Action Potential Analysis by Real Time DSP Hardware and Software for Odour Exposure Responses

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ABSTRACT

Tools for assorting and classifying neuronal action potentials can be either on-line or off-line. Here we examine on-line tools. We concentrate on an analysis of insect action potentials by using a DSP-based multi spike detector (MSD© by Alpha Omega Engineering). The MSD software is a modular program which is user-friendly and has optimal speed. In the action potential analysis neural firings are decomposed into waveforms, which are specific for each cell. Based on this comparative analysis and assorting of action potentials, many series of action potentials from the olfactory receptor neurones of small insects were analysed. The assorting algorithm compares the neural signals to three templates, which can be adaptively changed and fixed by the user. The action potentials are produced either spontaneously or at an odour exposure. In the response analysis they are further plotted as a function of the odour exposure sequence.

1. INTRODUCTION

Detection of action potentials (APs, spikes), which are large in relation to the electrode or membrane and other biological noise is unproblematic in sensory neurophysiology. There are two common methods for APs detection. However, until recently DSP-based devices have been the option of choice for AP assorting and further analysis. In the development of task-specific devices, it seems feasible to utilize an efficient on-line AP detector based on DSP hardware and its compatible software.

In the analysis of biological functions, it is important to distinguish exactly APs generated by other cells in the close vicinity of the cell from the primary olfactory receptor neurons (ORN) of the cell. There is free software available, which can separate action potentials from three different sources off-line and classify an action potential rate into three classes depending on the shape of each action potential. However, a separation into two classes was successful enough in practice. The recorded action potentials can be stored for further analysis and printing.

In the MSD, detection and assorting are simultaneous in each channel, which makes it possible to detect the interspike interval periods or action potential firing rates for each sensory neuron. A match between the AP and its template is found, when the local minimum of the sum of squared deviations between the template and AP is within the given criteria limit, and this means a detection, as reported in Fig.1 for three cells. The left window shows that the all AP matched, in the second window shows by the green color that there are double or triple match happened and in the third window three double match.

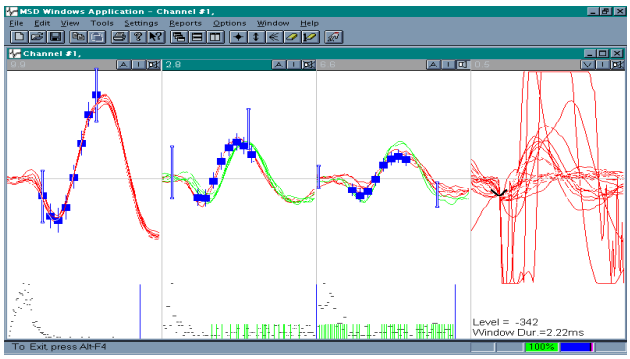

Figure 1. Three action potential shapes in the first three windows together with the laboratory noise in the fourth window. The match histogram shown by green sticks develops on the window bottom.

Fig. 2 shows action potential rates in pps (pulses per second) from two ORNs. A neuron is firing action potentials up to ca. 75 s around 45 - 75 pps and sometimes over, while the other neuron is firing about 5 pps continuously active without any break.

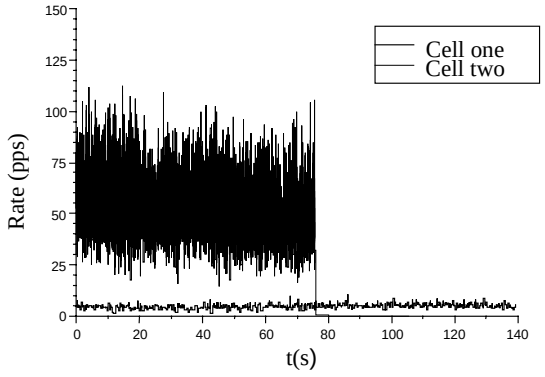

Figure 2. A high-rate action potential series together with a slow-rate at the bottom.

Before a response analysis of ORNs, it is important to

monitor the spontaneous activity of an ORN. The monitoring yields important information about the ORNs' activity. Therefore, our recordings last longer (up to 2 min) before and after the exposure to an odour.

2. MSD SOFTWARE OPERATIONS

Based on the differences in AP shapes obtained in single cell recordings from olfactory receptor neurons the APs can be assorted by means of the MSD hardware and software. It assorts up to 3 AP shapes per electrode signal. In a cascade it is possible to assort up to 3 multiplied channel numbers.

2.1. General Operations

The MSD environment is an advanced system designed to detect action potentials such as signals and assort them according to their shape. After detecting and sorting the AP shapes, the results are reported, allowing the user to measure the action potential rate, firing times, delays or construct histograms.
In the MSD software is based on modular sections. Its parameters, such as sampling rate, histogram bin, adaptive factor, double match, and polarity flag, can be changed through the dialog box in each module. Unsorted APs are displayed in two modes: All APs (like memory scope) or the last N APs (N=50 … 100) are displayed. A detection is indicated when the template and the corresponding AP match. APs are assorted simultaneously and saved on the basis of their match to each template. A typical AP pattern or template is defined to generate an optimal template for detecting and assorting the APs.

2.2. MSD Operations in AP analysis

The MSD software includes step by step instructions for the user to define clusters of APs, how to detect and isolate APs, the allowed noise level, and how to monitor the quality of multi-signal detection and isolation. The MSD hardware can filter the AP signal internally by a single-pole high-pass filter having a -3dB point at 200 Hz, and by a double-pole low-pass filter having a -3 point at 10 kHz. Because of this filtering the recorded signal bandwidth is also limited for the APs in the same or narrower band. However, this band contains all the information that is characteristic enough in order to assort AP signals.
The template definition program of the MSD software has three steps. In the first step, the input signal is displayed on the PC screen with the adjusted trigger level. In the second step, three groups of the APs are defined by the user. In the third step, the program computes and displays templates for all the selected AP groups. After these steps, the user switches to AP detection mode.
In the MSD software two separate programs cooperate together. One part operates the DSP, while the other operates the PC. The PC downloads a portion of the software from the logic board to the program-space. This program waits until a new value of the sampled input data is available. The new sample is transferred from the analog board into a buffer holding the last 100 samples. The first eight samples are compared to the first template and the sum of squared differences is computed. This sum is compared to the previous one. The minimum is reported by raising a flag in the program response, if this sum is a minimum and below the defined threshold.
In the PC the program examines if any data is available in the buffer. If the data is an AP, it displays it on the PC screen with appropriate options to define a new template if necessary.
In practice the MSD software functions as follows. Ongoing detected AP activity is displayed in three frames along with their eight template points and a line cursor. An additional graph develops at the bottom of each frame (Fig. 1). This graph contains an updated histogram, which displays the distribution of the sum of squared differences between the ongoing AP activity and the corresponding template. This sum of squared differences is called *spike distance*. The left point of the histogram is the zero distance or perfect match and it is used as a detection threshold. When the AP has its spike distance smaller than the detection threshold in two or three templates, a double or triple match is in process. These APs are shown in different colors, and the histogram is updated to show assessment of the situation.
In MSD operations it is recommended to assort as many AP shapes as possible in order to get used to the program's advantages and limits. In addition, one can develop his/her own strategy of satisfactory operations. The templates can be modified online without interrupting the sorting. Action potentials, which are detected and assorted the authentic spikes as they come and produces a 100 ms pulse following every real AP occurrence.
Action potential acquisition continues uninterrupted. The MSD software allows handling of AP intervals up to a memory size makes it possible.

3. DATA COLLECTION

We have reported elsewhere on several aspects of the AP responses to the odour exposures of individual olfactory receptor neurons [2]. Shortly, AP data were obtained from blowflies (*Calliphora vicina*), mosquitoes (*Culex pipiens*) and fruitflies (*Drosphila virilis*). The MSD collects the data on line. The analyzed data can be exported to other programs such as Excel™, Origin™ or MatLab™.

3. RESULTS

In the case of the blowfly ORNs the following histograms originate from two different olfactory receptor neurons, Figure 3. The histograms show that these ORNs have different distributions of AP intervals. One has longer intervals, while the other one has a normal distribution. The distribution function was fitted in the Origin™

software at the normal (red line) and Gaussian distribution (black line). The normal curve faithfully follows the measured values.

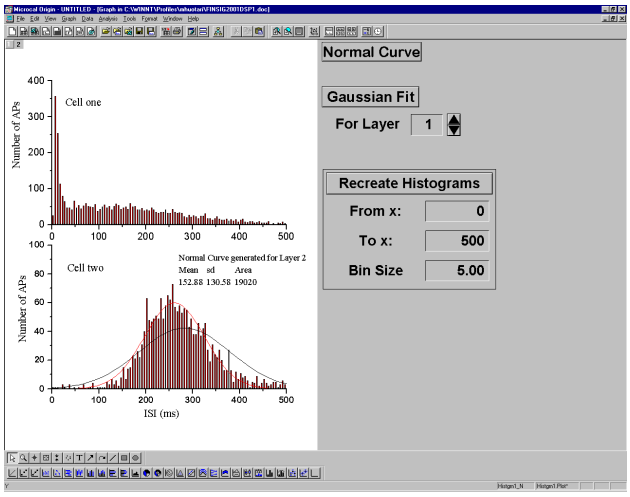

Figure 3. High-rate and a low-rate AP histograms of two ORNs.

5. CONCLUSIONS

Many studies of AP firing require the isolation of the AP generated by an ORN. The on-line peak detection method described in this paper facilitates the characterization of firing patterns during the recording of AP activity, for instance in ORNs. It makes it possible to extract information about the occurrence of APs from multiple sources. Unlike other much used techniques, the MSD method is user-friendly, economic, fast and its parameters are quantitatively defined in real time. In addition, this software and hardware do not require the use of a particularly powerful computer. This system provides the user with a whole set of graphical and statistical analyses of AP interval data, such as distribution, correlation and other functions.

Because AP trains can theoretically carry information in several ways (firing rate, interval distributions), it is important to record and save the actual AP interval history. These constitute the first step in any dynamic analysis, such as phase portraits, joint-interval distribution histograms and phase space of divided differences. The dynamic analysis makes it possible to make further evaluations on topological dimensions. On the basis of the histogram analysis ORNs were assigned to ten histogram classes. These AP interval histograms of each ORN class exhibit an obvious mathematical distribution function that is unambiguous.

Because the AP analysis is done partly by the researcher and partly by the computer, it is impossible to define any exact time or frequency characteristics for analysis purposes but we use common sense and utilize what we have learned.

ACKNOWLEDGMENT

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[2] Matti j. Huotari, Biosensing by insect olfactory receptor neurons Sensors and Actuators B 71, 212-222, 2000.