Detection and prediction of concentrations of neurotransmitters using voltammetry and pattern recognition

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***Abstract*— Neurotransmitters (NTs) are substances in the brain which are responsible for the transmission of neurological impulses. Changes in their concentrations are associated with numerous behavioral and physiological processes and neurological disorders. As opposed to the traditional chromatographic and capillary electrophoresis, using electrochemical sensors is a fast and inexpensive way to determine concentrations of NTs. In this study we measure the combination of dopamine (DA) and serotonin (SE) with glassy carbon electrodes and differential pulse voltammetry. The major challenge using this method is to differentiate between different NTs, since the signal obtained from the electrode represents the interactive effect of both NTs present. We address this problem through methods of pattern recognition which relate the voltammetric measurements provided by the sensor to the concentration of individual NTs. Two methods of pattern recognition were applied (PCR and PLS-regression). The best rates of correct classification for the validation sets ranged at 42-62% (DA) and 33-50% (SE). When the ranges for correct prediction were extended to include one level above and below the true concentration level, the rates values ranged at 81-91% (DA) and 91-100%(SE). These findings suggest that pattern recognition can be used to model the interaction between different neurotransmitters to predict actual concentrations of neurotransmitters using voltammetry.**

I. Introduction

NEUROTRANSMITTERS (NTs) are naturally occurring chemicals secreted in the brain which are responsible for the transmission of neurological impulses in the central nervous system. Changes in NTs levels are associated with numerous behavioral and physiological processes and neurological disorders including epilepsy, Alzheimer and Parkinson diseases, schizophrenia, depression, etc [1-3]. Examples include nitric oxide (NO), serotonin (SA), dopamine (DA), norepinephrine (NE), acetylcholine and glutamate. These NTs coexist in biological fluids (cerebrospinal fluid, blood plasma, urine and saliva) and their accurate measurement is of great practical importance in the field of biomedical chemistry, neurochemistry and clinical diagnosis. Development of efficient methods to accurately determine concentration of a specific NT or a group of NTs is, therefore, on the edge of the current biomedical research. Speed, sensitivity, cost and real time monitoring are the major factors responsible for the efficiency of methods for quantitative measurements of NTs in the central nervous system. Traditionally, concentrations of NTs are determined using chromatographic and capillary electrophoresis methods [4]. These techniques are complex, time-consuming and are not easily adaptable for real-time and *in-situ* monitoring. Moreover, their presence at very low concentrations and variable levels provide a great challenge for their detection, requiring that highly sensitive and selective methods be available. A number of NTs, such as the ones targeted in this project (e.g. DA, SA) are electrochemically active and therefore they can be determined directly by electrochemical means. A fast and inexpensive way to determine concentrations of NTs is to use electrochemical sensors, which are small and easily implantable. Measurement of neurotransmitters levels with voltammetry and carbon electrodes is an example of this method [5, 6].

In general the proposed method belongs to the electronic tongue technology which combines one or more electrodes with pattern recognition algorithms that separate the "true" signal from the detailed sensor response to complex, real-world samples that typically contain interfering species. Like the electronic nose, many of the applications of electronic tongues are related either to environmental monitoring, medical applications, or to food and beverage production. Electronic noses/tongues have been used in complex, real world environments where sensitivity as well as specificity must be considered, such as detection of chemical warfare agents, indoor air quality, and bacterial classification [7-9]. Many electronic tongues employ voltammetry for analyte detection at individual electrodes [10].

Electrodes have previously been used before for simultaneous detection of dopamine and serotonin *in vivo* [5]. Although previous analyses showed the existence of different patterns in the voltammetric readings for the

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experiments with only one (serotonin or dopamine) or both neurotransmitters present, they did not use this information to predict the exact concentrations of neurotransmitters. The contribution of this study is the ability to apply pattern recognition methods to voltammetry data to accurately and simultaneously predict the concentrations of several neurotransmitters.

II. Data

The data was obtained by Differential pulse voltammetry (DPV). Conventional glassy carbon electrodes (GCE) electrodes were used to obtain voltammetric data for dopamine (DA) and serotonin (SE) in three separate trials. The experiment was conducted in the laboratory settings at the Chemistry and Biomolecular Science Department. The whole set of DPV data consisted of three trials where each trial had 21 combinations of relevant concentration levels for the two neurotransmitters (serotonin and dopamine). The data was acquired with a bare GCE which had been polished for 3 minutes with 0.3μm alumina, sonicated in distilled water for 5 minutes, and rinsed three times with distilled water and then methanol each time. The differential pulse voltammograms were done in a 0.1M solution of PBS with a pH of 7.0. Pulse amplitude of 50mV, pulse width of 50ms and pulse period of 200ms were employed.

The concentration of each neurotransmitter was held constant started at 20 micromolars (μM), 40 and 60 μM while the concentration of other neurotransmitter was increased starting at 20 to 100 μM in with intervals of 20 μM. All together there were 63 voltammograms each representing a combination of the two controlled concentrations of DA and SE. An example of a voltammogram is given in Fig. 1.

III. Method

The goal of this study is to use the multidimensional data of the voltammogram to predict concentrations of both neurotransmitters (serotonin and dopamine) at the same time. This is a challenge since sensor produces mixed signal from both substances present in the sample.

Votammetric data contained over 1000 measurements (original features) per each combination of NTs. They all contribute differently to the response variable and if used for modeling altogether could cause overfitting and, therefore, inadequate prediction. The process of extraction of the few latent variables (dimensionality reduction) is a crucial step toward efficient predictive model. Principal component analysis (PCA) is a popular approach to this problem. During PCA the major principal components that lie in the direction of the greatest variance of the voltammetric data ***X*** (predictors) are extracted, thus, retaining the most information related to the data. Another suitable approach is the partial least squares (PLS) regression. The goal of PLS-regression is to extract components (latent variables) that lie in the direction of, i.e. explain most of the covariance

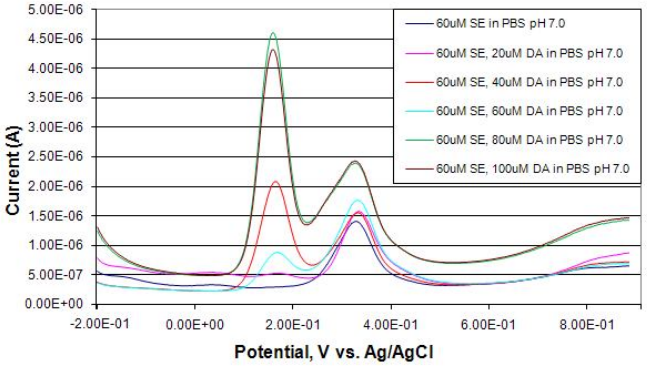

Fig. 1. Differential pulse voltammogram for a fixed SE level (60 μM), changing SE level (20-100 μM). The interactive action of the two NTs on the current's response makes it hard to distinguish between individual NT's actions.

between the predictors ***X*** (voltammetric measurements) and responses ***Y*** (NTs concentrations) variables. This approach is highly beneficial as captures the relationship between predictors and responses while performing dimensionality reduction.

The extracted features are then fitted into the simple linear regression model using the training set. The PCA followed by this step is called Principal Component Regression (PCR). Another approach applied to these data, PLS-regression, combines the two steps of dimensionality reduction and regression fitting. The constructed model is tested on its predictive power using the validation set.

IV. Results

For this study, two of the trials were taken for training and the remaining one trial was considered as a validation set. Although the controlled neurotransmitters concentration levels were discrete values (20, 40, …, 100 μM), the predictive models produced real numbers which were rounded to the nearest controlled level in order to estimate the models' performance.

The results of the application of PCR based on 7 principal components are shown in Table I. Separate models were

TABLE I
BEST PCR MODELS PERFORMANCE

	Training and validation sets	DA		
		P	**P***	**R^2**
PCR on normalized data	Training 1&2	0.5714	0.9286	0.6935
	Validation 3	0.6190	0.8095	0.6084
	Training 1&3	0.5000	0.9286	0.6855
	Validation 2	0.4762	0.9048	0.7101
	Training 2&3	0.5000	0.9286	0.6855
	Validation 1	0.4286	0.9048	0.6181
	Training and validation sets	**SE**		
		P	**P***	**R^2**
PCR on the original data	Training 1&2	0.8571	1.0	0.8896
	Validation 3	0.3333	0.9048	0.8390
	Training 1&3	0.8571	1.0	0.8896
	Validation 2	0.5000	0.9524	0.8502
	Training 2&3	0.6429	1.0	0.7987
	Validation 1	0.4762	1.0	0.8359

created for dopamine and serotonin. The normalization (by mean centering and standard deviation scaling) of the data for the dopamine models improved the results for the PCR method. Table I shows the best results for the DA and SE models using PCR.

The results of the simultaneous fitting and prediction of both neurotransmitters by the PLS-regression based on 7 extracted latent variables are shown in Table II.

The overall results from PCR and the PLS-regression

TABLE II
PLS MODELS PERFORMANCE

Training and validation sets	DA		
	P	**P***	**R^2**
Training 1&2	0.5952	0.9286	0.6459
Validation 3	0.5238	0.8571	0.5240
Training 1&3	0.4285	0.9048	0.6040
Validation 2	0.4286	0.8571	0.4710
Training 2&3	0.5952	0.9524	0.6003
Validation 1	0.1667	0.9286	0.5972
Training and validation sets	**SE**		
	P	**P***	**R^2**
Training 1&2	0.6905	1.0	0.9283
Validation 3	0.2857	0.7619	0.7933
Training 1&3	0.7857	1.0	0.9104
Validation 2	0.3333	0.8095	0.6509
Training 2&3	0.5476	0.9762	0.7518
Validation 1	0.3571	1.0	0.8745

provided the following accuracy for the neurotransmitters concentration prediction. For PCR models the rates of correct classification (***P***) for the validation sets ranged at 42-62% (DA) and 33-50% (SE) while the average rates were 50.7% and 43.64% respectively. When the ranges for correct prediction were extended to include one level above and below the true level, the correct classification rates (***P****) for

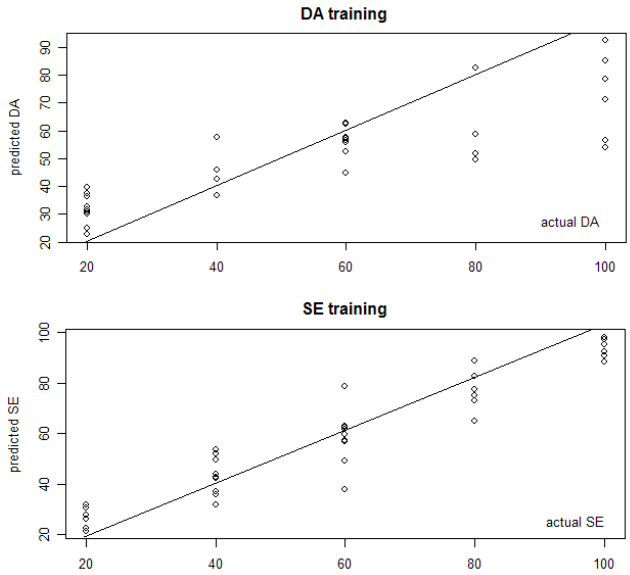

Fig. 2. Fitting of the training data set for the first model, PCR on 7 components. 45 degree reference line indicates the perfect fit.

the validation sets ranged at 81-91% (DA) and 91-100% (SE), with corresponding average values 87.3% and 95.24%. For PLS-regression correct classification rates (***P***) ranged at 16.7-52.4% (average 37.3%) for DA and 28.6-

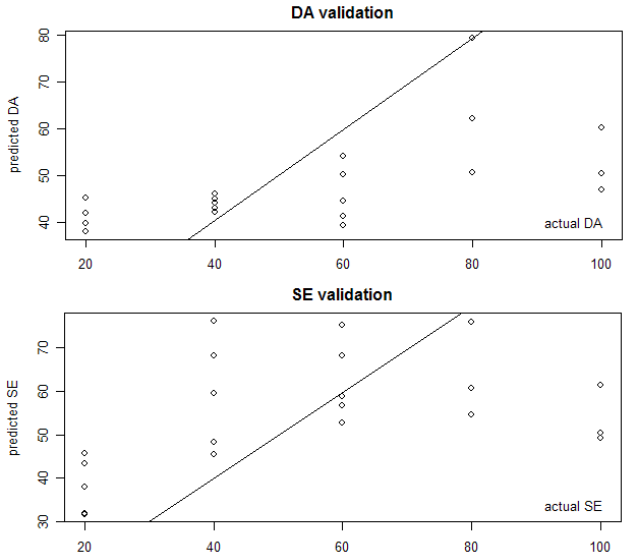

Fig. 3. Prediction of the validation data set for the first model, PCR on 7 components. 45 degree reference line indicates the perfect prediction.

35.7% (average 32.5%) for SE, whereas ***P**** values ranged at 85.7-92.9% (average 88%) and 76.2-100% (average 85.7%) respectively. Both methods provided very similar linear fit as shown by coefficients of determination (***R^2***) for the training data sets. Fig. 2 shows an example of the PCR model fitting of the original, untransformed data. The plotting of actual versus predicted concentration levels reveals good fit of the applied methods. Similar plots for the validation sets demonstrate relatively good predictive power of the models. An example is shown in Fig. 3 for the first PCR model. Additional results were obtained for prediction of the difference of the dopamine and serotonin concentrations (DA-SE) using the same data. Linear PCR provided a coefficient of determination of about 89%, thus, indicating a very good linear fit. These findings suggest using information on the interaction between different neurotransmitters in the prediction of actual concentrations of neurotransmitters. The overall results of the study are very encouraging and, in general, support the idea of accurate prediction of the neurotransmitters concentrations using voltammetry.

V. CONCLUSION

In this study two pattern recognition methods (principal component regression and partial least squares regression) were applied to the data obtained by differential pulse voltammetry using conventional glassy carbon electrodes for two neurotransmitters: serotonin and dopamine, for the purpose of prediction of neurotransmitters concentrations. Both methods showed similar results in the study for prediction of dopamine and serotonin with PCR providing slightly better results for the validation sets, resulting in the average rates of correct classification of 50.7% (DA) and

43.6% (SE). The average extended rates of correct prediction (including one level above and below the true level) were 87.3% and 95.2% respectively. These findings allow us to establish the lower bound for the subsequent analysis of the similar data.

The following problems have complicated the analysis. The experiments were subject to the passivation effect that was not completely accounted for. Passivation is the absorption of the reactive species to the electrode; when the same electrodes are used for the next trial this causes the reduction in sensitivity. This problem contributed to the errors in prediction. The next stage of the predictive model will incorporate the passivation of the electrode and thus eliminate some of the problems that generate inadequate analysis.

In addition, miniaturization of the sensor (e.g use of carbon-fiber microelectrodes, Pt wire) and another choice of the electrode material (carbon nanotubes, metal nanoparticles used as modifiers) together with a more careful optimization of the experimental conditions will contribute to the accuracy of prediction of the concentrations of neurotransmitters and are left for future work.

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