Evaluating Extracellular Cell-Typing Methods with Ground Truth Data

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Abstract

Multielectrode arrays can record information from thousands of cells, but with only their extracellular waveforms and location it can be difficult to cluster individual units and distinguish different cell types. In order to better understand the function of the brain and how it makes decisions, knowing what type of neuron each recorded signal originates from may prove to be a useful tool. Distinguishing exitatory and inhibitory neurons may allow for better understanding of neural circuits or improved decoding.

The first step in extracellular cell typing depends on generating informative representations of waveforms, these representations should allow for better clustering of similar waveforms while being mostly invariant to variables that do not originate from biological differences. These representations can then be clustered to generate groups of units that display similar extracellular dynamics.

However, it remains unclear how effective this strategy is at capturing the underlying biology of the brain. Even with perfectly clustered waveforms it may not be enough to distinguish the various cell types in the mouse brain. To answer this, ground truth data in the for of optotagging data from the Allen institute was used to provide information in the cell types that a subsection of the units recorded by a neuropixel probe corresponds to.

The results of this work show that some cell types are clearly distinguishable from others using their extracellular dynamics alone, however there are many other cell types that remain difficult to distinguish.

1 Introduction

Electrophysiology with Multielectrode Arrays

Multielectrode arrays (MEAs) have opened many avenues in the world of systems neuroscience. The ability to record from an unprecedented number of neurons simultaneously, across many brain regions and cell types, and with high temporal resolution allows for many experiments that would be impossible with optical (or other) methods.

One commonly used MEA, is the neuropixels probe. This device has 384 channels arranged along the face of a 10mm long shank. This shank can then be inserted into the brain to allow for simultaneous recording across along its length.

However, there MEAs are not without their drawbacks. A lack of spatial resolution makes identifying individual units a challenge, relying on characteristics of the recorded waveforms along with limited location information to cluster spikes into putative units. While the freedom from genetic labelling is an advantage in many ways, it also means that it is very challenging to identify the cell types that have been recorded from. This can make understanding neural circuits difficult as the roles of different units are unknown. [1]

Contrastive Learning

In order to generate lower dimensional representations of waveforms that can be better clustered, many methods have been proposed. While PCA remains a common choice its assumption of linearity and its sensitivity to nuisance variables makes it perform poorly on some data types. [2]

Another method that has become extremely popular in the world of computer vision is contrastive learning. This self supervised method allows the generation of representations that cluster similar objects. This is achieved through the utilisation of data augmentation and a loss function that draws augmentations of the same object closer together while repelling augmentations of different objects.

The software package CEED was used to generate contrastive embeddings, this method utilises augmentations specific to extracellular recordings to generate embeddings that are insensitive to nuisance variables such as noise and temporal jitter. [3]

Optotagging Data

In order to address the question of how well these representations are actually informative about underlying cell-types, the Allen Institutes optotagging datasets were used [4]. This data contains neuropixels recordings from the mouse brain taken taken from genetically engineered mice which expressed the light-gated ion channel channelrhodopsin-2 (ChR2) in different sub-populations of their

neurons. This allowed the experimenters to expose the cortex to blue light, activating these neurons, and allowing the identification of neurons which were tuned to the application of blue light. The assumption here is that these neurons are those expressing ChR2 and as a result, come from the specific population labelled.

This experiment was repeated across wild type mice and in 3 genetic strains, each of which expressed ChR2 in a different sub-population of inhibitory neurons: Somatostatin (Sst), parvalbumin (Pvalb), and vasoactive intestinal peptide (Vip) expressing neurons. These interneuons combined make up almost all of the interneurons in the neocortex.

2 Results

Effectiveness of Low Dimensional Representations for Clustering Waveforms

When evaluating cell types, a low dimensional representation of waveforms that allows for effective clustering is a common first step. There are many strategies for generating these embeddings and their effectiveness is evaluated based on the homogeneity of the clusters they produced.

As a first attempt at verifying the usefulness of CEED embeddings, its potential for improving spike-sorting methods was examined. In figure 1 it is clear that using a contrastive embedding would have merged these two clusters shown. However when using the kilosort spike sorting algorithm these clusters are identified as separate units, likely due to the small spatial separation between them. It is, however, difficult to know which interpretation is correct without ground truth data, this is a problem in cell-typing as well.

The effectiveness of clustering similar waveforms is also shown. Figure 2 shows the clusters generated using a gaussian mixture model (GMM) to cluster hand selected features from individual waveforms. These hand-picked features are: peak value, the largest value in the waveform; tip index, the index of the largest value before the absolute peak; tip value, the magnitude of this tip; trough value, the largest value after the peak; and peak to trough time, the time interval between the peak and the trough.

Finally, the effectiveness of using contrastive embeddings generated with CEED is shown in figure 3. These waveforms are also clustered with a GMM.

The clustered CEED embeddings seem, by eye, to be more homogeneous than those from hand picked features. Interestingly the CEED embeddings seem to be more robust to the sign of the waveform, and more representative of the overall shape of the waveform.

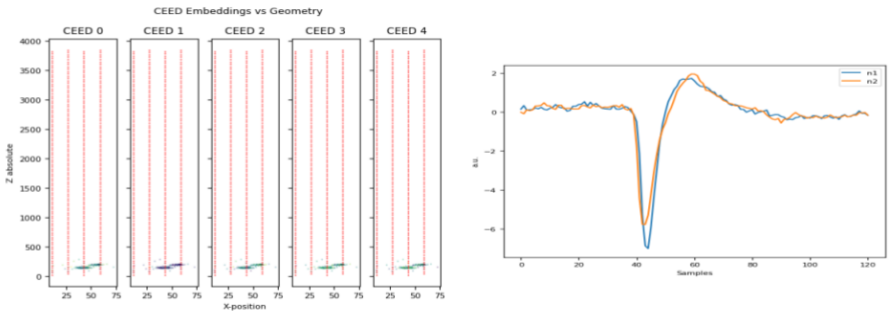

Figure 1: Ceed embedding values shown for two kilosort identified units shown over a neuropixels probe's geometry compared with their recorded wavefroms

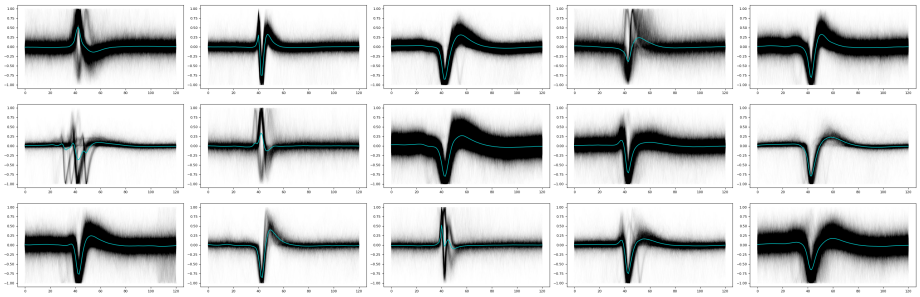

Figure 2: Waveforms clustered using hand selected features

Extracting Optotagging Data

Although clear that contrastive embedding provide a good low-dimensional representation of extracellular waveforms, it is less clear how informative waveforms are of cell type. Addressing this with the optotagging dataset requires the preprocessing and extraction of units that are tuned to optical stimuli.

In order to do this, for each session in the dataset the section of the recording that occurred during optical stimulation was extracted and each units instantaneous firing rate was found using a time-bin of 0.5 milliseconds. This firing rate was then compared to the unit's firing rate before optical stimulation, and units with an increase in firing rate of at least 2x were extracted. These units were then considered to be expressing ChR2 and labelled as the whichever cell

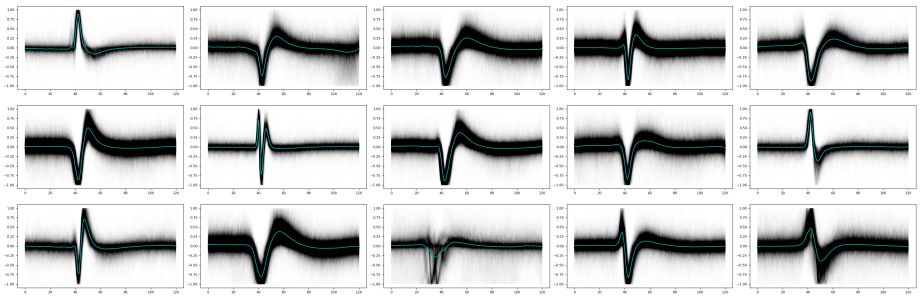

Figure 3: Waveforms clustered using CEED embeddings

type the animals was engineered to express ChR2 in (Sst, Vip, or Pvalb). The units that were not tuned to the stimulus were also extracted and labelled as untuned to serve as negative examples in the resulting dataset.

Figure 4 shows this process for a Pvalb tagged animal. Here the increase in firing rate is clear in a large number of units during optical stimulation, and the increase in firing rate versus baseline firing rate is plotted to show which units have a distinct increase during optical stimulation. Figure 5 shows the same analysis performed on wild type animals, where no units are expected to be tuned.

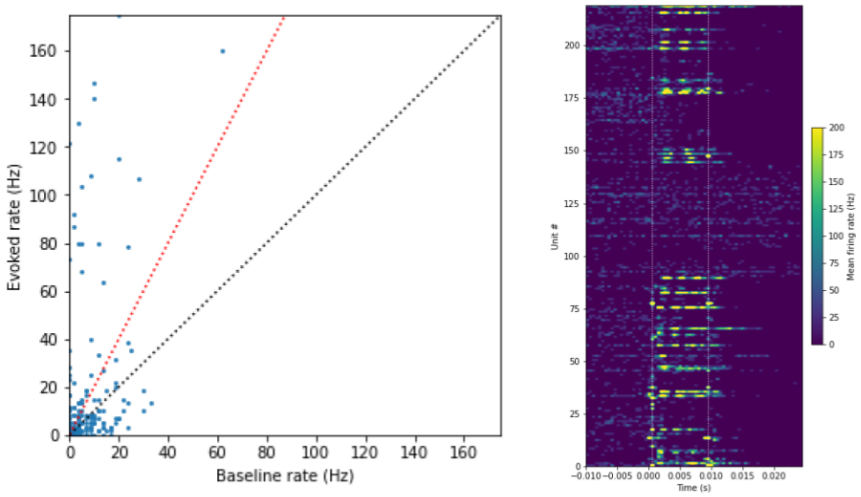

Figure 4: Pvalb neurons tuning response to optical stimulation

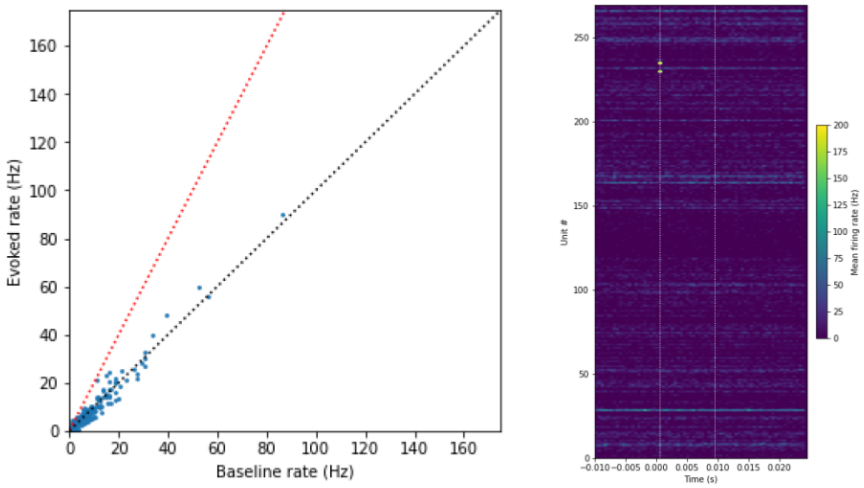

Figure 5: Wild type neurons tuning response to optical stimulation

Contrastive Embeddings to Identify Cell Types

Using this labelled dataset, we can revisit the issue of clustering using contrastive embeddings and evaluate its effectiveness at extracting cell types.

Once the waveforms were extracted, a CEED model was used to embed each waveform and a GMM used to cluster them. Before selecting the hyperparamters for the GMM, an evaluation was performed to identify the ideal number of clusters to use. This was performed using the Baysian information criterion (BIC) for the GMM with varying numbers of clusters. Figure 6 shows the result of this evaluation and indicates that the ideal number of clusters is four. This is encouraging as we know there are four distinct groups of units in the dataset (pvalb, vip, sst, and untuned units).

Following this evaluation, the units were clustered into four groups and the composition of each cluster evaluated 7. Using the labels for each unit along with the clustered waveforms, we can see that pvalb units are particularly distinguishable from untuned units. Although there is some separation between the other cell types - particularly sst cells, which are predominantly found in cluster number 3 - the ability to distinguish individual cell types is not particularly robust using this method. Vip units are especially hard to distinguish from untuned neurons, and on evaluating their waveforms it becomes clear that distinguishing these cell types based on waveform alone will be challenging.

The waveforms composing each cluster can be seen in figure 8. We can see that pvalb units (green) are noticeably different than any of the other cell types. The waveform is narrower and the first peak is consistently flatter. However, this figure also highlights some limitations of the clustering used. There are notice-

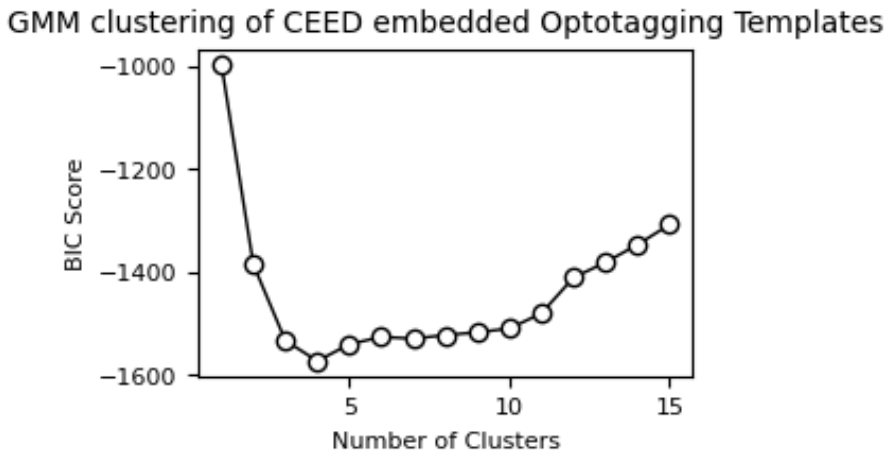

Figure 6: BIC scores for increasing numbers of clusters when using a GMM

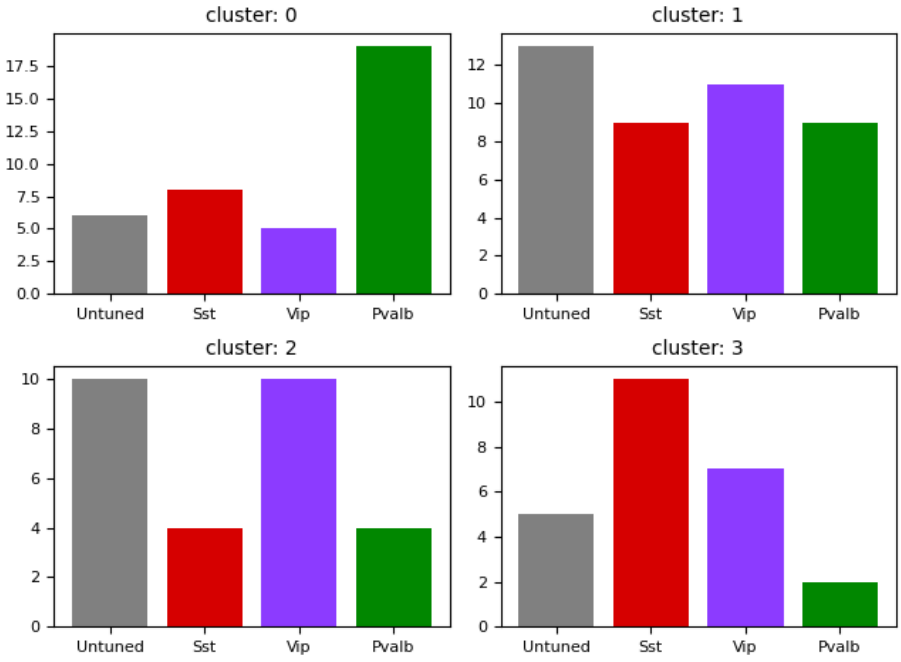

Figure 7: Cell type composition of each cluster

ably different waveforms in each cluster, and more importantly these different waveforms originate from units of different cell types. It seems likely that if waveform clustering is improved this will likely improve cell typing capabilities.

However, improved clustering will not likely allow for perfect cell typing as many different units display waveforms that are challenging to distinguish by eye despite originating from differently labelled units. It seems that while some cell types display distinct waveforms (Pvalb expressing cells in this dataset) not all cell types are so easily distinguished.

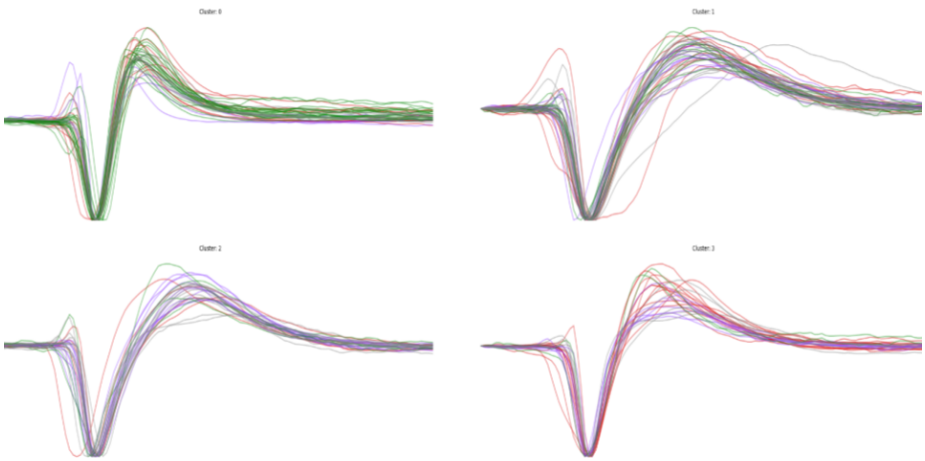

Figure 8: Waveforms found in each cluster, coloured by cell type

Finally, the CEED embeddings were compressed into two dimensions using principal component analysis (PCA) and plotted in figure 9. In this figure it appears that the different cell types are not easily separable, however there are multiple explanations for this. PCA may not provide a separable representation of the CEED features, and alternative visualisation methods such as UMAP may be used to improve this.

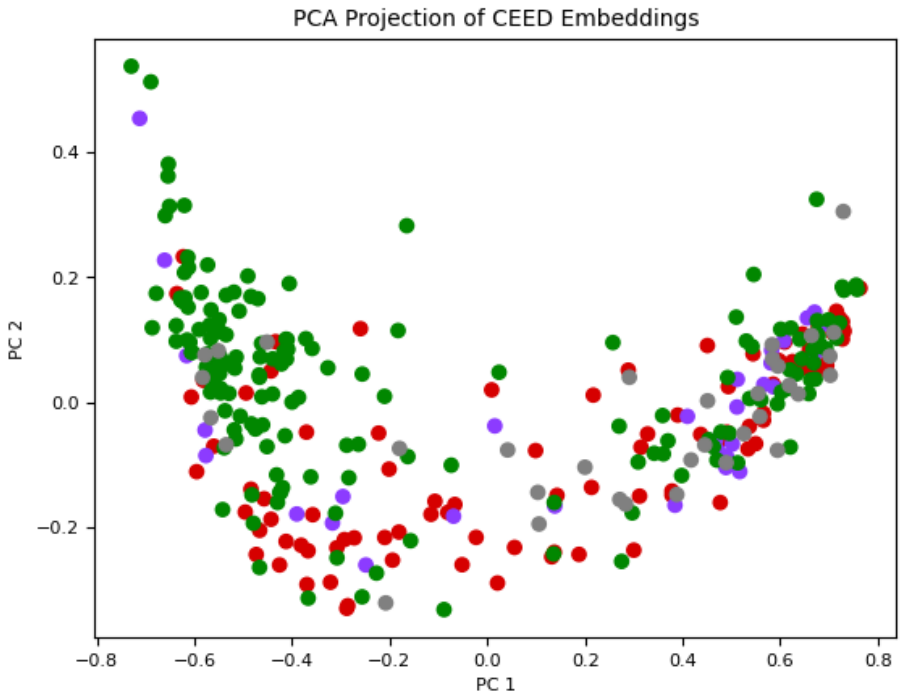

Figure 9: PCA embedded CEED features coloured by cell type

3 Discussion

While extracellular dynamics can be informative of cell types, it seems that it is unlikely that waveform information alone will not allow robust and accurate cell typing for all kinds of cells. However, for some cell types - particularly pvalb expressing cells - it can yield surprisingly accurate results.

Alternative Methods

One major improvement that could be made would be to use better clustering and embedding methods. While contrastive embeddings provide a good representation of a waveform, this should be compared to pca embeddings to attempt to confirm an improvement over the baseline. Additionally, this analysis could be used to inform better hand-picked interpretable features based on waveform features that best distinguish cell types.

In addition to improving featurisation, alternative clustering methods should be evaluated. While GMMs provide a good first attempt at clustering, they are not be able to capture clusters that are not 'gaussian' in nature, density based clustering methods such as HDBSCAN may provide an improvement here.

Using Additional Information

While this analysis has focused solely on waveform shapes, there are other forms of information yielded from extracellular recordings that could be used to identify different cell types.

One powerful indicator is a neurons firing rate, where we can assume diffident cell types have both different baseline firing rates, and different maximum rates. Additional information about spike distribution such as how 'bursty' a unit is (how likely the unit is to fire multiple times in a row, and for how long it will usually fire) are also strong indicators of cell type.

By adding this information into the analysis it is likely that cell typing accuracy will be improved.

References

[1] Steinmetz, Nicholas A., et al. "Challenges and opportunities for large-scale electrophysiology with Neuropixels probes." Current opinion in neurobiology 50 (2018): 92-100.

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[3] Vishnubhotla, Ankit, et al. "Towards robust and generalizable representations of extracellular data using contrastive learning." bioRxiv (2023): 2023-10.

[4] Saskia EJ de VriesJoshua H SiegleChristof Koch (2023) Sharing neurophysiology data from the Allen Brain Observatory eLife 12:e85550.