STATISTICAL MACHINE ANALYSIS OF THE IMPULSE ACTIVITY OF INDIVIDUAL NEURONS

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As has been shown in experimental works of recent years, an analysis of the activity of various structures of the nervous system is most promising with respect to data obtained from individual cells [5, 7, 11]. The sequence of the action potentials of constant amplitude and form for a given cell is the result of the activity of a single cell recorded at its output by means of a microelectrode. This permits the conclusion that coding of information in the nervous system is accomplished by a change of the time intervals between successive active potentials. The activity of a cell without applying an external action, which is called the random or background activity, also appears in sequence of the action potentials randomly distributed in time [2, 5, 7, 11]. The time intervals between action potentials for various areas of the central nervous system vary from 0.5 msec to 1 sec and more.

Thus for a statistical estimate (probability distribution, probability density function, etc.) of the intervals between impulses it is necessary to sort them out by groups and to count them. These operations until recently were done either manually from oscillograms or by means of counters [6, 10]. However, such methods require considerable time or limit the sampling of experimental material, which can lead to substantial errors.

General-purpose electronic computers have been used in a number of laboratories to automate the calculation work [4, 7, 9]. As experience has shown, owing to the small volume of the internal store of machines, which requires periodic stopping and starting of the input device, the accuracy of the results obtained does not exceed 3-5%, whereas the process of analyzing the data requires considerable machine time [1]. Therefore, it is of interest to use specialized equipment for statistical analysis of experimental data which is widely used in physical investigations.

Our industry is producing various multichannel amplitude and time (microsecond intervals) analyzers with a sufficiently large volume of the store. One of the most widespread analyzers is the 100-channel amplitude analyzer AI-100 [3]. Attempts have been made (including in our laboratory) to develop a time-amplitude converter which permits the direct use of such an analyzer to obtain histograms of interimpulse intervals. However, the appreciable nonlinearity of the converter, especially for large (up to 1 sec and more) time intervals forced us to reject it.

We chose another way. A new input unit for the AI-100 analyzer was developed and manufactured which permits obtaining a comparatively high (1%) accuracy of the measurements. The initial requirements in the development of the input unit were the following: the obtainment of histograms of interimpulse intervals, MII-histograms; histograms of the latent periods between the applied stimulus and a certain preassigned response impulse, LP-histogram; histograms of the intervals between the stimulus and a series of subsequent response impulses (all time intervals are counted relative to the stimulus). By analogy with the designation used in foreign literature [3] it can be called the PST-histogram (post-stimulation).

Furthermore, the unit developed should ensure, by analogy with the input unit of the AI-100 analyzer, self-locking during the time of recording the measured interval, cutting off the input of the analyzer when one of the

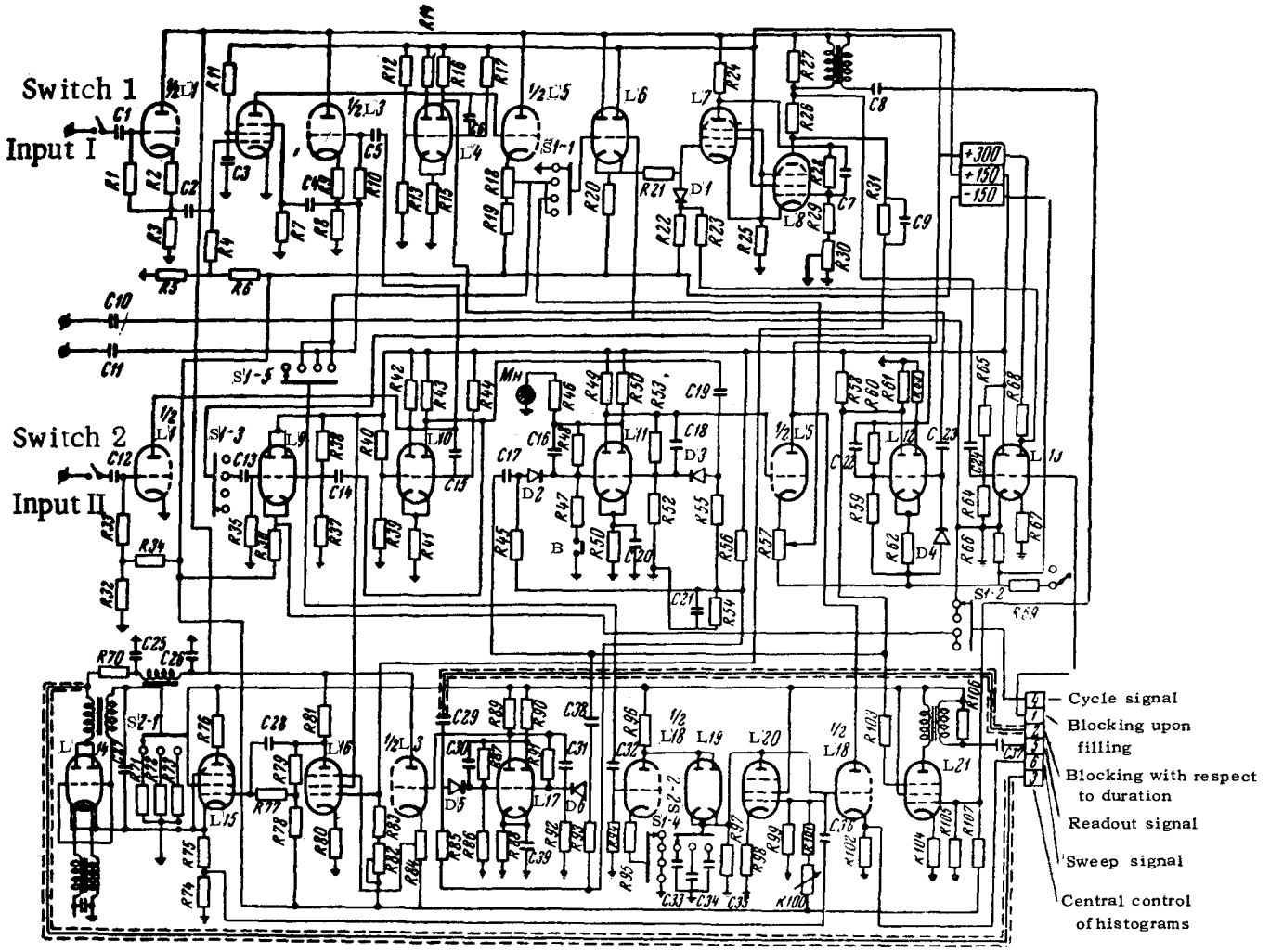

Fig. 1. Basic diagram of input unit. L1) $^1/_2$ 6N3P; L2) 6Zh2P; L3) $^1/_2$ 6N3P; L4) 6N3P; L5) $^1/_2$ 6N3P; L6) 6N3P; L7) 6Zh5P; L8) 6Zh5P; L9) 6N3P; L10) 6N3P; L11) 6N3P; L12) 6N3P; L13) 6N3P; L14) 6N15P; L15) 6Zh5P; L16) 6Zh2P; L17) 6N3P; L18) $^1/_2$ 6N3P; L19) 6Zh2P; L20) 6P1P; L21) 6Zh2P; R1) 330 kΩ; R2) 910 Ω; R3) 36 kΩ; R4) 200 kΩ; R5) 33 kΩ; R6) 330 kΩ; R7) 200 kΩ; R8) 33 kΩ; R9) 910 Ω; R10) 240 kΩ; R11) 110 kΩ; R12) 620 kΩ; R13) 100kΩ; R14) 16 kΩ; R15) 6.8 kΩ; R16) 18 kΩ; R17) 1.1 MΩ; R18) 100 kΩ; R19) 56 kΩ; R20) 51 kΩ; R21) 100 kΩ; R22) 33 kΩ; R23) 130 kΩ; R24) 10 kΩ; R25) 5.1 kΩ; R26) 10 kΩ; R27) 10 kΩ; R28) 91 kΩ; R29) 10 kΩ; R30) 10 kΩ; R31) 200 kΩ; R32) 33 kΩ; R33) 330 kΩ; R34) 360 kΩ; R35) 56 kΩ; R36) 33 kΩ; R37) 56 kΩ; R38) 910 kΩ; R39) 100 kΩ; R40) 620 kΩ; R41) 6.8 kΩ; R42) 16 kΩ; R43) 18 kΩ; R44) 1.1 MΩ; R46) 220 kΩ; R47) 56 kΩ; R48) 130 kΩ; R49) 16 kΩ; R50) 5.1 kΩ; R51) 16 kΩ; R52) 56 kΩ; R53) 130 kΩ; R54) 10 kΩ; R55) 100 kΩ; R56) 100 kΩ; R57) 100 kΩ; R58) 91 kΩ; R59) 8.2 kΩ; R60) 56 kΩ; R61) 9 kΩ; R62) 2 kΩ; R63) 5.1 kΩ; R64) 220 kΩ; R65) 3.9 kΩ; R66) 27 kΩ; R67) 510 Ω; R68) 130 kΩ; R69) 68 kΩ; R70) 270 Ω; R71) 100 kΩ; R72) 1 MΩ; R73) 10 MΩ; R74) 200 Ω; R75) 620 Ω; R76) 1.2 kΩ; R77) 330 kΩ; R78) 100 kΩ; R79) 100 kΩ; R80) 200 Ω; R81) 33 kΩ; R82) 47 kΩ; R83) 82 kΩ; R84) 150 kΩ; R85) 100 kΩ; R86) 56 kΩ; R87) 180 kΩ; R88) 5.1 kΩ; R89) 16 kΩ; R90) 16 kΩ; R91) 130 kΩ; R92) 56 kΩ; R93) 100 kΩ; R94) 220 kΩ; R95) 200 kΩ; R96) 5.1 kΩ; R97) 47 MΩ; R98) 2 kΩ; R99) 3.3 kΩ; R100) 15 kΩ; R101) 15 kΩ; R102) 30 kΩ; R103) 2 kΩ; R104) 100 Ω; R105) 24 kΩ; R106) 2.2 kΩ; R107) 330 kΩ; D1) D9E; D2) D9E; D3) D9E; D4) D9E; D5) D9E; D6) D9E; C1) 220 pF; C2) 56 pF; C3) 50 nF; C4) 50 nF; C5) 10 nF; C6) 330 pF; C7) 15 pF; C8) 1000 pF C9) 15 pF; C10) 200 pF; C11) 56 pF; C12) 220 pF; C13) 56 pF; C14) 56 pF; C15) 400 pF; C16) 15 pF; C17) 56 pF; C18) 15 pF; C19) 56 pF; C20) 50 nF; C21) 0.1 μF; C22) 68 pF; C23) 0.1 μF; C24) 510 pF; C25) 10 μF; C26) 10 μF; C27) 680 pF; C28) 15 pF; C29) 56 pF; C30) 15 pF; C31) 15 pF; C32) 56 pF; C33) 2 nF; C34) 10 nF; C35) 20 nF; C36) 1000 pF; C37) 5100 pF;

channels is filled, and generating the sweep signal of the oscilloscope in the programming. For the 100-channel analyzer to cover the entire range (0.1-1000 msec) while retaining a sufficiently high accuracy of the measurements, it was divided into three subranges: 0.1-10, 1-100, and 10-1000 msec. The appreciable overlapping of one subrange by another permits obtaining in most cases the complete histogram on one of them. The basic diagram of the new input unit is shown in Fig. 1.

The action potentials recorded by the microelectrode after preliminary amplification up to 10-20V are sent to the discriminating and shaping circuit analogous to that described in the literature [11] and which is separate from the analyzer. Impulses with an amplitude up to 30V, duration of 50-70 msec, and with a rise front of 3-4 μsec are sent to the input of the analyzer. Let us examine the sequential operation of the circuit for the three regimes of obtaining the histograms which are selected by switch S1 (see Fig. 1). In the diagram the switch is in the first position which corresponds to a disconnected input unit during operation of the analyzer in the regime "observation of the spectrum, counting, and check".

Histogram of the interimpulse interval (switch S1, second position). The input signal through the cathode follower L1 and the anticoincidence circuit L2 triggers univibrator L4. The duration of the square pulse generated by the univibrator is 60 msec. The univibrator operates Schmitt trigger L7, L8 through cathode follower L5. The signal with the Schmitt trigger operates the work of the blocking generator L14: through phase inverter L16 and tube L15. The pulse repetition frequency of the blocking generator is changed by means of switch S2 and is equal to 10.1 and 0.1 kc, which corresponds to the three selected subranges.* The circuit is regulated so that during the time of the impulse at the output of the univibrator L4 the blocking generator is blocked and readout (L13) and cycle (L12 and L21) impulses are generated for the distributor and the process of channel selection and recording occurs. The duration of the impulse of the univibrator L4 is equal to 60 μsec and is selected in conformity with the time of the internal operation of the analyzer (40 μsec). The tube L16 is controlled with respect to the suppressor grid by means of trigger L17 and the cathode follower on the right half of tube L3. The input pulse switches trigger L17 to one of the stable states in which tube L16 is opened. If the time interval before the next impulse exceeds the selected range, the impulse taken from the trigger of the 99th channel of the distributor through the univibrator and cathode follower of the controller (L37 is removed from resistor R38) L17 is switched to the second stable state in which the blocking generator is blocked.

The anticoincidence circuit L2 is introduced with respect to the following considerations. One of the most widespread methods of studying the nervous system is to analyze the responses of individual cells to a given electrical stimulus. However, in most cases (especially with closely spaced stimulating and recording electrodes) the impulse of stimulation, the so-called stimulation artefact, is sent to the input of the recording amplifier and can trigger the discrimination and shaping circuit. To eliminate this impulse we used an anticoincidence circuit. The stimulating impulses from the stimulator are sent to input II and trigger univibrator L10, which for 100 μsec (the width of one channel in the subrange 0.1-10 msec) forbids the transmission of the impulse with respect to input I. Thus, if the stimulation artefact is shifted relative to the signal of the stimulator by no more than 10 msec, it is disregarded by the analyzer. This circuit operates in all three regimes of analysis.

Histograms of the latent periods (switch S1, third position). This regime of operation was selected for an accurate measurement of the latent period between stimulation and one of the response impulses. The selection of the appropriate response impulse (1, 2, 3....) is done by means of a scaling circuit connected between the shaper and input I of the analyzer. The stimulating impulse is sent to input II through an external delay line and can be made close to the selected response impulse for a fixed time. All this permits obtaining histograms in a range with smaller width of the channel, which appreciably increases the accuracy of the measurements.

In this regime the circuit operates in the following manner. At the start of counting, trigger L11 by means of button B is switched into one of the stable states at which the Schmitt trigger L7, L8 blocks the blocking generator through tubes L11, L15. The stimulating impulse having arrived at input II after the delay line, triggers univibrator L10 through tube L1. The impulse of the univibrator trips trigger L11, as a result of which blocking of L14 begins to work. The selected response impulse having arrived at input I again trips trigger L11 and generates the readout signal of L21 and cycle signal of L9. If the stimulating impulse did not evoke a corresponding response impulse, the next stimulating impulse generates a cycle signal (L9) and there is no readout signal. This makes it possible not to introduce these intervals into the arithmetic unit.

*Theoretically this frequency can be different, which leads to a change in the width of each channel and range as a whole. Thus, in Fig. 3 the width of the channel is 3 msec, which corresponds to a frequency of the blocking generator of 0.3 kc.

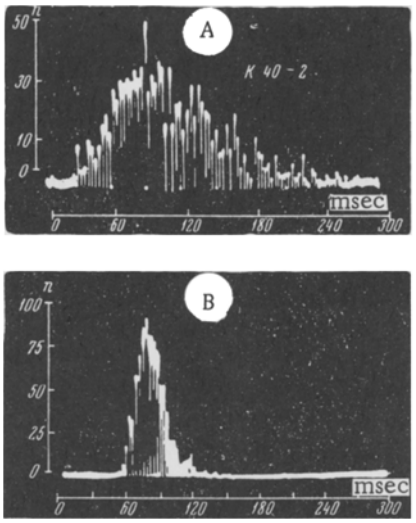

Fig. 2. Histogram of the spontaneous activity for a neuron of skin (A) and muscle (B) sensitivity.

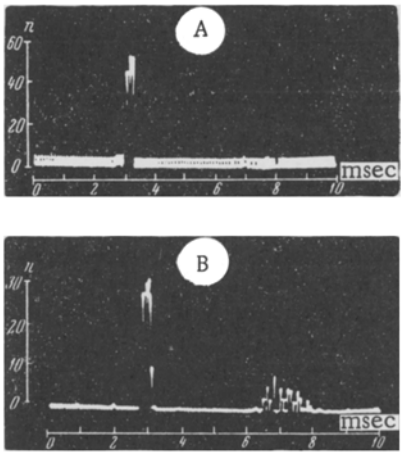

Fig. 3. Histograms obtained in latent period of (A) and poststimulation (B) regimes. Explanation in text.

Histograms of poststimulation (switch S1, fourth position). This regime differs from the preceding in that each response impulse generates a readout signal and the cycle signal is generated only by stimulating impulses (L9). The The possibility of the matching of one of the response impulses with the stimulating impulse is eliminated by the anticoincidence circuit at input I. Blocking of the analyzer upon filling of one of the channels is accomplished by a signal arriving from the controller which blocks the input of the Schmitt trigger through tube L13 and diode D1. The sweep unit for the oscilloscope did not need any substantial modification. For operation at various subranges the charging capacitors are switched (switch S2). In the first regime the booster charge of the capacitor through resistor R96 and diode L19 is produced by the response impulses and in the second and third regime by the stimulating impulses (switched by switch S1). The number of impulses arriving for analysis is controlled by external counters, for which appropriate outputs are provided for in the unit. Industrial models of the PS-100 type are used as counters.

Checking and adjustment of the unit in all regimes were accomplished by means of a GIS-2M paired pulse generator, OI-4 oscillograph, and counters. The results of a calculation showed that out of 4532 introduced impulses not a single one was lost.

The error in selecting the number of the channel for all subranges did not exceed the width of one channel, which corresponds to 1% of the range.

Figures 2 and 3 show histograms of the spontaneous and evoked activity of neurons of the cat spinal cord activated by skin and muscle nerves. Figure 2 shows histograms obtained in the interimpulse interval regime for a neuron of skin sensitivity from the dorsal horn of the lumbar area and a neuron of muscle sensitivity from Clarke's column; Fig. 3 shows histograms obtained in the latent period regime for this same neuron activated by a group of Ia muscle fibers and which responded with a single impulse to an afferent wave (see Fig. 3A) and a histogram in the poststimulation regime after connecting fibers of group Ib, as a result of which a second impulse periodically appeared in the response (see Fig. 3B).

The analyzer permits obtaining histograms on the screen of a cathode ray tube 5-7 sec after completion of the program, which is tenths of thousandths of times more rapid than when processing such data manually.

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