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Detecting states of ion channels on the cell membrane using neural networks

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Abstract. The problem of automating the process of analyzing the open states of channels on the membrane of a neuron of a living organism is considered. Taking into account that the registration of the electrical activity of the cell was made by the patch clamp method at various values of the applied potential, a division into intervals with a constant potential is carried out. Further, to eliminate noise, a notch filter, low-frequency and high-frequency Chebyshev filters are applied to the data. A neural network is applied to the normalized data, based on the results of which the data is changed and re-processed by the same neural network. As a result of the algorithm, the dynamics of channel states was obtained, which makes it possible to register up to several open channels simultaneously.

Key words and phrases: neural networks, ion channels, detecting states of channels, living organism

2020 *Mathematics Subject Classification*: 92B20; 97N80, 97R40

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Introduction

In recent years, information technology has shown significant progress. One of the breakthrough areas of their application is the development and use of machine learning methods in microbiology [1–3]. The use of machine learning allows making the procedures, which previously required a large number of man-hours, very routine [4]. Machine learning and artificial neural networks are actively used in fundamental science, in particular, in neurobiology [5–7].

One of the applications of machine learning is the detection of open states of ion channels on the membrane of excitable cells of animals (neurons, cardiocytes, myocytes, etc.). The most common method of functional registration of ion channels is the method of local fixation of the potential (patch-clamp technique), which allows recording the electrical activity of one cell, namely, the currents passing through its membrane [8]. The main mechanism that forms the current of ions through the channels is the electrochemical gradient.

The electrochemical gradient depends on the difference in ion concentrations outside and inside the cell, as well as on its membrane potential, and the detection of an open channel in some cases becomes a nontrivial task for the experimenter. In particular, at low values of the electrochemical gradient, the amplitude of the current passing through the channel decreases, which is accompanied by a deterioration in the signal-to-noise ratio. This means that the false-positive detection of the open state of the channel by the operator increases. In addition, the detection of open channel states is a routine procedure that requires scrupulous review of long-term records, which increases man-hours for analyzing the recorded data. In this regard, the use of machine learning in this area of neuroscience is more than justified. Here, various approaches and algorithms [9–12] are used to model and analyze the states of ion channels. However, the best results of tracking channel states on model data are shown by neural networks [13].

When registering ionic currents, interference of various nature also occurs, requiring data processing and parameterization [14–16]. Therefore, neural networks are applied after processing and normalizing the received data.

In the present paper, we propose an algorithm for automating the process of analyzing the open states of channels of a neuron of a living organism using a neural network. The analyzed record lasting 552 seconds was obtained by the patch-clamp technique on a neuron of the brain of a young rat at various values of the applied potential[1]. At the initial stage, the record is divided into separate sections corresponding to constant potential values and processed by filters to eliminate noise and unnecessary frequency intervals. Then a neural network [13] was applied to the processed data. Based on the results of the neural network, the original data is transformed and re-processed by the same neural network.

Graphs are shown demonstrating the change in the detection results at individual stages. The results obtained at the output showed the high efficiency of the neural network. The code is hosted on GitHub[2].

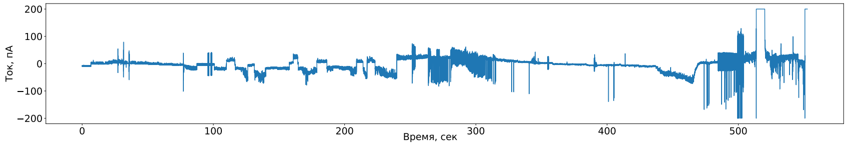
1. Ion channels and their registration

Ion channels are protein complexes on the cell membrane. Since these complexes are involved in the transport of inorganic ions, they are called ion channels. In terms of transport efficiency, ion channels have a throughput capacity, so that up to 100 million ions can pass through one open channel every second — at a rate almost 100 times higher than the highest transport rate mediated by any known carrier protein. However, channels cannot be connected to an energy source for active transport, so the transport they mediate is always passive. The main mechanisms that form the current of ions through the channels are the concentration gradient associated with the difference in the concentration of the ion outside and inside the cell, and the electrical gradient determined by the difference in charges located on opposite sides of the membrane. The combination of both gradients is called the "electrochemical gradient".

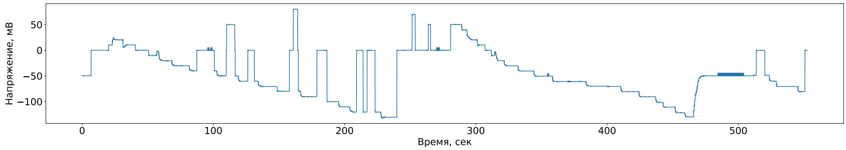
The characteristics of the electrochemical gradient determine the excitable cell properties, that is, the possibility of describing the parameters of the electrochemical gradient underlies our understanding of the functioning of excitable cells in a living organism. The patch-clamp technique makes it possible to register the electrical activity of one cell [13]. To do this, a fragment of the cell membrane is isolated using a special glass micropipette pressed against the membrane of a living cell. The resulting electrical access to the cell allows to control the potential difference outside and inside the cell, that is, to control and change the electrical gradient, and hence the severity of the ion current through the channels.

[1]Measurements were made with the Axopatch 200B device.

[2]https://github.com/DCel567/Patch-clamp-neuron



(*a*) current strength



(*b*) dynamics of potential values on the pipette

Figure 1. Synchronous recording of the dynamics of values

The controlled conditions of registration from a single cell make it possible to measure the currents passing through its membrane, which ultimately allows drawing conclusions about how ion channels respond to electrical and chemical effects [17].

Registration of the activity of single channels is one of the configurations of the patch-clamp technique. Electrical contact with the cell is carried out only through open ion channels located on the membrane under the pipette. The method of registration of ion channels is so sensitive that it allows describing the behavior of individual, single channels, namely, the moments and duration of their open state. At this moment, an electric current is observed, the amplitude and direction of which are determined by the electrochemical gradient of ions passing through the open channel.

2. Problem statement

From the obtained experimental data, containing the dynamics of changes in current values at different potentials applied to the pipette (external medium), it is necessary to determine the dynamics of opening and closing of ion channels. The sampling rate of the signal is 10,000 measurements per second. Let us consider one of the records of such signals with a duration of 552 seconds with a change in the value of the potential difference. Thus, in total for this record we have 5,552,000 measurements. Figure 1 shows the graphs of current values (top graph) and pipette potential values (bottom graph) versus time. We will present a further demonstration of the operation of the algorithm using this example.

Table 1. An example of data: current and potential values outside the cell

Current, pA	Potential value, mV
-8.81347643116897	-49.9572765072571
-8.77075182243061	-49.8962413496429
-8.69750963602201	-49.9267589284500
-8.67919908941986	-49.9267589284500
-8.73413072922631	-49.9267589284500
-8.75854479136251	-49.8962413496429

Thus, we have records of the values of the current strength and the applied potential. Table 1 shows a data fragment containing two columns: current values measured in picoamps (10^{-9} amps) and potential values measured in millivolts (10^{-3} volts).

Different potential values lead to different current amplitude values under the same external conditions. Therefore, we separate the time intervals with different potentials and consider them independently. We exclude the data obtained during the change in potential values from consideration, since it is impossible to reliably detect the opening or closing of channels at an unstable voltage.

It should be noted that the data itself is quite noisy in certain frequency ranges, so it is necessary to apply frequency filters to eliminate or smooth out these noises.

3. Neural network for synthetic data detection

Convolutional Neural Network (CNN) layers are a powerful deep learning component needed to extract patterns in complex datasets. The most common application of 2D convolutional layers is in machine vision [18, 19]. An adaptation of a 2D CNN is a 1D CNN. The one-dimensional variant was specially designed to use the capabilities of CNN to classify time series [20]. The most commonly used architecture for time series analysis is the deep learning architecture known as Recurrent Neural Networks (RNN) [21]. The problem with classical RNN is that the model starts to degrade when the output information depends on large time scales due to the vanishing gradient phenomenon. The development of the classical recurrent network was the emergence of a long-term memory network (LSTM) [22].

In the architecture used, convolutional layers are combined with LSTM blocks to improve the detection of long-term temporal relationships in time

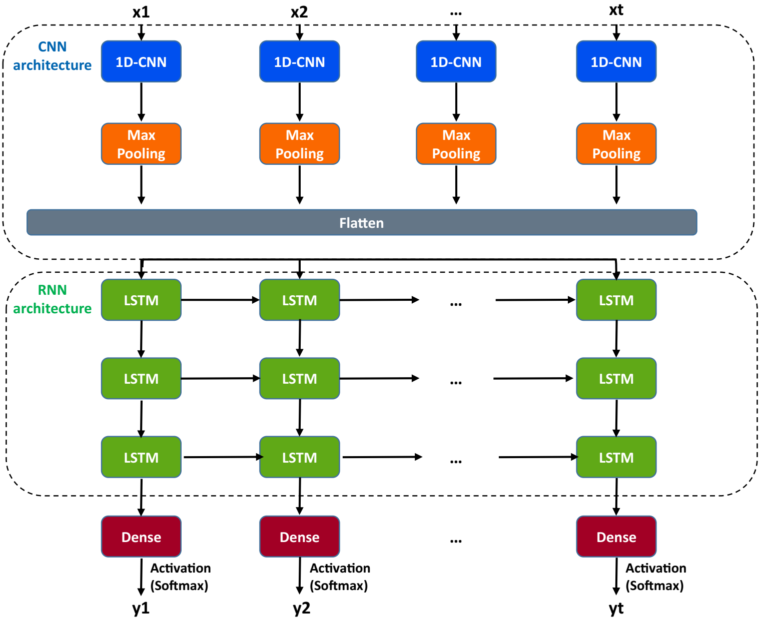


Figure 2. Neural network architecture [13]

series data (Figure 2). The neural network consists of one convolutional layer followed by a Flatten layer. The aligned data is fed to the input of the LSTM layer; there are three such layers in total. Each LSTM layer contains 256 LSTM cells. In addition, a dropout neural network regularization method with a coefficient of 0.2 is applied to each LSTM layer, which reduces the risk of model retraining. After three LSTM layers, we have a set of attributes at the output, which, in turn, is fed to the input of a fully connected layer with the SoftMax activation function. When passing data through the final activation function, we obtain the values of the probabilities of opening a certain number of channels. The last step in running the model is to select the maximum probability value from the list of obtained probabilities and to determine how many open channels this probability corresponds to.

We will train the neural network on existing data [13] hosted on GitHub[3]. It is possible to train the network to determine one, three or five possible simultaneously open channels. Due to the specifics of real data, for which the neural network is used in the present work, we will choose the

[3]https://github.com/RichardBJ/Deep-Channel.git

option of opening up to five channels. In addition, the greater freedom of the neural network in choosing the answer option will make it possible to modify the results more widely. For two epochs, the model is trained to accuracy = 0.9699, precision = 0.9700, recall = 0.9698, F1-score = 0.9699.

4. Data preprocessing

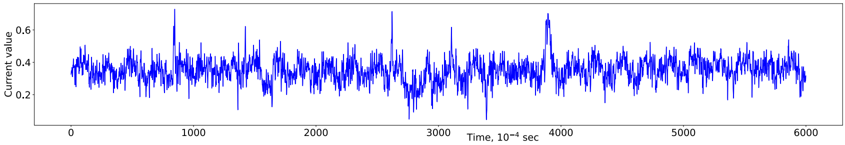
Before proceeding to the use of a neural network, the data must be divided into continuous blocks corresponding to the same potential, and then unnecessary frequency ranges and noise must be removed.

Let us describe an algorithm for partitioning data into segments with the same potential values. Let us fix the number of points on the time interval (frame), where the potential must be constant. We shift the frame from left to right according to the data with a step equal to the size of the frame, and if the swing in the frame turns out to be greater than a certain value (the voltage changes), then we discard such a frame and the signal points corresponding to it. If there are no strong voltage swings inside the frame, then the data contained in it will be left for further processing. Using such an algorithm, for a large frame size, one can get a high processing speed of the entire data set, while losing a lot of information in frames with voltage swings.

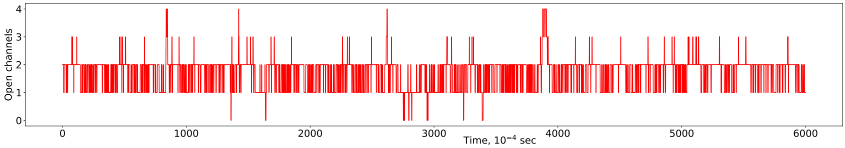
After the regions with changing potential difference are cut out, the remaining separated regions are saved for further processing. Before training the network, we normalize the data from zero to one and, as an example, demonstrate the same section with a length of 6000 points, which corresponds to the first 0.6 seconds of signal measurement.

In the following constructions, we will present figures each containing two graphs. The upper blue graph is a demonstration of the data section for which the neural network builds a "prediction" (the highest probability of the state of ion channels: no open channels, one channel is open, two channels are open, etc.). The lower, red graph shows the predictions made by the neural network. The points of the red and blue graphs correspond to each other in time, that is, the point with the ordinal number n on the blue graph has a prediction on the red graph at the point with the ordinal number n. The prediction is one of the numbers from zero to five, corresponding to the expected number of open channels at a given point in the upper graph.

The results of neural network processing show that most of the record is represented by states 1 and 2, which corresponds to the open state of one or, simultaneously, two ion channels. However, the probability



(a) the normalized current strength values



(b) the dynamics of opening and closing of channels

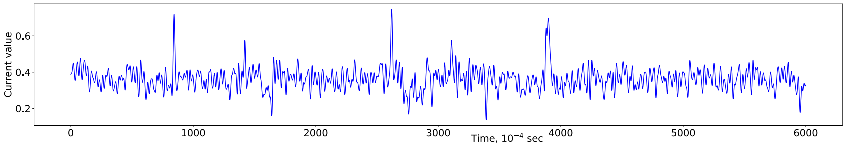
Figure 3. The first 0.6 sec of the filtered record from Figure 1 after normalization

of opening ion channels on the neuron membrane is much lower, which indicates an excessive detection of open states of ion channels by the neural network. The reason for excessive detection is the presence of parasitic fluctuations in current values (electromagnetic and power supply noises during registration). To reduce their contribution, it is necessary to keep the frequencies at which only changes in the states of ion channels are recorded. To do this, we apply low-pass and high-pass Chebyshev filters of the second kind to the data. Using a low-pass filter, we cut frequencies above 1 kHz (the function in python is `cheby2(4, 40, 1000, 'low', 10000)`, whereas using a high-pass filter, we cut frequencies below 5 Hz using the function `cheby2( 4, 40, 5, 'high', 10000)`. Figure 3 shows the periodic ups and downs of oscillations - these are standard noise pickups from 50 Hz electrical appliances. To get rid of this noise, we use a 50 Hz notch filter, implemented in python with the following code:

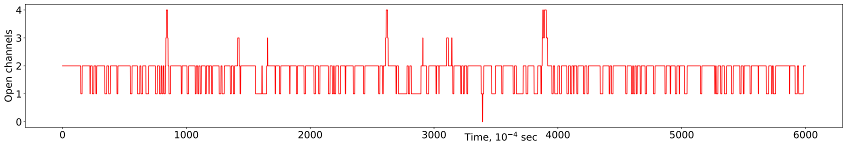
```
bnotcn, anotch = iirnotcn(10000, 30, 50)
filtfilt(bnotch, anotch, data).
```

Here `data` is filtered data with a base frequency of 10000 (Hz) and a quality factor of 30.

Let us pass the filtered data through the neural network; the result is shown in Figure 4. The blue graph shows the filtered data section in the interval of 0–0.6 sec. The red graph is the predictions of the neural network for this filtered signal. Comparing the lower graphs of figures 3 and 4, it is easy to see that the frequency of opening and closing of the channels has



(a) the filtered normalized current strength values



(b) the dynamics of opening and closing of channels

FIGURE 4. The first 0.6 sec of the filtered record from Figure 1 after normalization and a 50 Hz notch filter

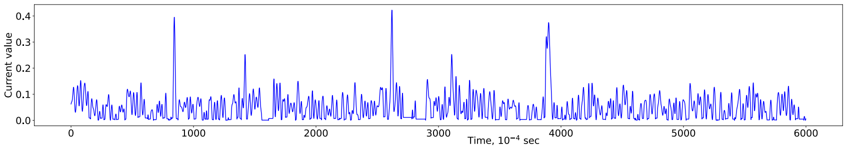
become significantly smaller, which is primarily due to the removal of the high-frequency spectrum.

From the viewpoint of microbiology, downward bursts in the upper graphs of figures 3 and 4 can be interpreted as the opening of anion channels [**23**]. We are primarily interested in cation channels [**24**], that is, areas on the graphs with upward bursts. On the other hand, the neural network is trained to process with cationic channels [**13**]. Therefore, for the correct operation of the neural network and the interpretation of the results, it is necessary to cut the current "downward" surges.

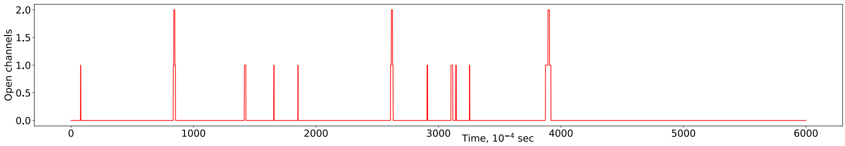
In fact, the bottom graph of Figure 4 clearly indicates that three channels are unequivocally open (the three largest peaks). In this section, it is required to detect three openings of the channel. The neural network marked such openings with the level of five simultaneous openings, which is not entirely true, but at least the openings were found.

5. Detection of ion channels in real data after preprocessing

For further data processing, we apply the following algorithm. We pass the data of interest to us through the neural network, then, having received predictions for this segment (red graph), we find the number of channels for which the cell opens the most. For example, for the lower graphs of Figure 3 and Figure 4, the maximum number of points has a value of two, which means that for points on the graph that take this value, we conditionally consider that the cell is inactive (does not have open channels). Let us call this level the baseline.



(a) the current strength values with the "anion currents" removed



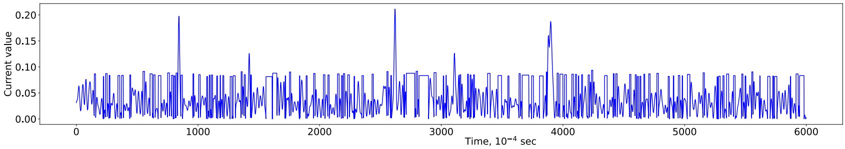
(b) the dynamics of opening and closing of channels

FIGURE 5. The first 0.6 sec of the filtered record from Figure 1 after removing of the "anion currents"

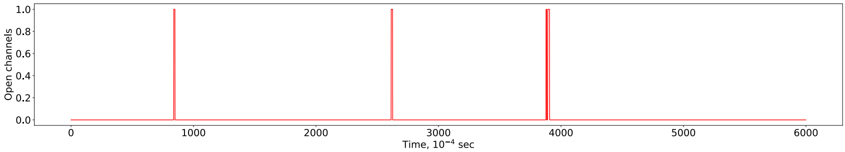
Now let us remove the points below the baseline according to the following algorithm. Having found the next range of points located below the baseline, we replace each of them with the arithmetic mean of the values of the two points that are the boundaries of this range. In this way, we are guaranteed to change the values below the baseline to values that exceed the values of the current on the baseline and remove information about possible openings of anion channels.

Now that the normalized current values have been changed in accordance with the requirements of the neural network for input data, we will send the obtained current values to the neural network again. Let us decrease the current values by subtracting the minimum value for the entire range from all values so that the minimum value is zero, and we "lower" the blue graph down in such a way so that the range of detected channel openings is from zero, and not from the baseline level. The result of the algorithm operation is shown in the upper graph of Figure 5. The lower graph of Figure 5 shows the detected openings and closings of channels.

After "cutting off" the anion channels, it is necessary to normalize the data by dividing the entire sample by two. In this case, it can be seen that the values of the upper graphs decreased when moving from Figure 5 to Figure 6. As a result, the number of open channels also decreased - only three ones remained. Note that the lower graph in Figure 5 in the vicinity of the value of 4000 indicates that at first one channel opened, then, while it was open, the second channel opened. From the lower graph of Figure 6, we can conclude that in the same time range one channel opened and

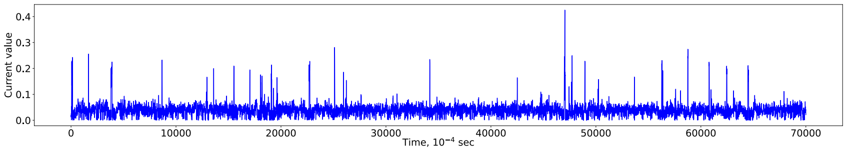


(a) the current strength values reduced to the requirements

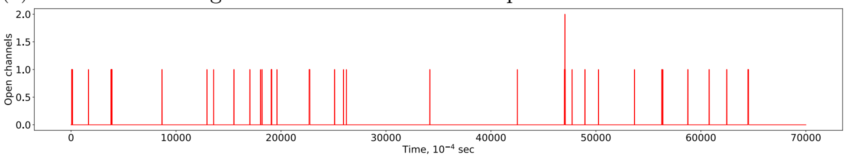


(b) the dynamics of opening and closing of channels

FIGURE 6. The first 0.6 sec of the record from Figure 1 after filtering to the requirements of the neural network



(a) the current strength values reduced to the requirements of the neural network



(b) the dynamics of opening and closing of channels

FIGURE 7. Filtered record from Figure 1 of the interval from the 70th to the 77th second

closed, then the channel immediately opened again, that is, there was not a simultaneous opening of two channels, but there was a sequential opening. Expert opinions confirmed the correctness of the conclusions drawn.

Let us now consider another interval of the upper graph of Figure 1 with a duration of 7 seconds: from the 70th to the 77th second. After processing the data, the normalized current values will take the form shown in the upper graph of Figure 7. In this case, the neural network outputs several open single channels and the simultaneous opening of two channels near the value of 45000.

6. Conclusion

The problem of determining the states of channels when recording current values at different voltages by the patch-clamp technique was considered. For channel detection, a neural network was used, which is a joint use of CNN and LSTM networks [13]. The general algorithm for processing and detecting data was divided into the following stages:

(1) Breaking down into intervals.

(2) Applying filters.

(3) Data normalization.

(4) Applying a neural network.

(5) Clipping data below the specified level. Multiplying by a factor.

(6) Reapplying the neural network.

The algorithm showed high stability to real data and accuracy of channel states detection.

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