Imperial College London

Planning Report for MSc Project

Experimental and Analysis Methods for Identification of Serotonin and Histamine Release from Skin and Hair Cultures

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Chapter 1

Project Specification

1.1 Background

The studies completed in the past decades on serotonin levels on psychiatric disorders suggest that there is a relation between the neurotransmitter and depression, schizophrenia, or autism [16]. Michael J. Owens et al. showed that the decrease in serotonin (5-HT) as well as of 5-hydroxyindoleacetic acid (5-HIAA), which is a metabolite of serotonin, is decreased in patients suffering from depression [16]. They reported that selective serotonin reuptake inhibiting (SSRI) medication is an effective way to treat depression [16]. Abi-Dargham et al. showed that serotonin plays a role is schizophrenia and the evaluation of medication used should be analysed more [5]. Studies have shown that administration of SSRI drug has an effect on how emotional information is processed in the brain [15]. Such effects have been studied in past 50 years and although SSRI medication was shown to be working, further analysis and research is needed to understand the relation [7]. Finally, a review of pharmacological treatment of anxiety disorders concluded that there are limitations and gaps in research of guided treatments in such disorders [10].

The development of new techniques for serotonin measurements in the brain is motivated by its role in mental disorders. As such, the detection of chemicals in the brain have been studied for 20 years. The fast-scan cyclic voltammetry (FSCV) was first applied for the detection of dopamine using Carbon Fiber Microelectrodes (CFM) [18]. The recent advances in FSCV allowed for measurements to be completed on substances such as serotonin or histamine [17]. Nevertheless, measuring serotonin still pose difficulties as 5-HT plays a role in fast deterioration of electrode quality [9]. Hashemi et al. applied Nafion coating to carbon fiber microelectrodes and showed that this layer is effective in reducing damage to electrodes [9]. Similar methods which include ambient serotonin levels have been developed. Fast-scan controlled absorption voltammetry (FSCAV) has been developed to measure ambient concentrations of substances. While FSCV can capture the concentration changes, the mentioned technology can provide insight into ambient concentration in cells [4].

The FSCV has been used to research serotonin relation to depression in mice. The measurements have been completed to determine a difference in the serotonin concentrations in the male and female mice brain. Research has found that there is little difference in serotonin concentrations between different genders [20]. Additionally, study was done to determine the SSRI as well as other medication effects on mice serotonin levels, showing the changes after acute dosage of SSRI [22]. However, these findings are based on research on mice and there is lack of research which would help to correlate them to humans.

The statistical analysis and data integration with machine learning algorithms pave the way for automated peak detection. However, these methods still pose significant challenges [17]. Given the importance of accurate and reliable technology for serotonin measurements, it is crucial to improve current microelectrodes and apply machine learning and statistical analysis on the collected data.

There has been attempts to use convolutional neural networks to detect chemical release automatically without a need of manual evaluation. Given that the manual evaluation of FSCV results is time consuming and requires a lot of effort, there is a motivation for development of automation tools [14]. Convolutional neural network has been built to detect the dopamine release in data collected from FSCV and achieved the result of around 98.31% accuracy during testing [14]. However, there is still a need to develop a more universal model which could be applied to serotonin or histamine release.

1.2 Aims & Objectives

While FSCV is an effective method of analysing the the dopamine levels in vivo, there is a need for further development of electrodes which would measure the 5-HT [9]. As described in previous section, there has been research completed on measuring serotonin levels in relation to depression using FSCV. However, these results are difficult to correlate to humans because they have been derived from experiments on mice. As such, the Hashemi Lab at Imeprial College London has started to measure the chemicals in human derived cells. Given that carbon fiber electrodes are complicated to use in cell cultures due to their shape, there is a motivation to develop planar electrodes where the cells would grow. Therefore, one of the aims of this projects is to develop carbon paste microelectordes.

The raw data of FSCAV/FSCV only shows levels of extracellular serotonin or the change in its concentration. Whereas, the development of neural network which would be applied to the cell measurements would allow to look at the excitability of neurons. Therefore, this project aims to construct a convolutional neural network (CNN) model to perform such analysis.

The objectives with a clear development steps are as follows:

- Research the work on serotonin effects on mental disorders, techniques for measurements in vivo and artificial neural network application in analysis of the data.

- Develop a neural network model to predict serotonin/histamine release in images obtained from microelectrode scans.
- Test and optimise the model to perform well on training data.
- Develop carbon paste microelectrodes.
- Perform measurements using newly developed microelectrodes.
- Process and analyse the collected data using neural networks.

In conclusion, the satisfactory results of the project will include microelectrodes which are able to measure serotonin release with high precision as well as the development of model which can be used to identify release of substances in a later research.

Chapter 2

Ethical Analysis

The experiments conducted during the development of the project are subject to ethical considerations. This chapter will describe the ethical implications on experiments and data used throughout the project and provide ways in which violation of College Ethics code is prevented [12]. The chapter is separated into 3 sections: considerations for experiments, data management, and long term effects of the project.

2.1 Experimental Ethics

The experiments are separated into two subgroups: lab and computational. The laboratory experiments are carried out by using FSCV measurements on serotonin and histamine measurements using carbon paste electrodes achieved in a flow cell analysis. As such, the substances are artificial and therefore does not include any subjects. Similarly, the technology used as well as all the substances are free from animal products.

Given that the carbon paste electrodes are developed from scratch, it produces considerable amount of waste. Specifically, such waste is produced from failed development of electrodes as well as iterative approach on development. For this reason, in accordance with College guidelines, following actions are taken: minimising energy use during the development, reuse and recycling of substances where possible, and disposal of any substance or material in an appropriate way.

According to Ordinance D17 [13], any misconduct relating to environmental damages will be reported to the supervisor.

2.2 Data Management

Computation experiments which are conducted on data gathered during laboratory experiments are subject to ethical considerations. Given that the data does not belong to any subject, the protection

of identity and personal data is not required. However, the care is taken to ensure that any data or analytical results are protected and do not leak. Additionally, precaution is taken to ensure that any experiments on data are tested and verified and does not produce false information.

2.3 Long term effects

As mentioned in Chapter 1, the analysis of serotonin release in images obtained by FSCV is time consuming and requires a lot of effort. Similarly, there is a gap in research which explains how SSRI medication works precisely. As such, Hashemi lab is conducting a long-term research on serotonergic dynamics in the brain. The development of carbon paste electrodes as well as analysis of data using convolutional neural networks will assist the research and therefore outweighs the damage done to the environment.

Chapter 3

Literature Review

3.1 Convolutional Neural Networks

The convolutional neural networks are used in wide range of problems as an autonomous way to classify data. Such artificial networks have shown accurate results in analysing image data [6, 19, 21].

There has been many research projects completed using CNNs to analyse breast cancer image data, EEG data on seizures, and dopamine release among other applications. In general, medical data analysis have seen a lot of success when using the method of convolutional neural networks to develop models [8, 11, 23]. The research on breast cancer image classification developed a neural network to classify image data and reported the results as being more accurate when compared to other machine learning methods used for similar tasks [21]. The structure of the convolutional network is shown in the Figure 3.1. The main layers contain convolutional layer in combination with pooling layer with varying kernel sizes. Final layers of the network contain dense layers which connect each neuron from preceding layer.

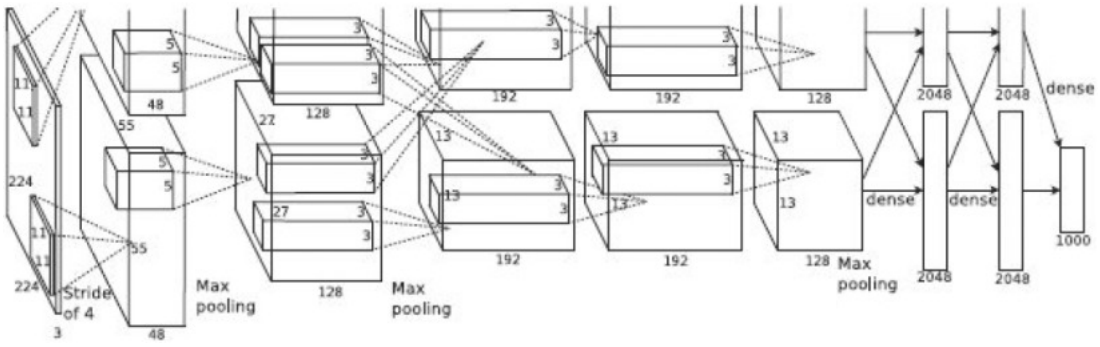

Figure 3.1: CNN architecture developed in [21]

Similarly, the first convolutional network based system to analyse EEG data in seizure cases [6] managed to achieve 88.67% accuracy using the model structure presented in Figure 3.2

As with CNN for breast cancer research, the structure of the model developed for EEG data is

Layers	Type	Number of neurons (output layer)	Kernel size for each output feature map	Stride
0-1	Convolution	4092 x 4	6	1
1-2	Max-pooling	2046x 4	2	2
2-3	Convolution	2042 x 4	5	1
3-4	Max-pooling	1021 x 4	2	2
4-5	Convolution	1018 x 10	4	1
5-6	Max-pooling	509 x 10	2	2
6-7	Convolution	506x 10	4	1
7-8	Max-pooling	253 x 10	2	2
8-9	Convolution	250x 15	4	1
9-10	Max-pooling	125x 15	2	2
10-11	Fully-connected	50	-	-
11-12	Fully-connected	20	-	-
12-13	Fully-connected	3	-	-

Figure 3.2: CNN architecture developed in [6]

similar. It consists of convolutional layers followed by pooling layer of different sizes. The final three layers are fully connected.

From the researches presented above there can be seen a pattern of how the neural networks are constructed; however, while dealing with image data, these experiments do not measure the release of substances.

The research has been conducted on dopamine release data using convolutional neural networks [14]. The researchers found a 98.31% accuracy in FSCV images for dopamine release. The structure for the model is similar to the ones reported above. While the results of the experiment are promising, there is a lack of further analysis on other chemical data such as serotonin. Similarly, the images from FSCV used to train the CNNs are not raw and have been manipulated. As such, that helps to gain better results in testing but does not necessarily generalise well. This provides an opportunity to fill the gap in this area of research.

Chapter 4

Implementation Plan

This section will present the design elements for development of the model to classify data into serotonin release and non-release categories. First, the general overview of the objectives of the neural network model is presented. Later, the brief overview of the data and preliminary design is discussed. Finally, the development plan and time estimates are developed with clear objectives and expected results.

4.1 Neural Network Design Considerations

The main objective of the project is to develop the neural network which would classify the data into two categories. Therefore, it is important to consider the design implications of the model. The categories would denote whether there has been a release of serotonin or histamine in the data sample. Given that the data is generated by using FSCV is an image, the use of convolutional neural networks was chosen. The CNN is a type of artificial neural network which is most commonly used on analysing image data, as described in Chapter 3.

The design of CNNs is important part of the development of an effective model. As such main consideration that have been addressed up to this point are described below.

4.1.1 Data Augmentation

The development of CNN model is effective when there is a large amount of data available. As such, the data augmentation is useful to expand the data set by creating new images from already existing ones. In general, data augmentation techniques include rotation or dilation of pixels in the image. For the purpose of this project, however, such techniques are not feasible as there is a specific pattern variation with time that has to be followed in each image. Therefore, two methods are used: transition of data along the time axis and combination of two images. The former is produced by shifting the image along the time axis. The latter combination of the two images with the same label is achieved

by multiplying the images by a random number and adding the two images to produce a new one. The equation for such summation is as follows:

$$C = r * A + (1 - r) * B$$

Where C is a new matrix, r is a random number between 0 and 1, and A and B are the original colour plots. Such augmentation allows the production of new data with varying features which enhances the model accuracy and helps avoid overfitting.

4.1.2 Overfitting

One of the most common problem that are encountered when developing a model is overfitting. It is a case when the model is trained to classify the training data accurately but fails to generalise to a new set of data. To tackle this problem, the data was split into two sets: training and validation set. This was achieved by using k-fold cross-validation - a technique which splits the data and uses the first part to train the model and the rest of the data to validate its performance. For example, the data is split into where 80% of the data is used for training and the remaining 20% is used for validation. Such split is performed for k times, using different subset for training and testing. Finally, the results are averaged to provide a final accuracy of the model.

Similarly, the early stopping is used to avoid overfitting. As the model is trained, there is a possibility that the NN will start to overfit the data. This method ensures that the training is stopped after the model passed the point where training data accuracy is maintained but validation accuracy sharply decreases.

4.1.3 Deep vs Wide Network

The network topology has impact on how the model captures the features of the data. The wide network can analyse large range of inputs that are present in the data whereas deep network is better at capturing a hierarchy of data. As such it is important to develop a network which is as small as possible in order to reduce computation time but can describe the given data effectively.

4.2 Plan

In this section, project plan and timeline is outlined. First, the brief description of the time frame is presented with reference to the Gantt chart (Figure 4.1). Later, the potential problems that could be encountered with the development of the project are discussed with reference to the mitigation.

The project timeline is separated into 3 section: research, development and report. The first section outline the time allocated for background research which is required to start the development

of neural network model. As such the majority of the research has been completed before March 2022 but is expected to continue throughout the project on smaller levels. The main part of the project is development. This part encompasses the implementation and training of neural networks, development of carbon paste electrodes, and testing. The initial part of the NN implementation has been completed with the results reported in the Chapter 7. The construction phase is expected to continue in parallel to the development of the electrodes. Afterwards, the testing is expected to be performed and improvements will be made based on the results. Finally, the project report is expected to span 3 months with the final review expected at the end of September 2022.

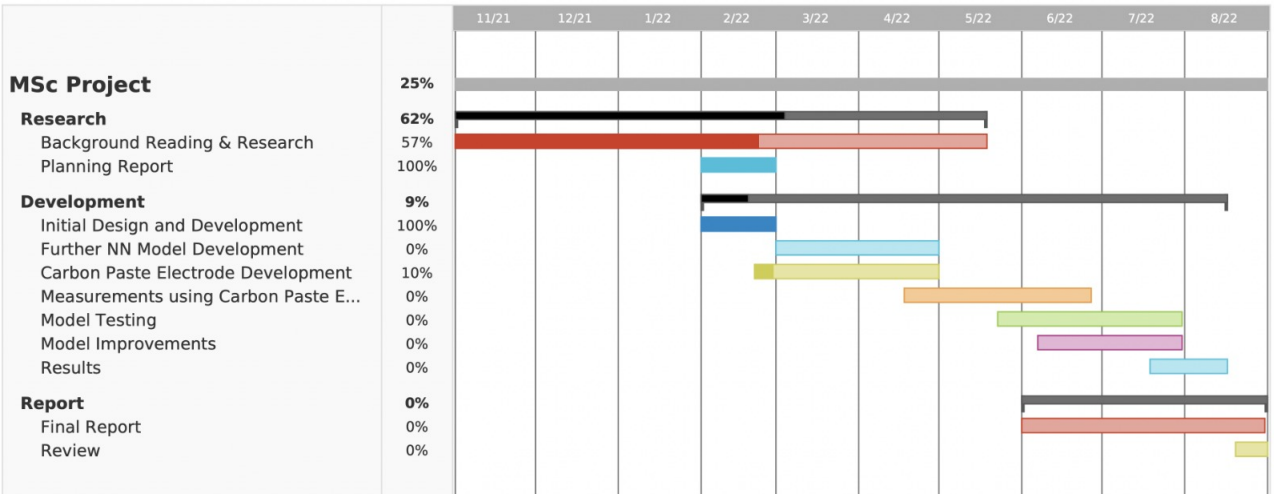

Figure 4.1: Gantt chart of project timeline showing the completion percentage up to this point

Chapter 5

Risk Register

In this section, the comprehensive risk analysis is developed with reference to the likelihood and impact of the risks. The severity of the risk is developed by using likelihood and impact and is presented in Table 5.1. Risks with the low likelihood and impact are considered to have low or negligible damage whereas high probability and severe risk can cause permanent damage or death. In Section 5.1, risks are identified, the severity is evaluated and mitigation is presented.

		Likelihood				
		1	2	3	4	5
Severity	1	IV	IV	IV	IV	III
	2	IV	IV	III	III	III
	3	III	III	II	II	II
	4	III	II	II	I	I
	5	II	II	I	I	I

Severity
Very High
High
Moderate
Low

Table 5.1: Risk severity table based on likelihood and impact.

5.1 Risk Assessment

The Table 5.2 presents the identified risks which are crucial to consider throughout the development of this project. The risks are separated into two categories: laboratory risk (E) and computational risks (C).

5.2 Conclusion

The results of the risk analysis show that project experiments are safe to conduct. Four risks are labeled as Very High or High severity and therefore are mitigated by usage of protective equipment and training. All other risks are labeled as Moderate or Low; therefore, with further mitigation do not pose any danger during the experiments.

ID	Description	Evaluation	Mitigation
E1	Electroshock from electrodes or any other electrical equipment	Very High	Examination of electrical equipment before experiments. Using protective equipment.
E2	Damaged electrical equipment due to improper use	Moderate	Mandatory laboratory induction and supervision. Following usage instruction. Examination of wiring before experiments.
E3	Burns from soldering or heat plate	Very High	Mandatory laboratory induction and supervision. Usage of protective equipment.
E4	Exposure to chemicals	High	Mandatory laboratory induction and supervision. Usage of protective equipment. Clear labelling.
E5	Alergic reaction	Low	Informing supervisors about potential allergies.
E6	Cuts with sharp objects or glass	Moderate	Handling sharp objects and glass with care. Usage of disinfectant.
E7	Aspiration of toxic fumes	High	Smoke extraction system usage when using equipment or chemicals which produce toxic fumes (soldering).
C1	Data leakage	Low	Following data storage standards.
C2	Data loss	Moderate	Usage of backup solutions.
C3	Insufficient computing resources	Low	Usage of college servers. Ensuring sufficient resources are available before starting experiments.
C4	Corrupted data	Moderate	Usage of backup solutions.

Table 5.2: Experimental and computational risk analysis with mitigation strategies

Chapter 6

Evaluation

This chapter will discuss the evaluation criteria for project deliverables with reference to implementation and testing methods presented in Chapter 4. The chapter is separated in two section: Computational Evaluation and Carbon Paste Electrode Evaluation. Former section will discuss testing method for development of Neural Networks whereas the microelectrode evaluation describes the testing of the results produced by the measurements using the electrodes.

6.1 Computational Results

Initially, the neural networks are developed using the training set which was collected using FSCV carbon fiber microelectrodes. The data is then normalised and reduced producing two data sets: full and reduced. The developed neural network is expected to have an accuracy of 75%-90% when trained on the full size data set and above 90% when trained on reduced. Such accuracy separation is due to the poor structure of some of the data plots which are manually removed in the reduced data set.

The evaluation of Neural Networks is conducted in two stages: training validation and testing. The validation is conducted during training by using 5-fold cross validation. The data is spilt into training and test sets and the latter is used to validate the performance of training. The validation accuracy is averaged over all testing splits and it provides an insight into how accurate the model is on classifying unseen data. In addition to accuracy, the validation data also shows the loss or cost of the objective function. The goal is to minimise the loss and it is used a another method to evaluate the model.

After the model is evaluated using the training/testing data, its performance will be tested on newly collected data using carbon paste microelectrodes. The trained NN will classify the seroton-in/histamine release images into release or non-release. Afterwards, sample of the images will be classified manually and the results will be compared. Based on that, the final evaluation of the model will be conducted. Finally, based on the results, the model is going to be improved and same

evaluation process is to be repeated.

6.2 Carbon Paste Microelectrodes

The Carbon Paste Electrode development is a crucial step for the project. The Neural Network model is supposed to be tested on a newly collected data from human derived cells and the conclusions are going to be determined for such evaluation. Therefore, it is important to test and verify the manufacturing of the Carbon Paste Electrodes. The length of the microelectrodes should be between $50\mu m$ and $300\mu m$.

Similarly, the calibration of the electrodes is tested by comparing the results to Carbon Fiber Microelectrode measurements which have been conducted before and expensively tested. The calibration is going to be examined using statistical analysis and the difference in characteristics of new electrodes will be determined.

Chapter 7

Preliminary Results

This chapter will describe the results of the development of Neural Networks that have been obtained up to this point in the project development. It will focus on accuracy and cost function of the models as well as present the further work and improvements.

7.1 Setup

The Neural Network model is developed using Python [2] programming language and computing servers at Imperial College London. Python has a wide range of open-source libraries which are used to train and test Neural Networks. Such libraries include Tensorflow [3] and Keras [1] as well as additional tools for data processing and plotting.

Initial neural networks were tested on a Apple Macbook Pro with M1 Max processor and 32 GB of RAM. Such networks are used as a proof of concept and are relatively small. As the size increases, the testing is conducted on the servers which are provided by Research Computing Service at Imperial College London.

7.2 Neural Network Development

This section describes the structure of Neural Networks as well as their iterations.

7.2.1 Baseline

The baseline model used to evaluate the performance was developed by Sergio Mena of Hasmeni Lab at Imperial College London. The network structure presented in Figure 7.1 consists of following layers:

- Layer normalisation
- Three 2D convolutional layers

- Two 2D Max Pooling layers
- Four Dense layers

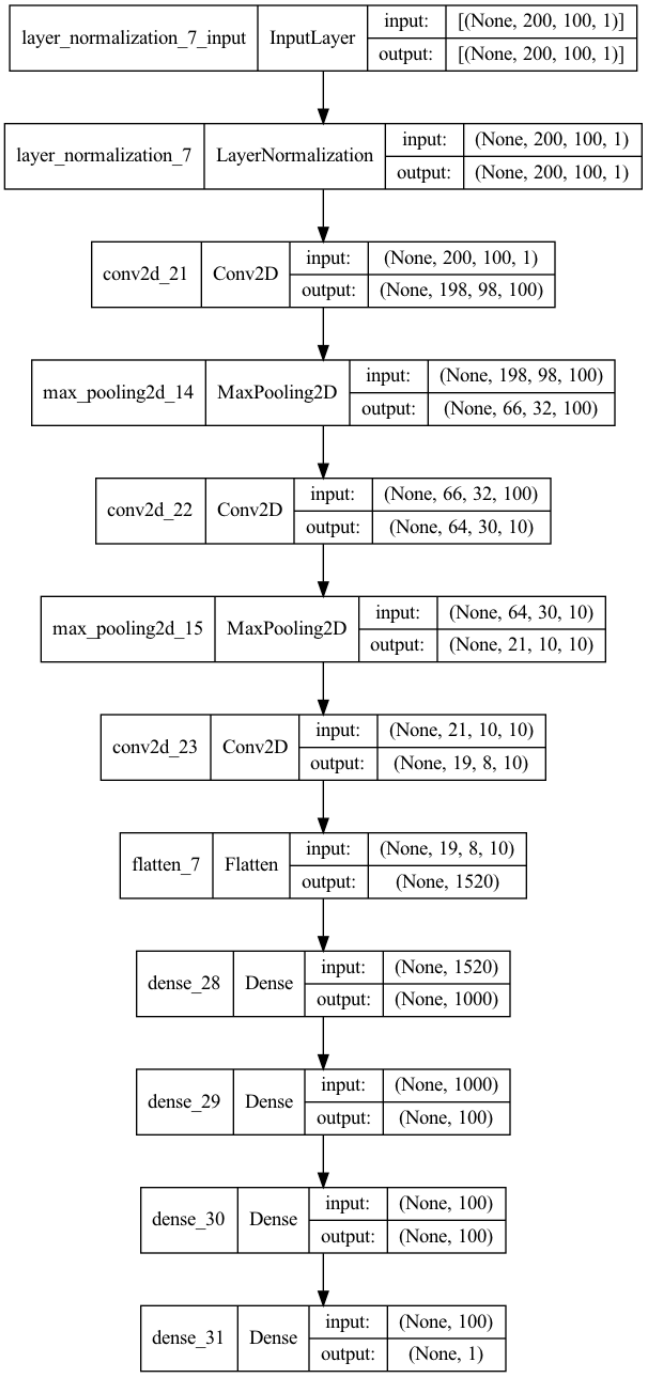

Figure 7.1: Baseline Neural Network Model Structure

The layer normalisation normalises the activation of layers independently in contrast to Batch normalisation layer which is described in the Section 7.2.2. The convolutional layer is one of the main layers in the CNN, it uses a set of filter which are smaller when compared to the input and produces the activation map. The convolutional layers are followed by max pooling layer, which select a maximum value from the activation map. This ensures that the most important values are selected

in the feature map. Finally, the dense layers which ensure that each neuron in the preceding layer is connected with every other neuron.

7.2.2 Model structure

The model developed to test the data classification is relatively small but similar structured models have been shown to perform well on image data. The Figure 7.2 provides a detailed structure of layers used in the model.

The difference between this model and the baseline model in terms of layer usage is that Dropout and Batch Normalisation layers are used. The former layer is used to normalise the activation of layers in batch. The Dropout layer randomly sets input units to 0 and is only used in the training environment. This layer helps prevent model overfitting.

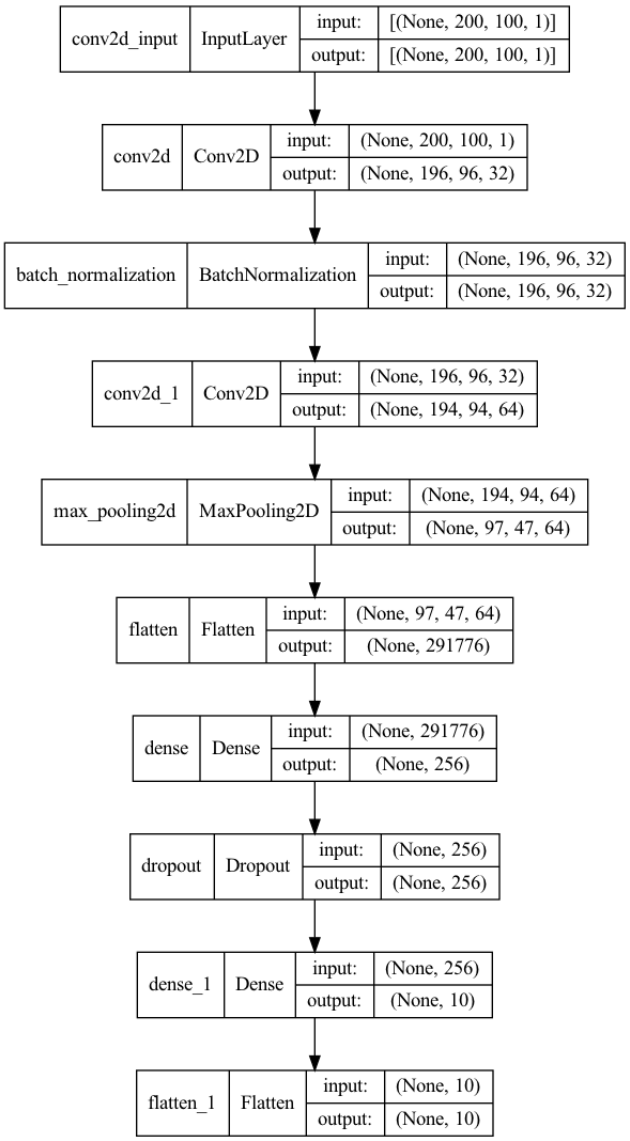

Figure 7.2: Neural Network Model Structure

7.3 Results

The initial testing of the neural network presented in Section 7.2.2 using 5-fold cross validation as well as running the model for 25 epochs provided the results described in this section. The training resulted to an average of training accuracy of 81.36% and validation accuracy of 85.62%.

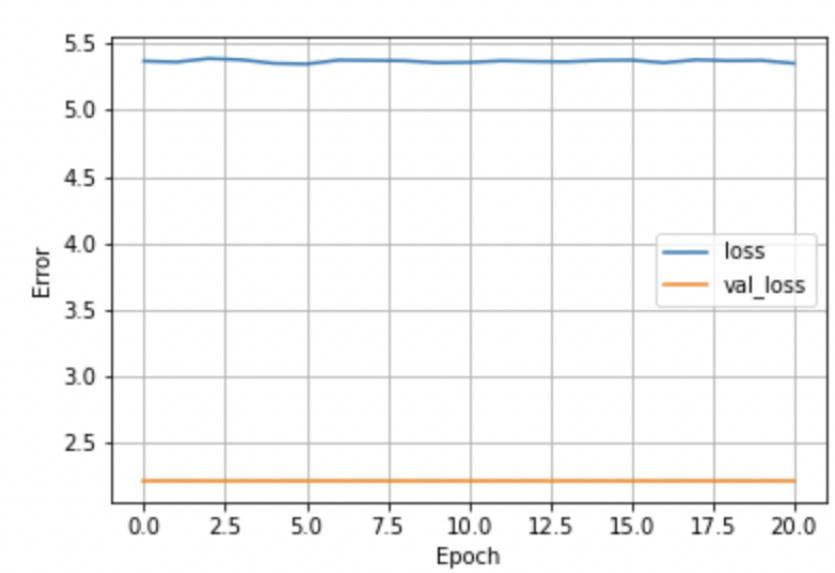

Figure 7.3: Error vs Epochs plot for model from Section 7.2.2

For each epoch, the error rate is plotted in Figures 7.3 and 7.4. It shows the error evolution for baseline model presented in Section 7.2.1 as well as the model from Section 7.2.2. It can be seen that the baseline model has a similar validation and training error up to epoch 10 and afterwards training error starts to decrease. This potentially shows that the model start to overfit. While such trend is not seen in a new model, this model has an issue of constant error. This is going to be solved by applying more dropout layers and reducing the learning rate.

Up to this point, training the newly developed model on the reduced data set did not yield any satisfactory results. Therefore, the model is going to be adjusted and improved to fit the data better.

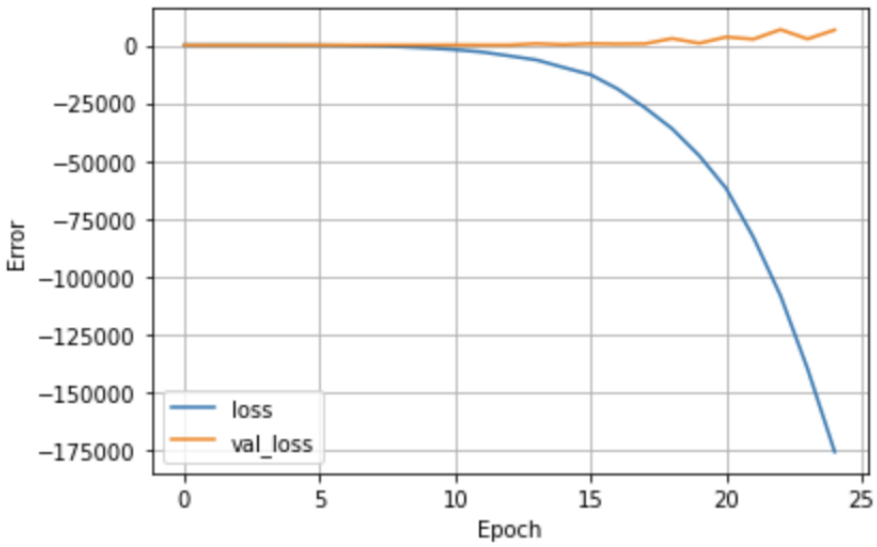

Figure 7.4: Error vs Epochs plot for model from Section 7.2.1

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