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Classifying GABAergic interneurons with semi-supervised projected model-based clustering

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ABSTRACT

Objectives: A recently introduced pragmatic scheme promises to be a useful catalog of interneuron names. We sought to automatically classify digitally reconstructed interneuronal morphologies according to this scheme. Simultaneously, we sought to discover possible subtypes of these types that might emerge during automatic classification (clustering). We also investigated which morphometric properties were most relevant for this classification.

Materials and methods: A set of 118 digitally reconstructed interneuronal morphologies classified into the common basket (CB), horse-tail (HT), large basket (LB), and Martinotti (MA) interneuron types by 42 of the world's leading neuroscientists, quantified by five simple morphometric properties of the axon and four of the dendrites. We labeled each neuron with the type most commonly assigned to it by the experts. We then removed this class information for each type separately, and applied semi-supervised clustering to those cells (keeping the others' cluster membership fixed), to assess separation from other types and look for the formation of new groups (subtypes). We performed this same experiment unlabeling the cells of two types at a time, and of half the cells of a single type at a time. The clustering model is a finite mixture of Gaussians which we adapted for the estimation of local (per-cluster) feature relevance. We performed the described experiments on three different subsets of the data, formed according to how many experts agreed on type membership: at least 18 experts (the full data set), at least 21 (73 neurons), and at least 26 (47 neurons).

Results: Interneurons with more reliable type labels were classified more accurately. We classified HT cells with 100% accuracy, MA cells with 73% accuracy, and CB and LB cells with 56% and 58% accuracy, respectively. We identified three subtypes of the MA type, one subtype of CB and LB types each, and no subtypes of HT (it was a single, homogeneous type). We got maximum (adapted) Silhouette width and ARI values of 1, 0.83, 0.79, and 0.42, when unlabeling the HT, CB, LB, and MA types, respectively, confirming the quality of the formed cluster solutions. The subtypes identified when unlabeling a single type also emerged when unlabeling two types at a time, confirming their validity. Axonal morphometric properties were more relevant that dendritic ones, with the axonal polar histogram length in the $[\pi, 2\pi)$ angle interval being particularly useful.

Conclusions: The applied semi-supervised clustering method can accurately discriminate among CB, HT, LB, and MA interneuron types while discovering potential subtypes, and is therefore useful for neuronal classification. The discovery of potential subtypes suggests that some of these types are more heterogeneous that previously thought. Finally, axonal variables seem to be more relevant than dendritic ones for distinguishing among the CB, HT, LB, and MA interneuron types.

1. Introduction

GABAergic interneurons of the cerebral cortex are key elements in many aspects of cortical function in both health and disease. Nevertheless, the classification of GABAergic interneurons is a difficult task and has been a topic of debate for a long time, since the

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pioneering work of Santiago Ramón y Cajal on the characterization and identification of interneurons [1]. The difficulty stems from the high variability of these cells according to morphological, electrophysiological and molecular features [2]. The scientific community lacks an accepted catalog of neuron names [3] which makes it difficult to organize and share knowledge [2]. There is some agreement on the set of morphological, molecular, and physiological features that can be used to distinguish among types of GABAergic interneurons [2]. However, a comprehensive classification according to those features is difficult to perform in practice [3]. A recent experiment enabled 42 expert neuroscientists from all around the world to classify interneurons by visual inspection and according to pre-selected neuron names [3]. It showed that the experts agree on the morphological definitions of some of the pre-selected types while disagreeing on the definitions of others. In particular, some types seemed to overlap in terms of the cells that were assigned to them by the experts. In [3], the authors also showed that supervised classification models can automatically categorize interneurons in accordance with the opinion of the majority of the experts.

Automatic classification of interneurons has mainly been done with (unsupervised) clustering; see, e.g., [4–8]. However, supervised approaches can be more accurate when there is prior knowledge about neuronal types [9]. In this study, such knowledge comes from the experts who participated in the experiment described in [3]. We can use this knowledge to guide classification and simultaneously discover subtypes using semi-supervised clustering, an approach that lies between the supervised and unsupervised approaches. In doing this, we follow the *cluster assumption* [10], i.e., we consider that the instances within a cluster are likely to belong to the same class whereas a class may consist of several clusters. In semi-supervised learning [10,11], some data instances are labeled whereas others are not. Since all our neurons were labeled by the experts, we fitted the semi-supervised scenario by removing the labels of (a) one type at a time; (b) two types at a time; and (c) half the instances of each type, simultaneously. By doing this we sought to discover possible subtypes and see if the types could be automatically discriminated. We used an adaptation of the semi-supervised projected model-based clustering algorithm (SeSProc) introduced in [12]. This is a probabilistic clustering algorithm which estimates the number of clusters and the relevance of each predictive feature for each of the clusters. The estimation of feature relevance within model-based clustering was introduced in [13].

We quantified the neurons with nine simple axonal and dendritic morphological variables, such as the axonal length close to the soma, and labeled them according to the choices of the expert neuroscientists. In [3] each instance was given up to 42 labels—coming from the 42 experts that concluded the study. Following a common practice in supervised learning [14], we reduced this vector of 42 labels to its mode (i.e., the most common value), thus obtaining a single label per neuron. However, since experts frequently disagreed, such labels were often not reliable, i.e., they were backed by few experts. To cope with the *label noise* [15,16] that expert disagreements may be introducing, we analyzed three subsets of our neuron population, each with a different minimum of 'label reliability', i.e., such that the label of each neuron in the subset was agreed upon by at least *th* experts, with *th* being a 'label reliability threshold'.

This paper is an extension of [17] and is the result of close collaboration between experts in neuroanatomy and machine learning.[1] We extend the mentioned paper by refining some of the predictor variables, adapting the SeSProC algorithm, and considering two additional experimental settings. The remainder of this paper is organized as follows: Section 2 describes the materials and methods we used; Section 3 reports and discusses the obtained results; while Section 4 provides conclusions.

[1] See the affiliations of the two institutions involved.

Table 1
Distribution of interneuron types with respect to label reliability threshold. Lowermost row shows total number of cells per dataset.

	th18	*th22*	*th26*
CB	49	24	9
HT	9	5	4
LB	27	19	12
MA	33	25	22
Total	118	73	47

2. Materials and methods

2.1. Data

We used 237 three-dimensional (3D) reconstructions of interneurons from several areas and layers of the cerebral cortex of the mouse, rat, and monkey. These neurons were used in [3], and were originally extracted from NeuroMorpho.Org [18]. From this population of neurons, we formed subsets by imposing minimums on the number of experts that agreed on the label of an included cell (i.e., a 'label reliability threshold'), considering that a higher threshold yields more confidence in the cells' labels. We used thresholds 18, 22 (half plus one out of the 42 experts), and 26 to build three databases: *th18*, *th22*, and *th26*, respectively. These data sets contained interneurons of four different types (classes): common basket (CB), horse-tail (HT), large basket (LB), and Martinotti (MA). Table 1 shows the distribution of different types at the three label reliability thresholds.

We characterized each neuron using nine features of axonal and dendritic morphology. While one may compute many morphological features (e.g., [3] used over 2000 features for classification), none are known, so far, as good predictors of interneuron type. Since detailed morphometric information on 3D reconstructed cortical interneurons is relatively scarce (a few hundred reconstructed neurons are available, comprising different types), it might be counterproductive to use many predictor variables. Therefore, we kept the number of variables low by defining variables which capture how, in our opinion, an expert classifies an interneuron upon visual examination.

We consider that an expert classifies an interneuron by estimating the distribution and the orientation of axonal and dendritic arborizations. We therefore measured the axonal and dendritic length according to the Sholl (5 features) and polar histogram (4 features) analyses from NeuroExplorer, the data analysis companion to Neurolucida [19]. Sholl analysis computes axonal and dendritic length at different distances from the soma whereas the polar histogram [20] describes the overall direction of dendritic growth; we only distinguished between two halves of the histogram, namely, the bifurcation angles falling in the $[0, \pi)$ interval and those falling in the $[\pi, 2\pi)$ interval. See Table 2 and Fig. 1 and for further details on predictor variables. We standardized all variables (transformed them so to have zero mean and unit standard deviation) prior to classification.

While an expert who classifies using a similar rationale can only roughly estimate these features, our classifier used exact values, thus possibly being more objective. This is important as some of the features that we use, such as the length of the axonal arbor at a certain distance from the soma, are rather hard for an expert to estimate.