Cultured Nerve Cell Networks as Biosensor using Artificial Neural Nets

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RÉSUMÉ

Les Réseaux neuronaux d'un mammifère qui se développent sur des microélectrodes représentent des transducers qui réagissent avec modifications de leur environement chimique. Leur réaction, qui dépend souvent de la substance ou de la concentration, peut servir de capteur biologique pour detecter les variations au niveau des substances chimiques. Avec des traitements singaux appropriés, ces systèmes biologiques peuvent être utilisés comme capteurs biologiques cellulaires. Dans cette publication, nous avons examiné la réaction dependant de la strychnine et de l'acide N-methyl-D-aspartic (NMDA) en utilisant des réseaux neuronaux artificiels. Nous avons étudié les singaux enrigistrés en utilisant la retro-propagation et la carte autoorganisante de Kohonen (SOM). En utilisant les informations contenues dans le réseau retro-propagation, nous avons appliqué FAGNIS (Fuzzy Automatically Generated Neural Inferred System) pour obtenir les règles fuzzy du système.

ABSTRACT

Mammalian neural networks grown on microelectrode arrays represent transducers that respond to changes in their chemical environment. Their response profile to a great variety of neuroactive compounds is often substance- and concentration-specific and might be used as a biosensor for the detection of a variety of chemical substances. Employing appropriate data processing and analysis, these biological systems may potentially be used for certain sensory tasks as cellular biosensors. In this paper, the concentration dependent response of the network for strychnine and N-methyl-D-aspartic acid (NMDA) are explored, using artificial neural nets (ANN). The recorded data has been investigated using Backpropagation nets as well as Kohonen's self-organizing map (SOM). Using the information containing within the trained Backpropagation net, FAGNIS (Fuzzy Automatically Generated Neural Inferred System) was applied to obtain fuzzy rules of the system.

1 Introduction

Neural networks from embryonic murine spinal cord tissue are very sensitive to changes in their chemical environment of the surrounding culture medium. Their response profile to a great variety of neuroactive compounds is often substance- and concentration-specific. Grown on planar multielectrode matrixes these neural networks can be used as transducers, to transform the highly sensitive and selective response of the biological system into electronic signals. These biological systems may potentially be used for certain sensory tasks as network biosensors [1]. In order to recognize and classify the spatio-temporal action potential patterns, associated with different conditions, we have tested two different artificial neural net (ANN) architectures. Those architectures are able to interprete nerve signal recordings [2, 3]. In this paper[1], we have compared an unsupervised learning algorithm (Kohonen's Self-Organizing Map (SOM)) with a supervised learning algorithm (Backpropagation). Additionally, the algorithm FAGNIS (Fuzzy Automatically Generated Neural Inferred System) [4] was applied to the backpropagation network to obtain fuzzy rules of the system.

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2 Experimental Setup

Spinal cord monolayer networks were cultured on transparent multimicroelectrode plates (MMEPs) with 64 photoetched electrodes in a central recording matrix. The technique used for MMEP fabrication as well as for cell dissociation, seeding and culture maintenance have been described elsewhere [5]. Briefly, MMEPs (5x5 cm) were prepared from 1.2 mm thick indium-tin oxide (ITO) sputtered barrier glass. The conducting ITO-pattern of the electrodes radiating from a 0.8 mm^2 central recording area with 4 rows and 16 columns was photoetched with standard procedures. The impedance of the recording microelectrode sites was lowered by electroplating a thin layer of gold on each exposed ITO tip.

Spinal cord neurons were obtained from fetal mice (E14-15) and cultured on MMEPs according to the methods of Ransom et al. [6], with the addition of an enzymatic dissociation step with papain and DNAse. Approximately $8x10^5$ cells were seeded on each MMEP. Thus the network develops in a central adhesion island (typically 1-8 mm^2) over the recording area. A typical low-density culture growing over the electrode sites in the central recording area of a MMEP is shown in figure 1.

For spike activity recordings the MMEP was mounted within a stainless steel chamber under 1 ml of conditioned medium. Two carbon-filled silicone elastomere, pressed

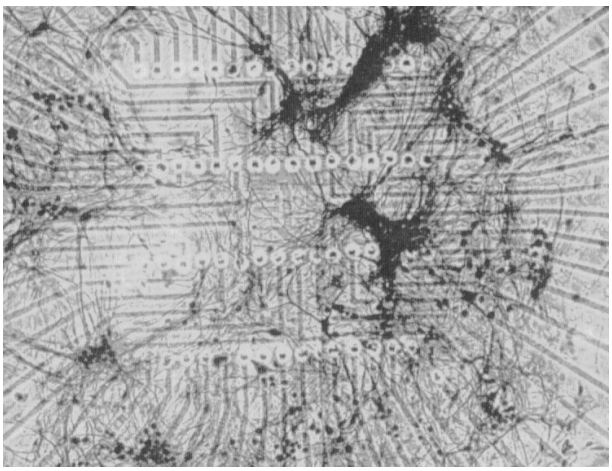

Figure 1 — A neural network of dissociated spinal cord cells grown on the recording array of a 64-microelectrode culture plate.

against the parallel ITO output strips, provide the electrical contact between the amplifier circuit board and the MMEP.

3 Data Acquisition

Electrical activity was recorded via a two-stage, 36-channel amplifier set. The amplifier bandwidth was usually set at 500 Hz to 6 kHz. Activity was displayed on oscilloscopes and recorded on a 14 channel Racal direct tape recorder. With a Masscomp 5700 platform spike data from active channels were digitized with sampling rates of 33 kHz and filtered. Actually, a maximum of 14 selected channels can be processed simultaneously.

The large amount of data from recordings of single action potential data requires simplification of the data acquisition. Since clustering of action potentials (spikes) into bursts is a prominent feature of signal patterns from spinal cord neural networks, spike trains were integrated into bursts. The recorded spike data was integrated with a double-integration method [5]. From the integrated data the main burst variables (burst maximal amplitude, burst duration, burst interval, burst period and burst area) were determined.

Most spinal cord networks show native spontaneous activity with loosely synchronized low frequency bursting. The electrical activity of the network can be stimulated respectively inhibited by adding chemical or medical agents to the culture. The data used were recordings under 6 different conditions: (1) the initial native activity of the cell in the medium, (2) after addition of 10 μM strychnine and 5 μM N-methyl-D-aspartic acid (NMDA), (3,4,5) after three medium changes, and (6) the final network state two hours after the medium changes. Each of the six experimental episodes is associated with specific spatio-temporal action potential patterns that must be recognized and classified [5]. As in [5] condition (4) and (5) are stated as one condition. This is reasonable since the chemical environment of the cellular network was similar which has been proved by normal chemical examinations.

The data sets have been recorded at 6 different electrodes out of 14. Obviously, some electrode sites are more suitable for the recognition of the concentration than others. This might be due to the circumstance that some electrodes are contacted by several different axons and some electrodes only by one. This may lead to superposed informations within the burst patterns, whereas the recordings from electrodes contacted by a single axon display an unequivocal information. Detecting those microelectrode sites allows a reduction of the complexity of the processing system and a decrease in the processing time. In this paper we concentrate on the data set of the most significant electrode.

In a preprocessing step, the main burst variables (burst maximal amplitude, burst duration, burst interval, burst period and burst area) for the integrated data were determined. In preliminary investigations we used all burst variables for the training of the ANNs to determine the most characteristic features within the burst variables. Best results have been obtained by using training vectors representing burst duration, interval between two bursts, and the maximum amplitude of the burst.

4 Kohonen's Self-Organizing Map

The same vectors as above have been used for the training of the SOM. The dimension of the map is 7 x 7 neurons. The obtained map is very well disposed. The SOM offers the possibility to estimate the conditions on the map.

The trained SOM is shown in figure 2. Each square represents a neuron. Within the neurons, the distribution of the conditions are encoded. Each color corresponds to a condition, whereas the square represents the color of the condition which hits the neuron most.

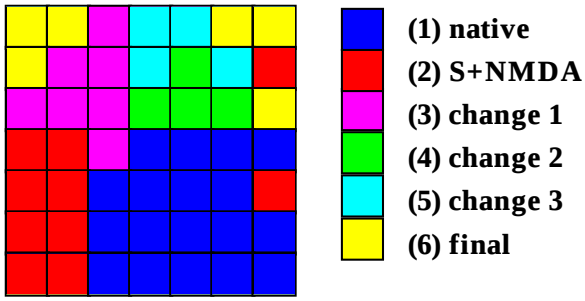

Figure 2 — Distribution of the conditions using Kohonen's SOM. The color of each square corresponds to the condition which hits the neuron most.

On the lower right hand side the initial native activity (1) is located. The condition (2), 10 μM strychnine and 5 μM NMDA, is located in the lower right hand side, whereas the medium changes are situated in the upper third of the map. Finally, condition (6), the final network state, is located in both upper corners of the map, whereas most hits are in the upper right hand side corner.

The distribution reflects the similar results as in [5] where classical methods have been applied. In contrast to this

method, the SOM organizes the condition in manner, that following conditions are neighbours, except the final network state, whereas the method presented in [5] have to cross other conditions following one condition after the other.

Condition	Accuracy
initial native activity	90 %
S+NMDA	78 %
medium change 1	75 %
medium change 2 & 3	72 %
final network state	78 %
total	79 %

Table 1 — Accuracy of different conditions for Backpropagation. For Kohonen's SOM. The accuracy has been calculated using the absolute error.

Even if the SOM is well disposed and generalized the data very well, the accuracy is 73 %. This is due to the fact, that some condition overlays others partially. Detailed results for channel 4 are shown in table 1, where condition (4) and (5) are stated as one condition.

5 Backpropagation

In a first attempt we have used Backpropagation [7] nets for the recognition and classification of the patterns associated with different conditions.

As mentioned above, the training vectors consist of three components representing the burst duration, the interval between two bursts and the maximum amplitude of the burst. From 750 vectors which have been examined for all concentrations, approximately one third has randomly been determined for testing. More precisely, 600 vectors have been used as training vectors and 150 as test vectors. The small number of vectors is due to short recording durations.

The best results have been obtained using a net with 3 input neurons, 10 hidden neurons and 6 linear output neuron. Each condition corresponds to one output neuron of the net. E.g. the condition (1), initial native activity, was coded by 0.5 at the first output neuron and -0.5 at all other output neurons; condition (2), addition of 10 μM strychnine and 5 μM NMDA, was coded by a 0.5 at the second output neuron whereas all other neurons were at -0.5 and so on. In order to calculate the accuracy, the condition with the highest value at the output neuron was judged as true. The accuracy for each condition has been calculated using the absolute error. The learning rate of the Backpropagation net was 0.001.

Using this architecture, an accuracy of 90 % was achieved for the most significant microelectrode site (channel 4). The detailed result using the assumption to state condition (4) and (5) as one condition is shown in table 2.

Furthermore, the result can be improved by taking a majority decision from all trained channels. Using the outputs for all microelectrode sites within a certain time slot, several different results will be obtained. Calculating a majority decision for these results of all microelectrode sites during a time slot, an accuracy of 96 % has been achieved.

Condition	Accuracy
initial native activity	90 %
S+NMDA	76 %
medium change 1	98 %
medium change 2 & 3	94 %
final network state	94 %
total	90 %

Table 2 — Accuracy of different conditions for Backpropagation. The accuracy has been calculated using the absolute error without majority decision.

6 Fuzzy Rule Extraction

Feedforward Neural Networks are often considered as black-box models because it is difficult to extract knowledge from them in a comprehensible way.

We want to present here the application of the fuzzy rule extraction to the nets trained with the backpropagation algorithm with the objective of validation of the neural network. The algorithm used to extract the rules was FAGNIS (Fuzzy Automatically Generated Neural Inference System) [4]. Only the main ideas of the algorithm in FAGNIS will be discussed. We refer to the literature for a detailed analysis. The main advantage of the algorithm is the possibility to extract Sugeno fuzzy rules from any network structure.

The fuzzy rules extracted by FAGNIS are the so called Sugeno fuzzy rules [9] and have the form "`IF` $x \in Z$ `THEN` $y = Ax+B$", where x is the input of the rule (and of the neural network), Z is a fuzzy set, y the output of the consequence part (and of the neural network), A and B are matrices respectively 6×3 and 6×1. Z is defined by a membership function $\mu_Z(x)$. $x \in Z$ is the premise of the rule and can be computed by the application of the defining membership function on x.

The application of the algorithm FAGNIS to the neural net with a structure 3-10-6 (10 hidden neurons) trained with the backpropagation algorithm for 500,000 epochs resulted in a Fuzzy Inference System (FIS) with 108 fuzzy rules. I will present here only the most important one:

$$\texttt{IF} \quad \bar{x} = \begin{pmatrix} 0.1 & -0.5 & -0.3 \end{pmatrix}$$

$$\texttt{THEN} \quad \bar{y} = \begin{pmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix} \begin{pmatrix} x_1 \\ x_2 \\ x_3 \end{pmatrix} + \begin{pmatrix} +0.5 \\ -0.5 \\ -0.5 \\ -0.5 \\ -0.5 \\ -0.5 \end{pmatrix}$$

Since the matrix A is a null matrix, the inputs inside a certain fuzzy set have no influence on the output. All entries of the matrix A are zero because there is no change in the response

of the network inside the fuzzy set (a plateau in the response of the network): the response of the network is a constant vector B (e.g. $B^T = [+0.5, -0.5, -0.5, -0.5, -0.5, -0.5]$ corresponds to condition (1), initial native activity). The fuzzy set Z is represented here by a vector $[0.1, -0.5, -0.3]$ that is located in the center of the fuzzy set. It is a kind of prototype of the fuzzy set. Since the input values are scaled to $[-0.5, 0.5]$, the prototype can also be interpreted as followed: burst duration is $MEDIUM$ and interval between two burst is $SMALL$ and maximum amplitude of the burst is $SMALL$.

The rules extracted from the net indicate that it was trained to the point where each class is sharply separated from each other. Since some patterns are overlapping, the network needs a lot of nonlinear regions to separate a pattern that belongs to a class but falls into a region of the input space that would belong alone to another class.

7 Conclusion

In this paper a basic approach for the development of biosensors is presented. Biological neural networks are very sensitive to changes in their chemical environment. The response of the neural network is often substance- and concentration- specific. These biological systems may potentially be used for certain sensory tasks as network biosensors.

Two different artificial neural nets, Kohonen's self-organizing map and Backpropagation, have been investigated for their usage to detect different concentrations based on the signals of cultured biological neural networks. Additionally, the algorithm FAGNIS (Fuzzy Automatically Generated Neural Inferred System) was applied to the backpropagation network to obtain fuzzy rules of the system.

Both algorithms yield the same result: the conditions can be detected with a certain accuracy. The Backpropagation net is able to detect the conditions with an accuracy up to 90 %. The result can be improved using a majority decision for all channels (96 %). A similar result has been obtained using Kohonens SOM. Although the accuracy of the SOM (79 %) is not as precise as the Backpropagation net, the SOM organizes the condition in manner, that following conditions are neighbours. The rules extracted from the net indicate that it was trained to the point where each class is sharply separated from each other. The network needs a lot of nonlinear regions to separate a pattern since some patterns are overlapping.

In addition, we have shown that the activity patterns recorded from some microelectrode sites are more suitable for the recognition of the concentration than the recordings from others. With backpropagation as well as with Kohonens SOM these distinct microelectrode sites can be detected. This important information allows us to reduce the complexity of the processing system and to decrease the processing time.

In conclusion, the results, obtained by using ANNs to interpret the electrical activities of a cell culture corresponding to different episodes are: i) after training the neural net, the evaluation of recorded data can be done on-line, ii) microelectrode sites which are highly correlated to the information about the concentrations within the recorded signals can be identified, iii) the recognition can be improved by using the artificial neural nets responses.

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