the waiting time \( \rho \)'s tended to be equal and thus were clearly discrepant with the measurements obtained from the closed time data. These results indicate that the waiting time data may emphasize different kinetics than the closed time data, which were obtained continuously along the voltage clamp pulse. This suggests that there is an
FIGURE 4.9
Waiting times at different potentials.
A and B. Waiting time histograms are shown at 0 and -20 mV from a single experiment. The experiments were done at room temperature with pulse durations of 50 ms. The smooth curves in both cases were obtained using Eq. A8 of Brown et al (1984), and the parameters for model $M_2$ as found in Fig. 13. The number of measurements at 0 mV was 151 with 12 failures, and at -20 mV the number of measurements was 81 with 9 failures. The computed curves were obtained by multiplying the PDF integrated across each time bin by the total number of records, including the failures. The presence or absence of the failures does not affect the position of the peak. Note that at -20 mV the predicted curve from model $M_2$ is somewhat "faster" than the measured response.

C. Waiting time histogram from another cell at -20 mV using 40-ms pulses. The smooth curve is from the same model and parameters as in B. The number of measurements was 62 and the number of failures was 24.
additional state in the activation process that has been uncovered by the waiting time measurements.

The above results prompted an analysis to determine whether the waiting time measurements were realizable with a 3-state model. In Appendix B, the relationship between the time of the peak and the size of the peak of the waiting time PDF is investigated. The analysis indicates that it is impossible to reconcile the waiting time measurements with a three-state model. First, in Fig. 4.10A the magnitude of the peak of the waiting time, \( f(\rho) \), is plotted versus \( \rho \) at a constant time to peak of 1 ms. An amplitude found on this curve will normally be associated with two \( \rho \) values. However, there is a maximum in this curve and only a single \( \rho \) value is obtained there. This explains why similar \( \rho \) values were obtained with the relatively "large" measured peaks. Also, it was found that the measured peak waiting times often fell above the maximum value in a plot such as that in Fig. 4.10A. Continuing with the analysis, equation B.8 relates the maximum permissible value for the peak of the PDF, \( P_{RO}^*(t_p) \), and the time of the peak, \( t_p \). This equation is plotted in Fig. 4.10B and an "acceptable" region is indicated where the data points must lie in order to be consistent with a three-state model. Note that all but one of the data points lie outside the acceptable region, indicating an inconsistency with the model. Moreover, one source of error would make the discrepancy larger. Peak values were taken from histograms with finite bin widths; such values would tend to be smaller than the true peak values.

4.3 Modeling of macroscopic activation

4.3.1 Parameter estimates from turn-on and turn-off
FIGURE 4.10

The measured waiting time peaks may not be realized by the 3-state model (M_2).

A. The solid line is a plot of the maximum value of the waiting time PDF given by f(p) defined in equation B.5. The time of peak was set at 1.0 ms. Given a t_p of 1.0 ms and a maximum value of the PDF corresponding to one of the three horizontal curves, we find either two distinct p values, a repeated p value, or no permissible p values.

B. The smooth curve is the maximum allowable value of the peak of the waiting time PDF as a function of t_p from equation B.8. Measured values for a 3-state model must lie in the "acceptable region". The ordinate shows the maximum probability density (1/ms). Potential and temperature are indicated. All but one of the data points lie in the "forbidden zone".
With regard to activation, the 6 models in Fig. 2.2 (page 27) were investigated. As described in Section 3.2 a set of three measurements was fit at each test potential. As examples, results from $M_2$ and $M_3$ are shown in Figures 4.11 and 4.12, respectively. The voltage-clamp protocols are drawn at the top of figures. In Table 4.1 are the parameter estimates obtained for models $M_1$ through $M_6$.

Two features common to all of the models used were of interest to the single channel work. First, the rate constant leaving the open state was quite fast in all cases ranging from approximately 3 to 6 ms$^{-1}$. This would predict a single channel mean open time (MOT) of .17 to .33 msec which is significantly faster than the measured MOTs ranging from .8 to 1.6 msec. The second feature is that the rate constant leaving the state adjoining the open state away from the open state ($k_{-1}$ in $M_2$ for example) tended to be constant as a function of voltage.

Below, the models are ranked in terms of their ability to fit the data in Figs. 4.11 and 4.12 as determined by the $\chi^2$ values listed in Table 4.1.

$$M_1 << M_2 < M_3 < M_4 \approx M_5 \approx M_6$$

Earlier, applying sum-of-exponentials-type models, large discrepancies were found between turn-on and turn-off time constants and this was used as a basis for indicating that $M_2$ was insufficient and higher-order models were required (Brown, et al., 1983). Similar arguments are found in Byerly et al. (1984). However, there was no good way to judge the actual discrepancy between $M_2$ and the measurements. The present method of using state models and multiple measurements is a much better way to make such a comparison. In fact
FIGURE 4.11
Fits of model $M_2$ to activation measurements as obtained from whole cell $I_{Ca}$. Three measurements obtained with the voltage clamp protocols shown comprised a data set for which a best fit was obtained. The test voltages are recorded on the left. Fits to the data were excellent and in most cases the smooth theoretical curve is not discernable from the data points. Parameter estimates are given in Table 4.1.
FIGURE 4.12.
Fits of model M₅ to whole cell I_{Ca}. Same as Fig. 4.11, except that model M₅ was used.
TABLE 4.1

Rate constant estimates for activation models $M_1$ through $M_6$ (Fig 2.2, page 27) from the data in Figs. 4.11 and 4.12. Parameter uncertainties calculated as described earlier were typically $<3\%$ of the point estimates (not shown). Units are $1/$msec for the rate constants and $A^2$ for $\chi^2$.

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$\Sigma = 5.05 \times 10^{-17}$

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as judged by eye, the fit of $M_2$ to the data was better than anticipated. However there is a significant deviation of $M_2$ from the data and, as shown later, under other experimental perturbations $M_2$ fails considerably. Between $M_4$, $M_5$ and $M_6$ the best model was $M_5$ as determined by this data set as well as the other types of experimental perturbations that follow. Thus, in the following I will focus on results from $M_5$ and where it is of interest, compare them to results from $M_2$.

In Fig. 4.13, various results from $M_5$ are shown. In A, the rate constant estimates are plotted as a function of voltage and smooth curves are drawn to indicate the splined curves used to approximate the voltage-dependence (see Section 3.4). The dashed portion of the curves indicate values that were extrapolated in order to meet with other results obtained, but from which parameters were not directly estimated (see figure legend). All of the models gave relatively smooth curves such as those shown in Fig. 4.13A; however, none, including $M_5$, gave curves that were well described by the usual Boltzmann description. Perhaps this indicates a complex, voltage-dependent conformational change, as opposed to a simple gating particle responding to changes in the electric field (Tsien and Nobel, 1969). In Fig. 4.13B, the instantaneous $I$-$V$ relation is shown; in Fig. 4.13C, the activation curve obtained from the steady-state value of $M_5$ is plotted; and in 4.13D, the peak $I$-$V$ curve is drawn. These all agree well with their experimentally determined counterparts.

4.3.2 Simulation of short 2-pulse experiments

Rate constant estimates for models $M_1$ through $M_6$ were obtained using data from the previously described voltage clamp protocols.
FIGURE 4.13
Various predictions of model \( M_5 \).

A. Rate constants for \( M_5 \) as a function of voltage. Data points were obtained from fits to the macroscopic currents. The smooth curves indicate the splined curves which were used to represent the data. Note that the rate constants change as a function of potential in a relatively smooth fashion. Dashed lines are drawn to show extrapolations required to meet constraints required by other measurements from which parameter estimates were not obtained directly. For example, at high voltages the forward rate constants had to be much larger than the backward ones in order for the activation curve to asymptotically reach one. At negative potentials the rate constants are relatively flat functions of potential because the tail current relaxation rate does not change over this range. Also, the rate of turn-off is independent of hyperpolarizations more negative than -50 mV.

B. Instantaneous I-V as obtained from the quantity \( N_i(v) \) in equation 2.28.

C. Activation curve as obtained from the opening probability at full activation.

D. The calculated peak I-V.
These were then used to simulate the responses from other test stimuli in order to test the appropriateness of the models. Examples are shown below and in the next section.

A short, 2-pulse protocol was particularly useful for testing the model order. Such test stimuli have been used in the case of Na currents (Hahn and Goldman, 1978a and 1978b) and in the case of Ca currents (Wilson, Brown & Tsuda, 1982; Byerly et al., 1984). The primary effect of the prepulse is a removal of the "phasic" delay during turn-on and a shorter time-to-peak as shown in the measured and simulated results in Fig. 4.14. Focusing on the results for a 3 msec interpulse interval, one finds that in the case of M_2 there is little effect of the conditioning pulse in contrast to the experimentally determined result. However, in the case of M_5 a significant acceleratory effect remains and the simulation agrees well with the measurements. Note that this result is obtained in spite of the fact that the tail currents for M_2 and M_5 are almost identical. Such a result is obtained simply because the additional state in M_5 allows channels to remain unopen but in the "active" or A pool for a longer period of time. In Fig. 4.15 are more results from measurements and simulations of M_5 using the same two-pulse protocol. In this case, the time to reach 1/2 of the peak value is plotted vs. the inter-pulse interval. Note that the parameters for M_5 were obtained from a different cell than the two cells used in Figs. 4.14 and 4.15. With this in mind, the agreement between the model and data is surprisingly good.

### 4.3.3 Simulation of temperature effects

Temperature has some interesting effects on the Ca channel in snail neuron which were modeled as described later. Since several
FIGURE 4.14

A 4-state model is required to describe results obtained from short 2-pulse protocols.

A. Currents obtained with the pulse protocol shown. In each panel the control current obtained without a prepulse is overlaid with the test current following the prepulse. The inter-pulse interval is written above. Note that the prepulse shortens the initial, slowly rising phase of the curves, or the "delay" of the current. Note additionally, that the time-to-peak is decreased. Since the final size of the current overlaps exactly, there was negligible inactivation with the 20 msec prepulse.

B and C Simulations obtained with the 3- and 4-state models shown. The currents shown were obtained using parameters as determined previously. In each pannel, test and control currents are overlaid and the interpulse interval is indicated. Note that in the case of the 4-state but not the 3-state model, an acceleratory effect of the prepulse remains with an interpulse interval of 3 msec. This occurs despite the fact the the current remaining at the end of the tail of the pre-pulse is identical in the two cases. One interesting feature of the simulations and data was initially observed from the simulations, indicating their predictive value. This feature was the small decrease in current immediately following the second pulse which is pratically apparent in the case of the 1 msec interpulse interval. This is due to a decreased driving force for current flow on a fixed number of open channels.
A

B

C
FIGURE 4.15
Effect of prepulse on the time to 1/2 the peak value \(t_{1/2}\). The pulse protocol is shown and the interpulse interval is plotted on the x axis. The points are measurements obtained from a different cell than that shown previously and the smooth curve, obtained from simulations of \(M_5\), agrees fairly well with the data. The non-normalized values of \(t_{1/2}\) were 1.43 ms as measured from the cell and 1.2 ms as obtained from the simulation. These were fairly close considering that the \(M_5\) parameters were obtained from a different cell.
different temperatures have been reported in the literature, $Q_{10}$ values are reported here where

$$Q_{10} = \frac{\text{Effect}_2}{\text{Effect}_1} \left( \frac{T_2 - T_1}{T_2 - T_1} \right)^{10},$$

4.2

and Effect$_2$ goes with temperature $T_2$, etc. With regard to whole cell current, turn-on kinetics slow markedly with decreases in temperature whereas turn-off or tail current kinetics do not. Brown et al. (1983) reported a $Q_{10}$ of $\approx 5-6$ for $\tau_m$ as obtained from $m^2$ fits to turn-on while turn-off was practically unchanged. Byerly et al. (1984) reported that the time to 1/2 the peak value in turn-on measurements changed with a $Q_{10}$ of $\approx 4.9 \pm 2$, and tail currents were somewhat slowed but this was not quantitated. Consistent with these observations were the single channel measurements of Lux and Brown (1984). The MCT was only slightly slowed following a decrease in temperature ($Q_{10} = 1.07$), and the closed time distribution was slowed primarily because of the relative increase in the amplitude and time constant of the slower of the two closed time components ($Q_{10}$ for this $\tau$ was 1.5). The waiting time was also markedly slowed (see Fig. 7 of Brown et al., 1984). In addition to these kinetic effects there was a decrease in the peak current amplitude with cooling as reported by Lux et al., 1984 ($Q_{10} = 1.8$); and Byerly et al., 1984 ($Q_{10} = 2.3 \pm 0.1$). In the case of Lux et al. (1984), this occurred due to a decrease in the peak $P_0$ ($Q_{10} = 1.5$) as well as a decrease in $i$ ($Q_{10} = 1.2$).
To model these kinetic features, the first transition in the state diagram was made to be very temperature sensitive. The modified diagram is

\[
\begin{align*}
R & \xrightleftharpoons{af_f}{a} A_1 & 2a & \xrightarrow{k_{+3}} O \\
& bF_b & 2b & \xleftarrow{k_{-3}} \\
\end{align*}
\]

where \( F_f \) and \( F_b \) are temperature-dependent multiplication factors as determined from thermodynamic considerations. The temperature dependence of the rate constants in most chemical reactions can be described by the activated complex theory, or Eyring theory, as given in the expression below (Espenson, 1981; Tsien & Noble, 1969).

\[
k = \frac{kRT}{Nh} e^{\frac{\Delta S}{R}} e^{-\frac{\Delta H}{RT}}
\]

In the above, \( \Delta S \) and \( \Delta H \) are the entropy and enthalpy, respectively, of the energy barrier associated with the activated complex; \( N, R \) and \( h \) are standard chemical constants; \( T \) is absolute temperature, and \( K \) is the "transmission" coefficient which is usually assumed to be unity. Using equation 4.4 a rate constant, \( k_2 \), at a new temperature \( T_2 \) can be evaluated from a previously determined value at \( T_1 \); i.e.

\[
k_2 = \frac{T_2}{T_1} \exp\left(\frac{-\Delta H (T_1 - T_2)}{RT T_1}ight)
\]

Thus, in the state diagram above [4.3] the values of the rate constants were determined initially from experiments done at room temperature; the \( F \)'s were given by the right-hand-side of equation 4.5; and the only free parameters were \( \Delta H_f \) and \( \Delta H_b \) for the forward and backward rate constants, respectively.
Assuming this description, the ΔH values were varied manually until reasonable agreement was obtained with all of the above observations. Whole cell current simulations done at 25°C and 15°C agreed very nicely with the measured results (Fig. 4.16). The time-course of the tail relaxations was unaffected by temperature at a return potential of -50 mV and very little affected at depolarized potentials. However, turn-on was affected greatly - the time to 1/2 peak had a Q_{10} of ~ 4. In Fig. 4.17 A&B it is shown that reducing the temperature 10°C decreased the peak whole cell current by a factor of 0.56 due to reductions in both the peak probability of opening and single channel current, i. The waiting times were slowed markedly and the closed-time PDF was also slightly slowed. The open-time PDF was unchanged. Thus, this simulation agrees nicely with the measured results. Note that this is not fortuitous; the multiplicity of measurements placed several constraints on these simulations. Agreement would not have been obtained by making other transitions strongly temperature dependent or by using a 3-state as opposed to a 4-state model.

The magnitude of the temperature dependence on the first transition step may be surprising. In the simulations, the forward and backward rate constants had ΔH, ΔS, and Q_{10} values of 3.7 x 10^4 cal/mole, 78 cal/mole^oK and 9 for the forward rate constant and 2.3 x 10^4 cal/mole, 16 cal/mole^oK and 3.9 for the backward rate constant, respectively. The other transitions must be relatively temperature insensitive. For example, the inverse MOT has a Q_{10} of only 1.07 (Lux et al., 1984). This latter value is not only low as compared to the Q_{10}'s above, it is also low as compared to the Q_{10}'s for other channel
FIGURE 4.16
Simulations of whole cell current relaxations at two temperatures. Note that the time-to-peak of the current is dramatically increased by the change in temperature at the 3 voltages shown on side A of the figure. The times to 1/2 peak were increased by ratios of ~ 2.6, 3.5 and 4.1 at -15, +10 and + 40 mV, respectively. The tail current relaxations at the holding potential were unaffected by temperature except that they were scaled. In B, tail currents obtained at -15 mV following a strong depolarizing pulse are shown, and again little effect on the tail relaxations are observed. These results compare favorably with data in Fig. 15 of Brown et al. (1983) and Fig. 3 of Lux and Brown (1984). Simulations were done using diagram 4.3 with \( \Delta H_f = 3.7 \times 10^4 \) cal/mole and \( \Delta H_D = 2.3 \times 10^4 \) cal/mole; \( v \) was reduced after cooling at all voltages by a factor of .82 and other parameters have been given previously.
FIGURE 4.17
Simulations of the effect of temperature on single channel parameters at 0 mV. In A, $p_o(t)$ curves are shown, and the maximum value obtained at 15°C is .68 of that at 25°C. The inset was drawn to indicate the measured decrease in $i$ by a factor .82. Both of these effects combine to give the reduction of the peak macroscopic current by .56 shown in B. In C, D and E the WCO PDF’s are shown. The inset in D is simply the same graph on a different scale. Qualitatively these results agree favorably with reports in the literature (see text). More quantitative comparisons were not made because results presented later indicated severe distortions in the measured WCO histograms. Refer to Fig. 4.16 for details of the simulation.
types (Tsien & Noble, 1969). According to equation 4.4 these transitions must correspond to reactions involving relatively more entropy than enthalpy. Tsien and Noble (1969) suggest that such reactions might involve the ordering of water molecules. At any rate, these thermodynamic values place constraints on the possible reactions and may help in future investigations of the gating conformations of the channel protein.

In general, given a complicated kinetic scheme, temperature effects on directly measurable parameters are relatively complicated to interpret (Espenson, 1981). For example, changes in macroscopic turn-on such as the time to 1/2 peak do not directly correlate to changes in $F_f$. Nor does the change in peak $p_o$ correlate with the ratio of $F_f/F_b$. Thus, an Arrheneous plot of such easily measurable parameters at various temperatures might give "break-points", or other peculiarities, which are simply an artifact of the complex kinetic scheme rather than a fundamental effect such as a membrane phase change.

There are other examples of the complexity of the effects of temperature. In simulations done at various potentials there was a relatively greater effect of temperature on macroscopic turn-on at depolarized potentials (Fig. 4.16). That was not observed experimentally (Brown et al., 1983). This might be due to a compensating effect that was unaccounted for in the present simulations. The enthalpy would be expected to have a contribution from a voltage dependent term, and this predicts a decrease in the $Q_{10}$ of a forward rate constant at depolarized potentials where it is faster (Tsien & Noble, 1969). The competition of this effect with the above one may
explain why no voltage dependence was observed. Another observation was that changes in $P_f$ could result in fairly substantial changes of the time-course of inactivation (not shown). Thus the measured effects of temperature on inactivation (Brown et al., 1983) may be due to an effect on the first transition rather than on "inactivation" transitions. Only in an "independent" model of inactivation could the temperature effects be readily separable.

Thus, the elucidation of temperature effects on single rate constants must involve parameter estimation and simulation studies as well as measurements.

4.4 Modeling of inactivation

4.4.1 Parameter estimates from single-pulse experiments.

$I_{Ca}$ measurements resulting from long voltage pulses were fit to 4 different models of inactivation (Fig. 2.3, page 29) and selected results are shown in Fig. 4.18. These pulses were sufficiently long for $I_{Ca}$ to display both the fast and slow phases of inactivation. All 4 models reasonably describe these data.

Parameter estimates are given in Table 4.2 for the experiment illustrated in Fig. 4.18. In some cases parameters were fixed at certain values in order to make the model response, more "reasonable". For example, at positive potentials (> 0 mV) $S_b$ was set to zero. This made the slow inactivating state to be absorbing and guaranteed that the current would eventually be zero. This is in accordance with previous measurements using longer pulse protocols (Brown et al., 1983). At potentials between -50 and 0 mV, $S_b$ was constrained to lie within the value at depolarized potentials and that obtained from recovery measurements. Yet another constraint contemplated was to
FIGURE 4.18
Examples of fits of models $M_{B3}$, $M_{B2}$, $M_C$ and $M_A$ (Fig. 2.3, page 29) to data obtained from 600 msec pulses at the potentials shown. Such pulses are sufficiently long for $I_{Ca}$ to display both the fast and slow phases of inactivation. A smooth curve is drawn to show the model result. The models in all 4 cases reasonably describe the data. Model $M_A$ deviates from the data more than the others, but it should be remembered that $M_A$ has fewer free parameters since only one parameter was allowed to change as a function of voltage. Activation parameters are shown in Fig. 4.13 and inactivation parameters are given in Table 4.2.
make the steady-state values equivalent to previously obtained $h_\infty$ curves (Brown et al., 1981). This was not done because the parameter estimates obtained here suggested that quite a long time would be required to reach steady-state. Perhaps this was not examined closely enough previously.

As described in Chapter 1, many experiments have indicated that $I_{\text{Ca}}$ inactivation depends on the presence of internal Ca, and these experiments have led to the Ca-accumulation-dependent model in Fig. 2.3, page 29. In terms of the alternative models, it is suggested that the presence of Ca may change the rates of transitions, but that they are held constant over the time-frame of a single pulse. For example, increasing internal Ca may act to induce another substance intracellularly which in turn acts as a catalyst to enhance inactivation. Perhaps such a mechanism would be relatively "slow" in developing or would saturated at relatively low concentrations of internal Ca. In such cases, the Ca entering through a single pulse would have negligible effect, yet the presence of Ca intracellularly or in the bath would make inactivation faster. In this regard, data obtained with and without EGTA in the intracellular solution were fit using model $M_{B3}$. It was found that parameter $F_b$ was most affected and was slower in the case of internal EGTA. Probably most differences could be reasonably described with a change in this single parameter (not shown).

It should be noted that even a Ca-accumulation-dependent model requires the intracellular action of Ca rather than an action at the internal face of the channel (Chad et al., 1984). This arises from the fact that the concentration of free Ca ions would saturate in the
TABLE 4.2
Parameter estimates for inactivation models as obtained from
the experiment in Fig. 4.18. In the case of $M_{b2}$, $M_{b3}$, and $M_C$
the value of $S_B$ was constrained as determined by the following
arguments. At depolarized voltages the current completely
inactivates (Brown et al, 1983); thus one of the states must be
absorbing (hence $S_B = 0$). The recovery data was best described
by the parameters shown at -50 mV. The $S_B$ parameter was
constrained at the other voltages to be between
these two values.

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<td>1.0</td>
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<tr>
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<td>$5.8 \times 10^1$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+10</td>
<td>$3.0 \times 10^1$</td>
<td></td>
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### Table M_B2

<table>
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<th>$F_f$</th>
<th>$F_b$</th>
<th>$S_f$</th>
<th>$S_b$</th>
</tr>
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<td>$3.5 \times 10^{-3}$</td>
<td>$2.0 \times 10^{-3}$</td>
<td>$4.0 \times 10^{-4}$</td>
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<td>-5</td>
<td>$3.7 \times 10^{-2}$</td>
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<td>$7.0 \times 10^{-3}$</td>
<td>$1.0 \times 10^{-5}$</td>
</tr>
<tr>
<td>0</td>
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<td>$6.5 \times 10^{-3}$</td>
<td>$6.0 \times 10^{-3}$</td>
<td>$1.0 \times 10^{-5}$</td>
</tr>
<tr>
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<td>0</td>
</tr>
<tr>
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<td>0</td>
</tr>
<tr>
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<td>$3.8 \times 10^{-2}$</td>
<td>$6.9 \times 10^{-3}$</td>
<td>0</td>
</tr>
</tbody>
</table>
vicinity of the channel almost immediately upon its opening; this response is not consistent with the integration of the current in the Ca-accumulation-dependent model.

From fits to single-pulse macroscopic data one cannot reasonably discriminate between the models suggested. This applies to models M_B1 and M_D as well as those used in Fig. 4.18. In order to discriminate between these models other measurements and analyses are required. Some suggestions follow.

4.4.2 Simulation of recovery from inactivation

I_Ca recovers from inactivation quite slowly in two phases (Yatani, Wilson, and Brown, 1983; Tillotson and Horn, 1978; Adams & Gage, 1979; Plant & Standen, 1981). This response is well described by a sum of two exponentials (Yatani et al, 1983). In Fig. 4.19 the recovery of the modeled peak current is shown and it agrees favorably with measurements reported previously (see Fig. 2 and Table 2 of Yatani et al, 1983).

In Fig. 4.20 are model results showing the time-course of the current occurring in the test pulse of the 2-pulse protocol used to obtain recovery data. The modeled results mimic the data quite well in that as the recovery time, t_R, varies from 0-100 msec the test pulse current becomes progressively more peaked. Over the next 1000's of msecs the slower phase of the current recovers. Observations such as these have been reported in Yatani et al (1983) and Chad et al (1984). The state variable plots in Fig. 4.20C show in detail why such a response is obtained.

The above recovery results were simulated using model M_B3, but models M_B1, M_B2, M_B3 and M_C would have worked just as well. Never-
FIGURE 4.19
Time-course of recovery from inactivation. Model currents were obtained from the voltage clamp protocol shown. The percent recovery was the ratio of the current in the test pulse to that of the control current without a conditioning pulse. In A & B the percent recovery is plotted as a function of $t_R$, the recovery time, and $t_c$, the length of the conditioning pulse. Note that there is a marked effect of $t_c$ and in all three cases recovery occurs in two phases. The model was $M_{B3}$ and parameters are given in Table 4.2. Actually, in the case of $M_{B2}$, the recovery time course is essentially equal to $Y = 100 - C_1 e^{-t_{F}} + C_2 e^{-t_{B}}$ where $C_1$ and $C_2$ are obtained from the initial probabilities of being in states $I_F$ and $I_S$, respectively.
FIGURE 4.20
Time-course of $I_{Ca}$ during recovery from inactivation. The voltage clamp protocol is drawn in A, and it consists of a long inactivating prepulse, a recovery pulse of length $t_R$, and a test pulse. In B modeled currents during the test pulse are shown following various recovery times. At $t_R$'s between 0-100 msec the recovery of the peak current is due to the appearance of the faster inactivation phase with no change in the current at the end of 200 msec. At longer $t_R$'s the slow phase recovers. This phenomena has been reported by Yatani et al (1984), and Josephson et al (1984). In C one can see why this phenomena occurs. The model on the right has been partitioned into 3 groups - the "available" states, the fast inactivating state, and the slow inactivating state; the probability of being in each of these groups as a function of time is $P_V(t)$, $P_F(t)$ and $P_S(t)$, respectively. These probabilities and $P_O(t)$ are plotted on the left as obtained from the voltage clamp protocol shown. Note that $P_O(t)$ and $P_V(t)$ decrease while $P_S(t)$ and $P_F(t)$ increase during the voltage pulse as expected. Following the return to the holding potential there is a rapid decrease of $P_F(t)$ and a rapid increase of $P_V(t)$ corresponding to the fast recovery phase from inactivation. Unloading of the $I_S$ state occurs much more slowly and gives rise to the slow recovery phase. It is the rapid unloading of the $I_F$ state which allows the current in a subsequent pulse to have a rapidly inactivating phase. The model was $M_{B3}$ and parameters have been reported previously.
theless, the recovery response helps eliminate certain types of models as described below. In the case of model $M_A$, one obtains a recovery response having a "delay" or a small initial derivative. This has been reported previously (Standen and Stanfield, 1982; Chad et al, 1984). A delay would also be obtained in the case of model $M_D$ as required by the following argument. In order for the current to eventually be zero, either state $I_1$ or state $I_2$ must be absorbing. Also, a 2 exponential inactivation phase is required. The diagram below is the only one fulfilling these requirements for the case where $I_2$ is absorbing.

```
R ← A_1 ← A_2 ← O
```

Also, transitions between $A_2$ and $I_1$ must be relatively much faster than from $O$ to $I_2$, or inactivation would be predominantly single exponential. During recovery, if transitions from $I_2$ to $O$ again remain slow, then the predominant path for fast recovery will be $I_2$ to $I_1$ to $A_2$ and this will give a phasic delay. Similar arguments apply when $I_1$ is made absorbing and the argument is complete.

Using recovery data it has been argued that model $M_A$ is incomplete and model $M_D$ is inappropriate. In the next section a classical 2-pulse protocol will be examined as a possible means of discriminating between models.

4.4.3 The 2-pulse test for a coupled model

The 2-pulse protocol for measuring inactivation has often been suggested as a means of discriminating between "coupled" and "independent" inactivation models for the case of Na currents (Goldman, 1976).
FIGURE 4.21

The "delay" in inactivation of a coupled model ($M_{B3}$) is not large enough to determine experimentally. Inactivation was measured using a 2-pulse protocol and the peak current during the test pulse at +20 mV is plotted following a variable length prepulse as shown on the diagram. The inset curve is simply drawn using a different scale. In the inset a very small delay is visible that is $\leq 1$ ms when the interpulse interval was 6 msec. However, when the interpulse interval was changed to be $\leq 2$ msec, it was found that a larger delay was obtained (not shown). This artifact occurs because insufficient time has been given for the activation states to unload as described previously (Kniffki, Siemen, Vogel, 1978). The model in these simulations was $M_{B3}$. The inactivation parameters were slightly different than those in Table 4, but not enough to alter the conclusion; they were, in units of ms$^{-1}$, $F_f = 2.1 \times 10^{-2}$, $F_b = 1.0 \times 10^{-2}$, $S_f = 3.2 \times 10^{-3}$ and $S_b = 0$. Other parameters have been given previously.
The pulse protocol is shown in Fig. 4.21, and the supposed feature of the coupled model is a phasic "delay" in the onset of the decrease of the test pulse current as the length of the conditioning pulse is increased. In the case of model $M_{B3}$ the delay was much too small to be measurable despite the fact that the model is coupled. Thus, such a protocol will not unequivocally discriminate between these models.

4.4.4 Inactivation can be ignored in studies of activation

In the case of short pulses used to study the activation of $I_{Ca}$, inactivation is sufficiently slow that it can be reasonably ignored. In Fig. 4.22, the time-course of $I_{Ca}$ for the first 6 msec does not appreciably depend on the presence or absence of inactivation. Note that this is not the case for Na currents (Wilson, Brown, Kunze and Lacerda, 1985; Kunze, Lacerda, Wilson, and Brown, 1985; and Aldrich, Corey, and Stevens, 1983).

4.4.5 Single channel tests for Ca-accumulation-dependent inactivation

In Fig. 4.23 are results on single-channel simulations from a test recently proposed for discriminating a Ca-accumulation-dependent process (Brown and Lux, 1984). The method consists of sorting a collection of traces into groups with low and high initial activity, identified as Lo and Hi in Figure 4.23. In the case of a Ca-accumulation-dependent process it was anticipated that the Hi curve would fall below the Lo curve at later times because on average more Ca had entered in the Hi group. In fact this is the case (Fig. 4.23D), but with a reasonable number of records, the result is not always unequivocal (Fig. 4.23B). There are various reasons for the insensitivity of this test. In Fig. 4.23E it is seen that the predominant reason
Inactivation of $I_{Ca}$ is slow enough that it can be reasonably neglected in the case of studying activation with short pulses. Model currents are shown on two different time scales with and without the presence of inactivation states. Note that for a pulse of $\leq 5$ msec the error in $p_o(t)$ due to neglecting inactivation is $< 4\%$. The model was $M_{b3}$ and the parameters were described previously with the exception that the inactivation parameters were in units of msec, $F_f = 1.87 \times 10^{-2}$, $F_b = 1.67 \times 10^{-1}$, $S_f = 4.47 \times 10^{-3}$ and $S_b = 0$. 
FIGURE 4.23

Testing of inactivation models by sorting single channel traces upon the initial probability of opening.

A. Average of 1000 traces for the Ca-accumulation-dependent (model $M_A$) and the voltage dependent (model $M_{B3}$) models. Note that the time-course of inactivation is approximately the same.

B. Ca dependent case: Two hundred traces were sorted on the basis of the fractional open time calculated for the first 60 msec in the trace. The Hi curve is the average of 65 traces with fractional open times $> .22$. The Lo curve is the average of 110 traces with fractional open times $< .2$. The fractional open time following the first 60 msec was .140 and .139 for the Lo and Hi curves, respectively. Unexpectedly, the Hi curve falls only slightly under the Lo curve at later times. Voltage dependent case: Same paradigm as above. Following the sort the Hi curve (70 traces) continued to lie above the Lo curve (112 traces). Fractional open times following the sort were .125 and .160 for the Lo and Hi groups, respectively.

C. Sort of 10,000 traces for the case of the Ca accumulation dependent model. The parameters for the sort were near optimum for model discrimination. The Hi curve is the average of 1634 traces, which in the first 80 msec have fractional open times $> .24$ and the Lo curve is from 5804 traces with fractional open times $< .2$. Fractional open times following the sort were .141 and .122 in the Lo and Hi curves, respectively. The sort method may distinguish between the two models, but an excessive number of records may be required.

D. The probability of being in the inactivated state for the Hi and Lo groups from the simulation in C. The primary reason for the Lo group having low initial activity is that it has a high probability of being inactivated.

Model parameters for these simulations were given previously with the exception of the voltage-dependent inactivation parameters which were, in units of ms$^{-1}$, $F_f = 3.9 \times 10^{-2}$, $F_b = 2.3 \times 10^{-2}$, $S_f = 7.4 \times 10^{-3}$, $S_b = 1.0 \times 10^{-5}$. 
for the Lo group having low initial activity is that in this group there is a relatively high probability of being in the inactivated state. Since the inactivated state is relatively long-lived, there is a predisposition of this group to have a low probability of opening at later times. Likewise, in the Hi group there is a greater chance for a channel being open at the end of the time period for sorting. These "leak-over" effects are in the direction to tend to cancel the desired effect. These phenomena are also apparent in the case of the voltage dependent model, \( M_{B3} \), where because of leak-over the Lo curve stays below the Hi curve throughout the record (Fig. 4.23).

Thus this test which is attractive on an intuitive level introduces biases which limit its usefulness. It is anticipated that the other tests described by Brown and Lux (1984) will suffer from the same types of deficiencies. These results led us to investigate certain other methods for testing nonhomogeneous Markovian behavior, but to-date all of these have been unsuccessful (not shown). In each case, the analysis introduced some rather obscure bias. It was only through testing these methods against simulations were the problems uncovered.

4.5 **Errors associated with single channel measurements.**

4.5.1 **Effects of filtering on single channels.**

The activation parameters obtained from the macroscopic currents and given in Table 4.1 were used to predict the single channel measurements, and these were compared to the measured results given earlier. The rate constant for leaving the open state (\( k_{-3} \)) was quite fast at all potentials and ranged from \(-3\) to \(6 \text{ ms}^{-1}\). These values predict single channel mean open times (MOTs) from .17 to .33 msec.
which are significantly faster than the measured MOTs ranging from .8 to 1.6 msec. In addition to the MOTs, the predictions for the waiting and closed time distributions were significantly faster than those measured (see Fig. 4.29). In order to evaluate such discrepancies more fully we used single channel simulations.

In such simulations, it was found that a significant number of fast openings and closures were missed under the normal recording condition of 1 Khz bandwidth (Fig. 4.24). The effects on the open and closed time histograms are complicated. For example, missing a fast opening not only distorts the open time histogram, it also distorts the closed time histogram. Similar distortions are obtained when a closure is missed.

In Fig. 4.25, closed and open time histograms obtained from such simulations are shown and compared to the apparent PDF theory developed Section 2.5. The distortion of the filtering is dramatic. Only 40% of the openings were detected, the MOT was increased from .25 to .55 ms, and the fast phase of the closed time distribution was practically eliminated. In Chapter 2, the detection system was modeled as having a dead time. This is an approximation as shown by the following argument. Consider the case of 3 pulses of width just less than the dead time separated by infinitesimally small gaps. The waveform obtained after filtering would cross the opening threshold, yet the analysis would not consider this to be a detected event. Nevertheless, the good agreement between the smooth curves obtained from the apparent PDFs and the simulated data indicate that such events are rare and that the dead time model is adequate.

4.5.2 More results from single channel simulations.
FIGURE 4.24
Filtering of single channel stimulations causes distortions in the measurement of open and closed time intervals. In each panel, 3 traces are shown which are (from bottom to top), the original simulation, the simulation after filtering, and the idealized record resulting from the threshold detection and amplitude measurement scheme. Channel openings are downward deflections. Panel A shows that three short openings were left undetected. The open time histogram would therefore be distorted as would the closed time histogram which would have one long interval in the place of four shorter ones. Panel B shows that several fast closures were missed, and only one long opening was measured. Once again both closed and open time distributions would be distorted. Thresholds were $.6$ and $.3$ of the channel amplitude for opening and closing, respectively. Other details of the simulation are in Fig. 4.25.
FIGURE 4.25
Effect of filtering on open and closed time histograms. The smooth
curves were obtained from the ideal PDFs in the case of the unfiltered
data and from the apparent PDFs calculated as described previously
for the filtered data case. In both cases the theory matched the
simulation quite well with no free parameters. Average values
measured (M) and computed theoretically (T) follow in units of msec:
MOT unfiltered (M = .247, T = .250); MOT filtered (M = .55, T = .58);
MCT unfiltered (M = 1.9, T = 1.8); and MCT filtered (M = 3.9, T =
4.6). The numbers of events were 1847 and 1150 for the unfiltered
and filtered data, respectively, as obtained from slightly different
lengths of continuous data (≈ 6 sec in the unfiltered case). After
correcting for record lengths, only 40% of the events were detected
after filtering. Parameters for the 3-state model are shown; the
minimum resolvable open and closed times were both .21 ms; and bin
widths are .3 and .03 ms for the closed and open times, respectively.
FIGURE 4.26

Single channel simulations showing the effect of noise and the effect of missed closures on amplitude.

A. In the top record are two idealized units with different amplitudes. The shorter unit resulted from missing a fast closure. Note that the length of an opening is not a good indicator of the reliability of the measurement - longer openings are not guaranteed to be accurate. That is, the unit measured in Fig. 29B is relatively long, but its amplitude is small due to the several missed closures it contains.

B. The addition of noise causes various effects on the detection and measurement of openings. The traces are: (1) original simulation, (2) trace 1 after filtering, (3) idealized record from detected events in trace 2, (4) record obtained after adding noise to trace 1 and filtering, (5) idealized record obtained from trace 4. Gaussian noise having a RMS-to-peak-signal ratio of .15 was added as described in Section 3.6. A comparison of traces 3 and 5 shows that noise affects both the amplitudes and intervals that were measured. A fast closure was missed near the time marked by "a" and an opening was added near "b". Thus the theory developed for the filtered noiseless trace (trace 2) would be expected to have some shortcomings when applied to noisy data.
In Fig. 4.26B single channel simulations are shown that include noise which was added to simulate the real recording situation. As a result of the noise, several distorted intervals were measured (compare traces 3 and 5). Note that this occurred despite the fact that false positive threshold crossings from the baseline would be rare. The latter may be calculated from

$$\lambda_F = k f_c \exp \frac{-\phi^2}{2\sigma_n^2}$$

4.6

where $k$ is $\approx 1$ but depends on the type of filtering and noise characteristics; $f_c$ is the corner frequency of the filter; $\phi$ is the detection threshold; and $\sigma_n$ is the standard deviation of the noise (Colquhoun and Sigworth, 1983). Evaluating $\lambda_F$ with $\phi/\sigma_n = 4$ and $f_c = 1$ Khz one obtains $\approx 3$/sec giving 18 false-positive openings in the above simulation consisting of 6 sec and $\approx 1800$ openings. Although the noise resulted in several distorted interval measurements, its effect on the WCO histograms tended to average out since some intervals were made larger and others smaller. Nevertheless, there was a "slowing" of the open time distribution which gave an error of $\approx 15\%$ in the maximum likelihood estimated of the MOT (Fig. 4.27).

"Small" openings were measured in those cases where fast closures were missed (Fig. 4.24 and 4.26A). Surprisingly, the amplitude distribution was not monotonic, as anticipated, rather it was bimodal (Fig. 4.27D, no noise case). This result is particularly interesting because it raises questions as to the validity of using such a distribution as a criterion for the existence of more than one type of channel (Nagy, Kiss and Hof, 1983; Carbonne and Lux, 1984). After
FIGURE 4.27

The effect of noise and filtering on WCO and amplitude histograms. Parameters for the simulations are described in Figs. 30 and 31.

A. The open time histogram had longer openings when noise was present. The smooth curve in each case is from a 1-exponential PDF having a MOT, or $\tau_0$, which was the maximum-likelihood estimate for the case without noise ($\tau_0 = .41 \pm .01$ ms). One can see an excess of longer openings in the case of the data with noise, and in this case a maximum likelihood estimate gave $\tau_0 = .47 \pm .02$. The addition of noise resulted in an additional 4.5% of detected openings.

B and C. The closed and waiting time distributions were also affected by the presence of noise, but perhaps not as markedly. In the case of the closed time, the smooth curves are from a 2-exponential PDF which was the maximum likelihood estimate for the case without noise. There was little deviation from the smooth curves in either case, but this is due partially to the increased bin width in these plots as compared to the open time histogram. The waiting time distributions were also distorted.

D. The amplitude distribution is not simply skewed to smaller values by filtering. Rather, as shown on the left, a bimodal distribution is obtained where the peak at lower amplitudes corresponds to apparent units having missed closures (Fig. 31). The average amplitude was 459 points as compared to the original amplitude of 500 points indicated by the arrow. On the right is the result obtained from a different simulation after adding noise. In this case the distribution was smeared sufficiently to obscure the bimodal distribution. Measured values fell above and below the original amplitude of 500 points, and the mean value was 410 points. The smooth curve is from a Gaussian fit to the data with mean and standard deviation parameters of 408 and 90 points, respectively. For comparison, the added noise had a RMS value of 75 points.
the addition of noise, the measured distribution was smeared and was not apparently bimodal (Fig. 4.27D, with noise case). It is anticipated that for lower values of noise, a compromise between the two cases could be obtained, and the distribution could be well described by a sum of two Gaussians.

Another interesting result was the distortion of the closed time distribution in the case of fixed-length records. In this case, the longest of the closed times were dropped, and this effect was quite marked for 30 msec epochs (Fig. 4.28). Using analyses similar to those for the dead time calculation, it may be possible to describe this effect analytically. This has not been done presently, but since most of the Ca single channel data has been recorded with longer data records, the effect may be minimal.

4.6 Comparison of macroscopic and microscopic measurements.

Using parameters obtained from the whole cell current measurements (model M5), predictions for the microscopic measurements were computed and compared to previously reported data. It should be noted that similar microscopic predictions were obtained with the other models investigated.

First, consider the results for the WCO PDF's (Fig. 4.29). The curves predicted from the macroscopic results without correction are much "faster" than the measurements. That is, the probability density at early times is larger for the former than the latter; in fact, on the open and closed time graphs the uncorrected macroscopic parameter curves are off the graph initially, indicating many fast events. In contrast, the corrected curves are probably within the experimental error of the single channel measurements. The corrected
FIGURE 4.28
Effects of fixed record lengths. Data obtained from fixed record lengths produce further distortions, particularly in the case of the closed time histogram. Shown are data points from simulations and smooth curves calculated from the standard theory which assumes continuous data. Insets are the same plots with different scales. Since the smooth curve is the same theoretical PDF in both cases, it provides a good reference for comparison. Note that there is a diminishing of long closed times in the case of the fixed record length data. This occurs because the longest closed times are selectively removed when the closed times approach the record length. In the case of the open times where the intervals were much smaller there was very little effect of the fixed record length (not shown). Likewise, closed times are less severely affected when longer pulses are used. The model is shown with the parameters used; the fixed record length was 30 ms and the channel began each record in the rest state; bin widths were .3 ms.

\[ R^{35}_{65} A^{16}_{4,0} O, CLOSED TIMES \]

CONTINUOUS

PULSED
FIGURE 4.29
Comparison of single channel measurements to predictions from macroscopic current measurements. As indicated in the legend, the figure contains: (1) predictions of parameters obtained from macroscopic current measurements, (2) predictions after correction for limited interval resolution, and (3) single channel measurements where a line indicates a single measurement and a dashed area indicates multiple measurements. Several details required in the construction of these curves follow. All data were obtained at 0 mV. The integral of the WCO PDFs is unity. The measured waiting and closed time curves were obtained from parameters in Fig. 5 of Brown et al., 1984. The dashed area on the open time curve corresponds to MOTs ranging from .7 to 1.25 msec (Lux & Brown, 1984; Hagiwara & Ohmori, 1983). Predictions from macroscopic current measurements were obtained using parameters reported previously and $t_o = t_c = .3$ msec.

The data were not obtained under exactly the same conditions. First, the temperature was 18°C in the case of the single channel measurements and 22°C in the case of the macroscopic data. The decreased temperature would tend to "slow" the waiting and closed time measurements but have relatively little effect on open time measurements (Lux and Brown, 1984). $C_{a_o}$ was 40 mM and 10 mM for the single channel and whole cell measurements, respectively. This would be expected to result in a voltage shift of the activation curve (Wilson et al., 1983); however no large activation time-course changes occurred when $C_{a_o}$ was changed from 10 to 20 mM (Brown et al., 1983). Inactivation was neglected in the single channel analysis and this might make the closed times slow. Considering the magnitudes and directions of these effects, it is believed that the agreement of the corrected curve with the single channel curve is fairly good.
open time curve falls close to the dashed area which represents the range of measurements reported. The corrected closed time curve compares favorably to a single measurement at this voltage, and it probably lies within the expected error, especially considering the temperature difference (see figure legend). Additionally, the corrected waiting time curve agreed with the single channel measurements better than did the uncorrected one.

Next, consider the tail current curves (Fig. 4.30). In the case of the prediction from the single channel data, the tail current relaxation did not reach steady-state for ~12 msec which is significantly slower than the measured result (Brown et al., 1983). This result would not be affected by the temperature difference (Brown et al., 1983), and it may be the clearest indication of a loss of kinetic resolution in the case of the single channel measurements.

Finally, we consider spectral measurements from many channel patches (Fig. 4.30). These reflect microscopic kinetic information and thus provide a good check on these analyses. Measurements reported to-date have had widely different parameters and are represented by the dashed area (Lux and Brown, 1984). At the high frequency end, the single channel prediction curve falls far below the dashed area while the macroscopic data curve falls within the range of measurement.
FIGURE 4.30
Comparison of macroscopic and microscopic measurements at 0 mV.
On the tail current graph, the broken line indicates the macroscopic measurement and the thin line indicates the prediction from single channel measurements. On the graph of the spectra, the dashed area indicates direct measurements and the broken and thin lines indicate predictions from macroscopic and single channel measurements, respectively. Curves were obtained as in Fig. 8 and spectral data were reported in (Lux et al, 1984). The tail current curves were obtained assuming an initial opening probability of 1. The spectral measurement parameters were obtained at 21°C in 40 mM Ca (Table 1 of Lux et al, 1984). The power in each spectrum is normalized to the same value.
Chapter 5: Discussion

In the following I have tried to discuss the important results. Particularly I have tried to indicate how the methods of parameter estimation and simulation have led to various interesting, and sometimes unexpected, results.

5.1 Separation of gating and permeation

Referring to equation 2.27, it is seen that the macroscopic current is composed of permeation properties, \( i(v) \), and gating properties, \( p_o(v,t) \). It is of interest to try to separate these effects. An early result was that the activation-voltage curve obtained from normalized, exponentially-extrapolated, tail current measurements did not saturate until \( \sim +40 \) mV (Fig. 4.4). On the other hand, the instantaneous current-voltage relation was first linear and then asymptotically approached the voltage axis as the voltage increased in a manner similar to the Goldman equation (Fig. 4.4). Other recent measurements have agreed with these observations (Fenwick et al., 1983; Brown et al., 1984; Byerly et al., 1984; and Eckardt et al., 1984) while earlier ones done with probably less kinetic resolution did not (Kostyuck et al., 1981 and Llinas et al., 1981). The linear-type result at negative potentials has been substantiated by recent single channel measurements (Lux and Brown, 1984 and Hagiwara and Ohmori, 1984).

Two important observations can be made. First, the instantaneous I-V results indicate that Ca ion movement is not restricted in a voltage dependent manner as the older measurements imply and some models have described (Llinas et al., 1981; Kostyuck, Mironov and
Doroshenko, 1982). This has important consequences for the characteristics of the channel molecule. The second point is that since the activation curve does not saturate until \( \sim +40 \text{ mV} \) one cannot determine effects of Ca channel modifiers on permeation by simply measuring peak macroscopic currents at a particular voltage. Such measurements may reflect changes in either \( p_o \) or \( i(v) \). This caveat holds for effects of drugs on the Ca channel as well as for examining the selectivity of the channel to different divalents as argued below.

From tail current measurements it was determined that the activation curve was shifted along the voltage axis whenever external divalent concentrations were changed (Figs. 4.6 and 4.7 and Wilson et al., 1983). As argued previously, this was thought to be due to a change in the surface potential. Additionally, Trautwein et al. (1983) found a voltage shift in the probability of opening as measured from single channels in heart cells when Ba replaced Ca. A ramification of the theory was that the free concentrations of the divalent species were markedly affected by such a surface potential (see Fig. 4.7 and Wilson et al., 1983). Such effects on \( p_o \) and available ion concentrations must be carefully investigated before one tries to characterize the channel's permeability. Recent investigations of the Ca channels' selectivity properties in heart cells have not taken such effects into account (Hess and Tsien, 1984). It should be noted however that in this latter report \( p_o \) was reported not to have been changed by the divalent species.

5.2 The kinetics of macroscopic activation

The model proposed for the macroscopic current activation kinetics (\( M_5 \) of Fig. 2.2, page 27) described all the basic properties
of the response to short-pulse protocols. That is, turn-on, tail current, and short 2-pulse measurements were all well described. Recall also that data from the latter type of measurement was not used to obtain parameter estimates. Thus the model has some predictive value. Additionally, effects of temperature were well described by a modification of this model. Previously there was speculation that a 4-state rather than a 3-state model was required (Brown et al, 1983, Byerly et al, 1984), but this is the first true demonstration. Actually the 3-state model worked better than previously supposed. This points out the importance of state modeling rather than exponential modeling as argued previously.

It should be noted that there most probably was some error in obtaining the tail current measurements because they were so fast. However, considerable effort was given to evaluating the errors, and they were expected to have small effects as described earlier. Also, several other reports have indicated very similar measurements. Thus, the errors in the rate constant estimates should be sufficiently small so as not to change the conclusions regarding the discrepancy between the whole cell and single channel measurements discussed below.

5.3 Are Ca single channels unresolved?

The most important result of this study is that parameters obtained from macroscopic measurements tend to agree with single channel measurements only if the effects due to limited interval resolution are taken into account. However, to a certain extent, this comparison is model dependent. Agreement between macroscopic and microscopic data may not be requisite as recent measurements of the Na current in squid axon have shown (Fishman, Leuchtag, and Moore, 1983).
Nevertheless, several lines of evidence indicate the appropriateness of the simple type of model suggested here. Note that "simple model" not only refers to model $M_5$, it also refers to the other activation models tested, all of which gave similar results. In order to make the first argument sounder, consider an alternative model in which most of the fast relaxation in the tail current following a step hyperpolarization was due to a time-dependent change in the elementary current and was not due to a change in the probability of opening as presumed. If this were the case, a very low slope conductance would be expected for single channels corresponding to the flat voltage dependence of the slow component in the tails (see Fig. 11 of Brown et al, 1983). Instead, the measured single channel conductance agrees favorably with the whole cell, instantaneous current-voltage relationship. In the case of single channel data, the slope conductance was 7 pS in 40 mM [Ca$^{2+}$] (Lux and Brown, 1984). From the macroscopic current tails, a value of 12 pS was obtained from the following calculation. Using a peak probability of opening of .55 at +20 mV, $5 \times 10^5$ channels were obtained for the cell in Fig. 11 of Brown et al (1983). Assuming all the channels to be open during the tail currents reported, the single channel slope conductance may be obtained by dividing the whole cell slope conductance of the instantaneous current-voltage relation by the number of channels. The agreement between these measurements indicates that the single channel current immediately following a voltage perturbation is the same as that obtained a few 100 μsec later. A second argument along the same line is that the activation curve is fairly close to the probability of opening curve. The third argument consists of the fact that the
spectral data which should reflect the microscopic properties of the current agree more closely with the macroscopic measurements than with the single channel measurements (Fig. 4.30). In addition, agreement has been found between macroscopic time constants and natural frequencies in Lorentzian functions used to describe spectral data (Fenwick et al., 1983). For these reasons, it appears that models such as those examined presently should describe the kinetic properties of the current.

Given the significant signal-to-noise problem in recording Ca single channels it is not surprising that the interval kinetics are significantly distorted. After all, the visual impression of the simulation in the presence of noise would not lead one to believe that 60% of the openings had been missed (Fig. 4.26). A quantitative analysis is required, and some suggestions arise from the results given here. One method might consist of measuring threshold crossings with various filter parameters and comparing results to analytical determinations. On the one hand, the number of threshold crossings due to noise at the baseline is given by equation 4.6, and on the other, the increase due to an improved interval resolution can be determined using the analyses in Section 2.5. Another technique might consist of measuring many intervals using a detection scheme with a known dead time, collecting the interval histograms, and fitting them with the apparent PDFs. In principle this should allow the determination of undistorted PDFs and rate constants from distorted measurements. Yet another technique would be to improve the spectral analysis of multichannel patch data. In this case, the power of the signal is appreciably more than the noise (Lux and Brown, 1984), and
the kinetic measurements should be fairly pure. Besides, the spectra may be corrected for background noise by subtracting records obtained when the channels are blocked or inactive or by using a variation of the method described by Fishman et al. (1983). In order to substantiate the argument that fast kinetics are present in the microscopic data, the microscopic measurements and analyses must be improved.

Other errors involved in the analysis of single channel measurements were examined. It was found that fixed-record-length recording of channels resulted in distorted closed time histograms. Unexpectedly, it was found that a single-amplitude channel simulation gave amplitude measurements that could be misinterpreted as arising from two channel types (Nagy et al., 1983; Carbonne and Lux, 1984), or a sub-conductance level (Auerbach and Sachs, 1983). In the case of severe filtering, many small events were measured that contained missed closures, and the amplitude distribution was bimodal (Figs. 4.26 and 4.27). Future investigations of this phenomenon can be done using simulations. For example, if each of the data points in a record are plotted, rather than the measured amplitudes, the resulting distribution may not display the bimodal behavior. Some analytical work on the shape of such distributions has recently been reported (Yellen, 1984). This may be the method of choice for discerning multiple channel types.

5.4 Towards a model of inactivation

A variety of results were shown regarding tests for discrimination of inactivation models. The most interesting one was the single channel test to discriminate between a Ca-accumulation-
dependent ($M_A$ in Fig. 2.3) and a voltage-dependent model (model $M_{B3}$). The results indicated that the Hi-Lo sort method recently described (Brown and Lux, 1984) may have some value, but it is not as sensitive as anticipated intuitively. Other methods were investigated but were found to introduce some rather subtle biases which obviated their usefulness (not shown). This might be an area for further research, since such analyses are important for determining other nonhomogeneous Markovian behavior.

It was found that the recovery experiments helped eliminate certain classes of models. Specifically, the Ca-accumulation-dependent model, $M_A$, requires modification in order to give a recovery curve without a "delay"-type response. Also, model $M_D$ was eliminated for the same reason.

A surprise was that the 2-pulse protocol for measuring inactivation did not give a significant phasic delay in the case of the "coupled" model, $M_{B3}$. This must be attributable to the values of the rate constants which were much different than those used earlier for the case of Na currents. This result underscores the usefulness of doing simulation analyses in conjunction with the planning of experiments.

It is concluded that models $M_{B1'}$, $M_{B2'}$, and $M_C$ are all candidates for describing $I_{Ca}$ inactivation. The other models in Fig. 2.3 all have bad attributes of one type or another. Careful consideration of the single channel parameters - failures, WCO interval measurements, etc. - might lead one to favor one of the remaining models over the others. An interesting result shown presently was the relative insensitivity of the inactivation rate constants to voltage in some
models (Table 4.2). This should be investigated more fully, and it may lead one to favor one model over another.

The method of Horn and Lange (1984) may be useful in further investigations of models that account for the single channel behavior. Another approach would be to improve the parameter estimation scheme used presently and include certain single channel parameters in addition to the macroscopic currents. This is probably more practical for the case of the Ca channel where sufficiently long recordings of single channels might be rare. Perhaps it should be noted that it will probably be impossible to discriminate between some models. That is, certain models are probably interchangeable in the sense that all the measured responses can be realized with them. This is an interesting theoretical question.

Leaving the search for the "true" model behind, a practical model is suggested below for describing most of the macroscopic results. A good choice would be model \( M_C \) with the activation model replaced by an \( m^2 \) model (\( M_1 \) in Fig. 4.1). This type of model is amendable to analytical solution and therefore could be placed in a rapid parameter estimation scheme. It is anticipated that such a model would fairly well describe most voltage clamp results with the exception of tail currents. This type of model would be useful for describing the Ca entering during a cardiac action potential, for example. With proper tuning, it could probably describe any changes that might occur with alterations in heart rate, etc.

5.5 Evaluation of the methods of parameter estimation and simulation.

The macroscopic current, parameter estimation scheme proved to have several advantages over the modeling methods normally found in
the ionic current literature. By using a set of measurements rather than a single measurement, parameters were reliably estimated from a variety of rather complicated kinetic schemes. This was shown by the model-to-model simulation tests. Note that multiple measurements were used despite the fact that all the parameters should have been identifiable from the tail current measurements alone. In practice, the additional measurements were required in order for the estimated parameters to describe all the data. Because the model output was generated numerically, complicated schemes were easily described. Another advantage was that rate constants were estimated rather than parameters from sum-of-exponentials type models. As shown presently, a particular advantage is that comparisons can be made to microscopic measurements. It is believed that these methods would be useful in various investigations of channel models.

In addition to the reasons given above, the mathematical analysis of identifiability is important as illustrated by the following example. Horn and Vandenberg (1984) recently analyzed Na current single channel records using 25 different models and the analysis method of Horn and Lange (1983). They found several models in which the rate constants were not identifiable as judged by the elements of the covariance matrix. I have analyzed one of those models (Model 1 of Table 2 in Horn and Vandenberg, 1984) using the WCO identifiability analysis described in Appendix A. It was found that an infinite set of parameters would describe the WCO histograms in the case of this mode. This analysis is important because it proves that the parameters were not identifiable simply because of insufficient data; rather, they were not identifiable as determined mathematically. Thus
the WCO identifiability analysis briefly described here, and in more
detail elsewhere (Wilson and Clark, 1985), should be used in such
parameter estimation problems.

Simulations proved useful in our analyses of ionic channels. The
single channel simulations pointed out the errors involved in interval
analyses at limited bandwidth, the effects of noise, and the
distortion of histograms due to finite record lengths of data. In the
case of inactivation, the simulations were used to evaluate the
usefulness of various tests for model discrimination. The single
channel simulations were also useful for testing our single channel
analysis program and for testing the appropriateness of the apparent
PDF calculations. But perhaps the most important function of such
simulations was not illustrated. The process of developing and
running such programs leads to a deeper understanding of the assump-
tions and mathematics involved in model building. Such an under-
standing enables one to plan and analyze experiments and test new
ideas.

In conclusion, it seems appropriate that such theoretical endeav-
ors continue in the study of ionic channels. It appears that only
through a combination of more and improved measurements and improved
analysis of the data can the true electrical model of the Ca channel
be obtained.
Chapter 6: Summary

In this section, each of the major results will be reviewed. First, I will give a brief synopsis and indicate how this work applies towards the general problem of understanding channels and how it may benefit other researchers in the field. Later, I will give a more detailed summary in the form of a list of results.

Parameter estimation and simulation techniques were used on several Markovian state models with the aim of determining how such results can be used to interpret experimental findings and plan new experiments and analyses. The most important result was that single channel measurements and interval analyses probably suffer from a significant lack of kinetic resolution due simply to the filtering required to obtain reasonable signal-to-noise ratios. This result was model independent since similar results were obtained with all of the models tested. Simulations indicated other possible errors in single channel measurements including the appearance of a bimodal amplitude distribution following the severe filtering of a record containing single amplitude openings. Models of activation and inactivation were investigated and some were eliminated on the basis of one or more experimental observations. For example, the short 2-pulse protocol (Fig. 4.14) and the temperature experiments (Figs. 4.16 and 4.17) gave convincing evidence for picking a 4-state rather than a 3-state model of activation. From simulations, certain analyses were found not to be as useful as previously supposed for discriminating models. Specifically, these include the 2-pulse inactivation test for coupled versus independent inactivation (Fig. 4.21) and the Hi-Lo sort method for Ca-accumulation-dependent inactivation (Fig. 4.23). Classes of
kinetic models were suggested that could describe all the experimental observations to-date. In addition to the work on channel kinetics, it was found that the fixed membrane surface charge would be expected to have large effects on permeation. This effect has often been overlooked.

Besides the above results specific to the Ca channel, there were some results of general importance to channel modeling. For instance, this is the first time that parameter identifiability has been described as it applies to channel kinetic modeling. Considering the complexity and the multiplicity of kinetic schemes presently being studied and the possible problems that can arise (see Appendix A and Section 5.5), this is of practical importance. Several computer programs were also described that are of general applicability. The method reported for the single channel Monte Carlo simulations is simple and quite easily adapted to non-linear models such as the Ca-accumulation-dependent model. The simulation program MODPRB may be used to simulate any modern kinetic measurement for practically any linear state model of interest. The parameter estimation routines developed for use with the deterministic whole cell and probabilistic single channel measurements are also generally applicable. The calculations of the effect of limited interval resolution on the single channel histograms is unique and can readily be applied to any linear kinetic scheme.

List of results

The following list briefly summarizes the important results in the order that they were given in the body of the thesis.
1. A theoretical development was given for predicting relaxation, WCO PDFs (waiting, closed, and open time probability density functions) and stationary fluctuation spectra from state models in terms of a state-variable description. Also a diagramatic representation of these calculations was developed.

2. The developments in (1) facilitated comparisons to theoretical results in the compartmental modeling literature. In this regard, identifiability of the models was investigated. A 3-state model was found to be identifiable from turn-off, but not turn-on measurements of macroscopic current. This model was also identifiable from a combination of closed and open time measurements. Other results were briefly described.

3. Computer methods for analyzing whole cell, noise, and single channel measurements were developed and used. It was found that such methods are necessary for studying the biophysical properties of channel currents.

4. Tail currents were studied in detail, and possible measurement errors were examined. It was found that Ca tail currents relaxed at the holding potential as a sum-of-two-exponentials, the $\tau$'s of which did not depend on the amplitude or duration of the preceding voltage clamp pulse. In many cells a Ca-blocker sensitive, contaminating current was found. The latter was identified on the basis of kinetics, divalent ion sensitivity, and temperature sensitivity.

5. Activation curves obtained from Ca tail current measurements indicated that the channels were not maximally open until $\sim +40$ to $+50$ mV.
6. The activation curve shifted along the voltage axis in response to changes in divalent concentrations. This effect was well modeled by the Gouy-Chapman diffuse double-layer theory modified to include specific binding. A prediction of these calculations was that the difference in current size of Ca, Ba, and Sr was not due to a difference in channel permeability, rather it was due to differences in the free concentrations of these species at the channel mouth.

7. Six models of activation were examined using a parameter estimation scheme that simultaneously fit a set of macroscopic current measurements obtained at a given test potential. This set included turn-off measurements in order to make the model identifiable as determined in (2). A best model was chosen (Model $M_5$ of Fig. 2.2, page 27) that had the lowest $\chi^2$ value and, more importantly, well described some additional 2-pulse protocol experiments.

8. The effects of temperature on the activation of macroscopic currents and on the WCO histograms were well described by a modification of $M_5$ that consisted of making the first transition very temperature dependent. This result gave additional support for a 4-state model of activation, such as $M_5$, as compared to a 3-state model.

9. Ca single-channel WCO measurements were examined and parameter estimates were obtained using a maximum-likelihood method on the PDFs of a 3-state model. These estimates were significantly "slower" that those obtained in (7).

10. Errors involved in single channel recording were examined
including the effects of limited interval resolution. Using parameter estimates obtained in (7) it was found that significant distortion would occur under the normal conditions for studying Ca single channels. This was determined both from Monte Carlo simulations of single channels and from an analytical result which assumed a channel-detection-system dead time. Single channel simulations with noise added showed qualitatively that it was not unreasonable that such resolution would be lost. An additional result showed the error due to fixed-record-length recording.

11. Six models of inactivation (Fig. 2.3, page 29) were investigated, 3 of which were discarded on the bases of macroscopic recovery-from-inactivation experiments or single channel observations. It was found that searching for a "delay" in inactivation as determined from a 2-pulse protocol would not be a particularly useful test for eliminating a "coupled" model of inactivation. The Hi-Lo sort method suggested for discriminating a voltage-dependent vs. a Ca-accumulation-dependent model (Brown and Lux, 1984) was not found to be particularly sensitive test.

12. The methods of parameter estimation and simulation were found to be very important in the study of ionic channels, and such methods helped identify several unexpected results in the case of the Ca channel.
Appendix A: Identifiability analysis of a 3-state catenary model.

As an example of identifiability analysis the 3-state model (M_2 in Fig. 2.2) will be analyzed using the Laplace-transformed equations. The identifiability properties are discussed below for the various measurements that one might use, i.e. single channel WCO measurements, noise fluctuations from a collection of channels, or macroscopic current relaxations.

**Macroscopic current measurements**

In the case of macroscopic current measurements under voltage clamp conditions, it is assumed that the current is given by

\[ I(t) = N_i \rho_0(t) \]  \hspace{1cm} A.1

where the variables have been described previously. It is assumed that the product \( N_i \) may be identified using single channel or tail current measurements. Thus \( \rho_0(t) \) may be obtained, and it is analyzed below.

First consider the identification problem for a turn-on measurement, i.e., \( P_3(t; P_1(0)=1) \). The initial condition vector is \( \underline{p}(0) = [1, 0, 0]^T \). In equations 2.15 - 2.19 Laplace transforms of conditional probability distributions were obtained. Similar results were used to obtain the result below.

\[
(sI - A) = \begin{bmatrix}
  s + k_{12} & -k_{21} & 0 \\
  -k_{12} & s + k_{21} + k_{23} & -k_{32} \\
  0 & -k_{23} & s + k_{32}
\end{bmatrix}
\]  \hspace{1cm} A.2

Below is an expression for the output, \( P_3(s) \), which is obtained as described in Section 2.25.
\[ P_3(s) = [0 \ 0 \ 1] \begin{bmatrix} sI - A \end{bmatrix}^{-1} = \frac{\text{cofactor } b_{13}(s)}{\det(sI - A)} \]

Substituting, one obtains

\[ P_3(s) = \frac{k_{12}k_{23}}{s[s^2 + s(k_{12} + k_{21} + k_{23} + k_{32}) + (k_{12}k_{23} + k_{12}k_{23} + k_{21}k_{32})]} \]

Identifiability analysis by the transfer function approach assumes zero modeling error (i.e. the model is capable of accurately mimicking the process) and zero measurement error; in addition a noise free measurement condition is assumed. Under these idealized conditions the data may be fitted using the analytical expression

\[ u = A_0 + A_1 e^{-t / \tau_1} + A_2 e^{-t / \tau_2} \]

Because of the idealizations above, equation A.4 may be uniquely Laplace-transformed to an expression similar in form to equation A.3.

One obtains

\[ Y(s) = \frac{n_0}{s(s^2 + d_1s + d_0)} \]

where \( n_0, d_0, \) and \( d_1 \) are functions of quantities in [A.4] and may therefore be considered as "measured"; they are related to the model parameters (the \( k \)'s) by the follow equations:

\[ n_0 = k_{12}k_{23} \]

\[ d_0 = k_{12}k_{32} + k_{12}k_{23} + k_{21}k_{32} \]

\[ d_1 = k_{12}k_{21} + k_{21}k_{23} + k_{21}k_{32} \]

It remains to be seen if the model parameters (the \( k \)'s) can be uniquely determined from the "measured" quantities, \( n_0, d_1, \) and \( d_0 \) using the non-linear mapping in equations A.5 - A.7. Upon inspection, it is determined that there are 3 equations which map 5 unknown parameters
to 3 measured quantities. Thus it is possible to determine the parameters uniquely. In fact, an infinite number of sets of parameter values would give the same response!

The expression for $P_3(s)$ above is called a driving point function in the linear systems literature because it is a response to an initial condition. It is easy to show that the result for $P_3(s)$ is unchanged by considering the system to have a unity impulse input at time zero, $\delta(t)$, on $P_1$ rather than a unity initial condition. Thus, the non-driven state model could have been considered as a system driven with an impulse input. Since the Laplace-transform of $\delta(t)$ is unity, $P_3(s)$ above is also the transfer function relating a "probability input" at state 1 with an output at state 3. In general, the initial conditions used here may be treated as impulse inputs and the results are identical to transfer function analyses. These remarks are prompted because the transfer function is almost always used in the identifiability literature. Here the terms driving point function and transfer function are practically interchangeable.

Continuing to analyze the subject of macroscopic current measurements, we now consider the turn-off or tail current experiment where the membrane has been pre-conditioned to load the channels into the open state. Equations 2.7 and 2.8 are thus solved subject to $P(0) = [0, 0, 1]$.

$$P_3(s) = \frac{\text{cofactor } b_{33}(s)}{\text{det } (sI - A)}$$

$$= \frac{s^2 + s(k_{12}k_{21} + k_{23}) + k_{12}k_{23}}{s(s^2 + s(k_{12}k_{21} + k_{23} + k_{32}) + (k_{12}k_{32} + k_{12}k_{23} + k_{21}k_{32}))}$$
The Laplace transform of the idealized tail current measurement may be expressed as

\[ I(s) = \frac{N_i (s^2 + n_1 s + n_o)}{s(s^2 + d_1 s + d_o)} \quad \text{A.9} \]

One notes from the initial value theorem that the product \( N_i \) may be obtained from \( I(t = 0) \). Equating coefficients in [A.8] and [A.9], the following relationships between the \( k \)'s and the \( n \)'s and \( d \)'s are obtained.

\[ n_1 = k_{12} + k_{21} + k_{23} \quad \text{A.10} \]
\[ n_o = k_{12} k_{23} \quad \text{A.11} \]
\[ d_1 = k_{12} + k_{21} + k_{23} + k_{32} \quad \text{A.12} \]
\[ d_o = k_{12} k_{32} + k_{12} k_{23} + k_{21} k_{32} \quad \text{A.13} \]

Given the non-linearity of this mapping a unique solution is not guaranteed. However, a unique mapping is obtained as shown presently.

From [A.10] and [A.12] one finds that

\[ k_{32} = d_1 - n_1. \quad \text{A.14} \]


\[ d_o = (k_{12} + k_{21}) k_{32}^* + n_o \quad \text{A.15} \]

where the \( * \) indicates the \( k_{32} \) has already been uniquely determined.

Further substitutions and rearrangements yield the following

\[ k_{23} = n_1 - \frac{d_o - n_o}{k_{32}} \quad \text{A.16} \]
\[ k_{12} = \frac{n_o}{k_{32}} \quad \text{A.17} \]
\[ k_{21} = d_1 - k_{12} - k_{23} \quad \text{A.18} \]

Thus, all 5 parameters may be uniquely determined.

Yet another extension of this problem is the case where all the channels are not open initially in a tail current measurement. We let
\( P(0) = [0, 1-P_3(0), P_3(0)] \) where \( P_3(0) \) is some value less than one and obtain

\[
P_3(s) = \frac{1}{\det(sI-A)} \left\{ [(1-P_3(0))\text{cofactor } b_{23} + P_3(0)\text{cofactor } b_{33}] \right\} \quad \text{A.19}
\]

\[
I(s) = \frac{N_i P_3(0)}{\det(sI-A)} \left\{ s^2 + [k_{12}+k_{21}+k_{23}/P_3(0)] s + k_{12}k_{23}/P_3(0) \right\} \quad \text{A.20}
\]

One sees immediately that \( I(t=0)=Ni P_3(0) \). Equating \([\text{A.20}]\) to an equation similar in form to \([\text{A.9}]\), the following set of equations are obtained.

\[
n_i = k_{12} + k_{21} + k_{23}/P_3(0) \quad \text{A.21}
\]

\[
n_o = k_{12}k_{23}/P_3(0) \quad \text{A.22}
\]

\[
d_1 = k_{12} + k_{21} + k_{23} + k_{32} \quad \text{A.23}
\]

\[
d_o = k_{12}k_{32} + k_{12}k_{23} + k_{21}k_{32} \quad \text{A.24}
\]

One observes that a unique mapping is impossible because there are 4 equations and 5 unknown parameters (4 k's and \( P_3(0) \)). Assume for the moment that \( P_3(0) \) is known from some other consideration. After considerable algebra one obtains a quadratic equation for \( k_{32} \) given by

\[
k_{32}^2 + b k_{32} + c = 0 \quad \text{A.25}
\]

where

\[
b = n_i P_3(0) - d_1 \quad \text{A.26}
\]

\[
c = \{1-P_3(0)\} \left[ d_o - n_o P_3(0) \right] \quad \text{A.27}
\]

Two solutions are obtained, namely

\[
k_{32}^*, k_{32}^{**} = \left[ -b \pm (b^2-4c)^{1/2} \right]. \quad \text{A.28}
\]

Positive, real rate constants are required. If the quantity \( b^2-4c < 0 \) then there is no solution. If \( b^2-4c > 0 \) then one must test to see if both \( k_{32}^* \) and \( k_{32}^{**} \) are positive. From \([\text{A.26}]\) and \([\text{A.27}]\) and \([\text{A.21}]\) - \([\text{A.24}]\) it is found that \( b < 0 \) and \( c > 0 \); from \([\text{A.28}]\) this requires
both solutions to be positive. Thus we arrive at the interesting situation where 2 physically reasonable values of $k_{32}^*$ may give an identical response. One can now proceed with the identifiability analysis as done previously. Unique values, $k_{12}^*$, $k_{21}^*$, and $k_{23}^*$ are found corresponding to the value $k_{32}^*$ and another set $k_{12}^{**}$, $k_{21}^{**}$ and $k_{32}^{**}$ are obtained corresponding to the value $k_{32}^{**}$. Thus 2 sets of rate constants are obtained which can fit the experiment equally as well!

**Fluctuation measurements**

The identifiability of the parameters will be analyzed assuming that the autocovariance has been measured. The results also apply to a power density spectrum measurement since one could in principle obtain the autocovariance from the spectral measurement. Laplace-transforming [2.11] one obtains

$$C(s) = N \int_0^2 P_3(\omega) \left[ P_3(s; P_3(0)=1) - \frac{P_3(\omega)}{s}\right]$$

A.29

where $P_3(\omega)$ refers to $P_3(t \to \omega)$. Substituting [A.8] and simplifying gives

$$C(s) = N \int_0^2 P_3(\omega) \frac{s^2[1-P_3(\omega)] + s[(1-P_3(\omega))(k_{12}^*+k_{21}^*+k_{23}^*)-P_3(\omega)k_{32}^*]}{s \left[ s^2 + s(k_{12}^*+k_{21}^*+k_{23}^*) + (k_{12}^*k_{32}^*+k_{12}^*k_{23}^*+k_{21}^*k_{32}^*) \right]}.$$  

A.30

where $P_3(\omega)$ can be expressed in terms of the $k$'s as determined from [A.8] and the final value theorem.

A noise measurement alone does not yield information about the steady-state occupancy of the open state; thus in general $P_3(\omega)$ is unknown. In [A.30] one can obtain only 3 equations relating measured qualities to the 4 unknown $k$'s. Thus the $k$'s cannot be obtained uniquely from a noise measurement alone.
However, in many cases one has a separate measurement of \( P_3(\infty) \) from a normalized tail current, etc. In fact, whenever a single channel current, \( i \), has been reported from stationary noise measurements an estimate of \( P_0(\infty) \) has been made as shown below. From [A.30] and the initial value theorem we obtain

\[
C(\tau=0) = ni^2 P_3(\infty) (1-P(\infty)).
\]

Substituting \( \langle I \rangle = NiP_3(\infty) \) and \( \sigma^2 = C(\tau=0) \) and rearranging the following well known expression is obtained.

\[
i = \frac{\sigma^2}{\langle I \rangle (1 - P_3(\infty))}.
\]

We thus see that an estimate of \( P_3(\infty) \), as well as \( \langle I \rangle \) and \( \sigma \), is required in order to obtain \( i \).

If \( P_3(\infty) \) and \( i^2N \) are known, then it can be shown, using [A.30] that all the \( k \)'s can be uniquely identified.

**Single-channel current measurements**

We now turn to an analysis of the identifiability properties of the single channel measurements as obtained from the conditional probability distributions described earlier. First consider the waiting time distribution. From [2.8] and the restriction that state 3 is absorbing (Fig. 2.1), the following matrix equation is obtained,

\[
(sI-A) = \begin{bmatrix}
s k_{12} & -k_{21} & 0 \\
-k_{12} & s k_{21} + k_{23} & 0 \\
0 & -k_{23} & s \\
\end{bmatrix}
\]

and from [2.15] - [2.19] one obtains

\[
P_{13}(s) = \frac{k_{12} k_{23}}{s^2 + s (k_{12} + k_{21} + k_{23}) + (k_{12} k_{23})}.
\]
We anticipate 3 Equations and 3 unknowns to analyze but find that the expressions for \( n_0 \) and \( d_0 \) are identical; thus only two independent equations in 3 unknowns are obtained and none of the \( k \)'s are uniquely identifiable.

The closed time distribution is obtained from \((\text{sI-A})\) found in [A.33] with a different cofactor (refer to [2.15] - [2.19] and Fig. 2.1). One finds

\[
P_{23}(s) = \frac{\text{cofactor } b_{23}}{\det (\text{sI-A})} = \frac{s k_{23} + k_{12} k_{23}}{s^2 + s(k_{12} + k_{21} + k_{23}) + k_{12} k_{23}} \quad A.35
\]

and obtains the following 3 independent Equations in 3 unknowns:

\[
\begin{align*}
n_0 &= d_0 + k_{12} k_{23} \quad A.36 \\
n_1 &= k_{23} \quad A.37 \\
d_1 &= k_{12} + k_{21} + k_{23} \quad A.38
\end{align*}
\]

It is easily shown that all 3 parameters are uniquely identifiable.

The open time probability distribution is given by

\[
P_{32}(s) = \frac{\text{cofactor } b_{32}}{\det (\text{sI-A})} = \frac{k_{32}}{s (s+k_{32})} \quad A.39
\]

and \( k_{32} \) is seen to be uniquely obtained.

**Summary of the identifiability results**

In summary, the combination of the closed-time and open-time measurements yield all the \( k \)'s uniquely while the waiting-time measurement alone yields no \( k \) values uniquely. In the case of macroscopic current measurements, the model parameters are uniquely identified from a tail current measurement only when we constrain \( P_3(0) = 1 \), and they are not identified from a turn-on measurement. These results are used in Chapter 4 to obtain the model parameters from the appropriate measurements.
Appendix B: Analysis of the time and amplitude of the peak of the waiting time PDF.

The waiting time PDF for model $M_2$ (Fig. 2.2) is given by Colquhoun and Hawkes (1981):

$$P_{RO} = \frac{\rho_3 \rho_4}{\rho_3 - \rho_4} (e^{-\rho_4 t} - e^{-\rho_3 t}), \quad B.1$$

where $\rho_3$ and $\rho_4$ are the system eigenvalues given by

$$\rho_3, \rho_4 = 0.5[b \pm (b^2 - 4c)] \quad B.2$$

and

$$b = k_{12} + k_{21} + k_{23} \quad B.3$$

$$c = k_{12} k_{23} \quad B.4$$

Equation B.1 has a maximum that occurs at time $t_p$ and it is found by differentiating [B.1], setting the result to zero, and solving for $t_p$. One quickly obtains

$$\frac{\rho_3}{\rho_4} = e^{-t_p (\rho_4 - \rho_3)}, \quad B.5$$

and after rearrangement

$$t_p = \frac{\ln(\rho_3/\rho_4)}{\rho_3 - \rho_4}. \quad B.6$$

We now wish to find the maximum value. Inserting [B.6] into [B.1], the following is obtained after considerable rearrangement.

$$P_{RO}(t_p) = \rho_4 \left[ \frac{\rho_4}{\rho_3} \right] \frac{\rho_3}{\rho_3 - \rho_4} \quad B.7$$

Using [B.5] and [B.7] we get

$$P_{RO}(t_p) = \rho_4 e^{-\rho_4 t_p} = \rho_3 e^{-\rho_3 t_p} = f(\rho). \quad B.8$$

In [B.8], $f(\rho)$ is the maximum value of the waiting time PDF, and it is plotted in Fig. 4.10 as a function of $\rho$ with $t_p = 1.0$ ms. Note that
given a waiting time histogram with a peak at 1 ms and the amplitude of the peak, the $\rho$ values can be obtained from this curve. Depending on the value of the peak, one can obtain two $\rho$ values corresponding to $\rho_3$ and $\rho_4$, or, at the peak of this function, two $\rho$'s of value $\rho_p$, corresponding to a repeated eigenvalue of the system.

We wish also to investigate the maximum amplitude permissible from this PDF. From the results above, this should occur in the case of a repeated root, i.e., $\rho_p = b/2$ from [B.2]. In this case, we solve the set of differential equations once again for the case of a repeat-ed root given by $\rho_p = b/2$. The result is

$$P_{RO}^*(t) = \frac{b^2}{4} t e^{-bt/2} \quad \text{B.9}$$

We now find the maximum value of this function as a function of time. The peak occurs at

$$t_p^* = \frac{2}{b} \quad \text{B.10}$$

and the value at the peak is

$$P_{RO}^*(t_p^*) = \frac{1}{t e^b} \quad \text{B.11}$$

Equation B.11 thus yields the maximum obtainable value of the waiting time PDF as a function of the time of the peak. Measured values must be less than or equal to this function and should fall in the shaded region of Fig. 4.10. Note that all but one of the data points fall outside the acceptable region, indicating an inconsistency with the model.
APPENDIX C: Computer programs.

C.1 CHPRB3: a computer program for calculating the predictions for various channel measurements from state models.

This is a program for computing relaxation, fluctuation, and conditional probability distributions for single channel measurements. The input data are the rate constants for a general 6-state Markovian channel model and the output consists of plots and/or equations describing these functions. Calculations are done using the method suggested by Colquhoun and Hawkes (1982), referred to as C&H below.

The subroutines listed here are linked with the MODPLT program developed by B. Little and D. Wilson in order to form the working program. The subroutines thus follow the conventions of MODPLT as given below.

IDMODL — fills the NAMES array with appropriate names for the parameters
IMODEL — initialization of model parameters
MODEL — called for each evaluation of the dependent variable

These subroutines may be used with any suitable driving program in a manner similar to that in the program segment shown below. The model parameters are passed via the array PARAME and appropriate names for the parameters are given in the array NAMES.

```
REAL Y(1000),PARAME(26)
BYTE NAMES(10,26)

CALL IDMODL(NAMES,...) !Get names.

CALL IMODEL(PARAME,...) !Initialize model.
DO 10 I=1,1000
   X = I*TPP !TPP is the time per point.
   CALL MODEL(X,Y(I),PARAME,...)
10   CONTINUE

CALL MODEL(X,Y(I),PARAME,...)

C !Plot the calculations.
```
4. There is a steering variable called 'MEAS TYPE' that determines the kind of calculation that is done and the convention follows.

1. Relaxation measurement
2. Fluctuation measurement
3. PDF for waiting times
4. PDF for closed times
5. PDF for open times
6. PDF for burst lengths
7. Discrete probability density for the number of openings, $R$, per burst or trace.

Note that there are two possibilities for measurement type 7:
1. The probability density given that the channel has opened, i.e. \( R \geq 1 \).
2. The probability density that includes failures to open, i.e. \( R \geq 0 \).

5. In the case of the waiting and closed times, states 'B' and 'C' are lumped together and considered as 'B'. This is required from the equations as they are written. If states in C are absorbing, then the order of the system is less than the number of state in 'B' and 'C'. To alleviate this problem a variable called 'C ABSORB' is included that allows for the proper calculation of model order. If 'C ABSORB'=1 then 'C' is absorbing; any other value indicates that 'C' contains no absorbing states.

6. There are two variables associated with initial conditions. The value of 'I COND' gives the state to which an initial condition of 1 is applied for relaxation and waiting-time calculations. The value of 'I CLOTO' sets the state for the initial condition in the case of the closed-time calculation. Only one such state can be given presently.
In the documentation that follows, I have listed only the main subroutines. Other subroutines come from either the IMSL (Houston, Tx.) package of numerical subroutines or the UTMB department of Physiology and Biophysics "MiscLib" library of subroutines. Subroutines and their sources are listed below.

IMSL:  EIGRF, LINV1F, VMULFF, VTRAN
UTMB:  CNDGTL, CNDGTR, FETCHR, FTSORT, SETMR, TELLI,
       TELLR, TYPE

The information given below is required in order to use these subroutines. Note that in most cases the variables referred to are passed via the array PARAME. Refer to subroutine IDMODL to match the names of these variables with the proper subscripts in PARAME.

1. The input to the program is a set of 14 rate constants. These form certain connections between the states of a 6-state model as shown below where the connections are bi-directional. Rate constants are named such that k12 refers to the rate constant for transfer from states 1 to 2, etc. Using this set-up it is possible to describe many models of interest including an m**2h Hodgkin-Huxley model. Rate constants can be zero allowing less complicated models to be analyzed.

   (1) + + (2) + + (3)
   +    +    +
   +    +    +
   (4) + + (5) + + (6)

2. The states are grouped as described by C&H as shown below.

   A states = open states
   B states = 'short-lived' closed states
   C states = 'long-lived' closed state

3. It is required that there is only 1 open state, i.e. only one 'A' state. Also, the groups of states must contain consecutively numbered states as follows: A={1}, B={2,3,...,j}, and C={j+1,j+2,...,6).
SUBROUTINE IDMODEL(MULTI, DERIV, DEP, NUMPAR, NAMES, PARAM)

C-------------------------------------------------------------------------------------
C PURPOSE: Load NAMES with appropriate names for the parameters and
C set some logical variables having to do with the model.
C INPUT: none
C OUTPUT:
LOGICAL MULTI   !IT : multiple independent values
LOGICAL DERIV   !IT : analytical derivatives
INTEGER DEP    !IT : present for curve fitting
INTEGER NUMPAR !IT : number of parameters
BYTE NAMES(18,26) !names of parameters
REAL PARAM(26) !parameters
C-------------------------------------------------------------------------------------

MULTI = .TRUE. !ALLOWS PLOTTING OF MULTIPLE DEPENDENT VARIABLES.
DERIV = .FALSE. !NEEDS NUMERICAL EVALUATION OF DERIVATIVES.
DEP = 1
NUMPAR = 26

CALL MOVEI('K12',NAMES(1,1),10)
CALL MOVEI('K21',NAMES(1,2),10)
CALL MOVEI('K22',NAMES(1,3),10)
CALL MOVEI('K23',NAMES(1,4),10)
CALL MOVEI('K36',NAMES(1,5),10)
CALL MOVEI('K63',NAMES(1,6),10)
CALL MOVEI('K65',NAMES(1,7),10)
CALL MOVEI('K36',NAMES(1,8),10)
CALL MOVEI('K54',NAMES(1,9),10)
CALL MOVEI('K45',NAMES(1,10),10)
CALL MOVEI('K14',NAMES(1,11),10)
CALL MOVEI('K41',NAMES(1,12),10)
CALL MOVEI('K25',NAMES(1,13),10)
CALL MOVEI('K52',NAMES(1,14),10)
CALL MOVEI('A STATES',NAMES(1,15),10)
CALL MOVEI('A A STATES',NAMES(1,16),10)
CALL MOVEI('B STATES',NAMES(1,17),10)
CALL MOVEI('C STATES',NAMES(1,18),10)
CALL MOVEI('MEAS TYPE',NAMES(1,19),10)
CALL MOVEI('INIT COND',NAMES(1,20),10)
CALL MOVEI('CLOSE TO',NAMES(1,21),10)
CALL MOVEI('C ABSORB',NAMES(1,22),10)

RETURN
END
SUBROUTINE IMODEL(X, PARAME, NUMPAR, Y, IDUM)

PURPOSE: Calculates the predictions for various measurements.
In all cases, the result of this calculation is an
 equation. The most common one has the form below.
Y = sum over I of ( WEIGHT(I)*EXP(-X*RLAMDA(I)) )
In the case of fluctuation and number of openings
per burst predictions other equations are used.
Values are passed out via a common area, PRB.COM.

INPUT:
REAL PARAME(1) MODEL PARAMETERS
INTEGER NUMPAR # OF PARAMETERS
REAL X,Y # DUMMY VARIABLES IN THIS CASE
INTEGER IDUM # DUMMY VARIABLE IN THIS CASE

OUTPUT: NONE

COMMON:
INTEGER IMEAS # TYPE OF MEASUREMENT TO CALCULATE
INTEGER KHSUM # OF EXPONENTIALS IN OUTPUT
REAL WEIGHT(6) # WEIGHTING FACTORS
REAL RLAMDA(6) # EIGEN VALUES
LOGICAL NORM # (T) NORMALIZE PLOTS FOR THE # OF FAILURES
COMMON PRB.COM. IMEAS, KHSUM, WEIGHT, RLAMDA

INTERNAL:
INTEGER KA, KB, KC # OF A, B, C STATES.
LOGICAL ABSORB (T) # C STATES ARE ABSORBING
LOGICAL CALPZ (T) # CALCULATE PROB(R=0) FOR # OF OPEN/BURST
INTEGER NSTATE # TOTAL # OF STATES FOR CALCULATION
INTEGER INCR # URED IN SOME OF THE INGL ROUTINES
INTEGER KW # OF EXPONENTIALS
REAL QMAT(6,6) # Q MATRIX OF C#H
REAL M4MAT(6,6) # M MATRIX OF C#H
REAL MMAT(6,6) # M MATRIX OF C#H
REAL WDGTC(6,6) # MATRIX OF C#H
REAL WGDTH(6) # MATRIX OF C#H
REAL WGDTH(6) # MATRIX OF C#H
REAL WGDTH(6) # MATRIX OF C#H
REAL RMMAT(6,6) # R MATRIX OF C#H
REAL RMMAT(6,6) # R MATRIX OF C#H
REAL ABMAT(6,6) # ABMAT(6,6), ACMAT(6,6) # PARTITIONED MATRICES OF C#H
REAL BMMAT(6,6), BMAT(6,6) # #
REAL CMAT(6,6) # CMAT(6,6), CMAT(6,6) #
COMPLEX EIGUAL(6) # EIGENVALUES
COMPLEX EIGVEC(6,6) # EIGENVECTORS
REAL REIGVA(12) # REAL EIGENVALUES= 2*6
REAL REIGVE(72) # REAL EIGENVECTORS= 8*6=2
REAL SCRCH(6,6) # SCRATCH MATRIX
REAL WDA(48) # WORK MATRIX FOR SUB EIGRF---12*6=6
REAL MCCOL(6,6) # SCRATCH AREA FOR M CALCULATIONS
REAL NROW(6,6) # SCRATCH AREA FOR N CALCULATIONS
COMPLEX IN # SCFLUC
REAL SCFLUC # SCFLUC FACTOR FOR FLUCTUATION OUTPUT

EQUIVALENCE (EIGUAL(1), REIGVA(1)) # REQUIRED BY SUB EIGRF
EQUIVALENCE (EIGVEC(1,1), REIGVE(1)) #

DATA LUM/5,
DATA NORMP/.TRUE.,/
DATA SCFLUC/1.03/
DATA TIMBEG/8.0/
DATA TEMEND/1.028/
DATA IN/6/
DATA 12/6/ # SQUARE DIMENSIONS OF ARRAYS

CALPZ = .FALSE.,
ABSORB = .TRUE.

LOAD VARIABLES FROM PARAME.
ICOND = IFIX(PARAME(28))
ICLTO = IFIX(PARAME(21))
NSTATE = IFIX(PARAME(15))
IMEAS = IFIX(PARAME(19))
KA = IFIX(PARAME(16))
KB = IFIX(PARAME(17))
KC = IFIX(PARAME(18))
IF(IFIX(PARAME(22)) .EQ. 1) ABSORB = .TRUE.

IF(KA .NE. 1) CALL TYPE(""KA .NE. 1 *** POSSIBLE ERROR"")
IF(KA+KB+KC .NE. NSTATE) CALL TYPE(""KA+KB+KC .NE. NSTATE *** ERROR"")
CONTINUE  \( \text{RETURN HERE FOR CALCULATION OF P(0)}. \)

C INITIALIZE ALL THE ARRAYS TO ZERO.
IDIM = IAIA
CALL SETM(HCOL,IDIM,0.0)
CALL SETM(HROW,IDIM,0.0)
CALL SETM(QMAT,IDIM,0.0)
CALL SETM(AMAT,IDIM,0.0)
CALL SETM(BMAT,IDIM,0.0)
CALL SETM(ACMAT,IDIM,0.0)
CALL SETM(BAMAT,IDIM,0.0)
CALL SETM(CMAT,IDIM,0.0)
CALL SETM(CCMAT,IDIM,0.0)
CALL SETM(WEIGHTC,IDIM,0.0)
CALL SETM(STRETCH,IDIM,0.0)
CALL SETM(MMAT,IDIM,0.0)
CALL SETM(NMAT,IDIM,0.0)
CALL SETM(WMAT,IDIM,0.0)
CALL SETM(HEMTB,IA,0.0)
CALL SETM(WEIGHT,IA,0.0)

C CHECK FOR C ABSORBING. FOR MOST MEASUREMENT TYPES, THE SYSTEM ORDER
C SHOULD NOT INCLUDE KC IF THE C STATES ARE ABSORBING. HOWEVER,
C FOR BURST-TYPE CALCULATIONS KC IS INCLUDED.
IF(ABSORB .AND. IMEAS.LT.6) KC = 0
IF(IMEAS.EQ.3 .OR. IMEAS.EQ.4) KB = KB+KC
IF(IMEAS.EQ.3 .OR. IMEAS.EQ.4) KC = B

C FILL THE Q MATRIX APPROPRIATELY.
CALL SETUP(PARAME,HNUMPAR,QMAT,IA)  \( \text{(LOAD A WITH THE RATE CONSTANTS)} \)
CALL UTRANSQMAT(IA,IA)  \( \text{Q IS THE TRANSPOSE OF A} \)

C PARTITION THE Q MATRIX AS IN EQT. 1.6 OF C&H (1982).
CALL MOPRT(QMAT,AAMAT,1,KA,1,KA,IA) \( \text{THE NAMES SHOULD BE OBVIOUS.} \)
CALL MOPRT(QMAT,ACMAT,KA+1,KA+1,KA,IA)
CALL MOPRT(QMAT,BMAT,1,KA+1,KA+1,KA+1,IA)
CALL MOPRT(QMAT,BMAT,KA+1,KA+1,KA+1,KA+1,IA)
CALL MOPRT(QMAT,BCMAT,KA+1,KA+1,KA+1,KA+1,KA+1,IA)
CALL MOPRT(QMAT,BMAT,KA+1,KA+1,KA+1,KA+1,KA+1,KA+1,IA)
CALL MOPRT(QMAT,CAMAT,KA+1,KA+1,KA+1,KA+1,KA+1,KA+1,KA+1,IA)
CALL MOPRT(QMAT,CCMAT,KA+1,KA+1,KA+1,KA+1,KA+1,KA+1,KA+1,KA+1,IA)

C OBTAIN THE B AND C MATRICES AS DEFINED IN EQT. 1.31 OF C&H (1982)
C AND PLACE THEM INTO WEIGHT AND HEIGHTC, RESPECTIVELY.
C THE VALUES PLACED INTO B AND C DEPEND UPON THE TYPE OF
C MEASUREMENT BEING CALCULATED AS DETERMINED BY IMEAS.
GO TO (110,120,130,140,150,160,160) IMEAS

110 CONTINUE  \( \text{MACROSCOPIC RELAXATION} \)
HEIGHTB(ICOND) = 1.0 \( \text{VECTOR B IS THE INITIAL CONDITION VECTOR.} \)
HEIGHTC(1,1) = 1.0 \( \text{OUTPUT IN A. ONLY ONE STATE.} \)
GO TO 190

120 CONTINUE  \( \text{FLUCTUATION} \)
\( \text{DON'T NEED B AND C VECTORS.} \)
GO TO 190

130 CONTINUE  \( \text{WAITING TIME} \)
HEIGHTB(ICOND-KA) = 1.0
CALL MOPRT(BMAT,HEIGHTC,1,IA,1,IA,IA)
GO TO 190

140 CONTINUE  \( \text{CLOSED TIME} \)
HEIGHTB(ILCLOSED-KA) = 1.0
CALL MOPRT(BMAT,HEIGHTC,1,IA,1,IA,IA)
GO TO 190

150 CONTINUE  \( \text{OPEN TIME} \)
HEIGHTB(1) = -1
CALL MOPRT(AMAT,HEIGHTC,1,1,1,IA,IA)
GO TO 190

160 CONTINUE  \( \text{BURST LENGTH AND } B/\text{BURST} \)
IF(KC .LE. 0) CALL TYPE(*** ERROR: NO C STATES DECLARED,...)
CALL SETB(AMAT,BMAT,ABMAT,BMAT,BCMAT,ACMAT,1STATE,IA,2
\( \text{KA,KB,IMEAS,HEIGHTB,STRETCH,WMAT,MCOL,HROW,HMAT,WORK,} \)
3 CAMAT,CBMAT,CCMAT)
GO TO 190

190 CONTINUE
C DETERMINE THE MEASUREMENT TYPE AND PLACE THE APPROPRIATE SUBMATRIX
C INTO THE WORK MATRIX FOR EVALUATION OF EIGENVALUES AND EIGENVECTORS.
C GO TO (210,220,230,240,250,260,260) IMEAS
C
210 CONTINUE !MACROSCOPIC RELAXATION
KW = KA+KB+KC
KWSUM = KW
CALL MOPRT(GMAT,WMAT,IA,1,IA,IA)
GO TO 230
C
220 CONTINUE !FLUCTUATION
KW = KA+KB+KC
KWSUM = KW
CALL MOPRT(GMAT,WMAT,IA,1,IA,IA)
GO TO 230
C
230 CONTINUE !WAITING TIME
CALL MOPRT(BBMAX,WMAT,IA,1,IA,IA)
KW = KB
KWSUM = KW
GO TO 250
C
240 CONTINUE !CLOSED TIME
CALL MOPRT(BBMAX,WMAT,IA,1,IA,IA)
KW = KB
KWSUM = KW
GO TO 250
C
250 CONTINUE !OPEN TIME
KW = KA
KWSUM = KW
CALL MOPRT(AAMAT,WMAT,IA,1,IA,IA)
GO TO 250
C
260 CONTINUE !BURST LENGTH AND #BURST
KW = KA+KB
KWSUM = KW
CALL MOPRT(GMAT,WMAT,IA,KA+KB,1,KA+KB,IA)
GO TO 290
C
290 CONTINUE
C IF(IMEAS .EQ. 7) GO TO 700 ! #BURST IS SPECIAL CASE
C CALCULATE THE EIGENVALUES AND EIGENVECTORS AND REPORT ANY PROBLEMS
C WITH THE CALCULATION. THE INPUT IS WMAT.
C NOTE THAT ON OUTPUT REIGUE WILL BE A MATRIX CONSISTING OF MSTATE COLUMN
C VECTORS THAT ARE THE EIGENVECTORS. IT IS EQUIVALENT TO THE M MATRIX
C OF CHE. REIGUA WILL BE A VECTOR CONTAINING THE MSTATE EIGENVALUES.
C IIJOB = 2
CALL EIGRF(WMAT,KW,IA,IIJOB,REIGUA,REIGUE,IZ,WORK,IER)
IF(IERR .NE. 0) CALL TELL ('*** ERROR IN EIGRF. IER=',IER,'**')
IF(WORK(1) .GT. .1) CALL TELL ('*** PERFORMANCE INDEX(IP) = ',WORK(1),'**')
C NORMALIZE THE EIGENVECTORS.
DO 305 J=1,KW
ZN = EIGVEC(1,J)
DO 305 I=1,KW
EIGVEC(I,J) = EIGVEC(I,J)/ZN
305 CONTINUE
C EXTRACT THE REAL PART OF THE EIGENVALUES AND EIGENVECTORS AND
C PLACE THEM INTO RLANDA AND MMAT, RESPECTIVELY.
C ALL RESULTS SHOULD BE REAL FOR COMPARTMENT-TYPE MODELS.
      DO 60 I=1,KW
      DO 55 J=1,KW
         RLANDA(I) = REAL(EIGVAL(1))
         MMAT(I,J) = REAL(EIGVEC(I,J))
      55 CONTINUE
  60 CONTINUE
C DO THE MATRIX INVERSION AND REPORT ANY PROBLEMS WITH THE CALCULATION.
      CALL LINIINV(SCRCH,KW,IN,MAT,B,WORK,IER)
      IF (IER .EQ. 129) CALL TYPE('*** SINGULAR MATRIX IN LINIINV.'
       IF (IER .EQ. 34) CALL TYPE('*** ACCURACY TEST FAILED IN LINIINV.'
C CALCULATE THE WEIGHT VECTOR USING THE APPROPRIATE ELEMENTS OF
C MATRICES A,B, AND C AS SHOWN IN EQU 1.33 OF CSH (1982).
C MATRICES B AND C ARE EQUIVALENT TO WEIGHTB AND WEIGHTC ALREADY DETERMINED.
C MATRIX A IS DETERMINED BELOW AND PLACED INTO SCRCH.
C THERE ARE KGSTATE PASSES THROUGH THIS LOOP.
      DO 600 NUMA=1,KW
         EXTRACT A VECTOR FROM MMAT AND A ROW FROM MMAT,
         DO 570 I=1,KW
            MCOL(I,1) = MMAT(I,NUMA)
         570 CONTINUE
         DO 575 I=1,KW
            NRROW(I,1) = MMAT(NUMA,I)
         575 CONTINUE
C GET A PER EQT. 15 OF CSH (1977) AND PLACE IT IN SCRCH.
C A IS OBTAINED FROM A VECTOR*ROW, REPORT ANY ERRORS.
      CALL UMULFF(MCOL,NRROW,KW,1,KW,IA,IA,SCRCH,IA,IER)
      IF (IER .EQ. 129) CALL TYPE('*** DIMENSIONING ERROR IN UMULFF.'
C IF (IMES .EQ. 2) GO TO 592
      IF (IMES .EQ. 2) GO TO 592 ! FLUCTUATION MEASUREMENT.
      ELSE...... FORM THE WEIGHT VECTOR.
      DO 590 I=1,KHSUM
         DO 585 J=1,KHNUM
            WEIGHT(NUMA) = WEIGHT(NUMA) + WEIGHTB(I)*SCRCH(I,J)*WEIGHTC(J,1)
         585 CONTINUE
  590 CONTINUE
C FOR FLUCTUATION MEASUREMENTS THE WEIGHTS ARE CALCULATED
C DIFFERENTLY. THE FOLLOWING GIVES THE STEADY-STATE
C PROBABILITY OF BEING IN EACH OF THE STATES. THE
C WEIGHT IS FURTHER MODIFIED LATER.
      DO 592 I=1,KHSUM
         WEIGHT(NUMA) = SCRCH(I,1)
  592 CONTINUE
C SORT THE RESULTS ON THE SIZE OF LAMBDA WITH THE SMALLEST
C LAMBDA VALUE AND ITS CORRESPONDING WEIGHT PLACED LAST.
      CALL FTSORT(RLANDA,WEIGHT,KW)
C************************************************************** ** OUTPUT ***
C DO SOME LAST MINUTE CALCULATIONS FOR SOME SPECIAL CASES OF IMES.
C CALL TELL(’THE MEASUREMENT TYPE IS ’, IMES) ;
C IF (CALPZ) GO TO 686 (CALCULATING P(B) FOR #BURST =) SKIP.
C CALL TYPE(’SUMS OF EXPONENTIALS OR Lorentzians CONSIST OF 2
C THE FOLLOWING TERMS;’)
C IF(IMES .EQ. 2)
C 2 CALL CNDGTR(’FOR FLUCTUATION SCALE FACTOR FOR HZ = ’, SCLFUC)
C DO 685 NUMA =1,KW
C IF(ABS(RLAMDA(NUMA)) .LT. 1.0E-15) RLAMDA(NUMA)=0.0
C 2 IF(NUMA.EQ.KW .AND. (IMES.EQ.1.OR.IMES.EQ.2) .AND. (.NOT.ABSORB))
C IF(ABS(RLAMDA(NUMA)) .LT. 1.0E-15) TAU=1.0/RLAMDA(NUMA)
C IF(ABS(RLAMDA(NUMA)) .GE. 1.0E-15) TAU=1.0E15
C IF((IMES.EQ.1) .AND. (RLAMDA(NUMA).NE. 0.0))
C 1 CONTINUE WITH THE CALCULATION OF WEIGHT FOR FLUCTUATION.
C IF(IMES.EQ.2) RLAMDA(NUMA) = RLAMDA(NUMA)*SCLFUC
C IF((IMES.EQ.2) .AND. (RLAMDA(NUMA).EQ. 0.0))
C 2 WEIGHT(NUMA) = -WEIGHT(NUMA) / RLAMDA(NUMA)
C IF((IMES.EQ.2) .AND. (RLAMDA(NUMA).EQ. 0.0))
C 2 WEIGHT(NUMA) = 0.0
C CALL TELL(’TERM ’, NUMAX)
C CALL TELL(’WEIGHT ’, ’WEIGHT(NUMA),’LAMBDA ’, ’RLAMDA(NUMA),’
C 2 TAU = ’, TAU,’_’)
C 685 CONTINUE
C 686 CONTINUE
C IF(IMES .LT. 3) GO TO 999 (MACRO CURRENTS => SKIP
C CALL TYPE(’BEGINNING AND END TRUNCATE THE FOLLOWING CALCULATIONS.’)
C IF(.NOT.CALPZ) CALL CNDGTR(’BEGINNING TIME = ’, TIMBEG)
C IF(.NOT.CALPZ) CALL CNDGTR(’END TIME = ’, TIMEND)
C IF(CALPZ) TIMBEG=0.0
C IF(CALPZ) TIMEND=1.0E20
C CUMPRB = 0.0
C RIDENS = 0.0
C AVERAGE = 0.0
C DO 650 NUMA =1,KW
C IF(ABS(RLAMDA(NUMA).NE.0.0) TAU = -1.0 / RLAMDA(NUMA)
C IF(ABS(RLAMDA(NUMA).EQ.0.0) TAU = 1.0E15
C CUMPRB = CUMPRB + WEIGHT(NUMA)*EXP(-TIMEND/TAU)*TAU
C 3 + WEIGHT(NUMA)*EXP(-TIMBEG/TAU)*TAU
C RIDENS = RIDENS + WEIGHT(NUMA)*EXP(-TIMEND/TAU)
C 2 + WEIGHT(NUMA)*EXP(-TIMBEG/TAU)
C AVERAGE = AVERAGE + WEIGHT(NUMA)*EXP(-TIMEND/TAU)*TIMEND+TAU*TAU**2)
C 3 + WEIGHT(NUMA)*EXP(-TIMBEG/TAU)*TIMBEG+TAU*TAU**2)
C 650 CONTINUE
C IF(CALPZ) GO TO 780 (CALCULATING P(B) FOR #BURST
C CALL TELL(’PROBABILITY DENSITY AT BEGINNING ’, ’RIDENS,’)
C CALL TELL(’CUMULATIVE PROBABILITY BETWEEN BEGINNING AND END = ’, ’CUMPRB,’)
C CALL TELL(’AVERAGE VALUE BETWEEN BEGINNING AND END = ’, ’AVERAGE,’)
C GO TO 999
C SKIP TO HERE FOR THE #BURST WHICH IS A SPECIAL CASE.
C 780 CONTINUE
C IF(CALPZ) GO TO 710
C CALL TYPE(’IN MODEL, CALPZ=TRUE, GO BACK TO 5_’)
C RHO = WEIGHT(1) SAVE THE VALUE
C CALL TYPE(’IN MODEL, RHO = ’, RHO,’_’)
C IMES = 3 (WAITING TIME
C GO TO 5 100 BACK TO CALCULATE P(B)
C CONTINUE
C 710 CONTINUE
C IF(IMES) 1-CUMPRB IPI (B)
C WEIGHT(2) = RHO IRHO
C IMES = 7 (RESET IMES
C CALL TYPE(’THE PROBABILITY OF R OPENINGS/BURST COMES FROM_’)
C CALL TELL(’B P(B) = ’, WEIGHT(1), ’RHO,’)
C CALL TELL(’MEAN OPENINGS IF IT OPENS = ’, 1.0*WEIGHT(1), ’_’)
C CALL TELL(’MEAN OPENINGS NORMALIZED FOR FAILURES = ’, 2 1.0*(1.0-RHO)**((1.0-RHO)**(1.0-WEIGHT(1))), ’_’)
C CALL CNDGTR(’NORMALIZE THE PLOT FOR THE NUMBER OF FAILURES = ’, 2 NORMP)
C GO TO 999
C**************************************************************
SUBROUTINE MODEL(X,PARAME,HUMPAR,Y)
C-----------------------------------------------------------------------------------
C PURPOSE: Evaluates the predictions at each value of X.
C INPUT: 
REAL PARAME(I)     !MODEL PARAMETERS
INTEGER HUMPAR      !# OF PARAMETERS
REAL X              !INDEPENDENT VARIABLE
C OUTPUT: 
REAL Y              !INDEPENDENT VARIABLE
C COMMON: 
INTEGER IMEAS      !TYPE OF MEASUREMENT TO CALCULATE
INTEGER KWSUM      !# OF EXPONENTIALS IN OUTPUT
REAL WEIGHT(6)     !WEIGHTING FACTORS
REAL RLAMDA(6)      !EIGEN VALUES
LOGICAL NORMP      !(IF)NORMALIZE PLOTS FOR THE # OF FAILURES
C INTERNAL: 
REAL PFR           !PROB OF FAILURE
REAL PZERO         !P(R=B)
C-----------------------------------------------------------------------------------
COMMON/PROCOM/IMEAS,KWSUM,WEIGHT,RLAMDA,NORMP
DATA RPI/3.1415927/
C
Y = 0.8
GO TO (610,640,610,610,610,610,610,610,610,670) IMEAS
C
610 CONTINUE !CALCULATE A CONDITIONAL DISTRIBUTION.
DO 625 I=1,KWSUM
   Y = Y + WEIGHT(I)*EXP(RLAMDA(I)*X)
625 CONTINUE
RETURN
C
640 CONTINUE !CALCULATE A NORMALIZED SPECTRUM.
DO 645 I=1,KWSUM-1
   IF(ABS(RLAMDA(I)) .LT. 1.0E-15) GO TO 645 !SKIP IF = 0
   FNOT = -RLAMDA(I) / (2.0*RPI)
   Y = Y + (WEIGHT(I) / (1.0+(X/FNOT)**2))
645 CONTINUE
RETURN
C
670 CONTINUE !CALCULATE PROBABILITY OF R OPENINGS / BURST
IR = IFIX(X) !INTEGER VALUED ONLY!
PZERO = WEIGHT(1)
IF(IR .GT. 0) GO TO 673 !SKIP
C ELSE....SPECIAL CASE OF R=0
IF(NORMP) Y = PZERO
IF(.NOT. NORMP) Y = 0.8
RETURN
C
673 CONTINUE
PFR = WEIGHT(2)**(IR-1) * (1.0-WEIGHT(2))
IF(NORMP)
   PFR = PFR / (PFR+PZERO) !NORMALIZE FOR FAILURES
   Y = PFR
RETURN
C
END
SUBROUTINE SETBUR(AAMAT, BEMAT, ABMAT, BAMAT, BCMAT, ACMAT, NSTATE, IA,
     2 KAKB, IMERS, WEHTC, WEIGHTB, AAINU, BBINU, GAB, GBC, GAC, WORK,
     3 SCRATCH, EB, COL)
C---------------------------------------------------------------------
C PURPOSE: Used in calculating the number of openings/burst.
C---------------------------------------------------------------------
C INPUTS:
REAL AAMAT(6,6), ABMAT(6,6), ACMAT(6,6)  !SEE IMOD(I)
REAL BEMAT(6,6), BCMAT(6,6)
INTEGER KAKB
INTEGER NSTATE  ! TOTAL # OF STATES FOR CALCULATION
INTEGER IA      ! SQUARE DIMENSIONS OF ARRAYS
C---------------------------------------------------------------------
C OUTPUTS:
REAL WEHTC(6,6), WEIGHTB(6) !USEFUL OUTPUT. SEE IMOD(I)
C WORK ARRAYS:
REAL AAINU(6,6), SCRATCH(6,6)  ! WORK SPACE ONLY. IN SOME CASES
REAL BBINU(6,6), EB(6,6)  ! THE ARRAYS HAVE BEEN RENAMED FOR
REAL BMAP(6,6)  ! CONVENIENCE.
REAL GAB(6,6), COL(6,6)
REAL GBC(6,6)
REAL GAC(6,6)
REAL WORK(4B)
C---------------------------------------------------------------------
C INTERNAL:
INTEGER I, J, IER
C---------------------------------------------------------------------
WEHTB(1) = 1.0
C---------------------------------------------------------------------
FORM MATRIX EB, CMH (1982). USE SEVERAL MATRICES AS SCRATCH.
CALL LINUFB(AAMAT, KA, IA, AAINU, 0, WORK, IER)
CALL LINUFB(BEMAT, KB, IA, BBINU, 0, WORK, IER)
C---------------------------------------------------------------------
CALL UMLUF(AAINU, ABMAT, NSTATE, NSTATE, IA, IA, GAB, IA, IER)
CALL UMLUF(BBINU, BCMAT, NSTATE, NSTATE, IA, IA, GBC, IA, IER)
CALL UMLUF(GAB, GBC, NSTATE, NSTATE, IA, IA, SCRATCH, IA, IER) ! GAB=GBC
CALL UMLUF(AAINU, ACMAT, NSTATE, NSTATE, IA, IA, GAC, IA, IER) ! GAC
---------------------------------------------------------------------
DO 165 I = 1, NSTATE
   DO 164 J = 1, NSTATE
      SCRATCH(I, J) = SCRATCH(I, J) - GAC(I, J) ! GAB=GBC + GAC
      COL(I, J) = 0.0  !MAKE UC, A COLUMN VECTOR
      COL(I, 1) = 1.0
   164 CONTINUE
165 CONTINUE
C---------------------------------------------------------------------
CALL UMLUF(SCRATCH, COL, NSTATE, NSTATE, IA, IA, EB, IA, IER) ! EB
C---------------------------------------------------------------------
IF(IMERS .EQ. 7) GO TO 59 ! IN-BURST
ELSE... CONTINUE WITH BURST LENGTH.
   CALL UMLUF(AAMAT, EB, NSTATE, NSTATE, IA, IA, WEHTC, IA, IER) ! C
   DO 18 I = 1, NSTATE
      DO 17 J = 1, NSTATE
         WEHTC(I, J) = - WEHTC(I, J)  ! C MATRIX
      17 CONTINUE
   18 CONTINUE
   RETURN
C---------------------------------------------------------------------
# BURST CALCULATION
59 CONTINUE
C GET GAB=SCRATCH
CALL UMLUF(BBINU, BMAP, NSTATE, NSTATE, IA, IA, SCRATCH, IA, IER)
C FORM RHO IN WEHTC(1,1)
CALL UMLUF(GAB, SCRATCH, NSTATE, NSTATE, IA, IA, WEHTC, IA, IER)
C FORM (1-RHO) IN WEIGHTB(1)
   WEIGHTB(1) = EB(1, 1)
C---------------------------------------------------------------------
CHECK FOR POSSIBLE ERROR BY CHECKING RHO BY TWO METHODS.
   IFABS(WEHTC(1,1)-(1.0-WEIGHTB(1))) .GT. 1.0E-5
      CALL TELLR("** POSSIBLE ERROR IN SETBUR. DELTA=",
               WEHTC(1,1)-(1.0-WEIGHTB(1)), ', _")
   RETURN
C---------------------------------------------------------------------
END
SUBROUTINE MUPRTR(ARRAYI,ARRAYO,IRBEG,IREND,ICBEG,ICEND,ISQR)
C PURPOSE: Move ARRAYI into ARRAYO. Useful in the partitioning of matrices.
C INPUT:
REAL ARRAYI(ISQR,ISQR)
INTEGER IRBEG,IREND !BEGIN AND END ROWS
INTEGER ICBEG,ICEND !BEGIN AND END COLUMNS
INTEGER ISQR !DIMENSIONS OF ARRAYI AND ARRAYO
C OUTPUT:
REAL ARRAYO(ISQR,ISQR)
C
DO 20 IRW=IRBEG,IREND
  DO 15 ICOL=ICBEG,ICEND
  ICNEW = ICOL-ICBEG+1
  IRENW = IRW-IRBEG+1
  IF(IRENW.LT.1 .OR. IRENW.GT.ICEND) GO TO 15
  IF(ICNEW.LT.1 .OR. ICNEW.GT.ICEND) GO TO 15
C
  ARRAYO(ICNEW,IRENW) = ARRAYI(ICOL,IRW)
C
15 CONTINUE
20 CONTINUE
C
RETURN
END
C
C
SUBROUTINE SETUP(PARAME,HUMPAR,QMAT,IA)
C PURPOSE: Load QMAT from the values of the rate constants.
C INPUT:
REAL PARAME(1)
INTEGER HUMPAR
INTEGER IA !DIMENSIONS OF QMAT
C OUTPUT:
REAL QMAT(IA,IA)
C
RK12 = PARAME(1)  !LOAD IT UP USING A RATHER 'BRUTE FORCE'
RK21 = PARAME(2)  !TECHNIQUE.
RK23 = PARAME(3)
RK32 = PARAME(4)
RK36 = PARAME(5)
RK33 = PARAME(6)
RK55 = PARAME(7)
RK56 = PARAME(8)
RK54 = PARAME(9)
RK45 = PARAME(10)
RK14 = PARAME(11)
RK41 = PARAME(12)
RK25 = PARAME(13)
RK52 = PARAME(14)
C
QMAT(1,1) = -(RK12+RK14)
QMAT(1,2) = RK21
QMAT(1,4) = RK41
QMAT(2,1) = RK12
QMAT(2,2) = -(RK21+RK23+RK25)
QMAT(2,3) = RK32
QMAT(2,5) = RK52
QMAT(3,2) = RK23
QMAT(3,3) = -(RK32+RK36)
QMAT(3,5) = RK53
QMAT(6,3) = RK36
QMAT(6,6) = -(RK63+RK65)
QMAT(6,5) = RK56
QMAT(5,2) = RK52
QMAT(5,5) = -(RK52+RK54+RK56)
QMAT(5,4) = RK45
QMAT(4,1) = RK14
QMAT(4,5) = RK54
QMAT(4,4) = -(RK41+RK45)
C
RETURN
END
C.2 ACT3ST: a computer program for making various calculations on state models.

This is a collection of routines for calculating whole cell current responses to various voltage pulse protocols. State models are used, and the version shown here is configured for the case of a 3-state model. Other more complicated models follow analogously. These routines have been linked to two different main programs. The program MODPLT has been used for doing simulations. This is described in Sections 3.4 and C.1. The same routines were also linked to MACEST for parameter estimation (see Section 3.2). However, these subroutines may be used with any suitable driving program in a manner similar to that described in Section C.1. In this regard, note that the subroutine names are similar to the ones given in Section C.1; i.e., IDMODL, IMODEL, and MODEL. Numerical integration is done using a subroutine that uses a Runge-Kutta algorithm. This general subroutine is RUNGE, and it is included.

In the documentation that follows, I have listed only the main subroutines. Other subroutines are from the "MiscLib" library of subroutines of the UTMB department of Physiology and Biophysics. These subroutines are listed below.

UTMB: TELL1, TELLR, TYPE, GTCOEF, MOVEB, and PPVALU
SUBROUTINE IDMODL(MULTI, DERIV, IDP, HUMPAR, NAMES, PARAME)

PURPOSE: Load NAMES with appropriate names for the parameters and
set some logical variables having to do with the model.
Also initialize some model parameters using the splined curves.

INPUT:
REAL PARAM(26) ! parameters
INTEGER HUMPAR ! # of parameters

OUTPUT:
LOGICAL MULTI ! IT => multiple independent values
LOGICAL DERIV ! IT => analytical derivatives
PRESENT FOR CURVE FITTING
INTEGER IDEP ! # of dependent values
BYTE NAMES(19,26) ! names of parameters

COMMON:
INTEGER KARY(6), LARY(6) ! variables for splined curve.
REAL COEF(30,6), BREAK(10,6) !

COMMON /SPLINE/ COEF, BREAK, KARY, LARY

DATA NCOEF/30/
   ! max # coeff for spline. =MAXBRK=ORDER
DATA MAXBRK/10/
   ! max # of break points

MULTI = .TRUE. ! allows plotting of multiple dependent variables.
DERIV = .FALSE. ! requires numerical evaluation of partial derivatives.
IDEP = 7
HUMPAR = 26

CALL MOVEB('K1') ! NAMES(1,1),10
CALL MOVEB('K2') ! NAMES(1,2),10
CALL MOVEB('K3') ! NAMES(1,3),10
CALL MOVEB('K4') ! NAMES(1,4),10
CALL MOVEB('K5') ! NAMES(1,5),10
CALL MOVEB('K6') ! NAMES(1,6),10
CALL MOVEB('SCALE IC') ! NAMES(1,7),10
CALL MOVEB('TYPE PLOT') ! NAMES(1,8),10
CALL MOVEB('TIME SS') ! NAMES(1,9),10
CALL MOVEB('K1-UH') ! NAMES(1,10),10
CALL MOVEB('K2-UH') ! NAMES(1,11),10
CALL MOVEB('K3-UH') ! NAMES(1,12),10
CALL MOVEB('K4-UH') ! NAMES(1,13),10
CALL MOVEB('K5-UH') ! NAMES(1,14),10
CALL MOVEB('K6-UH') ! NAMES(1,15),10
CALL MOVEB('SHIFT ALPHA') ! NAMES(1,16),10
CALL MOVEB('UNUSED') ! NAMES(1,17),10
CALL MOVEB('STEP MIN') ! NAMES(1,18),10
CALL MOVEB('STEP DEL') ! NAMES(1,19),10
CALL MOVEB('STEP Tau') ! NAMES(1,20),10
CALL MOVEB('DUR') ! NAMES(1,21),10
CALL MOVEB('DUR') ! NAMES(1,22),10
CALL MOVEB('DUR') ! NAMES(1,23),10
CALL MOVEB('DUR') ! NAMES(1,24),10
CALL MOVEB('DUR') ! NAMES(1,25),10
CALL MOVEB('DUR') ! NAMES(1,26),10

PARAM(1) is used as a flag to determine when parameters are to be
initialized using the splined curves. PARAM(1) is set elsewhere.

IF PARAM(1).LT.-100) GO TO 900 ! skip initialization

Fetch the files containing the coefficients for the splined curves.
DO 100 I=1,6
    CALL TELL('H=COEFFICIENT VS VOLTAGE: NUMBER ',I,'.')
    CALL GETCOEF(NCOEF,MAXBRK,KARY(I),LARY(I),BREAK(I),COEF(I))
100 CONTINUE

Evaluate the rate constants at the desired voltage and place the
result into the appropriate positions in PARAM(1). These values
will serve as initial guesses for the parameter estimation routine. Note that this can be done for 1 through 6 only.

UM = PARAM(24) ! voltage for rate constant evaluation.

PARAM(1) = PPVALU(BREAK(1),COEF(1),LARY(1),KARY(1),UM,0)
PARAM(2) = PPVALU(BREAK(2),COEF(2),LARY(2),KARY(2),UM,0)
PARAM(3) = PPVALU(BREAK(3),COEF(3),LARY(3),KARY(3),UM,0)
PARAM(4) = PPVALU(BREAK(4),COEF(4),LARY(4),KARY(4),UM,0)
PARAM(5) = PPVALU(BREAK(5),COEF(5),LARY(5),KARY(5),UM,0)
PARAM(6) = PPVALU(BREAK(6),COEF(6),LARY(6),KARY(6),UM,0)

RETURN

CONTINUE
CALL TYPE(\"THERE WAS NO INITIALIZATION IN IDMODL\")
RETURN
END

SUBROUTINE IMODEL(PARAME, NUMPAR, ITRACE)

PURPOSE: Initialize the variables for the model.

Voltage clamp pulses are prescribed in the following manner.

<-- DUR1 --><-- DELAY2 -->

VOLT(2) VOLT(4) VOLT(2)

VOLT(3)

INPUT:

REAL PARAM(1) ! model parameters
INTEGER NUMPAR ! dimension of PARAME
INTEGER ITRACE ! trace I--identifies type of measurement

OUTPUT: NONE

COMMON:

REAL CONT(21), PROBAB!
REAL TIME, TIMEOL!
REAL TIMES(4), VOLT(4)
REAL ROUT(7) ! OUTPUT ARRAY
REAL KEND, STPSIZ!

INTEGER NORDER ! order of diff. eqs., input to RUNGE
INTEGER IND, NW, IER, K ! more input to RUNGE, includes dummy variables
COMMON /XPULSE/ UM, ITYPE
COMMON /MODEL/ RTF, PSICEN, CONBLK, RP, NW, NSYTE, TOL,

2 IND, SINCOL, STPMIN, STPDEL, STPTAU, TIMEOL, CONT,
3 TIMES, VOLT

EXTERNAL ACTIVA ! for function evaluations in RUNGE

Set constants for the instantaneous I-U calculation.

DATA RTF/25.0/ !H
DATA PSICEN/15.0/ !H
DATA CONBLK/10.8E-3/ !MOLAR
DATA CONC1/1.0E-3/ !MOLAR
DATA RP/2.76E-3/ !NAMP/(MwMOLAR)
DATA NSYTE sets the order of the system of diff. eqs. This should
be the only statement change required in IMODEL or MODEL when the
state model is to be changed. All other changes occur in the
routines ACTIVA, EVALA, and ACTA. Note that for these calculations
NSYTE = 3 states - 1.

DATA NSYSTE/2/ ! order of the system of diff. eqs.

Set some miscellaneous variables.

SINCOL = $1234566.0E10 !flag for the first call to modelC
STPMIN = PARAME(19) !variable step size information
STPDEL = PARAME(19) !
STPTAU = PARAME(20) !
DUR1 = PARAME(21) ! see diagram
DELAY2 = PARAME(22) !
VOLT(1) = PARAME(23) !
VOLT(2) = PARAME(24) !
VOLT(3) = PARAME(25) !
VOLT(4) = PARAME(26) !
C Set up TIMES, an array which gives the intervals for the pulses.
TIMES(2) = DURI
TIMES(3) = DURI + DELAY2
TIMES(4) = DURI + DELAY2 + 1.0E10
C
C Initialization in preparation for initial conditions.
SCALIC = PARAHE(7)
TIMEOL = 0.0
DO 20 JS=1,21
CONTA(J) = 0.0
20 CONTINUE
C Initial condition depend on ITRACE.
GO TO (50,60,70) ITRACE
CALL TYPE("*** ERROR IN IMODEL, BAD ITRACE ***")
GO TO 950
C
50 CONTINUE 10N response
CONTA(1) = 1.0
GO TO 950
60 CONTINUE 10FF response
C Surprise...nothing needed! From the conservation relation
C all the channels are already in the last state, or the open state.
C Also allow initial conditions such that the next to last state will
C contain a fraction of the channels.
IF(SCALIC .GT. .99999) GO TO 55
C Else... get the concentration in the next to last state.
CONTA(NSSTB) = 1.0 - SCALIC
55 CONTINUE
GO TO 950
C
70 CONTINUE (TURN OFF AT VH
CONTA(1) = 1.0
C Integrate the model up to 4 msec. This will load CONTA with the
C appropriate initial conditions for the subsequent repolarization.
CALL MODEL(4.0,PARAME,NUMPAR,ROUT,1)
TIMEOL = 0.0
950 CONTINUE
C
C RETURN
END
SUBROUTINE MODELTIME, PARAM, NUMP4, ROUT, ITRACE

C------------------------------------------------------------------------
C INPUT:
REAL PARAM(1) !model parameters
INTEGER NUMP4 !dimension of PARAM
INTEGER ITRACE !trace N--identifies type of measurement
REAL TIME
C OUTPUT:
REAL ROUT(7) !output array
C INTERNAL:
INTEGER NORDER !order of system of diff. eqts.
C COMMON:
REAL CONTA(21) !state variables
REAL PROBB !probability of opening
REAL TIMES(4), VOLT(4) !see IMODEL
REAL STPSIZE !step size for integration
REAL KPI, KPH, KPH2, KPH3, KMH !rate constants
INTEGER IYPE !plot type
LOGICAL TRANS !IT => voltage transition
C COMMON /RMODEL/ RTF, PSICEN, CONBLK, RP, NN, NSYSTE, TOL,
COMMON /RATCON/ KPI, KPH, KPH2, KPH3, KMH, TS, TSCALE
COMMON /MODEL/ RTF, PSICEN, CONBLK, RP, NN, NSYSTE, TOL,
2 IND, SINCOL, STPMIN, STPD, STP, TIME, CONTA,
3 TIMES, VOLT
C EXTERNAL ACTIVA

C------------------------------------------------------------------------
C Check the plot type and take action.
C If an activation curve is desired the time is set large.
C If sensitivities are not to be evaluated NORDER is set equal to
C the order of the solution vector, NSYSTE.
IYPE = IFIX(PARAM(9))
IF(IYPE .NE. 4) NORDER = NSYSTE !Sensitivity NOT evaluated.
IF(IYPE .NE. 3) GO TO 15 !NOT an activation curve
WM = TIME
TIME = PARAM(9) !TIME to steady state
GO TO 150
15 CONTINUE
C For plotting, usually want 0.0 before TIME=0.0.
IF(TIME .LE. 0.0) ROUT(1)=0.0
IF(TIME .LE. 0.0) GO TO 999
C Find in which interval TIME lies. Also keep track of SINCE, the
time that has elapsed since a voltage transition.
TRANS = .FALSE. !TRUE when transition has occurred.
IF(TIME .GE. TIMES(2)) GO TO 65
SINCE = TIME
ITIME = 2
GO TO 100
65 IF(TIME .GE. TIMES(3)) GO TO 70
SINCE = TIME-TIMES(2)
ITIME = 3
GO TO 100
70 IF(TIME .GE. TIMES(4)) GO TO 100 !CHECK IF NEED MORE TRANS
ITIME = 4
SINCE = TIME-TIMES(3)
100 CONTINUE
C IF(SINCE .GT. SINCOL) GO TO 200 !NO transition
IF(TIME .EQ. 2) GO TO 120 !FIRST VOLT in RECORD.
C Else... transition. Integrate the differential equations to the
transition time with the parameter values at the old voltage.
C Then change the values and integrate to the next display time.
TRANS = .TRUE.
TIMS + = TIME
TIME = TIME-SINCE !THIS SHOULD BE THE TRANSITION TIME.
ITIME = ITIME - 1 !THIS SHOULD BE THE LAST INTERVAL
SINCE = SINCOL !APPROXIMATELY TRUE
120 CONTINUE
WM = VOLT(TIME) !EVALUATE THE VOLTAGE
C 150 CONTINUE !NECESSARY FOR ACTIVATION PLOTTING.
KP1 = PARAM(1)  !SET THE PARAMETERS
KP2 = PARAM(2)
KP3 = PARAM(3)
KP4 = PARAM(4)
KP5 = PARAM(5)
K1 = PARAM(11)
K2 = PARAM(12)
K3 = PARAM(13)
K4 = PARAM(14)
K5 = PARAM(15)
TS = Param(7)  !TEMPERATURE SCALE FACTOR

IF (ITRC .NE. 3) GO TO 153

C Set up for the special case of turn-off at UM.
UM = -50  !NEEDED FOR INSTANT IV CALCULATION
KP1 = PARAM(10)
KP2 = PARAM(11)
KP3 = PARAM(12)
KP4 = PARAM(13)
KP5 = PARAM(14)
K1 = PARAM(15)
CONTINUE

153 CONTINUE

C UMB = UM  !SKIP TO HERE WHEN NO TRANSITION.  !!!!!!!!!!!!!!!
CONTINUE

C Step size for integration is variable.  The step size increases
C exponentially following a voltage transition.  Good values
C are STPMIN=1.E-5, STPDEL=1.E-2, and STPTAU=.4
C STPSIZ = STPMIN + STPDEL*(1.0 - EXP(-GINC/STPTAU))

C Do the integration.
C CALL RUNGECORDER,ACTIVA,TIMEOL,TIME,RDUM1,RDUM2,RDUM3,RDUM4,
C PROB = 1.0 - CONTA(1) - CONTA(2) - CONTA(3)  !PROB. OF OPENING
C
C Check for a transition and act accordingly.
C SINCO = SINCO
C IF (.NOT. TRANS) GO TO 388
C ELSE.... Go back to integrate to next display point.
C SINCO = TIMSTO-TIME
C TIME = TIMSTO
C TRANS = .FALSE.
C ITIME = ITIME + 1
C GO TO 130
C
C***********************************************************************

C 388 CONTINUE

C Calculate the value of the current at the present voltage.
C UCHAN = UM - PSICEN
C CONCO = CONBLK - EXP(-2.0*PSICEN/RTO)
C RPART2 = CONC/CONBLK
C RPART2 = 1.0 - EXP(2.0*UANCH/RTO)
C IF (ABS(RPART2) .LT. 1.0E-10) RPART2 = 1.0E-10
C INSTA = RP * UCHAN / RPART2
C RINTA = RINTA + 1.0E-8  !VALUE OF INSTANTANEOUS IV IN AMPS.
C CURREN = PROB * RINTA  !CALCULATE THE CURRENT !!!!!!!
C
C Set up the output.
C GO TO (210, 220, 230) ITYPE

C 810 CONTINUE  !STATE VARIABLE PROBABILITIES
C ROUT(1) = PROBAB
C ROUT(2) = CONTA(1)
C ROUT(3) = CONTA(2)
C ROUT(4) = CONTA(3)
C IF (ITYPE .EQ. 3) TIME = UM  !TO PLOT ACTIVATION CURVE
C GO TO 890

C 820 CONTINUE  !CURRENT
C ROUT(1) = CURREN
C GO TO 950

C 830 CONTINUE  !CURRENT AND PARAMETER SENSITIVITIES.
C ROUT(1) = CURREN
C ROUT(2) = (CONA(4) + RINTA)
C ROUT(3) = (CONA(7) + RINTA)
C ROUT(4) = (CONA(18) + RINTA)
C ROUT(5) = (CONA(12) + RINTA)
C ROUT(6) = (CONA(16) + RINTA)
C ROUT(7) = (CONA(19) + RINTA)
C
C 890 CONTINUE

C IF (IND.LT.0 .OR. IER.GT.8) GO TO 980
C IER = IER + IER
C CONTINUE

C 999 CONTINUE
C CALL TELL("**ERROR IN ACTIVATE OR RUNGE" .IND=",IND," IER="",
C 1 = IER,"ITRACE="ITRACE,"_"
C RETURN
C
C END
SUBROUTINE ACTIVA(NORDER,TIME,CONTA,DERIVA)

PURPOSE: Calculate the derivatives of the state variables for numerical integration. 3-state model.

The solution vector and parameter sensitivity vectors are placed in CONTA, and the corresponding time derivatives are placed in DERIVA according to the following scheme:

- R = CONTA(1) (prob. of being in the rest state)
- A1 = CONTA(2)
- A2 = CONTA(3)

- B = 1.0 - R - A1 - A2

The sensitivity to KPI is placed in CONTA(4) TO CONTA(7), etc.

INPUT:
- INTEGER NORDER (order of system of diff. eqts.
- REAL CONTA(21) (state variables)
- REAL TIME

OUTPUT:
- REAL DERIVA(21) (derivative of each state variable

COMMON:
- REAL KPI,KM1,KP2,KM2,KP3,KM3 (rate constants)
- INTEGER ITYPE (plot type)

COMMON /XPI/ UM,ITYPE
COMMON /RATCON/ KPI,KM1,KP2,KM2,KP3,KM3,TSCALE

Evaluate the derivatives for the solution vector at 'TIME'.

CALL EVALA(DERIVA(1),CONTA(1))
DERIVA(2) = DERIVA(2) + KM2

IF (ITYPE .NE. 4) GO TO 990 !DON'T WANT SENSITIVITIES.

Evaluate the derivatives for the KPI parameter sensitivity vector.

CALL EVALA(DERIVA(4),CONTA(4))
DERIVA(4) = DERIVA(4) - CONTA(1)
DERIVA(5) = DERIVA(5) + CONTA(1)

Evaluate the derivatives for the KM1 parameter sensitivity vector.

CALL EVALA(DERIVA(7),CONTA(7))
DERIVA(7) = DERIVA(7) + CONTA(2)
DERIVA(8) = DERIVA(8) - CONTA(2)

Evaluate the derivatives for the KP2 parameter sensitivity vector.

CALL EVALA(DERIVA(10),CONTA(10))
DERIVA(10) = DERIVA(10) - CONTA(3)
DERIVA(12) = DERIVA(12) + CONTA(3)

Evaluate the derivatives for the KM2 parameter sensitivity vector.

CALL EVALA(DERIVA(13),CONTA(13))
DERIVA(13) = DERIVA(13) + CONTA(3)
DERIVA(15) = DERIVA(15) - CONTA(3)

Evaluate the derivatives for the KP3 parameter sensitivity vector.

CALL EVALA(DERIVA(16),CONTA(16))
DERIVA(16) = DERIVA(16) - CONTA(3)

Evaluate the derivatives for the KM3 parameter sensitivity vector.

CALL EVALA(DERIVA(19),CONTA(19))
DERIVA(21) = DERIVA(21) - CONTA(1) - CONTA(2) - CONTA(3) + 1.0

990 CONTINUE
RETURN
END
SUBROUTINE EVALA( DER, CON )

C PURPOSE: Used to calculate the derivatives of the state variables for numerical integration. 3-state model.
C NOTE: The arrays DER and CON are used here as subsets of the arrays that are passed. The variables operated upon will be determined by the first element given in the passed array. This is useful for sensitivity calculations.
C INPUT:
   REAL   CON(1) state variables
C OUTPUT:
   REAL   DER(1) derivative of each state variable
C COMMON:
   REAL   KP1, KM1, KP2, KM2, KP3, KM3 lrate constants
   COMMON /RATCON/ KP1, KM1, KP2, KM2, KP3, KM3, TSCALE

C DER(1) = -KP1*CON(1) + KM1*CON(2)
C DER(2) = (KP1-KM2)*CON(1)
C Z = -(KM1+KP2+KM2)*CON(2)
C RETURN
END

FUNCTION AETA( CON )

C PURPOSE: Used in sensitivity calculations. The case of a 3-state model as shown here is rather trivial.
C NOTE: Subsets of array CON are used as in EVALA above.
C REAL   AETA
C REAL   CON(1)

C AETA = -CON(1) - CON(2)
C RETURN
END
SUBROUTINE RUNGE(ORDER, FUNC, XOLD, Y, XNEW, DUM, DUM1, DUM2, IDUM1, DUM3, IDUM2, SIZINI)

C---------------------------------------------------------------------
C PURPOSE:
C Routine for integration of a system of differential equations
C using the Runge-Kutta integration method. Nomenclature
C follows that used by Hornbeck in his book, "Numerical Methods".
C
C NOTE:
C - XOLD and XNEW should contain the x values for the beginning and end
C of the integration period. Upon output XNEW will be placed in XOLD.
C - This is useful for repeated calling.
C - The array Y should contain initial values on input. These will be
C replaced by the new values for output.
C - Values are obtained exactly at XNEW.
C - Checked rigorously against the subroutine DVERK of the IMSL package.
C
C PARAMETER IORDER=5 !set .GE. ORDER
C INPUT:
C INTEGER ORDER !order of the diff. equats.
C REAL XNEW !independent variables, see above
C REAL FUNC !function for evaluation
C INPUT AND OUTPUT:
C REAL XOLD !independent variables, see above
C REAL Y(1) !independent variables, see above
C INTERNAL:
C REAL Q1(ORDER), Q2(ORDER) !see reference above
C REAL Q3(ORDER), Q4(ORDER) !
C REAL DERIVY(ORDER), YSTAR(ORDER) !
C INTEGER NSTEP !number of integration steps
C---------------------------------------------------------------------
C IF(XNEW-XOLD .LE. 1E-38) GO TO 200
C XNEW=XOLD => return
C IF XNEW-XOLD is less than the step size then the integration may
C not be accurate enough.
C IF(XNEW-XOLD .LT. SIZINI) GO TO 900
C ELSE...Calculate a step size so that a solution value will
C be obtained at XNEW. It should have a value close to SIZINI.
C RSTEP = (XNEW-XOLD) / SIZINI
C IF(RSTEP .GT. 32000) GO TO 900
C NSTEP = IFIX(RSTEP)
C STPSIZ = (XNEW-XOLD) / NSTEP
C---------------------------------------------------------------------
C INTEGRATE THE SYSTEM OF DIFFERENTIAL EQUATIONS.
C DO 200 NSTEP=1,NSTEP
C X = XOLD + (ISTEP-1)*STPSIZ
C CALL FUNCORDER,X,Y,DERIVY)
C DO 58 IEQT=1,ORDER
C Q1(IEQT) = STPSIZ*DERIVY(IEQT)
C YSTAR(IEQT) = Y(IEQT) + Q1(IEQT)/2.0
C 58 CONTINUE
C CALL FUNCORDER,X+STPSIZ/2.0,YSTAR,DERIVY)
C DO 58 IEQT=1,ORDER
C Q2(IEQT) = STPSIZ*DERIVY(IEQT)
C YSTAR(IEQT) = Y(IEQT) + Q2(IEQT)/2.0
C 58 CONTINUE
C CALL FUNCORDER,X+STPSIZ/2.0, YSTAR, DERIVY)
C DO 78 IEQT=1,ORDER
C Q3(IEQT) = STPSIZ*DERIVY(IEQT)
C YSTAR(IEQT) = Y(IEQT) + Q3(IEQT)
C 78 CONTINUE
C CALL FUNCORDER,X+STPSIZ, YSTAR, DERIVY)
C DO 88 IEQT=1,ORDER
C Q4(IEQT) = STPSIZ*DERIVY(IEQT)
C 88 CONTINUE
C DO 100 IEQT=1,ORDER
C Y(IEQT) = Y(IEQT) +
C (Q1(IEQT) + 2*Q2(IEQT) + 2*Q3(IEQT) + Q4(IEQT)) / 6.0
C 100 CONTINUE
C DO 200 NSTEP=1,NSTEP
C XOLD = XNEW
C RETURN
C 900 CONTINUE ! Report errors.
C CALL TELL('#####ERROR IN RUNGE####: RSTEP=',RSTEP,'SIZINI=',
C SIZINI,' XNEW-XOLD=',XNEW-XOLD,'')
C RETURN
C END
REFERENCES


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