Artificial neural networks in action for an automated cell-type classification of biological neural networks

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Abstract—In this work we address the problem of neuronal cell-type classification, and we employ a real-world dataset of raw neuronal activity measurements obtained with calcium imaging techniques. While neuronal cell-type classification is a crucial step in understanding the function of neuronal circuits, and thus a systematic classification of neurons is much needed, it still remains a challenge. In recent years, several approaches have been employed for a reliable neuronal cell-type recognition, such as immunohistochemical (IHC) analysis and feature extraction algorithms based on several characteristics of neuronal cells. These methods, however, demand a lot of human intervention and observation, they are time-consuming and regarding the feature extraction algorithms it is not clear or obvious what are the best features that define a neuronal cell class. In this work we examine three different deep learning models aiming at an automated neuronal cell-type classification and compare their performance. Experimental analysis demonstrates the efficacy and potent capabilities for each one of the proposed schemes.

Index Terms—Artificial neural networks, calcium imaging, neuronal cell-type classification

1 INTRODUCTION

To understand the function of neural circuits we first need to identify and characterize the various subtypes of neurons that comprise these circuits. Different cell types typically exhibit different anatomy, connectivity and/or biophysical properties which in turn influence their specific function and role in pathologies such as epilepsy [1], [2], anxiety disorder [2], [3], the Tourette syndrome [4], autism [5], the Rett syndrome [6] and schizophrenia [7], [8]. Thus, neuronal cell-type classification is a crucial step in understanding the function and dysfunction of neuronal circuits.

Despite the fact that a systematic classification and accepted nomenclature of neuron types is much needed, neuronal classification still remains challenging, primarily because it is unclear how to designate a neuronal cell class and what are the best features that define it. Traditionally, neuronal cell-types have been classified using qualitative descriptors, such as the expression of specific proteins in combination with their anatomy and laminar localization [9]. Recently, quantitative methods using supervised and unsupervised classifiers have become standard for neuronal classification based on morphological, physiological, molecular, or electrophysiological characteristics [10], [11], [12]. They provide quantitative and unbiased identification of distinct neuronal subtypes when applied to selected datasets.

In this work we aim at the automated cell-type recognition using a real-world dataset, which consists of timeseries data describing the activity of four neuronal cell-types in the CA1 subregion of the hippocampus. The dataset consists of pyramidal cells and three GABAergic interneuron subtypes, which are parvalbumin-positive (PV), somatostatin-positive (SOM) and vasoactive intestinal polypeptide-positive (VIP) cells. The method used to record neuronal activity is calcium imaging, which is a powerful means for monitoring the activity of distinct neurons in brain tissue in vivo [14]. Calcium imaging allows simultaneous recording of numerous cells without destroying their components (i.e., dendrites, axons, somata), as opposed to other recording methods, which use electrodes, such as tetrodes, octrodes and silicon probes, which are highly intrusive. Compared to these methods, calcium imaging is also more stable, as the same neurons can be recorded over time periods that extend from days to months. Despite its advantages, calcium imaging also has some limitations. Due to its slow kinetics, the signal produced is not ideal for resolving single spikes nor for reliably inferring the neuronal cell-type in the behaving animal. Instead, molecular markers are typically used to identify cell types in post-hoc analyses. Our main motivation is to replace existing methods with reliable, fast, cell-type classification, which is based on calcium imaging recordings using an appropriate Deep Learning architecture. Towards this goal, we use the raw data fluorescence signal
without any preprocessing. To our knowledge, this is the first successful attempt to classify cell-types based solely on the raw calcium activity signal.

Since their origin in 1946, Artificial Neural Networks (ANN) have been used in many real-world applications, such as speech [15] and handwriting recognition [16], audio-visual speech enhancement [17], video dynamic detection [18] and also for applications in emerging areas such as, real time sign-language translation [19] or for smart cities [20]. The rise of the “golden age” of Deep Learning networks is attributed to their ability to derive important characteristics from the raw data by learning intermediate representations and by structuring different levels of abstraction, replacing the existing conventional machine learning models, which heavily rely on the development of efficient feature extractors, which is a non-trivial and very challenging task. Their recent success has been facilitated by their capacity to harness big data and the cutting-edge hardware technologies that are exploited by these networks.

With the advent of deep learning, models for time-series analysis and forecast have also been developed. For different fields and applications, suitable algorithms and models vary depending on the nature and purpose of the data. Thus, for the task of neuronal cell-type classification we review the most appropriate models of 1-Dimensional Convolutional Neural Networks (1D-CNNs), Recurrent Neural Networks (RNNs) and Long Short-Term Memory Networks (LSTMs), which are specialized in time-series analysis. The motivation behind using the 1D-CNN model is that this architecture exploits spatially local correlations, while the other two architectures reveal if there are any long-term dependencies in the calcium activity data. Thus, the performance of these models will show us if the specific data are mainly locally correlated or if there exist long-term dependencies. The contributions of our work are summarized as follows:

- We conduct a comparative research analysis on the 1D-CNN, RNN and LSTM deep learning models, in the domain of time-series analysis and forecasting for the task of neuronal cell-type classification, where such an analysis is missing from the existing literature.
- To the best of our knowledge, this is the first time that algorithmic models use raw calcium signals to classify different neuronal types. The models are based solely on the calcium imaging signatures of the neuronal types, replacing existing post-hoc techniques.

The remainder of the paper is organized as follows: In Section II, we describe and analyze the proposed approaches. Experimental results are presented in Section IV and conclusions are drawn in Section V.

2 PROPOSED APPROACHES

In this work we explore the extent to which various types of neuronal cells, such as pyramidal neurons (PY), PV, SOM and VIP interneurons, whose activity is described with time-series of raw calcium imaging data, can be correctly classified. To that end we employ the following classification models and we compare their performance on neuronal cell-type classification.

- 1-Dimensional Convolutional Neural Networks
- Recurrent Neural Networks
- Long Short-Term Memory Networks

2.1 Convolutional Neural Networks

A Convolutional Neural Network (CNN) is a class of deep neural networks, most commonly applied to imaging applications that consists of input, output and hidden layers of neurons along with their respective connections that encode the learnable weights of the network. CNNs are regularized versions of traditional Multilayer Perceptrons (MLPs), which usually refer to fully connected networks, i.e. each neuron in one layer connects to all neurons in the next layer. This characteristic of MLPs makes them prone to overfitting during training. Thus, one of the distinguishing features of CNNs compared to MLPs is the local connectivity among neurons. When dealing with high-dimensional inputs such as calcium imaging time-series data, it is impractical to connect neurons to all neurons in the previous volume because such a network architecture does not take the spatial structure of the data into account.

Convolutional networks exploit spatially local correlation by enforcing a sparse local connectivity pattern between neurons of adjacent layers, i.e. each neuron is connected to only a small region of the input volume. Namely, in a convolutional layer, neurons receive input from only a restricted subarea of the previous layer and this input area of a neuron is called its receptive field. Another distinguishing feature of CNNs is the shared weights. In CNNs, each filter is replicated across the entire receptive field. These replicated units share the same parameterization (weight vector and bias) and form a feature map, i.e. all the neurons in a given convolutional layer respond to the same feature within their specific receptive field. Replicating units in this way allows for features to be detected regardless of their position in the visual field, thus constituting a property of translation invariance.

2.1.1 Typical Architecture of a 1-Dimensional CNN

A typical 1D-CNN architecture, as shown in Fig. 1(c) is formed by a stack of distinct layers that transform the input volume into an output volume (holding the class scores) through a differentiable function. The core building block of a CNN is the convolutional layer, whose parameters consist of a set of learnable filters (or kernels), which have a small receptive field. Given an input vector \( x \in R^{1 \times N} \) and a trainable filter \( f \in R^{1 \times K} \), the convolution of the two entities results in an output vector \( c \in R^{1 \times M} \), where \( M = N - K + 1 \). The value of \( M \) may vary based on the stride of the operation of convolution, with bigger strides leading to smaller outputs.

The trainable parameters of the network, i.e. the filter and the bias are initialized randomly, but as the network is trained through the process of backpropagation, they are optimized and are able to capture interesting features from the given inputs. In order to construct a reliable network that will be able to capture complex and abstract features
from the input data, we need to build a deep architecture comprised with more than one convolutional layer. As we go through layers in deep architectures, the features not only increase in number (depth size) but also in complexity. In other words, the network builds a hierarchical representation of the input, as the first layer represents the input in terms of elementary features and as we go deeper, layers can recognize more abstract features. The capture of such more complex features requires also to introduce some non-linearity in our system. Thus, non-linear functions are interjected between adjacent convolutional layers, and as a result a two-layer CNN can be proven to be a universal function approximator [21], [22], while the identity activation function does not satisfy this property and generally, when multiple layers use the identity activation function, the entire network is equivalent to a single-layer model. Typical choices for the non-linear function, also known as activation function include the logistic (sigmoid) function, the hyperbolic tangent (tanh), the Rectified Linear Unit (ReLU) and its variations [23].

Another important concept of CNNs is the pooling layer, which is a form of non-linear down-sampling. There are several non-linear functions to implement pooling, among which max pooling is the most common. Intuitively, the exact location of a feature is less important than its rough location relative to other features. This is the idea behind the use of pooling in convolutional neural networks. The pooling layer serves to progressively reduce the spatial size of the representation, to reduce the number of parameters, memory footprint and amount of computation in the network, and hence to also control overfitting. It is common to periodically insert a pooling layer between successive convolutional layers in a CNN architecture.

Eventually, after several convolutional and max pooling layers, the high-level reasoning in the neural network is done via the Fully Connected layers (FC), also known as dense layers. As its name implies, neurons in a fully connected layer have connections to all neurons in the previous layer leading to a very dense connectivity. Essentially, when the fully connected layer is inserted at the end of the architecture, it looks at the output of the previous layer, which represents the activation maps of high level features and determines which features most correlate to a particular class.

As a final classification step we use the softmax activation function, which extends the idea of logistic regression into a multi-class world. That is, softmax assigns decimal probabilities to each class in a multi-class problem using the following equation:

$$\sigma(x)_i = \frac{e^{x_i}}{\sum_{j=1}^{C} e^{x_j}} \quad \text{for } i=1,...,C$$

where \(x\) is the input of the fully connected layer and \(C\) is the total number of the distinct classes related to the problem at hand. This probabilistic approach renders possible to quantify the level of confidence for each estimation and provides a lucid view on what has been misconstrued in the case of misclassification.

### 2.2 Recurrent Neural Networks

Recurrent Neural Network (RNN) is a kind of neural network that specializes in processing sequences and has shown great promise in many Natural Language Processing (NLP) tasks [24]. The idea behind RNNs is to make use of sequential information, and they are called recurrent because they perform the same task for every element of a sequence, with the output being depended on the previous computations. Another way to think about RNNs is that they have a memory, which captures information about what has been calculated so far.

The RNN model in our proposed method accepts an input vector \(x\) and gives an output vector \(o\), which in our case are the input time-series and the cell-type label respectively. The diagram in Fig. 1(a) shows an RNN being unrolled (or unfolded) into a full network. By unrolling we simply mean that we write out the network for the complete sequence. At timestep \(t\), \(x_t \in R^{1 \times d}\) is the input entry, where \(d\) is the dimensionality of that entry and \(h_t\) is the hidden state at timestep \(t\). Hidden state \(h_t\) can be considered as the memory of the network, given the fact that it captures information about what happened in all the previous timesteps. It is calculated based on the following equation and utilizes the previous hidden state as well as the input at the current timestep \(t\):

$$h_t = f_1(W_1x_t + W_2h_{t-1} + b_h)$$  \hspace{1cm} (2)

where function \(f_1\) is usually a non-linearity, such as tanh or ReLu, \(W_1\) as well as \(W_2\) are the weight matrices used for all \(x_t \rightarrow h_t\) and \(h_{t-1} \rightarrow h_t\) links respectively and \(b_h\) is the bias added when calculating \(h_t\). Hidden state \(h_{t-1}\), which is required for the calculation of the first hidden state, is typically initialized to all zeroes. Eventually, at the final timestep \(T\), a dense layer calculates as shown in Fig. 1(a) the output \(o_T\), given by the following equation:

$$o_T = f_2(W_3h_T + b_o)$$  \hspace{1cm} (3)

where function \(f_2\) is usually a softmax activation function (as in the case of 1D-CNNs, and generally when the task is a classification problem), which takes the logits of the dense layer and turns them into probabilities, and \(b_o\) is the bias added. Note that depending on the task, Fig. 1(a) could have an output \(o_t\) at each timestep \(t\), but for our application, which outputs a cell-type label given an input time-series, only one output is calculated at the final timestep. Moreover, it is worth noting that unlike a traditional deep neural network, which uses different parameters at each layer, an RNN model shares the same parameters \((W_1, W_2)\) as shown in Fig. 1(a) across all timesteps. This reflects the fact that we are performing the same task at each step, just with different inputs, which greatly reduces the total number of parameters that the network needs to learn.

Despite the advantage of the RNNs to remember information through time, training an RNN is not a very simple task, in terms of the backpropagation, which is used as in the traditional neural networks but with a little twist. Because the parameters are shared by all timesteps in the network, the gradient at each output depends not only on the calculations of the current timestep, but also on the previous timesteps. For example, in order to calculate the gradient at
Fig. 1. Proposed Deep Learning architectures for the neuronal cell-type classification: (a) RNN architecture: An RNN layer unfolded in $T$ RNN Cells, where $T$ is the total number of timesteps. Every cell accepts at timestep $t$, the current value $x_t$ and the previous hidden state $h_{t-1}$ as inputs and in our case only the last cell outputs a vector $o_T$, which represents the 4 distinct classes of our problem. (b) LSTM architecture: An LSTM layer unfolded in $T$ LSTM units. At timestep $t$ the gates $I$, $F$ and $O$ calculate their activations (i.e. $I_t$, $F_t$ and $O_t$ respectively) considering the current value $x_t$ and the activation of the memory cell at the previous timestep $C_{t-1}$. Circles containing an $X$ symbol represent an element-wise multiplication between its inputs. The rectangles containing a $σ$ symbol represent the application of the sigmoid differentiable function. At final timestep $T$ the last LSTM unit outputs the vector $o_T$ with the 4 classes of the problem. (c) 1D-CNN architecture: The input vector $x$ is convolved with a trainable filter $f$ (stride equal to 1) resulting to a vector $c_t$, to which a non-linear activation function is applied, resulting to another vector $c_t′$ with the same size. A max pooling layer of size 2 is also applied to $c_t′$, in order to down-sample the input representation reducing its dimensionality. The number of the output neurons $C$ equals to the number of the classes (4 in our case).

$t = 4$ we would need to backpropagate 3 steps and sum up the gradients. This is called Backpropagation Through Time (BPTT) and vanilla RNNs trained with BPTT have difficulties learning long-term dependencies (i.e. dependencies between steps that are far apart) [25], due to what is called the vanishing/exploding gradient problem. Thus, in order to address these issues, certain types of RNNs, like LSTMs, which are described in the next subsection were specifically designed to deal with these problems.

2.3 Long Short-Term Memory Neural Networks

Long Short-Term Memory is an artificial recurrent neural network model [26] used in the field of deep learning, which was developed to deal with the exploding and vanishing gradient problems that can be encountered when training traditional RNNs. An LSTM has a similar control flow as a recurrent neural network. It processes data passing on information as it propagates forward. The differences with traditional RNNs. An LSTM has a similar control flow as a recurrent neural network. It processes data passing on information as it propagates forward. The differences with traditional RNNs. An LSTM has a similar control flow as a recurrent neural network. It processes data passing on information as it propagates forward. The differences with traditional RNNs. An LSTM has a similar control flow as a recurrent neural network. It processes data passing on information as it propagates forward. The differences with traditional RNNs.

A common architecture of an LSTM unit at timestep $t$, as shown in Fig. [1](b) is composed of a cell state $c_t$, which is the memory part of the LSTM unit, and three “regulators”, usually called gates that control the flow of information inside the LSTM unit: a forget gate $F_t$, which decides what is relevant to keep from prior steps, an input gate $I_t$, which decides what information is relevant to add from the current step and an output gate $O_t$, which determines what the next hidden state $h_t$ should be. Some variations [29], [30] of the LSTM units do not have one or more of these gates or maybe have other gates.

The first step of an LSTM unit is to decide what information it is going to throw away from the cell state. This decision is made by a sigmoidal layer called the forget gate layer. It looks at $h_{t-1}$, which is the hidden state vector also known as output vector of the LSTM unit and the current information $x_t$, and outputs a number between 0 and 1 for each number in the cell state $c_{t-1}$. A 1 represents completely keep this information, while a 0 represents completely get rid of this. The equation for the forget gate layer is as follows:

$$F_t = σ_g(W_Fx_t + R_Fh_{t-1} + b_F)$$

(4)

where $σ_g$ denotes the gate activation function, $W_F$, $R_F$ and $b_F$ are the learnable weights of an LSTM layer, i.e. they are the input, the recurrent weights and the bias respectively for the forget layer component.
The next step, which consists of two parts is to decide what new information will be stored in the cell state. First, a sigmoid layer called the input gate layer decides which values will be updated and a tanh layer creates a vector of new candidate values, $c_t'$ that could be added to the cell state. The equations describing these components at timestep $t$ are the following:

$$I_t = \sigma_g(W_Ix_t + R_Ih_{t-1} + b_I)$$

(5)

$$c_t' = \sigma_c(W_cx_t + R_c h_{t-1} + b_c)$$

(6)

where $\sigma_c$ denotes the candidate cell state tanh activation function and $W_I$, $R_I$, $b_I$ as well as $W_c$, $R_c$ and $b_c$ are the learnable input and recurrent weights, and the bias for the input gate and cell candidate respectively. These two steps are combined in order to update the old cell state $c_{t-1}$ into a new cell state $c_t$ based on the following equation:

$$c_t = F_t \ast c_{t-1} + I_t \ast c'_t$$

(7)

Eventually, the LSTM unit needs to decide what it is going to output. The output will be based on the cell state, but will be a filtered version. Firstly, a sigmoid layer called the output gate layer decides what parts of the cell state it is going to output. Then, the cell state is put through a tanh, so that the values are pushed between $-1$ and $1$ and is multiplied with the output gate layer so that it outputs via the hidden state $h_t$ only the parts that it decided to. The equations describing these components are the following:

$$O_t = \sigma_g(W_Ox_t + R_Oh_{t-1} + b_O)$$

(8)

$$h_t = O_t \ast \sigma_c(c_t)$$

(9)

where $W_O$, $R_O$, $b_O$ in eq. [8] are the learnable input and recurrent weights and the bias for the output gate. Note that depending on the task, LSTMs could also output a label at each timestep $t$, but for our application, which outputs a cell-type label given an input time-series, only one output is calculated at the final timestep.

### 2.4 Regularization Methods

Deep neural network architectures are very powerful machine learning systems but with a large number of parameters, which makes them quite complex models. As a neural network learns, neuron weights settle into their context within the network. Weights of neurons are tuned for specific features providing some specialization. Neurons of neighboring layers rely on this specialization, which if taken too far can result in a fragile model too specialized to the training data. This reliance on context for a neuron during training is referred to complex co-adaptations and can lead to overfitting of the training data, meaning that the network produces over-optimistic predictions throughout the training process, but fails to generalize well on new data leading to a decaying performance.

Dropout [31] is a regularization technique, which is essentially used to prevent overfitting while training neural nets. The term dropout refers to dropping out units in a neural network, and thus these units are not considered during a particular forward or backward pass. More specifically for the 1D-CNNs, at each training stage, individual nodes of a specific layer are either kept with probability $p$ or dropped out of the net with probability $1-p$, so that a reduced network is left with the incoming and outgoing edges of the dropped-out node to be removed. Regarding the RNN and LSTM architectures, dropout can be also applied to the recurrent connections of these networks (i.e. the connections related to the hidden states), so that the recurrent weights could be regularized to improve performance. For any of the three architectures each layer can be associated with a different $p$ value, meaning that dropout can be considered as a per-layer operation with some layers discarding neurons in a higher percentage compared to others dropping neurons in a lower rate or not at all.

### 3 Experimental Analysis and Discussion

The Deep Learning models that were used in our analysis were implemented with the help of Tensorflow [32] and Keras open-source libraries written in Python code. Tensorflow is a general-purpose machine learning framework for numerical computations using data flow graphs, developed by Google, and Keras is a higher level Deep Learning-specific library, which utilizes TensorFlow as a backend engine with support and frequent updates on the most state-of-the-art deep neural network models and algorithms. Both TensorFlow and Keras can run calculations on GPU, dramatically decreasing the computational time of the networks training. For our experiments we used Python 3.6, the Tensorflow version 1.9 and NVIDIAs GPU model, GeForce GTX 750 Ti, running on Windows 10 operational system.

### 3.1 Data Set

The data set we used in order to train the classifiers were collected during a goal oriented task in awake, behaving mice. Specifically, head-fixed mice ran on a 2-meter long treadmill belt equipped with a water delivery port and the neural recordings were performed using two-photon imaging. The mice learned the reward location after training on the belt for a few days. The recordings were made in CA1, a hippocampal subarea responsible amongst others for the spatial memory formation. The data were then processed in order to translate the video recordings into fluorescence signals over time. Four different neuronal types were recorded during the aforementioned task. Namely, the excitatory pyramidal cells, the PV, the SOM and the VIP inhibitory neurons making the problem a four-class classification task. Therefore, our design matrix consists of signals in time (time-series) of four different neuronal types across all sessions/days and different animals.

### 3.2 Impact of Regularization and Network’s Depth

In this subsection we study how the architecture of each model, such as depth and dropout regularization, affects the performance of each model as well as the training run time corresponding to 20 epochs, and we present the results in Table 7. We have used a fixed number of 3157 training and 790 testing examples, where each example is a time-series that corresponds to a specific neuronal cell-type consisting...
of 4000 time-steps (we have used the minimum length of all time-series). Each experiment is repeated 10 times and in every experiment we randomly select 1000 PY examples out of the 11200 available, 1000 SOM examples out of the 1478 available, 1000 VIP examples out of the 7747 and all the 947 PV available examples. These 3947 examples are split into a training and testing set, and after the split we apply a z-score normalization to both sets, based on the mean and standard deviation of the training set. Thus, we report the mean accuracy and training run time of the 10 experiments and their corresponding standard deviations. The hyperparameter values for all the models have been selected after several pre-experiments, which showed that they were the desirable values for the network's training.

Regarding the architectures of the 1D-CNN model, which are presented in Table 1, each convolutional layer is followed by a relu activation function and the pipeline ends up with a Fully Connected (F.C.) layer. The optimizer that is used in order to train is the Adam gradient-descent based algorithm with learning rate 0.001. The first convolutional layer of each architecture is convolved with 32 filters of kernel size 10 and stride 1, while as we go deeper, namely from the second convolutional layer onwards, layers are convolved with 64 filters of kernel size 10 and stride 1. We can see from Table 1 that the best architecture is the fifth one, whose mean accuracy is in bold, with 2 convolutional layers followed by a max pooling layer of size and stride equal to 2, which is followed by a last convolutional layer and a dropout layer.

We observe that the architecture consisting of 2 convolutional layers gives a better classification performance compared to the architectures consisting of 1 and 3 convolutional layers, as by using only 1 layer, we create a very shallow network, which cannot be trained properly, while 3 layers lead to overfitting. By using the dropout regularization technique combined with a max pooling layer in order to control overfitting, we observe that the 5th architecture, where the dropout layer is inserted just before the F.C. layer is the most effective one, as F.C. layers are more prone to overfitting due to their large number of connections. Regarding the training run time, as expected, this increases by adding more convolutional layers.

Concerning the RNN as well as the LSTM model, each time-series $X$ of length $N$ is unfolded in a number of time-steps $T$ with each time-step $x_t \in \mathbb{R}^d$, where $d$ is the input dimensionality and $t = 1, ..., T$, such that $N = d \cdot T$. Namely, RNN and LSTM models accept input time-series $X$ consisting of $T$ time-steps, where each time-step is of dimensionality $d$. In our case, where each time-series has a length of $N = 4000$ time-steps, we break each time-series as shown in Table 1 in $T = 2$, $T = 5$ and $T = 10$ time-steps, where each time-step $x_t$ is of dimensionality $d = 2000$, $d = 800$ and $d = 400$, respectively. Each RNN and LSTM layer is followed by a relu activation function and the pipeline ends up with a F.C. layer. Moreover, each RNN and LSTM layer consists of 100 hidden units, which is essentially the dimensionality of the output space, and the optimizer used for training the network is the Adam gradient-descent based algorithm with learning rate 0.001.

More specifically, the best architecture for the RNN model, as shown in Table 1 is obtained in the case of 2 time-steps for a single RNN layer. Moreover, for all different values of time-steps, the single RNN layer architecture gives better classification results compared to the stacked RNNs (i.e. an RNN with more than one layer), which lead to network overfitting. In order to prevent overfitting, we added a dropout layer, but as shown in Table 1 the performance was not improved. We also experimented by adding a recurrent dropout layer together with the dropout layer, and also tried adding only a recurrent dropout layer, but the accuracy performance was worse (i.e. 1% – 3% lower). As far as the training run time is concerned, we observe again that as we increase the complexity of the architecture by adding extra RNN layers, the training run time is also increased. Regarding the LSTM model, the best architecture as shown in Table 1 is obtained in the case of 2 timesteps with 2 stacked LSTM layers. In general, as in the case of RNN, the various combinations of dropout and recurrent dropout layers among the LSTM layers did not improve the classification performance.

We also demonstrate in Fig. 2 the normalized confusion matrices that were obtained by the best architectures of the models, as they were reported in Table 1 (architectures whose accuracy is highlighted in bold). We observe that for all models, most of the mistakes occur in the identification of the VIP cells, while the PY and PV cells are the ones most correctly identified.

From the results presented in Table 1 and Fig. 2 we observe that 1D-CNN is identified as the best model giving the most accurate predictions. The intuition behind this finding is that the input data probably do not have notably long-term dependencies, as the models which are capable of identifying such dependencies, also using them for future prediction, are the RNN and LSTM models, while CNN architectures mainly focus on the given input sequence and
do not use any previous history during the learning process. This finding is also strengthened, as shown in Table 1, by the fact that as we increase the number of time-steps (and thus automatically reducing the input dimensionality), there is a clear decrease in the accuracy performance of the RNN model. The LSTM model has more fluctuations regarding its performance, but if we exclude the architecture of the single RNN layer, all the rest have better performance when 2 time-steps are used. We also experimented with the extreme case of \( T = 4000 \) time-steps, which practically means that we feed the network by giving a scalar value at each time-step. The results regarding the performance were very poor, as the maximum classification accuracy that was achieved was around 45\% for both models. Thus, we conclude that for these two models a higher input dimensionality is preferable than an unfolding of too many time-steps, which indicates that no significant long-term dependencies exist in the input data. Eventually, we observe that the LSTM model has a worse performance compared to the RNN model, regarding both the classification accuracy and the training run time. The worse performance can be justified by the fact that the LSTM models are more complex than the RNNs, and as it was proved in our case, given also the fact that we did not use a big volume of training data, this complexity is unnecessary for the modeling of the specific data. This was also revealed by the training accuracy, where 1D-CNN and RNN models achieved a training accuracy of 95\% – 100\% during the last epochs, while the LSTM model, depending on the architecture achieved a lower training accuracy of 88\% – 97\%.

We also experimented with number of epochs that the network needs to be trained, by training the best 1D-CNN model with 60 and 100 epochs and the achieved accuracy was 0.8787 and 0.881, respectively. This demonstrates that the number of 20 epochs that we used for training is adequate, as more epochs lead to overfitting and they would probably make sense only if we trained the model with much more data. For the best-case performance of the RNN model, we also used 60 and 100 epochs, in order to train the network and we obtained an accuracy of 0.8188 and 0.8169, respectively. Eventually, by training the best LSTM model with 60 and 100 epochs, we achieved an accuracy of 0.7911 and 0.7894, respectively, which again confirmed the adequacy of 20 epochs, given the specific number of 3157 training examples used here.

| Models | Depth | Mean Acc. | St. Dev. | Mean Tr. Run Time (sec.) | St. Dev. |
|--------|-------|-----------|----------|--------------------------|----------|
| 1D-CNN | 1 Conv. Layer | 0.8368 | 0.0116 | 50.2281 | 0.8775 |
|        | 2 Conv. Layers | 0.8674 | 0.0116 | 124.0125 | 2.7903 |
|        | 2 Conv. Layers-Max P-1 Conv. Layer | 0.8739 | 0.0125 | 155.3015 | 1.9314 |
|        | 2 Conv. Layers-Max P-2 Conv. Layer | 0.8746 | 0.0102 | 188.139 | 2.9593 |
|        | 2 Conv. Layers-Max P-1 Conv. Layer-Dropout | 0.8807 | 0.0119 | 161.9093 | 2.3241 |
|        | 2 Conv. Layers-Max P-1 Conv. Layer-Dropout-1 Conv. Layer | 0.8683 | 0.0114 | 197.5203 | 3.5911 |
|        | 2 Conv. Layers-Dropout-2 Conv. Layers | 0.8394 | 0.0219 | 345.664 | 2.3541 |
|        | 3 Conv. Layers | 0.8502 | 0.032 | 226.925 | 2.5193 |
| RNN 2 timesteps | 1 RNN Layer | 0.8205 | 0.011 | 38.6984 | 0.6726 |
|        | 2 RNN Layers | 0.804 | 0.03 | 55.1843 | 1.1724 |
|        | 2 RNN Layers-Dropout-1 RNN Layer | 0.8067 | 0.0141 | 71.5421 | 0.9292 |
| RNN 5 timesteps | 1 RNN Layer | 0.8167 | 0.01627 | 49.925 | 0.6941 |
|        | 2 RNN Layers | 0.8065 | 0.0151 | 80.2203 | 1.1572 |
|        | 2 RNN Layers-Dropout-1 RNN Layer | 0.7975 | 0.0227 | 109.6156 | 1.2772 |
| RNN 10 timesteps | 1 RNN Layer | 0.8026 | 0.0194 | 71.8468 | 0.7897 |
|        | 2 RNN Layers | 0.8024 | 0.0273 | 124.6187 | 0.979 |
|        | 2 RNN Layers-Dropout-1 RNN Layer | 0.7969 | 0.0133 | 170.7687 | 1.6552 |
| LSTM 2 timesteps | 1 LSTM Layer | 0.7734 | 0.0166 | 69.35 | 0.8836 |
|        | 2 LSTM Layers | 0.7915 | 0.015 | 102.8062 | 1.7754 |
|        | 2 LSTM Layers-Dropout-1 LSTM Layer | 0.7897 | 0.01 | 141.7281 | 2.1877 |
| LSTM 5 timesteps | 1 LSTM Layer | 0.7869 | 0.0211 | 91.5953 | 1.3077 |
|        | 2 LSTM Layers | 0.7822 | 0.0158 | 156.3843 | 2.9283 |
|        | 2 LSTM Layers-Dropout-1 LSTM Layer | 0.7648 | 0.018 | 228.5359 | 3.7028 |
| LSTM 10 timesteps | 1 LSTM Layer | 0.7911 | 0.0111 | 140.9765 | 1.3509 |
|        | 2 LSTM Layers | 0.7896 | 0.0171 | 256.5265 | 1.333 |
|        | 2 LSTM Layers-Dropout-1 LSTM Layer | 0.6464 | 0.1118 | 381.5828 | 4.6384 |

TABLE 1
Mean accuracy performance of Deep Learning architectures for neuronal cell-type classification and their corresponding mean training run time for 10 runs per experiment-architecture.
| Model      | Mean Accuracy | St. Dev. |
|------------|---------------|----------|
| 1D-CNN     | 0.8867        | 0.0115   |
| RNN        | 0.7915        | 0.015    |
| LSTM       | 0.8184        | 0.0108   |
| Linear SVM | 0.6168        | 0.0125   |
| Gaussian SVM| 0.7715   | 0.0152   |
| Polynomial SVM| 0.8205 | 0.0108 |

TABLE 2
Comparison between the best-case performance proposed methods with the SVM classifier.

3.3 Comparison with other classifiers

We next compared the optimal performance of the proposed models against the popular Support Vector Machine (SVM) classifier for the cases of linear, gaussian and polynomial kernels. Table 2 corroborates the claim that 1D-CNN is the most efficient algorithm for the task at hand. The main competitor is the gaussian SVM, which actually outperforms all the other models except from the RNN. The parameters related to the Gaussian and polynomial SVM classifier are the gamma and penalty parameters and the degree parameter for the polynomial SVM, while the parameter related to the linear SVM classifier is the penalty parameter. Gamma is a parameter for non linear hyperplanes. The higher its value the harder the classifier tries to fit the training data set. The penalty parameter of the error term controls the trade off between a smooth decision boundary and classifying the training points correctly. Degree is a parameter used with a polynomial kernel and is essentially the degree of the polynomial used to find the hyperplane to split the data. After extensive experimentation, we found that for the gaussian SVM, gamma equal to 0.0001 and the penalty parameter equal to 100 are the best-performing combination. For the polynomial SVM, which has lower accuracy performance, the best combination of values is a polynomial degree equal to 3, the gamma and penalty parameters equal to 0.001 and 100, respectively. Eventually, for the linear classifier, which has the lowest accuracy performance, the optimal penalty parameter equals to 10. In general, pre-experiments showed that increasing the values of gamma and the penalty parameters leads to overfitting, as the classifier tries to perfectly fit the training data, while for smaller values, the classifier is not trained properly making more errors during the testing phase.

3.4 Impact of the number of time-steps

In this subsection, we examine the degree to which the classification accuracy and the training run time of each best-case performance architecture (according to Table 1) is affected, when we reduce the number of time-steps of the input time-series. As expected, the results in Table 3 confirm that decreasing the number of time-steps results in decreased accuracy performance and training run time for all models. However, for 2000 time-steps the classification accuracy (especially for the 1D-CNN model) has a small drop compared to the 4000 time-steps, while the training run time has a sharp decrease, suggesting that this value maybe acceptable for fast cell-type detection.

The above indicate that accurate results can be obtained even when using half of the recorded time-series, achieving a faster training and cell-identification process.

3.5 Testing the models on a new dataset

To assess the generalization performance of our models, we used a completely new dataset, obtained from an entirely different experiment in mice. The dataset consisted of 119 time-series, each one of length 2606 time-steps, 91 of which corresponded to the activity of SOM interneurons, while the rest to the activity of PV interneurons.

To ensure a fair comparison, we reduced the length of the time-series in the first dataset from 4000 to 2606 time-steps, in order to be of equal length with the time-series of the new dataset. We then re-trained our best models on the first dataset and tested them, separately, on both datasets. The accuracy that was achieved by the 1D-CNN model on the new dataset was 81.65%, while the RNN and LSTM models achieved an accuracy of 78.3% and 73.28% respectively, which are very promising results, given the fact that the models were trained and tested on completely different datasets.

As shown in the normalized confusion matrices of Fig. 3, all models make most of their mistakes in the identification
of the VIP and PV neurons, which are mainly misclassified as SOM cells. More specifically, the VIP neurons are again misclassified with a SOM label, as in the case of the first dataset (Fig. 2). Regarding the PV neurons, they are also misclassified as SOM cells, but only when we use the second dataset. This could be explained by the fact that the newly added PV cells are only 28, which is a quite small sample that probably does not allow the network to recognize this type correctly, compared to the 191 newly added SOM cells, where there is more information that can be exploited.

### 4 Conclusion

In this work we propose a deep learning-based formalization for the task of neuronal cell-type classification. We employed the 1D-CNN, RNN and LSTM models, and as it was shown, the specific deep neural network architectures are capable of capturing hidden dynamics and also making accurate predictions. For the specific task of neuronal cell-type classification, 1D-CNN is identified as the best model, which shows that no significant long-term dependencies exist in the given data. The great potential of the proposed methods that was unveiled by the experimental results demonstrate that 1D-CNN could potentially replace dyes used with calcium imaging as well as feature extraction algorithms making neuronal cell-type classification an automated procedure.

Moreover, these results trigger the need for a deeper study, which will include more neuronal cell-types and also their subtypes (i.e. basket cells, axoaxonic and bistratified cells that are subtypes of the PV cells) examining if their recognition is possible. Another avenue of application involves the development of a continuous learning artificial neural network. Namely, this network will be able to understand if during the testing procedure new neuronal cell-types appear (i.e. cell-types that were not used during its training), and should incorporate them in the network being able to recognize them in a next appearance but without being trained from beginning. These two cases will be investigated in our future work.

### Acknowledgments

This research is co-financed by Greece and the European Union (European Social Fund-ESF) through the Operational Programme ‘Human Resources Development, Education and Lifelong Learning’ in the context of the project ‘Strengthening Human Resources Research Potential via Doctorate Research’ (MIS-5000432), implemented by the State Scholarships Foundation (IKY).

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