ARTIFACT IDENTIFICATION WITHIN LARGE DATA SETS FROM CORTICAL CALCIUM IMAGING USING DEEP LEARNING

By

Samiksha Patil

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APPROVED

Dr. Edward Korzus
Department of Psychology

Dr. Richard Cardullo, Howard H Hays Jr. Chair
University Honors

Abstract

Fear is an innate emotion that influences how humans interact and respond to their surroundings. It is an aversive response that protects humans from dangerous situations and environments. However, overgeneralized fear can be detrimental to humans' state of mind and has been correlated to neurological disorders such as Post-Traumatic Stress Disorder (PTSD). Studies have revealed that the medial prefrontal cortex (mPFC) circuit-level mechanisms are involved in fear discrimination learning; however, the mechanisms that underlie safety learning to overcome generalized fears remain unclear. A combination of computation and neuroimaging have been used to shed insight on the mechanisms of safety learning involving fearful but harmless stimuli. One-photon calcium imaging requires several stages to obtain neuronal fluorescence extraction. Currently, extraction of data is a manual process that is tedious and time-consuming given the number of neurons extracted, the number of trials, and the number of subjects for a behavioral experiment. The purpose of this study is to provide a mathematical and statistical analysis to automate the process of identifying good neurons vs bad neurons based on the activity of calcium fluorescence. This study uses a Deep Neural Network, DNN, a subset of Artificial Neural Network to precisely identify artifacts. The DNN code, known as Artifact Identification Software (AIS) is constructed and modified for neuronal data, using the programming language MATLAB. The code (AIS) was provided by the Korzus lab. This initial data demonstrate that this software has potential to be used for automatized appropriate neuronal signals.

Acknowledgements

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Introduction

Anxiety disorders are a prevalent issue affecting a significant portion of the American population. According to research, approximately 19.1% of American adults experience an anxiety disorder within a given year, while 31.2% suffer from such disorders at some point in their lifetime (U.S Department of Health). Post-Traumatic Stress Disorder (PTSD) and Generalized Anxiety Disorder (GAD) are examples of such anxiety disorders. Research has suggested that the circuit-level mechanisms in the medial prefrontal cortex (mPFC) are involved in fear discrimination learning. However, the mechanisms underlying safety learning, which involves encoding behaviors in response to safety and danger into memory to overcome irrational fears, remain unclear (Korzus, 2015). Understanding these mechanisms could help develop more effective treatments for anxiety disorders, which often involve a dysfunctional fear response.

By investigating the neural mechanisms involved in safety learning, researchers can gain insights into how the brain processes and responds to fear, and how this process can be modulated to alleviate anxiety disorders. This could lead to the development of new treatments, such as cognitive behavioral therapy, that target specific neural circuits involved in fear and safety learning. Ultimately, a better understanding of the neural basis of anxiety could improve the lives of those suffering from these debilitating disorders.

The prefrontal cortex plays a critical role in integrating information from higher cortical areas, sensory regions, and subcortical structures such as the hippocampus and amygdala, to generate output signals that modulate cortical network function (Courtin, 2013). Advances in microscopy technology, such as the use of calcium biosensor GCAMP and head-mounted microscopes in mice, have enabled researchers to record and analyze neuronal activity at the

single-cell level in raw video footage (Jacob et al, 2018). However, behavioral data analysis is subject to bias, and thus, a MATLAB program called *Constrained Nonnegative Matrix Factorization for microEndoscopic* data (CNMFE) has been developed to facilitate data analysis. CNMFE identifies the location and quantity of neurons in a video and generates individual neuron files for further analysis (Zhou et.al 2018). Despite its efficiency, the program often includes artifacts, which are cells that are not suitable for data analysis due to their structure and function. Artifact removal has traditionally been a manual process because artifacts often appear similar to desired neurons in studies. However, this process is time-consuming and subject to bias due to the large number of neurons per mouse. Therefore, the aim of this study is to use Deep Learning, a form of machine learning, to classify real neuronal signals vs artifact neurons in a more efficient and unbiased manner.

Understanding the role of the medial prefrontal cortex (mPFC) in fear discrimination learning is a critical research goal. This study aims to contribute to that goal by developing a Deep Learning model that can distinguish real neuronal signals from artifact neurons in mice models. The data used in the study were obtained through mice models that were conditioned via a fear conditioning model. Fear conditioning is a type of classical conditioning that involves pairing an aversive stimulus with either a neutral setting or another independent stimulus (LeDoux et al, 2000). Fear acquisition occurs when the stimulus is identified as fearful, while Fear Extinction is the reduction in the conditioned fear response due to a decrease in reinforced exposure to the stimuli (Hofmann, 2008). Differentiation between a conditioned fear response to safe versus dangerous stimuli is referred to as Fear Discrimination (Bergstrom, 2020). Conversely, the unsuccessful differentiation between the two stimuli is known as fear generalization (Pearce, 1987). Understanding the mechanisms that underlie Fear Discrimination

and Fear Generalization is crucial for developing treatments for anxiety disorders such as PTSD and GAD, which affect a significant proportion of the American population (Zhu et al, 2022).

This study aims to advance our understanding of the neural circuitry patterns that underlie fear discrimination learning in the medial prefrontal cortex. By developing a Deep Learning model to classify real neuronal signals versus artifact neurons, the study aims to streamline the data analysis process and reduce potential biases. The ultimate goal is to develop more effective treatments for anxiety disorders, which can have a significant impact on the lives of those who suffer from them.

Literature Review

The study of safety learning is guided by three core principles: fear conditioning, fear acquisition, and fear extinction (Kong et al, 2014). Researchers aim to understand the neural circuitry underlying the encoding of fear, which has important implications for the treatment of anxiety disorders (Maren et al, 2013). Prior research has identified three key areas of the brain involved in the fear circuit: the amygdala, hippocampus, and medial prefrontal cortex (mPFC) (Marek et al, 2013). These regions are highly interconnected and often regulate each other (Sotres et al, 2010). Fear learning and extinction are initiated in the amygdala, and the expression of learned fear and extinction is differentially mediated by two regions of the mPFC: the prelimbic (PL) and infralimbic (IL) cortex (Hoover & Vertes, 2007). The mPFC is widely known to play a role in planning and decision making (Euston et al 2012). The medial prefrontal cortex consists of two areas; the prelimbic (PL) and the infralimbic cortex (IL) (Heidbreder & Groenewgen, 2003). The PL is dorsal to the IL in the brain, and due to their proximity, they have numerous connections with each other (Giustino et al, 2015). Studies prior have shown that optical stimulation of IL reduces PL activity, and separating these two areas will affect IL

activity (Ji et al, 2012). Damage to the mPFC has been linked to the onset of anxiety disorders such as PTSD and GAD (Koenigs & Grafman, 2009). It has been suggested that the PL is involved in fear learning, whereas the IL is responsible for fear extinction (Sotres et al, 2010). Therefore, neuronal circuits in the mPFC are crucial for accurate fear behaviors (Koenigs & Grafman, 2009).

Anxiety disorders can be debilitating conditions that cause the brain to struggle with differentiating between threatening and non-threatening stimuli (Alexandra et. al, 2022). However, fear extinction, a type of learning adaptation, has been shown to be effective in mitigating these disorders (Milad & Quirk, 2012). Recent research has revealed that fear extinction memories and fear generalization memories are stored in the same regions of the brain (Sah et al, 2003). However, the encoding of these memories differs due to separate neuronal circuits (Milad & Quirk, 2002). In particular, inhibitory and excitatory neurons in these circuits play a crucial role in regulating the consolidation and expression of fear (Dejean, 2016). The mPFC, which is responsible for fear extinction, has been the focus of numerous studies in recent years (Milad & Quirk, 2002). Within the mPFC, the PL is known to play a critical role in fear extinction and is connected to the IL through excitatory neurons (Dixsaut & Gräff, 2021). Activation of these excitatory neurons connecting PL and the IL has been shown to enhance fear extinction, whereas inhibition of these neurons can impair it (Marek et al, 2018). These findings suggest that the balance between inhibitory and excitatory neuronal activity in the PL-IL circuit is a key determinant of successful fear extinction (van Aerde et al, 2008).

The insights gained from studying fear circuits could have important implications for the treatment of psychiatric and neurological disorders (Maren et. al 2013). Many of these disorders

are associated with dysfunction in fear extinction and learning, and understanding the neuronal circuits involved could provide new targets for therapeutic intervention (Jovanovic et al, 2012).

The Korzus Laboratory is focusing on the fear circuitry patterns of the medial prefrontal cortex (Corches & Korzus, 2019). Based on previous research, the laboratory has implemented in vivo calcium imaging of the mPFC using head-mounted microscopes to measure fluorescence of neural activities in behavioral mice (Jacob et al, 2018). The data acquired was modified through several MATLAB programs and used to train a Deep Learning Network (Pastore & Korzus, 2023).

Deep Learning is a rapidly advancing subset of machine learning that is revolutionizing the way we process and analyze data (Alzubaidi et al, 2021). It has proven to be extremely effective at solving complex problems that were previously considered unsolvable, such as image recognition, speech recognition, and natural language processing (Benali Amjoud & Amrouch, 2020). The technology has also found applications in a wide range of fields, including finance, healthcare, transportation, and entertainment (Liu et al, 2017). At its core, Deep Learning is based on artificial neural networks that are modeled after the human brain (Marblestone et al, 2016). These networks consist of multiple layers of interconnected nodes that process and transform data as it passes through them (LeCun et al, 2015). The idea behind this architecture is that by adding more layers, the algorithm can learn to recognize increasingly complex patterns in the data (Hornik, 1991). This approach has proven to be extremely effective, particularly in applications where the data is unstructured or where the relationships between different features are not well understood (Alzubaidi et. al 2021).

In a typical Deep Learning model, the first layer is the input layer, which receives the raw data (LeCun et al, 2015). The output layer generates the classification or prediction, while the

layers in between are referred to as hidden layers (Hassoun, 1995). Signals in the network travel between nodes and assign corresponding weights, which determine the relative importance of each feature (Andrews et al, 2014). A heavily weighted node will exert more influence on the next layer of nodes, allowing the algorithm to recognize and classify complex patterns ("Deep Learning in MATLAB"). Deep learning models are first trained with a set of data known as a training data set. Based on optimal combinations of variables the algorithm will produce a good predictive model that can generalize to new and unknown data (Krizhevsky et al 2017.) There are challenges associated with Deep Learning, particularly in terms of overfitting (Bengio et al 2012). Overfitting occurs when the algorithm becomes too specialized to the training data, and is unable to generalize to new data (James, 2013). This can occur when the network is too complex, or when there is not enough training data to properly train the model (Bishop, 2006). Having a validation data set can overcome this problem (James, 2013). Validation data sets are data sets used to tune hyperparameters of a classifier but affect the model indirectly (Xu & Goodacre, 2018). Lastly, testing data set is data independent of the training data set and used to evaluate the model (Ripley, 1996). Generally, 80% of data should be training data and 20% should be testing data. Within the 20% of testing data, 15% of the data is validation data (Gholamy et al, 2018).

Another challenge of Deep Learning is the computation time required to train a Deep Learning model can be quite significant, particularly for large datasets (Justus, 2018). To overcome these challenges, researchers have developed a range of techniques and algorithms to optimize the performance of Deep Learning models (Srivastava, 2014). These include regularization techniques to prevent overfitting, as well as techniques to optimize the learning rate, number of layers, and initial weights (Bengio et al 2013). Researchers are also exploring

new approaches to hardware design, such as using GPUs and specialized chips, to speed up computation time and reduce energy consumption ("Classify Videos Using Deep Learning").

Classifying and labeling neurons using deep learning algorithms is a challenging task due to the intrinsic complexity of these neural structures (Yip et al, 2021). Unlike other objects in image and video datasets, such as animals and food, neurons appear very similar to each other, making it difficult to distinguish one from another (Tyagi & Rekha, 2020). This poses a significant challenge for researchers attempting to develop artificial neural networks capable of accurately detecting and classifying neurons (Najafabadi et al, 2015).

Methods

This project consists of two main stages: Data Acquisition and Data Analysis. In the Data Acquisition stage, mice were used as animal models and underwent Safety Learning conditioning paradigms. Specifically, mice were placed in cages and were exposed to shocks at specific intervals while being presented with either a neutral context or a tone. The mice exhibited freezing behavior, an innate response to threatening stimuli, upon receiving the shocks. After conditioning, mice were able to display freezing behavior solely in response to the tone or neutral context. Prolonged exposure to the stimuli resulted in fear generalization among fear-conditioned mice. In Data Analysis, several MATLAB programs provided by the Korzus Lab were run.

Data Acquisition

Prior to the behavioral experiments, mice underwent brain surgery to allow for the study of specific areas of the brain, including the medial prefrontal cortex, basolateral amygdala, and the hippocampus. These regions are known to be involved in consolidating fear memories (Marek et al, 2013). The medial prefrontal cortex is further divided into the infralimbic and

prelimbic cortex, which are the primary focus of the research goal (Heidbreder & Groenewgen, 2003).

Several techniques were utilized during the experiments, including electrophysiology and neuronal imaging. Electrophysiology involves measuring the electrical activity of neurons to ensure that the neurons of interest are involved in the behavior (Cavanagh, 2019). Neuronal imaging was used to record neuronal activity in vivo while mice were behaving. This technique takes recordings of the activity of neurons in real-time, providing valuable insights into the neural mechanisms underlying behavior (Yang & Yuste, 2017). Together, these techniques allowed for the detailed study of the neural circuitry involved in fear discrimination learning in mice, with a particular focus on the role of the medial prefrontal cortex.

Data Analysis

In this project, experimental mice have mini microscopes surgically attached to their skull to record neuron activity. Viruses encoding for calcium sensitive proteins, also known as GCAMP, are injected into neurons of interest to image neuronal populations in deep regions of the brain. One of the main challenges with this design is the difficulty in extracting single neuron activity from the recorded data. To address this challenge, the video recordings taken from the behavioral experiments are processed through several MATLAB programs to extract calcium signals from the data.

One of these programs is CNMFE, which determines the quantity and location of neurons in a video and then identifies individual neurons. The program takes into account the noise from adjacent neurons and overlapping neurons when isolating individual neurons (Zhou et.al 2018). However, CNMFE can sometimes over classify neurons that are actually poor in quality, which

should be considered artifacts. Removing artifacts from the data is necessary to draw accurate conclusions about the neuronal circuitry patterns of particular regions in the brain

Currently, the process of artifact removal is done manually through an app on MATLAB, which involves researchers analyzing footprints and traces of neurons to identify fluorescence and peaking. However, given the number of neurons per mouse, this manual process is time-consuming and subject to bias.

To address this issue, this project aims to utilize machine learning, specifically a deep learning model, to automate the process of neuron classification. The deep learning model is trained to learn how to classify neurons based on predetermined sets of videos that highlight real neuronal signals vs artifact neurons. The predetermined parameters in the deep learning model are manipulated through a MATLAB program (Pastore & Korzus, 2023). Raw footage of neuronal activity is converted to a certain number of frames to highlight maximal fluorescence, which includes frames before, during, and immediately after peaking. This process ensures that there is no false labeling of peaks. By automating the process of neuron classification through machine learning, this project aims to significantly reduce the time and bias involved in artifact removal from data, thus improving the accuracy and efficiency of the analysis of neuronal circuitry patterns in specific regions of the brain.

Good Neuron Vs. Bad Neuron

Examples of real neuronal signals and artifact neurons are shown below, provided by the Korzus lab (**Table 1**). In the classification process of “Good Neurons” (real neuronal signals) versus "Bad neurons” (artifacts), fluorescence is one of the key criteria used. Fluorescence refers to a bright flash of light that is observed in the video recording of neurons. This can be clearly seen in image 2 in the figure below. “Good Neurons” generally exhibit clear fluorescence, while

artifacts show no fluorescence. In addition to fluorescence, shape and overlapping of neurons are also taken into consideration when classifying artifacts. Neurons with irregular shapes and sizes and those with significant overlaps are generally considered artifacts. In this project, fluorescence will be the primary indicator used to identify artifacts.

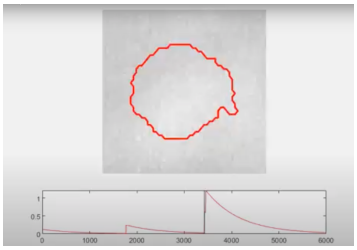
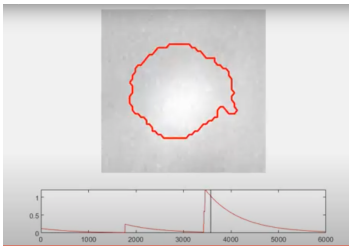
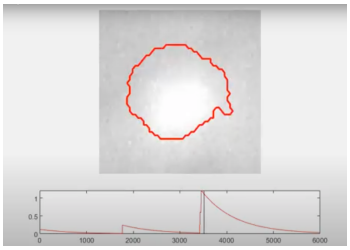
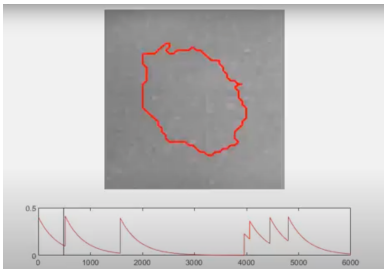
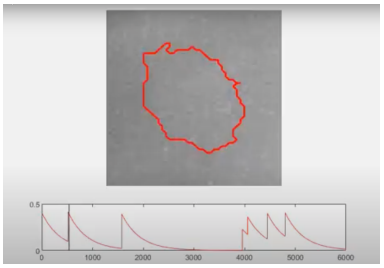
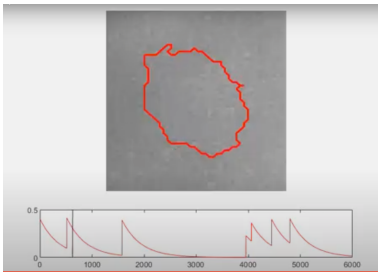
	Time Before Fluorescence	Time During Fluorescence	Time After Fluorescence
"Good Neuron"	 1.	 2.	 3.
"Bad Neuron"	 4.	 5.	 6.

Table 1: Classification of Neurons. Image 1 and 3 are the times before and after fluorescence of a "Good Neuron" so there is no fluorescence. Image 2 is the time during fluorescence as it highlights clear fluorescence in the neuron. Image 4 and 6 are the times before and after fluorescence of a "Bad Neuron" so there is no fluorescence. Image 5 is the time during no fluorescence and is classified as a "Bad Neuron", otherwise known as an artifact.

Deep Learning is a powerful class of machine learning algorithms that is employed in this project. It utilizes multiple layers to extract high-level features directly from data, and there are several types of deep learning models such as Convolutional Neural Networks (CNNs) and Long

Short-Term Memory (LSTM) that are designed to create artificial neural networks (Nanni et al, 2022). These models are inspired by the information processing of the human brain, but they differ in their approach to learning from data (Nanni et al, 2022). CNNs are particularly effective at detecting features in images that are useful for recognition and classification, while LSTMs are well-suited for processing both individual data points and entire sequences, making them useful for training networks with video files ("Classify Videos Using Deep Learning").

It's worth noting that while deep learning networks are static, unlike the dynamic processes of the biological brain, they can still achieve high levels of accuracy (often above 90%) in identifying and classifying images when configured with the right parameters (Marblestone et al, 2016).

For this project, the MATLAB program, provided by the Korzus lab, uses a convolutional network, from MATLAB's *DNN ToolBox*, *Version 2018*, ("Deep Learning Toolbox"), to train with video files (Pastore & Korzus 2023). The code, known as Artifact Identification Software (AIS) was written, used and edited from MATALB's Deep Learning "Classification Videos" page ("Classify Videos Using Deep Learning"). The program creates avi files of raw neuronal footage and converts the files to frames. The frames are set to only times of maximum fluorescence, identify boundaries around cells to isolate neurons, find the overlap between them and their neighboring neurons and eliminate neurons that had more than 50% overlap (Pastore & Korzus, 2023). After classification the frames of data are converted to feature vectors, using the CNN, for training and then prepared for the LSTM network ("Deep Learning Toolbox"). Specific parameters for the network are chosen, which include miniBatchsize, learning rate, and validation patience. The miniBatchsize is the amount of data included in each training set to evaluate the gradient of the loss function and update the weights (Kandel & Castelli, 2020). The

miniBatchsize is set to a value of 8 as small batch sizes achieve high performance. The learning rate value, which controls how quickly the model adapted to previous errors, was set to 1e-4. A value too low could lead to time-consuming training while a high value could lead to inaccurate results (Senior et al, 2013). The validation patience is set to 5. Validation patience represents the number of times of a loss in the validation set that can be larger than or equal to the previously smallest loss before the training stops. After 5 iterations of no improvement in validation error, the training process will stop ("Options for training deep learning neural network").

The network is trained and the algorithm assembles the video classification network, which adds convolutional layers, sequence input layers and adds LTSM layers. The Deep Learning code was modified for video classifications of "Good Neurons" and "Bad Neurons" in vivo of experimental behaving mice (Pastore & Korzus, 2023). The videos were converted using a pre-trained convolutional neural network, GoogLeNet, to extract features of each frame. The LSTM network is then trained on the sequences to predict labels and finally, a network is assembled to classify videos by combining the layers from previous networks ("Deep Learning Toolbox").

This project focuses on improving the accuracy of the network by altering parameters of the avi files created. The parameter that was manipulated was framerate in the creation of the avi files (Pastore & Korzus, 2023). Frame rate refers to the number of frames per bin with the bin representing seconds. The bins range from 1 to a finite number. The mini-scope videos are recorded 30 frames per second, so a framerate of 10 would have the avi files create 300 frames. Deep learning is known to improve with more training data. Increasing the number of neurons for the training data will increase the accuracy of the neural network because there is more training data for the network to work with (Andrews et al 2014). Altering the parameter of

framerate is proposed to improve the consistency between training and testing accuracy of the DNN.

The study is designed into 3 trials of varying frame rates: 5, 10, and 20. Trial 1 was a framerate of 5, trial 2 was 10, trial 3 was 20. Neuron video files of Mouse 22 and Mouse 8 in the behavioral experiment were provided by the Korzus lab. The region of interest (ROI) in the behavioral experiment was the prelimbic cortex of the brain. The network was trained with 520 bad neurons and 520 good neurons for each trial (1040 total neurons). After training, the model was then tested with data they had not been previously tested with. 57 "Good Neurons" and 57 "Bad Neurons" were tested during each trial for accuracy. An accuracy report given after the completion of training in how well the network can identify "Good Neurons" and "Bad Neurons".

Results

Each trial was trained with 520 bad neurons and 520 good neurons. Trial 1 resulted in 77.88% in accuracy of labeling Good and Bad neurons **(Figure 1)**. Trial 2 resulted in 86.54% in accuracy of labeling Good and Bad neurons **(Figure 2)**. Trial 3 resulted in a 69.23% accuracy of labeling Good and Bad Neurons **(Figure 3)**. Each model was then tested with 114 neurons (52 good and 52 bad) that were not exposed to the training model. Trial 1 had a testing accuracy of 33.34% of Bad Neurons and 96.49% of Good Neurons. The overall testing accuracy was 64.75% with 16.86% error. Trial 2 had a testing accuracy of 85.96% of Bad Neurons and 96.49% of Good Neurons. The overall testing accuracy was 91.23% with 5.15% error. Finally, trial 3 had a testing accuracy of 26.32% of Bad Neurons and 98.24% of Good Neurons. The overall testing accuracy was 62.28% with 10.04% error. A table summarizes the findings **(Table 2)**.

	Frame Rate	Number of Frames	Accuracy in Labeling Good vs Bad Neurons (%)	Testing Accuracy Of Bad Label Neurons	Testing Accuracy of Good Label Neurons	Testing Accuracy Overall (%)	Percent Error (%)
Trial 1	5	150	77.88%	19/57 (33.34%)	55/57 (96.49%)	64.75%	16.86%
Trial 2	10	300	86.54%	49/57 (85.96%)	55/57 (96.49%)	91.23%	5.15%
Trial 3	20	600	69.23%	15/57 (26.32%))	56/57 (98.24%)	62.28%	10.04%

Table 2: Summary of Labeling accuracies in Trials 1-3. Overall testing accuracy remained similar for trials 1 and 3. Trial 2 shows the highest overall testing accuracy and both high Bad and Good Neurons testing accuracy.

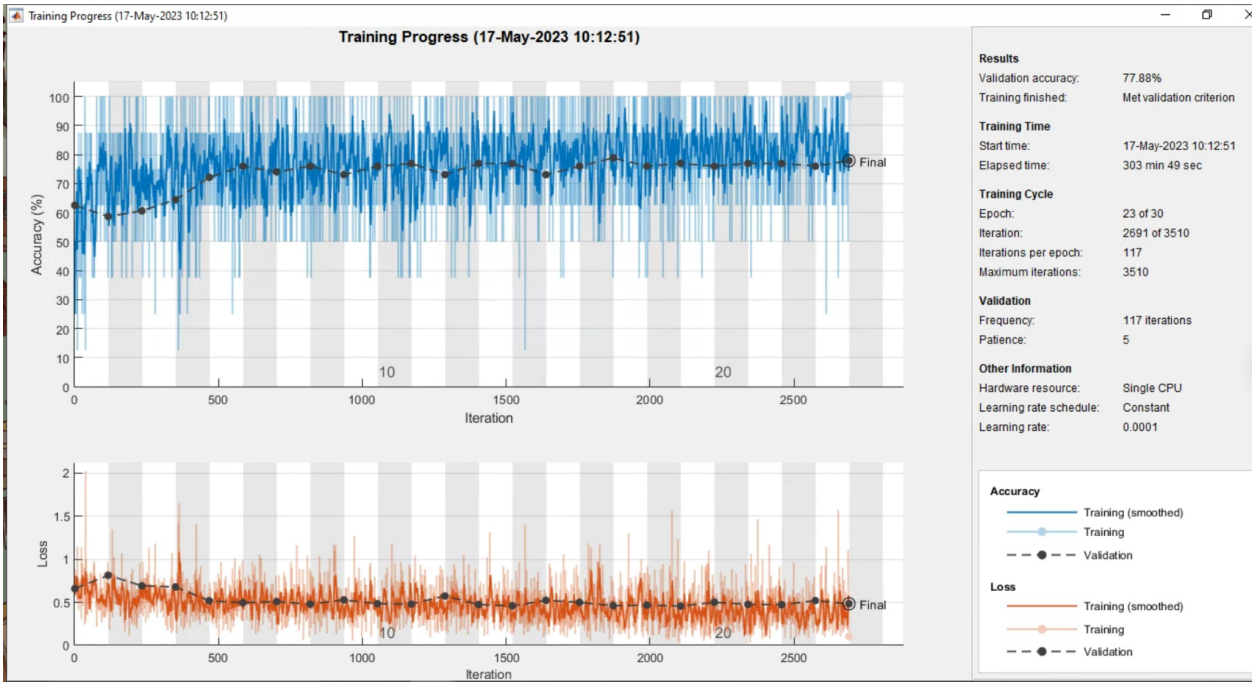


Figure 1 *Frame Rate of 5 with 77.88% accuracy in labeling of 1040 Good and Bad neurons. Neurons tested on this model should result with the desired precision.*

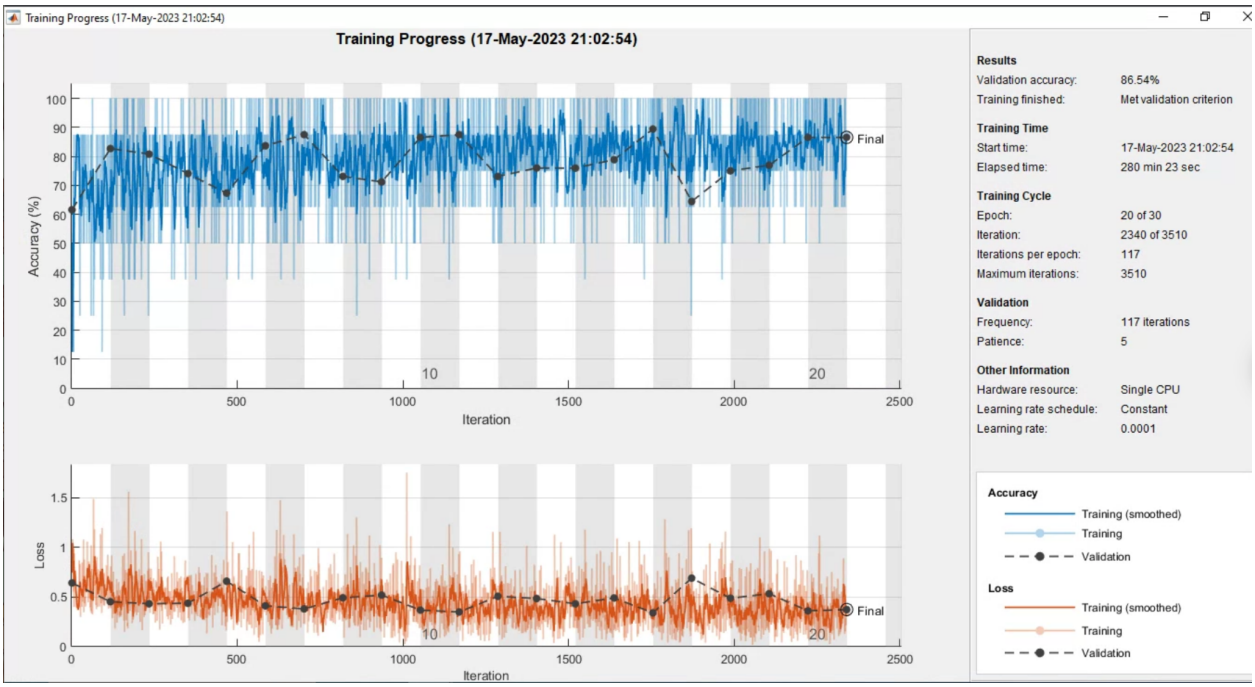


Figure 2 *Frame rate of 10 with 86.54% accuracy in labeling of 1040 Good and Bad neurons.*

Neurons tested on this model should result with the desired precision.

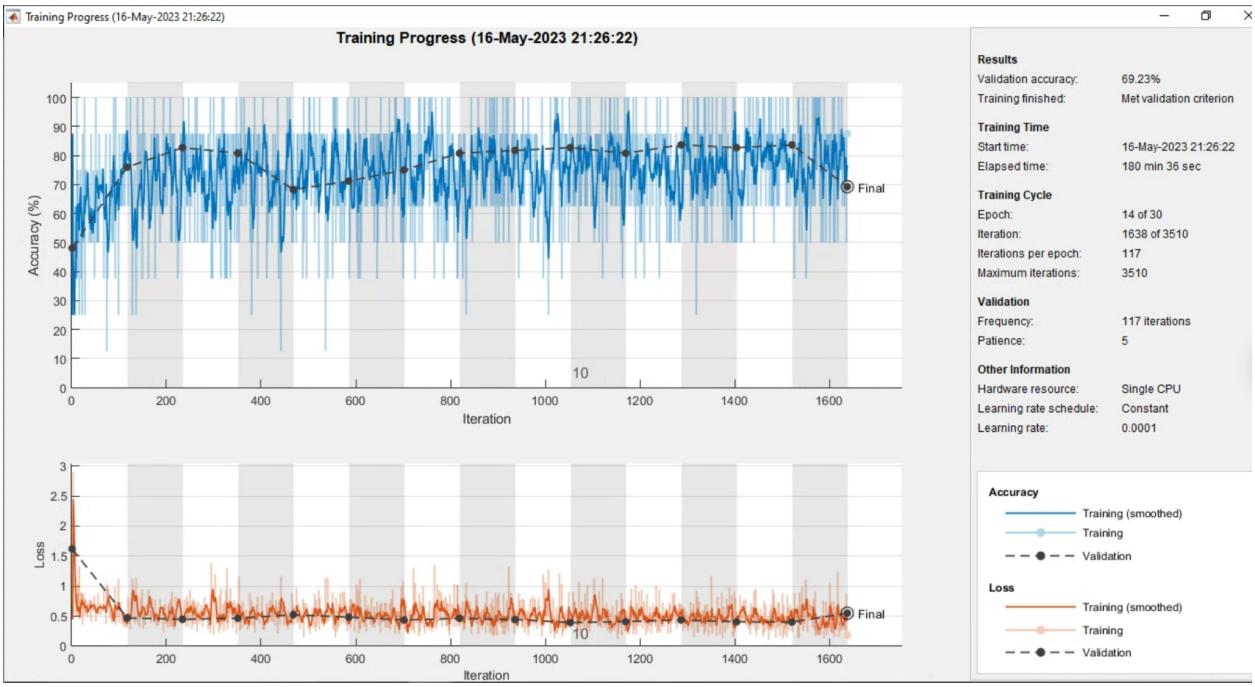


Figure 3 *Frame Rate of 20 with 69.23% labeling accuracy of 1040 Good and Bad neurons. Neurons tested on this model should result with the desired precision.*

Discussion

This research project aimed to develop a method for artifact removal using Deep Learning to identify Good and Bad neurons in a neural network of safety learning behavior. The study discovered that Deep Learning can be used for identification analysis, and adjusting the parameters of the learning algorithm can improve its accuracy. The parameter of interest in this study was the frame rate, and the results showed that a frame rate of 10 could have the Deep Learning model classify testing videos accurately with the approximate desired precision as the model (percent error of 5.15%).

The study found that an optimal frame rate could exist, which indicates a certain number of frames that would have minimum noise and minimum similarities of luminosity, making training hard for the algorithm. The results indicated that a frame rate of 10 could be the optimal frame rate for the algorithm (testing accuracy of 91.23%). A range of 6-19 frame rate can be tested to determine at which frame rate the testing labeling accuracy peaks. A follow up study could determine if the frame rates give a high and consistent labeling accuracy output between model and testing that is statistically consistent amongst all the behavioral mice in the experiment.

The study also revealed that increasing the frame rate would lead to an increase in the number of frames per neuron video, resulting in a higher number of images that the convolutional neural network trains with. A frame rate of 20 indicates 600 frames of a given neuron video. 600 frames represent 600 images of a neuron fluorescence or an absence of fluorescence that the convolutional neural network trains with. According to the results, a frame

rate of 20 had the lowest testing labeling accuracy of Good and Bad neurons (62.28%). Whereas a frame rate of 10, had the highest testing labeling accuracy (91.23%). The increase in frame rate did not consistently improve the labeling accuracy of Good and Bad neurons. The significant variation in labeling accuracy and frame rate could be due to excess noise and the algorithm unable to detect a pattern with the high amount of varying neuron video frames.

The study was conducted using data from 1040 neurons, which is a small percentage of the total number of neurons in the experimental data set. Of a given mouse there's approximately 10,000 to 15,000 neurons in the ROI that would need to be run through the algorithm. Therefore, future studies should increase the number of neurons per trial to reduce the impact of confounding variables. One of the limitations of the study is the limited computer memory available, which limits the number of frames that can be used for analysis.

Additional studies, to improve labeling accuracy, can consist of modifications to miniBatch size and learning rate. The model focuses on greater than 50% overlapping neurons; alterations of isolating all overlapping neurons could possibly contribute to improvements of the algorithm.

While training deep learning algorithms to classify and label neurons is challenging, researchers are making progress in developing new techniques that can better model the complex structure of these neural networks. Deep Learning is a powerful tool that has the potential to transform the way we process and analyze data. As the technology continues to evolve, we can expect to see new applications and use cases emerging, as well as new techniques and algorithms for optimizing performance.

This initial data demonstrates that this software has potential to be used for automatized appropriate neuronal signals. The results suggest that an optimal frame rate could exist, and

further studies are necessary to determine if this holds true for other mice and to increase the number of neurons per trial to reduce confounding variables. The findings of this study could potentially lead to the development of more accurate and efficient methods for artifact removal and identification analysis using Deep Learning.

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