Microcomputer program for automated action potential waveform analysis

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Abstract
A program for action potential waveform analysis based on a PC compatible computer is described. Single or averaged action potentials are analyzed by obtaining its first derivative and using criteria which allow automatic measurement of several action potential components, including: depolarization rate, repolarization rate, amplitude, duration, resting membrane potential and afterhyperpolarization amplitude and slope. Data can be imported from pClamp™ (Axon Instruments) and exported to other software such as Excel™, Sigmaplot™ and MatLab™ for example.

Key words: intracellular recording, ionic currents, intrinsic properties, data acquisition, waveform analysis, Helix aspersa.

Introduction

Intracellular recording of neuronal activity allows information to be obtained at three different levels of neuronal integration. Interspike intervals statistics, the characteristics of action potential waveform, and response of neurons to electrical currents [1, 2]. This analysis allows to characterize the intrinsic properties of neurons [3]. It is currently understood that neurons can no longer be conceived as passive threshold elements in which action potential are generated. Action potential waveforms will help to elucidate the mechanisms underlying neuronal activity, as well as contribute to defining the role of neuronal sets and circuits in the generation of central nervous system integrative properties.

This work describes a procedure for action potential waveform analysis. It allows automatically defining and measuring the action potential depolarization rate, repolarization rate, amplitude, duration, membrane potential and afterhyperpolarization amplitude and slope. Other parameters of the spike train, such as interspike timing and pattern of discharge have been also implemented and software here presented form part of a system which allows an extensive analysis of neuronal spike activity [4, 5, 6].

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Implementation

The software described in this work was designed to run under Windows™ (3.11, 95, 98, 2000 or NT) in a PC compatible microcomputer, with a clock speed of at least 33 MHz. The system requires the use of an analog to digital (AD) converter (LabMaster from Scientific Solutions, Digidata 1200 from Axon Instruments and the IBM AD converters are supported). The program was written using Delphi™ 2.0. Routines for analog to digital data acquisition and display are essentially the same as previously described [6]. The software allows, to select up to ten data segments, centered on an action potential. The user may average the arrays, thus having a mean action potential or acquire a single action potential and analyze it without averaging (Figure 1A).

The program calculates the first derivative of the voltage by using the Newton-Raphson method; the first derivative of the data and the raw recording are subsequently displayed. The software allows, to select up to ten data segments, centered on an action potential. The user may average the arrays, thus having a mean action potential or acquire a single action potential and analyze it without averaging (Figure 1A).

The program calculates the first derivative of the voltage by using the Newton-Raphson method. The mean and the variability of the register are calculated in a running average procedure, along the data set:

$$\text{mean} = \frac{\sum_{i=1}^{n} dv_t}{n}$$

$$\text{dev} = \sqrt{\frac{\sum_{i=1}^{n} (dv_t - dv_{t-1})^2}{n}}$$

up to finding a $dv_t$ (the derivative $dv/dt$ of the digitized data at time $t$) for which its difference with the previous data is greater than three times the deviation (dev) of the whole data segment that have been already analyzed: $dv_t - dv_{t-1} > 3 \cdot \text{dev}$. This data point defines the beginning of the action potential, and is referred as the spike threshold level ($V_{th}$). Defining a threshold level has no a strict physiological sense, but this data is used to perform some of the calculations needed to define the action potential. Membrane potential ($V_m$) was obtained as the mean value of the whole data recording. The user is prompted to accept or to reject membrane potential reading. Once the membrane potential value has been established, action potential peak ($V_{peak}$) is identified and the amplitude of the action potential is measured. Since action potential can be generated by a current pulse injection, its amplitude is also calculated in reference to the threshold $V_{th}$ and is referred as SP (Figure 1B). The action Potential duration is calculated by linear interpolation of the time ($t_c$) at which the record intersect downwards the $V_{th}$ line:

$$t_c = t - \frac{(v_t - v_{th})}{m}$$

where $t$ is the time of the last point greater than the threshold value and $m$ is the slope for the $v_{th}$ to $v_t$ line. Duration at 50% (Dura 50) is also calculated using a similar procedure.

Maximum depolarization and repolarization rates of the action potential are found by searching for the maximum of the derivative for those data that are to the left and to the right of the peak value.

Calculation of the afterhyperpolarization (AHP) slope is interactively performed. Two cursors are displayed at the bottom of the screen. The user can move these cursors as
necessary to define the boundaries under which the AHP slope is to be measured. The amplitude of the AHP is obtained by finding the minimum between the two cursors minus the membrane potential. The slope of decay of the AHP is found by:

\[
\text{AHP slope} = \frac{V_{\text{cursor}2} - V_{\text{cursor}1}}{t_{\text{cursor}2} - t_{\text{cursor}1}}
\]

Where \(t_{\text{cursor}1}\) and \(t_{\text{cursor}2}\) are the position of two cursors. We decided to use a linear approximation for measuring the slope of the AHP because other type of functions, such as an exponential or parabolic, implies making assumptions that seems not valid for the AHP slope trajectory. However, we have also implemented an exponential fit.

Data are graphically displayed along with their numerical values (Figure 1B) and they can be stored in an ASCII disk file.

**Figure 1.** A) Intracellular recorded action potentials from a snail (*Helix aspersa*) neuron. Up to ten action potentials can be selected from the register and displayed as shown in the graph. The user is allowed to average the recordings to perform the measurement of typical components. B) Display showing an average action potential from the records shown in A. Data are numerically displayed and stored in ASCII files. AD, sampling rate; Gain, total recording gain; Vmem, membrane potential; AP amp, amplitude of the action potential in reference to membrane potential; SP amp, amplitude of the action potential in reference to threshold level; AP dura, duration of the action potential; Dura 50, duration of the action potential at 50% of repolarization; d+, depolarization rate; d-, repolarization rate; AHP, amplitude of the afterhyperpolarization; AHPs slope, slope of the afterhyperpolarization; Avrg, number of records in the average. Marks from which measurements have been obtained are shown with numbers: 1, action potential duration; 2, membrane potential; 3, AHP amplitude; 4 AHP slope.
Conclusions

Neurophysiologists have paid significant attention to the statistics of action potential intervals. Indeed, theories of neuronal activity have established that the nervous system represents signals by sequences of identical action potentials [3, 4]. Particularly after the development of voltage clamp techniques, little attention has been paid to the action potential morphology. However, important information can be gained regarding the role of action potential morphology on the spike interval statistics of neurons [7].

The use of visual estimation procedures for analyzing transient events, like the action potential, introduces considerable uncertainty with respect to the definition of its parameters. The software here presented allows to obtain numerical data about action potential morphology. Statistical comparison of action potentials under different experimental conditions is therefore feasible, with variations in measurement error minimized. In addition, studies of action potential waveform and its correlation with discharge pattern of neurons are more efficient when the program is used. The program maintains a constant graphical feedback for the user to supervise its operation. Since the program is specifically designed to analyze the action potential waveform, it requires a minimum of user interaction, operating in an automated form, thus constituting a useful tool particularly for those experiments demanding the comparison of large sets of action potentials. Other commercially available software programs permit similar measurements to be made, but because of their multipurpose design, they demand from the user an important participation in defining the action potential regions from where measurements are going to be made.

Availability

The program and User's guide are available from the authors.

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