The Application of an Averaging Method to Intermittently Modified and Endogenously Generated Spike Activities of Neurons in Mollusc Ganglia

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Abstract

The averaging method was applied for the analysis of the effect of intermittent light as well as for the analysis of the spontaneous spike activities of burster neurons in Helix pomatia and Aplysia depilans ganglia. Owing to such analyses by means of which the unwanted effects of permanently present “noise” (i.e., irregular frequency modulation of neuron impulse activity) were diminished, the dynamics of the neurons reaction to light and the longer time effects of intermittent illumination on neurons spontaneous activity could be observed. The functional characteristics of burster neurons were determined by averaging and the least squares and equalization methods. The results were presented in the table and in two average curves which are asymmetrical with a horizontal asymptote. From these data it was concluded that the dynamics of the spontaneous activity of the two burster neurons in both specia is similar. The possibilities and limitations of the method in physiological analyses are discussed.

Introduction

In experiments on gastropodes, Arvanitaki and her collaborators (1961, 1968) discovered photosensitive neurons in Aplysia and Helix ganglia. Studies on photosensitive neurons are the present interest of our research group (Pašić et al., 1972, 1974a, b). Testing the effects of light on the spontaneously active neurons in parietal and visceral ganglia of Helix pomatia, we noticed that their spike frequencies underwent specific changes during illumination. These changes were, however, often obscured by a permanently present “noise” (i.e., irregular frequency modulation of neuron impulse activity). In order to diminish the unwanted effects of this “noise”, the averaging method (C.B.G.R.L.E. and Siebert, 1959) was applied for the analysis of microelectrode recordings of the neurons spontaneous activity in the course of intermittent photostimulation.

The averaging method was also applied here for analysing the spontaneous activity of a neuron in the right parietal ganglion of Helix being characterized by periodical activities separated by intervals of silence. This neuron was previously identified by Sakharov and Salánki (1969) as the Br-type neuron and was also described by us (Zečević et al., 1973). The activity of Helix burster neurons was here also compared with that of R 15 (PB) neuron in Aplysia visceral ganglion being spontaneously activated in a similar way (Frazier et al., 1967; Zečević and Pašić, 1972).

The aim of this analysis was to extend the application of averaging method to the neuron spike activity, as a method for describing characteristics of spontaneously active neurons. Besides, the similarities and differences among similarly behaving neurons in the two mollusc specia are also of interest, since these cells could play an identical or similar role in regulating some functions (Willows, 1973).

According to Strumwasser (1965) who investigated the activity of R 15 neuron of Aplysia, the time course of the successive spike intervals during a burst could be readily approximated by the equation for a parabola. Salánki and his collaboratours (1972) reached a similar conclusion on Helix burster-type neuron. In the analysis of functional characteristics of these cells we applied, as stated before, the averaging method choosing, however, the average number of spikes per minute or second (Pašić et al., 1974a) instead of the interspike interval. Using this method, quantitative results, elaborating further the data and conclusions of Strumwasser and others, were obtained.

Materials and Methods

a) Data Recording

The experiments were carried out on subesophageal ganglia of Helix pomatia. Prior to the experiments the animals were kept under laboratory conditions of natural light. In the experiments on sea hares the Southern Adriatic Aplysia depilans was used. The small brains were dissected by usual dissecting technique and mounted individually in a small recording chamber with
circulating snail solution (Magura, 1969) at room temperature. Neurons in left and right parietal and visceral ganglia were penetrated with KCl filled glass capillary microelectrode with a resistance of 10–15 MΩ. A Gruss microelectrode amplifier (P 16), a Tektronix 502 oscilloscope and a dual channel Beckman Dynograph recorder were used for recordings.

In experiments where the effects of intermittent photostimulation were investigated, a Zeiss microscopic lamp was used as the light source. The light was focused on the penetrated cell by a system of lenses. The diameter of the spot was about 0.5 mm. The possible thermal effects of light were avoided by a transparent water filter. As many as possible light/dark periods, each lasting 1, 2 or 3 min, were applied in the experiments. Prior to the application of intermittent light the ganglia were kept at least 30 min in darkness.

The spontaneous activity of the Helix Br cell was recorded as long as possible in constant light and at constant temperature. In experiments on R 15 cell in Aplysia the isolated visceral ganglia were also kept at constant temperature and light in a chamber containing sea water.

b) Data Processing

Statistical analysis of the spike activity of Helix neurons in the course of intermittent photostimulation during a longer period of time was first carried out. The mean number of spikes per minute was chosen as the random variable characterizing the impulse activity (Pašič et al., 1974a). Each recording of such artificially induced activity was first divided into samples which were in correct time relation to the periods of illuminations and darkness. Each of these samples was further broken down to small specimens of arbitrary length (usually lasting 15 sec). After that, according to the formula \( y = 60n/T \), the mean number of spikes per minute, \( y \), was calculated for each specimen and the obtained number was attributed to the moment belonging to the centre of the specimen. Here, \( T \) is an arbitrary time interval placed symmetrically round the central moment of each specimen, and \( n \) the number of spikes in the time interval \( T \). The mean numbers of spikes, so obtained and taken after an identical delay from the moment of the light onsets, were added and then divided by the number of specimens (C.B.G.R.I.E. and Siebert, 1959). The corresponding graphs were plotted according to the standard methods of graphical interpolation.

An identical processing was also applied for analysing the spike activity of both Br neuron in Helix, and R 15 neuron in Aplysia, except that in this case, beside the averaging method, the least squares and equalization methods (Bronstein and Semendyev, 1964) were also used. The mean frequency (i.e., the mean number of spikes per second) was now chosen as the quantity describing the spontaneous bioelectrical activity of neurons. This frequency was calculated in equidistant moments 0.4, 1.2, 2.0, and 2.8 sec according to the formula \( f = n/T \) by placing symmetrically the time interval \( T = 0.8 \) sec. The number of spikes in this interval was also denoted by the letter \( n \). The obtained four points were interpolated by the curve the shape of which is readily fitted by the empirical equation

\[
 f = a + b e^{-ct},
\]

where \( a, b, \) and \( c \) are the parameters, \( e \) the basis of the system of natural logarithms, and \( t \) the time (in seconds) taken from the moment of the first spike appearance in the discharge period.

In order to apply the method of least squares to these data, logarithms, to the basis \( 10 \), of both sides of (1) are taken:

\[
 \log f = \log a + b \log t - c \log e.
\]

If Eq. (2) is applied to the two adjacent moments, \( t \) and \( t' \), taken from the arithmetic progression consisting of four chosen moments mentioned above, and the differences of both sides of these equations are found, the following expression can be obtained:

\[
 A \log f = b A \log t - c h \log e,
\]

where

\[
 A \log f = \log f - \log f'; \quad A \log t = \log t - \log t'; \quad h = t - t'.
\]

Equation (3) can be though of as the equation of a straight line containing only two parameters \( b \) and \( c \). For that reason the method of least squares can be applied to the set of points the coordinates of which are \( y = A \log f \) and \( x = A \log t \).

In order to obtain the parameter \( a \), the following approximate method (the method of equalization) was applied:

The coordinates of all experimental points are replaced in Eq. (2) and approximate equations of the straight line so obtained are added:

\[
 \sum \log f = k \log a + b \sum \log t - c \log e \sum t.
\]

From the last equation the approximate value for the parameter \( a \) can easily be calculated since the parameters \( b \) and \( c \) were already estimated by the method of least squares.

Results

1. Averaged Reactions of Helix Neurons to Intermittent Light

As previously described, most photosensitive neurons in Helix ganglia reacted to light onset by an increase of their spike frequency (Pašič et al., 1974b), although one on-off as well as some off neurons were also found. The averaged reactions of these three types of cells to light as well as to consecutive dark periods are presented in Figs. 1 and 2. Beside the overall effects of illumination, the dynamics of the mean frequency changes during the light and dark periods could also be estimated, as seen in Figs. 1 and 2.

In several experiments, where microelectrode recordings and application of intermittent photostimulation could be carried out for a longer period of time, differences in the course of the frequency changes were demonstrated at successive stages of the experiment (Fig. 1). The spike activity of the neuron belonging to the first 17 periods of light and dark, each lasting one minute, was in this case averaged separately from the activity of the next 16 periods belonging to the second part of the experiment. It can be observed (Fig. 1, upper graph) that the dynamics of the frequency increase differed significantly in the two averaged periods belonging to light, whereas the frequency decrement during darkness remained nearly the same. From these facts the conclusion was drawn that the reactivity to light of some neurons can be modified in the course of...