J. theor. Biol. (1992) **154,** 475–484

A 3-D Approach to Voltage Clamp Data

SHIMON MAROM†

Department of Physiology and Biophysics, Faculty of Medicine and the Rappaport Family Institute for Research in the Medical Sciences, Technion, P.O. Box 9697, *Haifa* 31096, *Israel*

(*Received on* 20 *November* 1990, *Accepted in revised form on* 10 *September* 1991)

A simple transition from two-dimensional (2-D) handling of voltage clamp data to three dimensions (3-D), uncovered some stimulating relationships between the graphic representation of the *I–V–t* space of electrical activity of the membrane and physiological and biophysical functions of the plotted ionic currents.

Introduction

The purpose of this report is to introduce a holistic approach to voltage clamp data. By voltage clamping excitable membranes, in the whole-cell or single-channel level, it is possible to define four basic types of parameters: *kinetic* (e.g. time and rate constants), "*size*" (e.g. current, conductance and permeability), *selectivity* to certain ions over others and *dependence of these parameters on various stimuli* (e.g. membrane potential and drugs). Analysis of voltage clamp data is based on two elementary viewpoints: (1) current induced by clamping the membrane to a specific holding potential as a function of time (*I–t*) and (2) an *I–V* curve which is the current as a function of holding potential at a specified point in time. These parameters and the techniques to derive them have served elecrophysiologists very successfully for more than 40 years now and the amount of data and characterized conductance published so far is immense. The approach presented here is different, aimed at illustrating the gestalt nature of excitable membrane activity under voltage clamp conditions, beyond time and voltage domains. It is based on transition from 2-D handling of voltage clamp data to 3-D, defining the *I–V–t* space of electrical activity of the membrane‡.

It is very important that the reader see the following discussion and examples as a possible extension of the conventional way of voltage clamp data analysis, rather than an alternative.

Computations and Description of the Contour Approach

Currents were calculated using the Hodgkin–Huxley formulation. Most simulations were built by direct implementation of the Hodgkin–Huxley equations (1952)

† Present address: Graduate Department of Biochemistry, Brandeis University, Waltham, MA 02254-9110, U.S.A.

‡ The author is indebted to one of the reviewers for pointing out that David Goldman used a plastic cast 3-D model for teaching purposes. This model was not published (D. Goldman, personal communication).

0022-5193/92/040475 + 11 $03.00/0

(describing the squid axon) and the DiFrancesco–Noble equations (1985) (describing the cardiac conducting fibers). At indicated places, currents were simulated using eqns (1) and (2) to compute rate constants of a hypothetical inward-going inactivating channel. Gates were treated as first-order reactions and their rate constants were expressed as

$$A = A_0 \exp(gZ\Delta E) \tag{1}$$

$$B = B_0 \exp[-Z(1-g)\Delta E] \tag{2}$$

where A_0 and B_0 are the values of the rate co-efficients at the voltage $V_{0.5}$ at which $A = B$ and $\Delta E = \Delta VF/RT$; $\Delta V = V - V_{0.5}$; g is the fraction of membrane potential that influences the gate; Z is the activation equivalent charge of the gate (Jack *et al.*, 1975). All simulations were generated on a Macintosh IIx computer using Mathematica™ (Wolfram, 1988) (a system for doing mathematics by computer, Wolfram Research Inc.).

The transition from a 3-D view to a contour plot is straightforward. Figure 1(a) and (b) describes the conventional representation of voltage clamp data generated by computer simulation of a squid giant axon inward-going inactivated Na current. A family of command voltages was applied to the membrane and in Fig. 1(b), I–V curves of the channel at different points in time are shown. Since I–V curve shape depends on time, time should be added as a third parameter in order to study the ensemble conductance and kinetics active in the membrane. This procedure is demonstrated in Fig. 1(c) where currents, command potentials and time are shown simultaneously. This type of presentation is referred to as a "3-D view". Another, more objective, way to present the data is demonstrated in Fig. 1(d), where curves connect points having the same current magnitude, similar to the way isobars are plotted to create a synoptic map, and referred to as the "contour" of the data. A South to North slice of the contour at a particular point of time recovers a conventional I–V curve of the channel. A West to East slice recovers standard traces of current vs. time at a particular holding potential. When studying the gating process of a given conductance it is more appropriate to use the gate state as a parameter instead of the current, which is influenced by gate state, ionic concentrations, holding potential, and number of channels in the clamped membrane.

In the following sections, some characteristics of the contour of voltage clamp data, related to biophysical and physiological functions of ionic curents, are demonstrated.

The contour predicts time–voltage domains for the physiological activity of a conductance. To demonstrate this point, contours of three types of inward-going inactivating currents were simulated; TTX-sensitive fast sodium current and "slow inward" current of the cardiac action potential [both based on the DiFrancesco & Noble model (1985)], and the squid Na current [as formulated by Hodgkin & Huxley (1952)]. The contours are shown in Figs 1(d) and 2(a) and (b). There are marked differences between the currents. This is evident in their different time–voltage activity domains and their areas of steepest descent. Consider the area of steepest descent (voltage–time) as an area where minimal changes in time produce a maximal effect on current generation. One can predict, based on steepest descent area, 6that the fast Na channel of the cardiac cell will contribute to membrane conductance changes significantly in the range of −40

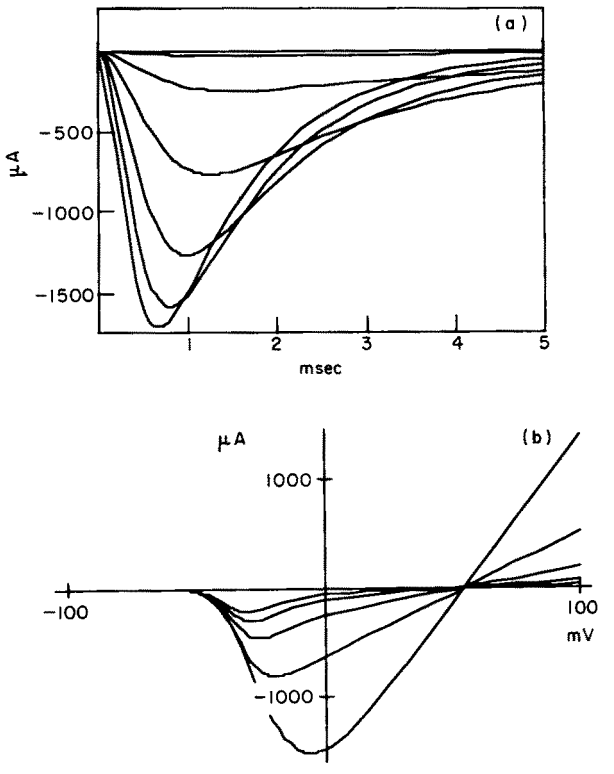

FIG 1. (a), (b) Conventional representation of squid sodium currents as current-time and current-voltage curves. In (a) data were generated by applying a family of command voltages (−70-0 mV, step = 10 mV) to the clamped membrane while currents were calculated as a function of time. In (b), current was calculated for a voltage range of −100 to 100 mV at different points of time (1-5 msec, step = 1 msec). In (c) and (d) currents, voltages and time are presented simultaneously to produce a 3-D view (c) and the contour plot (d) of channel activity. Initial settings and equations as in Hodgkin & Huxley (1952). Currents in μA cm^{-2}.

to 0 mV and 0·1 to ~3 msec whereas the slow inward channel will contribute to membrane conductance changes mainly in the range of −30 to +30 mV in the first 50 msec of an action potential. We can also predict that a steady-state component of the fast Na current in the cardiac cell will be generated around −40 mV. This current is manifested by a pathologic repolarization of the DiFrancesco-Noble cardiac action potential (DiFrancesco & Noble, 1985). We can also say that the slow inward current has a noticeable window conductance that is limited to a −30 to −10 mV range. This current would be a major component in the action potential plateau. Another interesting difference is between the fast cardiac Na channel [Fig. 2(a)] and the squid Na channel [Fig. 1(d)] contours. The latter is active in the >0 mV range for up to ~2 msec. To further emphasize the different time-voltage activity domains of these conductances, outlines of their contours are presented together (on the same time-voltage scale) in Fig. 2(c). The differences are self evident.

A most dominant feature of the contour map is the volume entrapped between

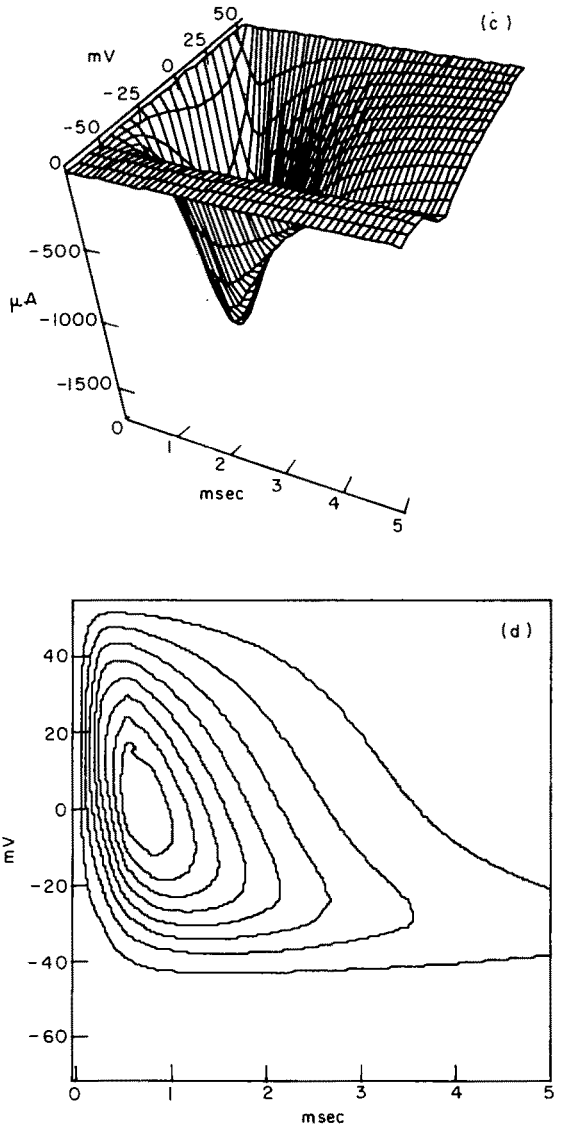

FIG. 1 (c), (d).

the contour and zero level ("current volume"). The term "current volume" (s) refers to the "volume" entrapped between the surface of the current contour and zero current level. Dimensions of this volume are in Joules (watt . second), calculated by a multiple integral of the current:

$$s = \int_{(t=0 \text{ to } t_{max})} \int_{(v=v_{min} \text{ to } v_{max})} I \, dt \, dV. \tag{3}$$

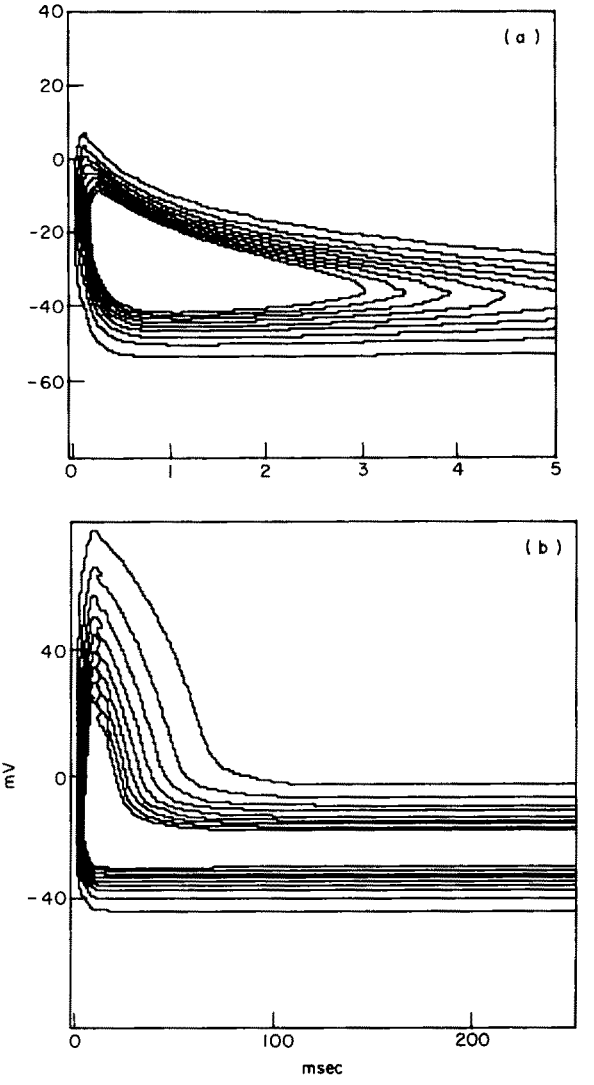

FIG. 2. The "fast" sodium current (a) and the "slow" inward current (b) of a cardiac cell, simulated according to DiFrancesco & Noble (1985) to demonstrate marked differences between contours of these channels. Compare to Fig. 1(d). (c) A comparison of time voltage domains of the squid Na current (NS), slow inward current (SC) and fast Na current of cardiac cells (FC). See text for remarks concerning these contours. Note time scales.

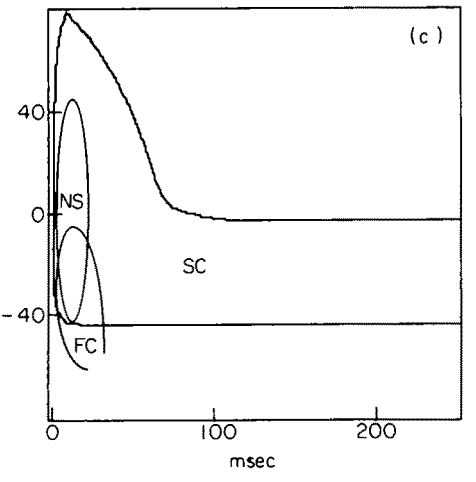

FIG. 2 (c).

Current volume is a space that relates to the amount of energy a particular conductance is capable of dissipating within a defined range of time and voltage applied under voltage clamp conditions†. What are the parameters that influence current volume of a channel? Apart from conductance, the most obvious, current volume appears to be very sensitive to the value of the activation equivalent charge (Z). In Fig. 3(a) and (b) two contour plots of the same conductance are demonsrated, differing in respect to their equivalent charges. These contours were generated using eqns (1) and (2) to compute rate constants of a hypothetical inward going inactivating current. The general "look" of these contours is different. The lower the equivalent charge, the more dispersed the contour becomes, creating a second, non-inactivating, open current volume at more negative potentials. This additional volume contains a window current (and represents $m \cdot h$ infinity product that is higher than zero). In Fig. 3(c) current volume of this model channel was computed as Z was changed from 1 to 8 (time and voltage were limited to contain the main activity of the channel, see legend). Current volume becomes greater upon increase in Z values. The value of Z represents voltage dependence of channel activation and we expect it to narrow regions of activity the higher it becomes. In other words, the higher the value of Z, the more energy is dissipated by the current in a more defined area within the contour. The shape of the curve in Fig. 3(c) suggests that the range of Z values that is effective in "concentrating" the energy dissipation to a defined

† In case of inward and outward current generated by the same conductance, there are two ways to deal with current volume calculations, depending on the question asked; the first is to subtract the "outward" from the "inward" volume (e.g. when studying the amount of membrane energy "dissipated by" a certain conductance); the second approach is to dissect, if possible, the conductance into its components and to compute a current volume for each separately (e.g. when studying the contribution of each component to the overall activity).

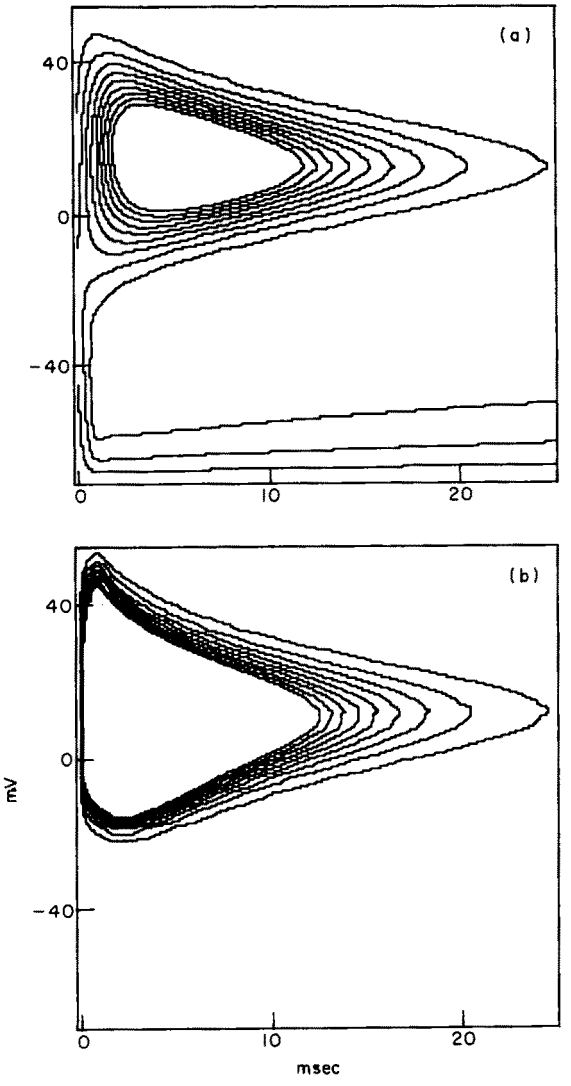

FIG. 3. The effect of changing the activation equivalent charge (Z) on a model channel contour. (a) $Z=1$. (b) $Z=6$. A curve describing the relation of "current volume" to activation equivalent charge is shown in c. Current volume in units of Joule . 10^{-7}. See Appendix for computations.

area within the contour is 1–~8, which is in agreement with measured values (Hille, 1984). In other words, viewing current volume as a parameter characterizing the conductance capability to "use up" membrane energy in a limited time-voltage range (e.g. the fast sodium channel), the investment in adding to a channel protein more than ~8 equivalent charges is not profitable.

The contour of a certain conductance is a visual representation of its activity space. As such, it constitutes a basis for comparison between membrane conductances. For

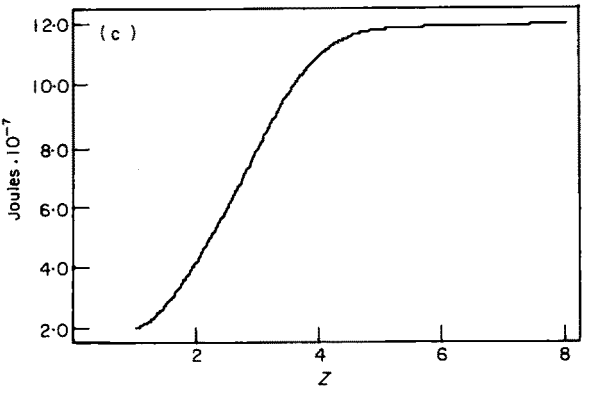

FIG. 3 (c).

example, channels that are "designed" for ionic transport proper (e.g. the amiloride sensitive epithelial Na channel [Sariban-Sohraby & Benos (1986)] have a dispersed "open" contour that does not have any closed iso-current lines. Such a channel is expected to dissipate energy in an infinite time range since its job is to enable a constant and stable flow of Na ions through the membrane. These channels are, therefore, classified as "energy dispenser" channels. At the other extreme of the scale one finds channels that have a voltage dependent fully inactivated state (e.g. squid Na channel). These channels demonstrate almost† finite "closed" contours that are concentrated in a limited area (time–voltage) of the activity on the contour plot [Fig. 1(d)] in accordance with their role in the membrane—i.e. electrical signaling while minimizing intracellular electrolyte disturbances. The salvage of energy, in these cases, is achieved by time and voltage dependence of the channel's gate state. The "slow" inward current of the cardiac muscle is an intermediate case where the conductance (mainly for Ca ions) plays an electrical signaling role (as in the case of Na channels) as well as an ion transport mechanism (Ca is needed for activation of contractile processes). Indeed, the contour of the "slow" inward channel [Fig. 2(b)] is composed of two distinct structures—the early, almost closed, part that represents conductance dedicated to electrical signaling and the second, ion transporting phase, that represents the activity dedicated to Ca ion influx.

A further possible use of an I–V–t contour plot relates to action potential threshold computation. The use of a current–voltage relation to quantify threshold potential accurately, necessitates extensive computations. There are ways to overcome this difficulty, mainly by assuming that some reactions occur infinitely quickly or infinitely slowly [based on large differences in speed of gating reactions (Jack *et al.*, 1988)]. While these methods are suitable for the action potential of cardiac cells, the approximations are not valid in some other tissues (e.g. squid). For the present discussion

† Squid Na conductance has a small window current, negligible in comparison to the cardiac slow (Ca) current (see below).

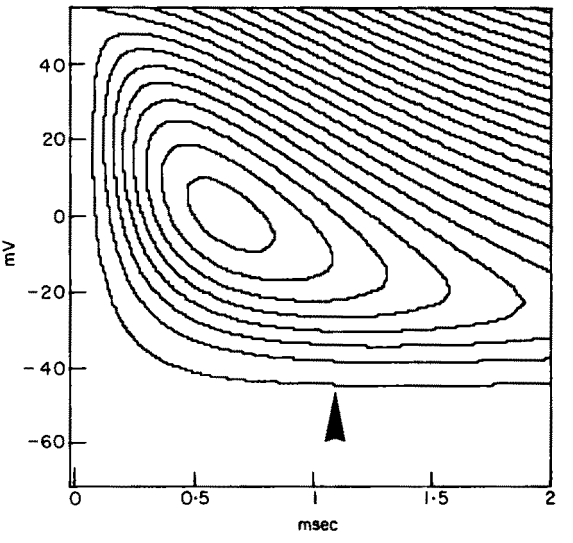

FIG. 4. Contour of the total current of a squid membrane. Arrow indicates the point where an inward current is developed in response to a minimal membrane potential held for ~1 msec.

threshold potential is defined as a minimal membrane potential held for a sufficient time to generate a negative slope conductance inward current. Using the contour plot approach, deriving a threshold potential is straightforward—it is the southernmost point where a descent is detectable. In Fig. 4, the contour of the total current of squid membrane is plotted. An arrow indicates the point where an inward current develops in response to a minimal membrane potential held for ~1 msec.

Concluding Remarks

Unlike the traditional biophysical trend that dissects membrane electrical activity (under voltage clamp conditions) to its basic components mentioned above (kinetic, "size", selectivity and dependence of these parameters on various stimuli), the 3-D approach is aimed at "seeing" the picture as a whole. A few demonstrations were used to convey this message: the contour predicts time–voltage domains for the physiological activity of a given conductance; the "volume" entrapped between the surface of the current contour and the zero current level is a measure of the ability of the conductance to dissipate energy; contours form a basis for channel classification; from a contour plot of the total current flow through the membrane, under voltage clamp conditions, an "instantaneous" action potential threshold can be predicted. Many other points, that arise from the 3-D approach, await a more quantitative treatment.

The author is grateful to Dr D. Dagan, in whose laboratory this work was carried out, for his advice and support, and to Drs S. Mager, C. Miller and Y. Palti for their comments on

this manuscript. This work was supported by the Lady Davis Foundation post doctoral fellowship to Shimon Marom and by a US-Israel Binational Science Foundation grant 88-00233 to Daniel Dagan.

REFERENCES

DiFrancesco, D. & Noble, D. A. (1985). A model of cardiac electrical activity incorporating ionic pumps and concentration changes. *Phil. Trans. R. Soc. Lond.* **B307**, 353–389.

Hodgkin, A. L. & Huxley, A. F. (1952). A quantitaive description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* **117**, 500–544.

Hille, B. (1984). *Ionic Channels of Excitable Membranes*. Sunderland, MA: Sinauer Associates.

Jack, J. J. B., Noble, D. & Tsien, R. W. (1975). *Electric Current Flow in Excitable Cells*. Oxford: Oxford University Press.

Sariban-Sohraby, S. & Benos, D. J. (1986). The amiloride-sensitive sodium channel. *Am. J. Physiol.* **250**, C175–190.

Wolfram, S. (1988). *Mathematica™, A System for Doing Mathematics by Computer*. Reading, MA: Addison-Wesley Publishing.

APPENDIX

(1) Following is a complete *Mathematica™* description of computation to generate Fig. 3(a) and (b) (*time in sec, voltage in volts*)

$\alpha[a\_, z\_, v_{0.5\_}, \gamma\_, v\_] := a \exp[\gamma z(v - v_{0.5})37{\cdot}4]$
$\beta[b\_, z\_, v_{0.5}, \gamma\_, v\_] := b \exp[-z(1-\gamma)(v - v_{0.5})37{\cdot}4]$
$\text{gate}_\infty[z\_, v_{0.5\_}, v\_] := 1/(1 + \exp[-z(v - v_{0.5})37{\cdot}4])$
$z1 = 1$ (*for* Fig. 3(b) $z1 = 6$*);
$t = .$; $e1 = .$; $a1 = 100$; $b1 = 0{\cdot}1$; $a2 = .1$; $b2 = 100000$; $z2 = 4$;
$m_{V0.5} = -0{\cdot}03$; $h_{V0.5} = -0{\cdot}08$; $\gamma = 0{\cdot}5$; $E_{Na} = 0{\cdot}055$;
$V = -0{\cdot}07$;
$m_0 = \text{gate}_\infty[z1, m_{V0.5}, V]$;
$h_0 = \text{gate}_\infty[-z2, h_{V0.5}, V]$;
$\alpha m = \alpha[a1, z1, m_{V0.5}, \gamma, e1]$;
$\beta m = \beta[b1, z1, m_{V0.5}, \gamma, e1]$;
$m_\infty = \text{gate}_\infty[z1, m_{V0.5}, e1]$;
$m = m_0 - ((m_0 - m_\infty)(1 - \exp[-(\alpha m + \beta m)t]))$;
$\alpha h = \alpha[a2, z2, h_{V0.5}, \gamma, e1]$;
$\beta h = \beta[b2, z2, h_{V0.5}, \gamma, e1]$;
$h_\infty = \text{gate}_\infty[-z2, h_{V0.5}, e1]$;
$h = h_0 - ((h_0 - h_\infty)(1 - \exp[-(\alpha h + \beta h)t]))$;

Contour Plot $[m\hat{}\,3\, h(e1 - E_{Na}), \{t, 0, 0{\cdot}025\}, \{el, -0{\cdot}07, 0{\cdot}055\}]$.

(2) A numerical integration of eqn (3) (see text) was performed to generate Fig. 3(c) NIntegrate $[m\hat{}\,3\, h(e1 - E_{Na}), \{t, 0, 0{\cdot}03\}, \{e1, -0{\cdot}07, 0{\cdot}05\}]$.