

Analysis of Single Channel Currents with a Microprocessor Based Device

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Data evaluation of single channel currents obtained from artificial black lipid membranes and with the patch clamp method is an important part of every single channel study, but it is a time consuming part often exceeding the time for experimentation and recording by far.

We describe here a microprocessor based device, which allows the experimenter to analyse in a simple way the distribution of current levels in a single channel trace (amplitude-histogram analysis of single channel currents) either online, or offline.

Current levels are sampled at a constant frequency of 6 kHz and the relative frequencies of occurrence of these current levels are displayed as a histogram on the screen of an analog or digital storage oscilloscope.

The data reducing algorithm of this analyser eliminates the requirement of large amounts of mass storage that normally is needed for digital amplitude-histogram analysis of single channel recordings.

Examples of evaluation for both a voltage operated cation-channel and a blockage of a potassium channel by tetraethylammoniumchloride (TEA) are given.

Introduction

Data evaluation of single channel currents obtained from artificial black lipid membranes and with the patch-clamp method is an important part of every single channel study, but it is a time consuming part often exceeding the time for experimentation and recording by far.

An attractive alternative to manual single channel analysis is the evaluation of data using minicomputers. This alternative is quite expensive as it involves the use of sophisticated hard- and special purpose software and is time consuming as well, due to frequent user interaction. We describe here a microprocessor based device, which allows the experimenter to analyse the distribution of current levels in a single channel trace (amplitude histogram analysis of single channel currents), either online, or offline, when the single channel trace is replayed from a FM-tape or a modified videotape-recorder [1].

Current levels are sampled at a constant frequency of 6 kHz and the relative frequencies of occurrence of these current levels are displayed as a histogram on the screen of an analog or digital storage oscilloscope, as the signal is fed into the analyser.

The data reducing algorithm of this analyser eliminates the requirement of large amounts of mass stor-

age that normally is needed for digital amplitude-histogram analysis of single channel recordings.

Description of the electronic circuit and the program

The main purpose of the described instrument is to reduce the huge amount of input data (ca. 20 Mbytes/h) by employing an algorithm which produces an amplitude histogram of the currents and shows the graph on the screen of an oscilloscope as the analysis is running. The analyser was realized using the microprocessor based circuit shown in Fig. 1.

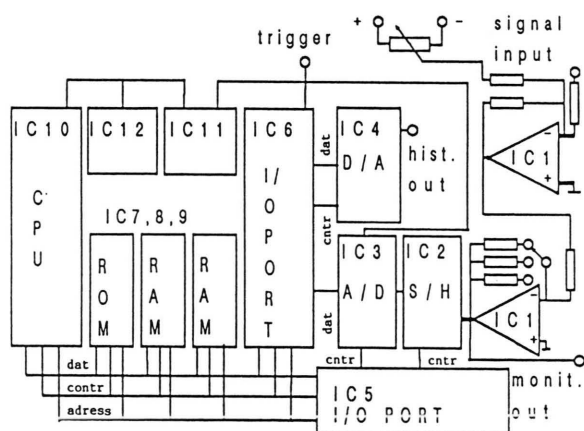


Fig. 1. Block diagram of the analyser.

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The signal from the current-to-voltage converter is preconditioned by an input stage consisting of two operational amplifiers (IC1: UA747, Texas Instruments) by which offset and amplification can be manually adjusted. The output signal of this preamplifier is connected to a monitor output. Sample-hold amplifier (IC2: AD583, Analog Devices) and A/D converter (IC3: AD7574, Analog Devices) transform the analog value to a digital 8-bit-word. This binary word is transferred *via* an I/O port (IC6: R6522, Rockwell) to the microprocessor data bus and is immediately processed by the program. A/D converter and sample-hold amplifier are controlled *via* I/O port (IC5: R6522, Rockwell).

The actual histogram stored as a variable with 256 elements is converted to an analog signal (IC4: AD1408, Analog Devices) and sent to an analog screen by the output program each 30 ms. Triggering of the oscilloscope is controlled by program *via* I/O port (IC6: R6522, Rockwell). The use of a digital oscilloscope allows documentation of the graph with a xy-chart-recorder. The resolution of the output is limited to 8 bits. Automatic range selection for the histogram output is controlled by the output program. The sample rate for the A/D converter is 6.2 kHz. The program is written in assembler for the MOS 6502 microprocessor (IC10: 6502, MOS Technology) and stored on 2 kByte EPROM (IC7: MSL2716, Mitsubishi). Variables for the program and the data of the histogram are stored in two RAM chips (IC8: M58725, Mitsubishi and IC9: TMM314, Toshiba). The system clock (IC12: NE555, Signetics) of 1 MHz is divided by two (IC11: HEF4013, Phillips) to provide a clock for the A/D converter. The instrument is build as a plug-in-unit of a standard rack system with the dimensions of 160 × 100 × 50 mm.

The software consists of a main program (output program) and an input program. A timer interrupts the output program each 160 μ s and starts the input program. To hold the actual histogram, pressing a stop key avoids additional data input by switching off the timer. The histogram is represented as an array of 256 counters, each one with a range from 0 to 16777215. When the program starts, all counters are set to zero. If we assume a maximum input voltage range of 0–256 mV, each input signal in the range of $z \pm 0.5$ mV will cause counter z to be incremented by one. In a similar fashion for other voltage ranges, each input voltage is mapped to one of the counters, which is incremented by one each time a value is

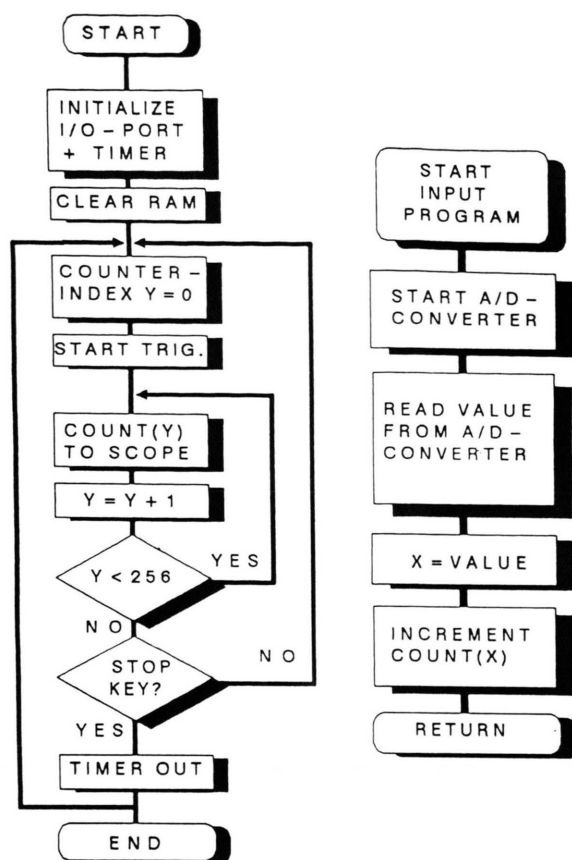


Fig. 2. Flow chart of output program (main program) and input program. The invocation of the input program is timer controlled.

mapped to it. Counter overflow is avoided by the large word length of 24 bits. A flow chart of the program is shown in Fig. 2.

Data evaluation and discussion

Two examples of analysis of single channel records are given: First the analysis of a voltage-gated cation-channel recorded with the patch-clamp method [2] from rat cardiac ventricular cells is shown. The second example is the blockage of a potassium-channel [3, 4] from the same preparation with tetraethylammoniumchloride (TEA).

Voltage-gated cation-channel

Adult, calcium-stable cardiac myocytes from rat ventricular tissue were prepared as described by

Piper *et al.* [5]. A small glass pipette (open-tip-diameter 0.1 to 1 μm) was pressed against the cell membrane, then gentle suction was applied to obtain a high gigaohm-seal between glass pipette and cell membrane [2]. The small membrane patch under the opening of the glass pipette was isolated from the cell according to the excised patch technique [6], the outside of the cell membrane faced the extracellular medium (outside-out membrane patch).

The extracellular medium contained (in mM): NaCl 131, KCl 4, CaCl_2 0.5, MgCl_2 2 and Hepes 10, buffered with NaOH to pH = 7.4.

The electrolyte solution in the pipettes interior simulating the intracellular medium contained (in mM): NaCl 7, KCl 128, CaCl_2 0.5, MgCl_2 2 and Hepes 10 buffered with KOH to pH = 7.4.

Under these recording conditions several potassium-selective ion-channels and a strongly potential-

dependent cation-selective channel, which did not discriminate between sodium and potassium-ions could be observed.

An ionic channel from cardiac cells with similar permeability features was first observed by Colquhoun *et al.* [7]. Recordings from experiments where the small membrane patch contained only this one type of channel were selected for subsequent analysis (left side of Fig. 3).

Steady-state single channel recordings at three different potentials (-70 , -50 and -30 mV) were fed to the input of the amplitude histogram analyser (1 min for each record). The resulting amplitude histograms are shown on the right side of Fig. 3. The voltage dependence of single channel fluctuations is seen much more clearly in the histogram than by just inspecting the single channel records directly. Whereas at -70 mV the occurrence of channel openings could

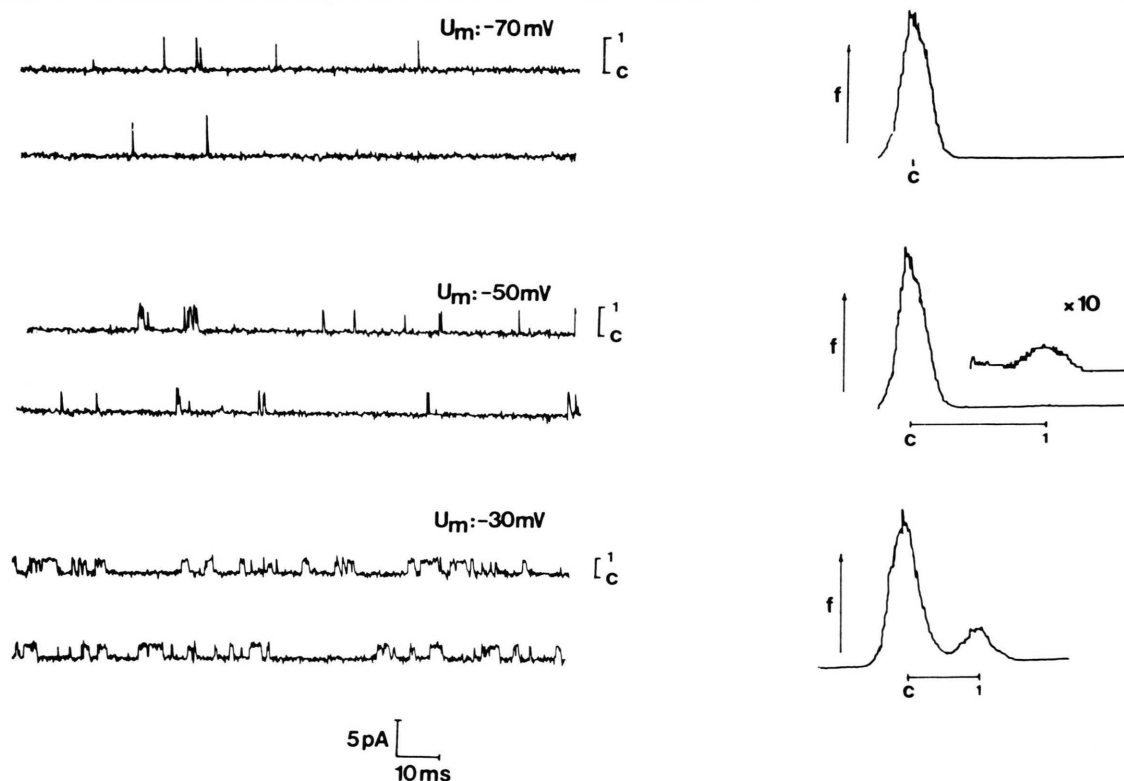


Fig. 3. Single channel recordings from unselective cation-channels from isolated adult rat ventricular cells. Upward-going deflections represent inward currents of cations in a physiological sense. On the right hand the amplitude histograms corresponding to the single channel traces are plotted. x-Axis: conductance; c = closed; 1 = open; single channel conductance is 75 pS. y-Axis: relative frequency of occurrence of the conductance levels.

not be detected in the amplitude histogram, they could be seen at 10-fold magnification of the y-axis at -50 mV and were pronounced at -30 mV.

In addition, the single channel conductance can be calculated with much higher accuracy from the amplitude histograms than from single channel openings and the mean current flowing through the channel within the observed time interval can be calculated (see below).

Blockage of a voltage-dependent potassium channel by internally applied TEA

Excised membrane patches were made from the same preparation but in this case the side of the membrane normally facing the cell interior faced the extracellular medium. The pipette was filled with the same extracellular solution and contained (in mM): NaCl 131, KCl 4, CaCl_2 0.5, MgCl_2 2 and Hepes 10 buffered with NaOH to pH = 7.4.

When the voltage-dependent potassium channel was present in a given patch under these recording conditions, only small single channel currents could be observed (not shown). When only this type of single channel activity was present in a given patch, the extracellular medium in the perfusion chamber was exchanged for a medium with higher potassium-concentrations, the composition of which (in mM) was as follows: NaCl 7, KCl 128, CaCl_2 0.5, MgCl_2 2 and Hepes 10 buffered with KOH to pH = 7.4.

After perfusion pronounced current steps resulting from the opening and closing of several of these channels could be observed (upper left part of Fig. 4a). Steady-state single channel currents at three different potentials (0, +25 and +50 mV) were recorded (1 min for every given potential). Then the electrolyte solution in the perfusion chamber was replaced with the same solution plus 10 mM TEA. After this procedure the single channel activity decreased markedly (lower left part of Fig. 4a). Again,

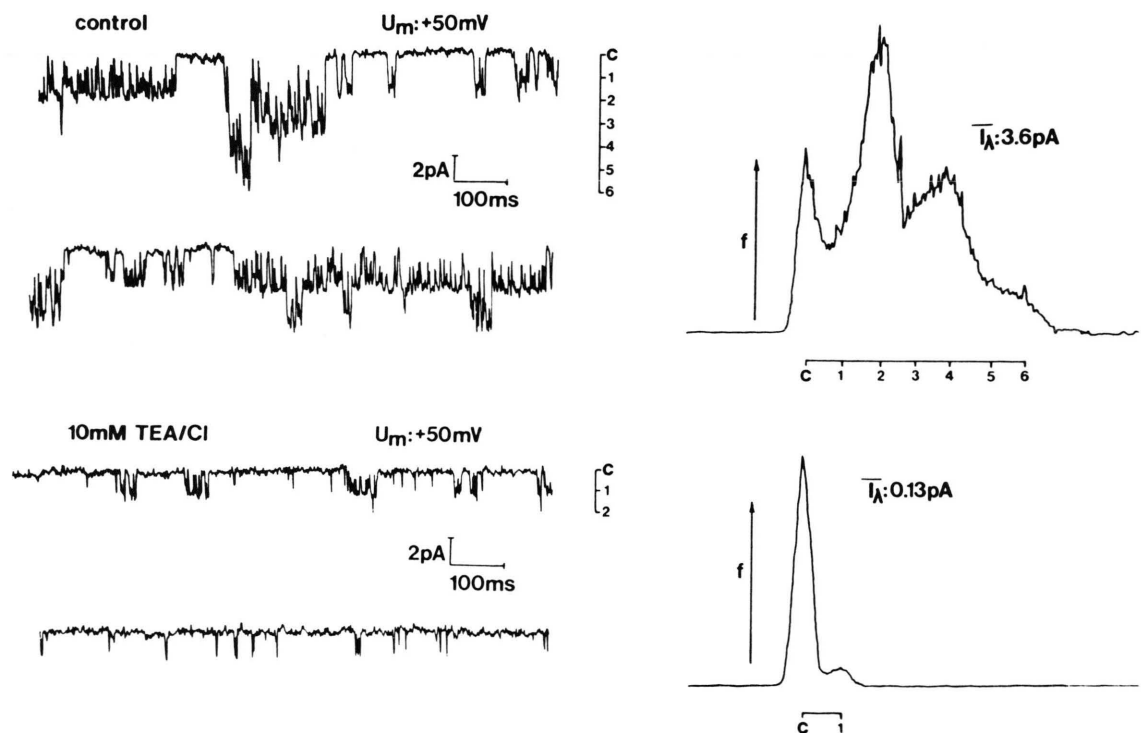


Fig. 4a. Potassium channel recordings from isolated adult rat ventricular cells at $+50$ mV. Physiological outward potassium currents are deflected downwards. Upper 2 traces: single channel activity in physiological salt solutions. Corresponding amplitude histogram is shown in the upper part of the figure. Lower 2 traces: single channel activity after addition of 10 mM TEA. Corresponding amplitude histogram is shown in the lower part of the figure. c = closed; 1, 2, ... 6 = number of individual pores open simultaneously.

single channel activity was recorded for 1 min for every potential. When the electrolyte solution was again replaced with the potassium-rich medium but without TEA, a washout of the effect was possible (not shown). Amplitude histograms of the recorded single channel traces were produced and showed clearly the blockage of this type of potassium-channel by internally applied TEA (right part of Fig. 4a).

The mean current produced by the potassium-channels during the observed time interval was calculated according to the equation $\bar{I}[\text{pA}] = \sum i(i) \times f(i)$, where \bar{I} represents the total calculated average current produced by single channel activity; $i(i)$ is the current level of an observed conducting state of the channel in pA and $f(i)$ is the relative frequency of occurrence of the given conductance level.

The mean currents produced by the given membrane patch with and without TEA blockage at different potentials are shown in Fig. 4b.

With these examples, we have shown that quantitative evaluation of single channel records is possible with this simply constructed, inexpensive microprocessor based device, which obviously will also be useful for evaluation of single channel records obtained from *trans*-membrane channels reconstituted into artificial planar lipid bilayers [8, 9].

$$\bar{I}[\text{pA}] = \sum i(i) \times f(i)$$

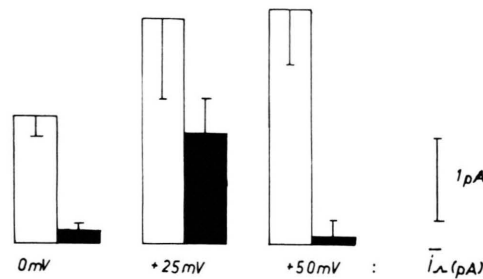


Fig. 4b. Average patch currents calculated from the formula is shown in the upper part of the figure with standard deviations for three different test potentials. White bars: control. Black bars: after addition of 10 mM TEA.

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