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The Use of Artificial Neural Networks as a Component of a Cell-based Biosensor Device for the Detection of Pesticides

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Abstract

The present study describes an artificial neural network (ANN) system that uses a cell-based biosensor based on the Bioelectric Recognition Assay (BERA) methodology, for the detection and classification of pesticide residues in food commodities. The insecticidal compounds carbaryl and chlorpyrifos as well as the pyrethroid group were used as models for the training of the ANN. The biosensor was based on neuroblastoma N2a cells, which are targets of the pesticides due to the inhibition of the enzyme acetylcholine esterase by them. The response of the biosensor to different concentrations (samples) of either pesticide was recorded as a time-series of potentiometric measurements (in Volts). The feedforward methodology was used for the development of the ANN, which was trained with the backpropagation training algorithm. The results of the application of the developed system indicate that the novel classification methodology exhibits promising performance as a central component of a rapid, high throughput screening system for pesticide residues.

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1. Introduction

Food safety control is a major economic activity with a volume of \$2 billion and a growth rate of 12 %.

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There is a considerable demand by the European farming and food industry for technologies, which will allow the testing of agricultural products for the presence of pesticide residues at the site of production. Therefore, rapid pesticide residue testing is necessary, especially in view of the new EU and international regulations for minimal residue concentration in agricultural products.

The Bioelectric Recognition Assay (BERA) refers to a generic biosensor method based on a unique combination of living, physiologically active cells immobilized in a matrix with an electrical sensor system. Cells are selected to specifically interact with the analyte under detection. In this way, when a positive sample is added to the probe, a characteristic, ‘signature-like’ change in electrical potential occurs upon contact between the target molecule and the cells in the gel matrix.

A highly sensitive cellular biosensor has been already developed based on the BERA working principle for detecting organophosphate and carbamate pesticide residues in food commodities [1,2]. The main drawback of this system is the employment of an empirical way to determine the presence of a pesticide in a sample, by examining the biosensor’s response data curve. To overcome this problem, we herewith report the development of a computational classifier system able to interact with the biosensors as a pesticide classification software, able to learn during use and therefore to improve its classification accuracy.

Artificial neural networks (ANNs) are computational models that try to estimate or approximate a function from sample data [3]. In other words, they can be trained with a sufficient number of data to “learn” the process that has produced these data. Their popularity in a large number of application areas was drastically increased after the development of the Backpropagation training algorithm [4].

Two different biosensor-based experimental approaches were taken, targeting either the individual pesticides carbaryl and chlorpyrifos or the pyrethroid group.

2. Experimental Setup

In both experimental approaches, the biosensor was based on neuroblastoma N2a cells, which are targets of the pesticides due to the inhibition of the enzyme acetylcholine esterase by them. Under this condition, treatment of the cells with the neurotransmitter acetylcholine (ACh) would lead to extensive membrane depolarization [1].

2.1. Electrode preparation and cell culture

For the single individual pesticide detection, N2a cells in suspension were mixed with 1,2% (w/v) Bactoagar[®] solution at 37°C and then the mixture was transferred to a specially fabricated 96-well plate (ordered in 12 eight-well arrays), where it was left to solidify at room temperature. Each well contained 0,335ml Bactoagar, 100µl (50 x 10³) cells and 65µl medium. A pair of carbon electrodes was screen-printed on the bottom of each well.

For the pesticide group screening N2a cells in suspension were placed on the top of 8X carbon screen-printed electrodes (DROPSSENS DRP-8X110), 50 x 10³ cells/single electrode.

N2a cells were routinely cultured as reported previously [1]. After cell detachment from the culture were concentrated by centrifugation (2 min, 1200 rpm, 25°C), at a density of 2,5 x 10⁶ ml⁻¹. During each assay (see below, 2.2) cells were used at a density of 1000/µl .

2.2. Electrochemical experiments with cells

The response of the biosensor to different concentration (samples) of either pesticide was recorded as a time-series of potentiometric measurements (in Volts). Three different concentrations of either carbaryl or chlorpyrifos we measured: 0 ppm (Control), 0.005 ppm and 0.01 ppm. The pyrethroid group was composed of a mixture of eight pyrethroid pesticides (acrinathrin, cyfluthrin, cyhalothrin-lambda, cypermethrin, deltamethrin, fenpropathrin, fenvalerate, flucythrinate), each at the concentration

corresponding to the lowest Minimum Residue Level (0.01 ppm). The sample volume ranged from 5 μ l (pyrethroids) to 20 μ l (carbaryl, chlorpyrifos). Ach (10mM) was added at respective volumes.

The 96-well plate (“disposable biosensor plate”) was placed on a customized device (Conductive Technologies, PA), which comprised the PMD-1608FS A/D card (Measurement Computing, Middleboro, MA). Consequently, 12 converters were required in order to simultaneously measure signals from all 96 wells of the sensor. The software responsible for the recording of the signal and processing of data was InstaCal (Measurement Computing). For the screening of pyrethroids group a customized UNISCAN (Buxton, UK) potentiostat with an 8-channel DROPSENS (Asturias, Spain) adaptor was used. The duration of each measurement was 180sec and 360 values/sample were recorded.

2.3. ANN design and training

Individual pesticides: Based on the recorded potentiometric measurements of the responses of the biosensor, two data sets were created, consisting of 104 and 121 time-series for carbaryl and chlorpyrifos, respectively. Each time-series contained 181 data samples. From each time-series, we constructed specific meta-data that constituted the inputs of the ANN model. The meta-data that gave the best performance were the following:

- average and standard deviation of all data samples (timeseries).
- dividing the data samples into 4 equal length segments and taking the average and standard deviation of each segment
- minimum value of all data samples
- maximum value of all data samples

Thus, these 12 characteristics of each time-series constituted the inputs of the developed ANN. The two outputs of the ANN corresponded to each one of the two possible cases (existence of either carbaryl or chlorpyrifos). The feedforward NN methodology was chosen, while the backpropagation training algorithm was used for the training of the developed model.

Several experimentations were conducted with the training set, for the discovery of the best ANN architecture, focusing on three main aspects of the ANN: i) the design of the network (number of hidden nodes of one or two hidden layers), ii) the algorithm used by the backpropagation methodology in the error-minimization process during training (steepest-descent, quasi-Newton, Levenberg-Marquardt or conjugate-gradient algorithms) and iii) the type of activation function in the hidden nodes (logistic or hyperbolic tangent (*tanh*) functions). We concluded that the best model was a one-hidden-layer ANN with 5 hidden nodes trained with the Levenberg-Marquardt algorithm.

Pyrethroid group: the time-series contained 360 data samples. The ANN regarding the detection of pesticides of the pyrethroid group used two additional inputs: the age of the cells (in days) and their generation number (four different generations of clonal propagation). The best neural model consisted of one hidden layer with 48 nodes, trained with the Levenberg-Marquardt algorithm.

3. Results

The best combinations of ANN architectures and minimization algorithms for the training process were further trained and tuned, leading to the development of the final ANNs. The performance of these models was evaluated using new data, different than those used for the training process (namely, the testing sets).

Concerning the ANN for the individual pesticides detection, the results on the first column of Table 1 show its performance using the logistic activation function in the hidden nodes of the network. The performance of the model in the detection of carbaryl is significantly high (correct classification on 29 out of 30 time-series, a 97% success rate), but the corresponding success rate in the case of chlorpyrifos is quite low (27%). By changing the activation function of the ANN from logistic to hyperbolic tangent

(*tanh*), the overall performance of the system was drastically improved. As it can be seen in the second column of Table 1, with this change in the activation function, a substantial increase in the correct classification of chlorpyrifos (from 27% to 53%) was achieved, while the decrease of the already quite high percentage of correct classification of carbaryl was rather low (from 97% it went down to 90%).

Table 2 shows the percentages of correct detection of pesticides of the pyrethroid group. The correct classification of samples with no pesticides was 93.3%, while the success rate was 76.7% in the case of samples containing some pesticide, giving an overall correct classification percentage of 85%.

Table 1. Correct classifications for carbaryl and chlorpyrifos (number of samples and corresponding percentages)

	ANN with <i>logistic</i> act. fn.	ANN with <i>tanh</i> act. fn.
carbaryl	29 / 30 (97%)	27 / 30 (90%)
chlorpyrifos	8 / 30 (27%)	16 / 30 (53%)
Overall	37 / 60 (62%)	43 / 60 (72%)

Table 2. Correct classifications for the pyrethroid group (number of samples and corresponding percentages)

Control (negative) sample set	28/30 (93.3%)
Positive sample set	23/30 (76.7%)
Overall	51 / 60 (85%)

4. Conclusions

A novel methodology based on the classification capabilities of artificial neural networks is proposed, as a replacement of the empirical way to determine the presence of a pesticide in a sample by examining the biosensor's response data curve. The results of the application of the proposed ANN system indicate that the novel classification methodology exhibits promising performance as a central component of a rapid, high throughput screening system for pesticide residues. Its performance could be further improved by the introduction of a sophisticated decision support system (DSS) that would perform the final classification based on the exact output values of the ANN.

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