

Supporting Information for

Novel, User-friendly Experimental and Analysis Strategies for Fast Voltammetry: 2. Next Generation FSCAV with Artificial Neural Networks

Sergio Mena,¹ Marco Visentin,¹ Colby E. Witt,² Lauren E. Honan,² Nathan Robins,¹ & Parastoo Hashemi^{1,2}

¹ Department of Bioengineering, Imperial College London, SW7 2AZ UK.

² Department of Chemistry and Biochemistry, University of South Carolina, SC, 29208 USA.

1. GitHub code repository of the web application. The web application code, including the code used for FSCAV analysis can be accessed at the following [link](#).

2. Scatter graphs of features used to predict serotonin absolute concentration.

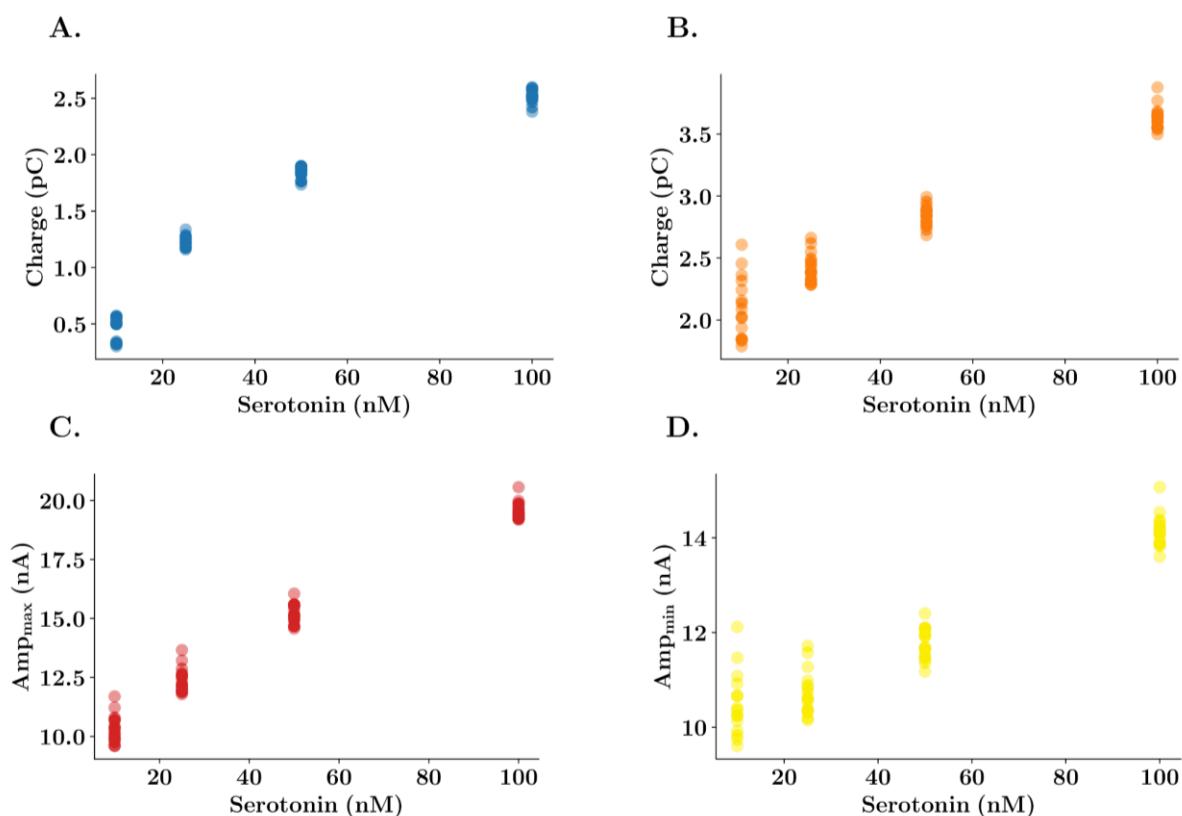


Figure C.1: Scatter graphs of cyclic voltammogram features of the serotonin faradaic peak (see Figure 5 of manuscript) for a representative post-calibration. (A) Charge vs. concentration for the charge above the baseline of the integration points (Pearson's $r = 0.95$). (B) Charge vs. concentration for the charge calculated below the baseline of the integration points, which commonly has a higher interference from the capacitive peak (Pearson's $r = 0.97$). (C) Current vs. concentration for the maximum amplitude of the serotonin faradaic peak (Pearson's $r = 0.99$). (D) Current vs. concentration for the valley point between the faradaic and the capacitive peak. In all cases, the correlation between the feature and serotonin absolute concentration *in vitro* is clear (Pearson's $r = 0.95$).

3. Training results of standardised neural network.

Figure C.2 shows a representative example of the training of the standardized neural networks for the last cross validation split with and without the background current charge as input parameter. The training loss per epoch show a similar trend in both cases, and the testing predictive error is also similar and not statistically significant across all the k-fold dataset splits ($n = 5$ cross-validation splits, RMSE = 3.84 ± 0.24 nM vs. 4.10 ± 0.48 nM, $p = 0.6452$). Training results of electrode-specific neural network models depend on the specific electrode used and are available upon request.

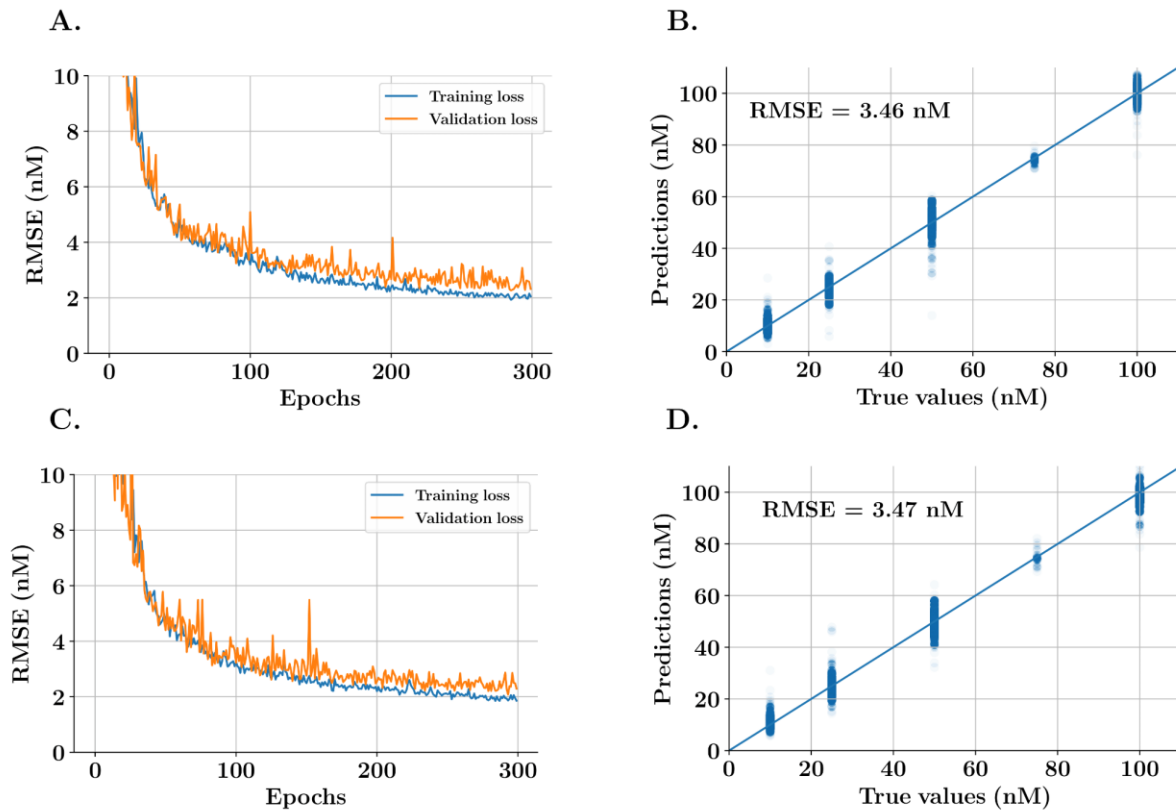


Figure C.2: Training and validation loss and test predictions for standardised neural networks training. (A, C) Root mean square error (cost function) progression by iteration of the training and validation datasets for the last k-fold of the standardized neural networks training. (B, D) True vs. predicted values of the test dataset for the last k-fold of the standardised neural network training. The blue vertical line shows the ideal response, where true values are equal to predicted values. In panel A and B, the results are shown for the standardised neural network, while panel C and D show the results after the inclusion of the background current charge feature at the input.

4. ANOVA analyses on the error of the estimate and variability of FSCAV calibration methods. Five representative electrodes were used to compare the predictive error and variability in the predictions of the linear regression and neural networks for the calibration of FSCAV signals. After training, the root mean square error (RMSE) between predictions and true concentrations of serotonin *in vitro* was calculated across the models.

Table S.1: List of names of groups and indexes of the analysis of variance.

Group	Number
Linear regression	1
Single electrode neural network	2
Pretrained neural network	3
Standardised neural network	4

Table S.2: Analysis of variance results for the RMSE of predictions (Figure 3).

Source	Sum sq.	d.f.	Mean sq.	F statistic	Prob > F
Model	102.2995	3	34.0985	12.4015	1.9111e-04
Error	43.9927	16	2.7495	-	-
Total	36.3537	19	-	-	-

Table S.2 shows that a significant effect of the model used for calibration was found in the variability of the error of the estimation obtained from *in vitro* postcalibrations. After that, a *Tukey-Kramer post-hoc* multiple comparison test was performed. The full matrix of multiple comparisons p values is shown in **Table S.3**, and p values in bold are reported in the main manuscript.

Table S.3: Analysis of variance results for the RMSE of predictions (**Figure 3**). Probabilities in bold text are of interest.

Tukey-Kramer post-hoc multiple tests Pr > t for H ₀ : <i>Mean RMSE (i) = Mean RMSE (j)</i>				
i/j	1	2	3	4
1	-	0.0023	1.6306e-04	0.0029
2	0.0023	-	0.5449	0.9996
3	1.6306e-04	0.5449	-	0.4869
4	0.0029	0.9996	0.4869	-

As shown in **Table S.4**, the effects of electrodes and model applied do not have a significant effect on the variability (standard deviation of repetitions for same concentration *in vitro*) of the predictions. The reduction of the error of prediction comes from a better fit of the model to the experimental data.

Table S.4: Two-way analysis of variance results for the standard deviation of predictions for equal concentration.

Source	Sum sq.	d.f.	Mean sq.	F statistic	Prob > F
Electrode	95.4993	3	31.8331	1.7611	0.1643
Model	25.3395	4	6.3349	0.3505	0.8427
Interference	21.3619	12	1.7802	0.0985	0.9999
Error	1.0845e+03	60	18.0758	-	-
Total	1.2268e+03	79	-	-	-

5. ANOVA analyses on the *in vivo* basal estimations using linear regressions and the standardised neural network model. Five *in vivo* acquisitions calibrated with electrode-specific linear regressions and the standardised neural networks were used to compare the basal predictions of both calibration methods. To achieve this, a repeated measurements ANOVA with features of time after treatment and calibration model were used.

Table S.5: List of names of groups for the analysis of variance.

Within groups
Control (0-30 min)
Saline (30-60 min)
ESCIT 120 min
Effects
Linear Regression
Neural Network

Table S.6: Analysis of variance results for the estimations of absolute concentration of serotonin (**Figure 5**).

Source	Sum sq.	d.f.1	d.f.2	Mean sq.	F statistic	Prob > F
Time	3.7487e+04	61	244	614.5550	2.1816	0.000015
Model	2.0919e+03	1	4	2.0919e+03	0.3644	0.578607
Interference	1.2479e+04	61	244	204.5806	1.1859	0.185256

Table S.6 shows that a significant effect of the model used for calibration (linear regression and standardised neural network) and the treatment (control, saline and ESCIT (10 mg/kg) administration) was found in the basal *in vivo* estimations of serotonin ambient levels. After that, a *Tukey-Kramer post-hoc* multiple comparison test was performed. An extract of the matrix of paired multiple comparisons p values are shown in **Table S.7**, and p values in bold are reported in the main manuscript.

Table S.7: Analysis of variance results for the *in vivo* predictions of absolute serotonin concentration. Probabilities in bold text are of interest. An extraction of the pairwise comparison within and between groups is provided for ease of read (large number of groups were compared, including the interactions). All probability values are available upon request.

Tukey-Kramer post-hoc paired multiple tests		
Pr > t for H₀: Mean serotonin (i) = Mean serotonin (j), within groups		
Group i	Group j	P-value
Control (0-30 min)	Saline (30-60 min)	0.9981
Control (0-30 min)	ESCIT 120 min	0.0124
Saline (30-60 min)	ESCIT 120 min	0.0243
Pr > t for H₀: Mean serotonin (i) = Mean serotonin (j), between groups		
Group i	Group j	P-value
Linear Regression	Neural Network	0.7200
Pr > t for H₀: Mean serotonin (i) = Mean serotonin (j), interactions		
Group i	Group j	P-value
Linear Regression, Control (0-30 min)	Neural Network, Control (0-30 min)	0.5731
Linear Regression, Escit 120 min	Neural Network, Escit 120 min	0.6841
Linear Regression, Control (0-30 min)	Linear Regression, Escit 120 min	0.0314
Neural Network, Control (0-30 min)	Neural Network, Escit 120 min	0.0257

6. Video Tutorial of *The Analysis Kid* FSCAV Application. A video tutorial of the FSCAV application was hosted in YouTube at the following [link](#). The video tutorial intends to show users the main features of the FSCAV application, supported files and how to navigate the different features of the application.

7. Experimental and Computational Requirements for *The Analysis Kid* FSCAV Application. As described in the video tutorial, the FSCAV application has 4 different calibration methods available. These calibration models are explained in the manuscript (see Computational Methods section). Below, we explain the experimental and computational requirements of each of the models. Example files, as well as information on supported browsers to run the application are available in the [homepage](#).

Linear regression model

The linear regression is a generic model; in theory it can be used for any experimental procedure (e.g., carbon fibre microelectrode characteristics, solution buffers) and analyte as long as cyclic voltammograms have a Faradaic peak correlated to tonic concentration. This model requires an FSCAV *in vitro* post-calibration in the form of background subtracted FSCAV cyclic voltammograms for a range of analyte concentrations. The video tutorial mentioned above provides guidance in how to get a calibration curve and apply the calibration factors to cyclic voltammograms with unknown concentration (e.g., *in vivo* acquisitions).

Single electrode neural network

The single electrode neural network is also a generic model and can effectively be used as the linear regression for any experimental paradigm and analyte. As the linear regression, it is electrode specific. This means that it is trained with a post-calibration from an electrode. There is, however, one difference with respect to the linear regression: the complexity of the model is much higher. This means that the NN model is much more susceptible to overfitting when the training set is small. To avoid this, the number of repetitions per concentration should be enough (e.g., in our case, 15 repetitions are obtained for each of the serotonin solutions prepared). The layer size of the neural network, number of iterations, learning rate can also be modified to adapt to the needs of the user. Gaussian dropout and Gaussian noise can also be configured in the application to avoid overfitting. As for the linear regression model, the video tutorial provides a step-by-step guide in how to get started by fitting the model and use it to predict other signals.

Pretrained neural network

The pretrained neural network, as described in the manuscript, has been pretrained with 140 post-calibrations of tris-buffered serotonin from different electrodes. After that, it is expected to be finely tuned (trained for a limited amount of iterations) with a post-calibration from a particular electrode. Since the neural network has been pretrained with specific parameters and signals, there are computational and experimental requirements to follow in order to use it. A summary of the main specifications required can be found below.

1. Carbon fibre microelectrodes used in the training dataset are cut to a length of 150 μm and coated with electrodeposited Nafion polymer. See the Experimental Section for a full description of the fabrication procedure.
2. Four tris-buffered serotonin solutions (10 nM, 25 nM, 50 nM and 100 nM) were used to generate the training dataset. This means that when using this calibration model only serotonin can be detected, and the predictive error would be optimal for that range of concentrations.

3. The “Jackson” waveform with a frequency of acquisition of 500 kHz was used to register the cyclic voltammograms. This gives cyclic voltammograms of 1100 samples.¹ See the Experimental Section for more information on the waveform application.
4. Cyclic voltammograms in the training set are background subtracted with an average ($n = 10$) background voltammogram from the 2 seconds of acquisition before the adsorption period (see Experimental Section). Both the cyclic voltammograms for training and prediction used with this model should have an analogous background subtraction.
5. The neural network is pretrained with a learning rate of 0.001 and layer size of 64 nodes. The fine-tuning process should then be performed with the same training parameters.

Standardized neural network

The standardized neural network is fully trained with the same dataset as the pretrained neural network. This means that the required specifications stated above remain necessary for this model. Although no post-calibration is needed for this calibration model, it is paramount that the experimental procedure followed for the training signals is also followed for the signals uploaded for prediction to minimize the error of the predictions.

¹ When a different acquisition frequency is used, which results in a lower number of samples, interpolation can be used to convert the cyclic voltammograms to 1100 sample time series.