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A proposal for the morphological classification and nomenclature of neurons*☆

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Abstract

The morphological and functional characteristics of neurons are quite varied and complex. There is a need for a comprehensive approach for distinguishing and classifying neurons. Similar to the biological species classification system, this study proposes a morphological classification system for neurons based on principal component analysis. Based on four principal components of neuronal morphology derived from principal component analysis, a nomenclature system for neurons was obtained. This system can accurately distinguish between the same type of neuron from different species.

Key Words: neuron; geometry; principal component analysis; back-propagating neural networks; morphological classification; neural regeneration

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INTRODUCTION

The brain, containing billions of neurons, is certainly the most complex biological organ in terms of both structure and function. Its basic element is the neuron-a cell whose geometric morphology varies greatly, from one type to another. Currently, the classification and nomenclature system for neurons is tremendously complicated and confusing, due to the diversity of neurons in different categories as well as the variety of neurons in the same category. Neurons function to transmit signals, and the geometric morphological features of neurons primarily consist of its spatial structure, which comprises the signal-receiving dendrite, the signal-processing soma, and the signal-sending axon. The complexity and diversity of neuronal shapes are a major reason for the lack of a comprehensive approach for identifying and classifying neurons. In particular, there is the need for a uniform methodology for the classification of neurons[1-4].

The morphological characteristics of neurons have attracted increasing attention[5-16]. Ascoli *et al* [17] studied the growth of neurons, and designed a number of neuronal characterization indices, which include stem number, branch number and bifurcation number. However, they did not study classification or nomenclature.

The existing classification system of neurons is based primarily on function and the number of dendrites, and a few other characteristics[18]. However, there is no satisfactory definition of neuronal cell type, with terms like *class*, *subclass*, *type*, and *subtype* often used interchangeably and without proper definition[19-20]. Ristanovic *et al* [21] directly divided neurons, based on size, into large, medium and small. Classification and identification based on a neuron's morphological and physical characteristics have yielded a relatively good classification scheme[22]. However, it does not provide for the normalized nomenclature of neurons. Only a few studies have focused on nomenclature[23-26]. In this study, we analyzed the spatial geometric features of neurons, and we proposed a neuronal morphological classification model based on a back-propagating (BP) neural network. Moreover, to resolve neuron naming problems, we proposed a normalized nomenclature of neurons derived from the statistical analysis of morphological data of known neurons.

RESULTS

Classification results based on principal component analysis (PCA) and the BP neural network

Neuron data from references[27-29] were used as the test dataset to evaluate the classifier proposed in this study. After statistical analysis and PCA on the test dataset, the input vectors of the classifier were acquired. In order to eliminate particularity, we

performed the experiments ten times, and then obtained the average outputs of the classifier (Figure 1). As shown in Figure 1, the proposed classifier appropriately classified about 95% of the neurons in the test dataset. However, the morphologies of some neurons were not stably classified in the ten experiments by the proposed classifier. Take an unknown neuron-A, for example-with four colors in its horizontal histogram (indicating that neuron A may be estimated to four different categories). Among the four colors, there were three histograms of the same length, indicating that neuron A may be estimated to these three categories with the same probability. Although we ultimately classified neuron A as a multipolar interneuron, it simply meant that interneuron A displayed morphological features characteristic of multipolar interneurons to a slightly greater extent, but it also exhibited features of neurons from other categories.

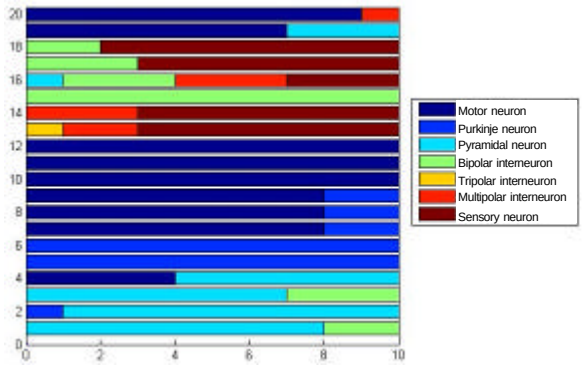

Figure 1 Distribution of classification results from ten experiments. In order to eliminate particularity, we performed ten experiments and then obtained the average outputs of the classifier. Different colors represent different types of neurons, while the length of each horizontal histogram with a color represents the account of the same type classified in ten experiments.

Nomenclature results for different types of neurons

A number of tests were performed to verify our classification and nomenclature system. First, five different neurons were selected according to a previous report[27] - a motor neuron, a Purkinje neuron, a pyramidal neuron, a bipolar interneuron, and a sensory neuron. Detailed information for these neurons and their PCA values are presented in Table 1.

Table 1 Classification of different neurons

Data file(.swc)	F_1	F_2	F_3	F_4	Neuron type
Cat motoneuron-2	9.025 5	0.919 0	-0.554 7	-0.540 8	A?-V5
Mouse Purkinje neuron 2	-1.976 0	4.718 6	-0.866 0	-0.163 2	Fa-V4
Rat pyramidal 3	0.422 0	0.987 8	1.903 9	2.008 9	D?-II2
Bipolar interneuron 1	-0.954 7	0.635 4	1.232 1	0.951 1	E?-III3
Sensory neuron 7	-2.250 1	-1.129 3	2.969 7	-2.321 4	Fe-I6

F_1, F_2, F_3, and F_4 are the four principal components of the neuron.

Based on these values, the neuron type was obtained. Test results showed that the proposed method can classify neurons with different morphologies correctly, and that the classification results can also provide a significant evaluation of morphological characteristics. It is very important to be able to distinguish between the same type of neuron from different species. The guinea pig and mouse Purkinje neurons were selected according to a previous study[27]. Detailed information for these neurons and their PCA values are given in Table 2. The pictures of these neurons are shown in Figure 2. The profile information of the guinea pig Purkinje neurons is more prominent. As a result, the proposed method has the ability to distinguish between the same type of neuron from different species. Based on these values, the type of Purkinje neurons from guinea pig and mouse was obtained. Test results demonstrated that Purkinje neurons from different organisms can be classified into different types.

Table 2 Classification of Purkinje neurons from guinea pig and mouse

Data file (.swc)	F_1	F_2	F_3
Guinea pig Purkinje neuron 1	-1.291 1	4.339 4	-1.231 7
Guinea pig Purkinje neuron 2	-1.369 5	3.463 8	-0.762 3
Guinea pig Purkinje neuron 3	-1.472 9	3.478 0	-1.282 3
Mouse Purkinje neuron 1	-2.445 9	4.750 8	-0.602 5
Mouse Purkinje neuron 2	-1.976 0	4.718 6	-0.866 0
Mouse Purkinje neuron 3	-1.968 9	3.314 4	-1.271 0

Data file(.swc)	F_4	Neuron type
Guinea pig Purkinje neuron 1	-1.425 7	Ea-V5
Guinea pig Purkinje neuron 2	-1.515 9	Ea-V5
Guinea pig Purkinje neuron 3	-1.477 2	Ea-V5
Mouse Purkinje neuron 1	-0.696 2	Fa-V5
Mouse Purkinje neuron 2	-0.163 2	Fa-V5
Mouse Purkinje neuron 3	-0.532 7	Fa-V5

F_1, F_2, F_3, and F_4 are the four principal components of the neuron.

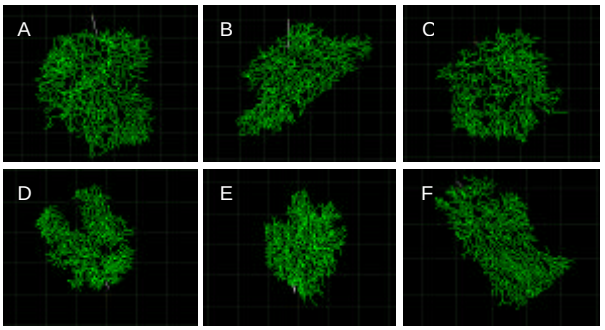

Figure 2 Purkinje neurons from different organisms. The pictures were obtained from neuromorpho.org. The outline of Purkinje neurons from guinea pig was not evident, but clearer than that from the mouse. Morphologically, the outline information of Purkinje neurons was more abundant from guinea pig compared with mouse. (A-C) Guinea pig Purkinje neurons 1-3; (D-F) mouse Purkinje neurons 1-3.

Neuronal nomenclature based on PCA

A neuronal nomenclature system based on PCA was proposed, based on the geometric characteristics of neurons, regardless of their other physical features, such

as action potential properties.

Neuronal classification

Geometric morphology is the most important feature of neurons. Thus, this study utilized this characteristic to classify neurons. In order to illustrate our nomenclature scheme, we introduced biological classification first. Biological classification is a method by which biologists group and categorize organisms by biological type, such as genus or species; it is a form of scientific taxonomy. There are seven major ranks in this nomenclature system: kingdom, phylum, class, order, family, genus, and species. The most basic rank is that of species, the following higher rank is genus, then family. For example, the classification of humans is as follows: Animalia, Chordata, Mammalia, Primates, Homonidae, Homo, H. sapiens. Following the principles of this biological classification scheme, we divided neurons into four ranks based on geometric characteristics. Each rank was divided into several sections, with neurons in each particular section having similar geometries.

Nomenclature of neurons

The four principal components characterize the morphology of neurons in four different aspects: the first principal component, F_1, represents 12 features of neurons (Soma Surface, Number of Stems, Width, Height, Depth, Diameter, Length, Surface, Volume, Euclidean Distance, Path Distance, and Rall's Ratio) which provide the profile information for each neuron; the second principal component, F_2, represents four features (Bifurcation Number, Number of Branches, Branch Order, and Fragmentation) which provide the branch information for the neuron; F_3 represents three features (Contraction, Bifurcation Angle Local, and Bifurcation Angle Remote) which provide the contraction information for the neuron; F_4 reflects one feature (Partition Asymmetry) that provides information on the symmetry pattern for the neuron. As shown in Table 3, the PCA values for different neurons are located at different intervals.

Table 3 Principal component analysis (PCA) value of various neurons

Data file (.swc)	PCA value			
	F_1	F_2	F_3	F_4
Motor neuron	7.428 8	0.178 2	-0.000 1	-1.1155E-08
Purkinje neuron	-2.336 5	3.969 0	-0.999 5	-0.783 2
Pyramidal neuron	-0.198 6	0.311 6	0.477 1	1.309 4
Bipolar interneuron	-1.413 6	0.422 0	2.060 8	0.985 3
Tripolar interneuron	-1.090 6	-1.485 3	-0.681 1	0.893 0
Multipolar interneuron	-0.682 7	-1.887 8	-2.432 9	-0.055 8
Sensory neuron	-1.706 8	-1.853 3	1.471 4	-1.988 5

F_i denotes the value of the i^{th} principal component. SPSS was used to reduce the dimension of original morphological data (.swc) and to calculate the four principal components.

For example, value F_1 for motor neurons is much bigger than for other neurons, indicating that the profile information for motor neurons is very prominent. Value F_2 for Purkinje cells is much bigger than for other neurons, indicating that branch information for this particular neuron is very important. Thus, there are four ranks in our classification system. First of all, the neuron is classified according to its profile information (value F_1). Then it is successively classified according to its branch information (value F_2), its contraction information (value F_3), and its symmetry information (value F_4).

In order to define the neuronal morphology grading standards, we performed statistical analysis, characteristic index Z standardization, and principal component analysis on morphological data for different types of neurons.

Ranking standards of neurons are as follows:

F_i	[-8, -2.5]	[-2.5, -1.5]	[-1.5, -0.5]	[-0.5, 0.5]	[0.5, 1.5]	[1.5, 2.5]	[2.5, +8]
Ranking	G, ?, VII, 7	F, ?, VI, 6	E, e, V, 5	D, d, IV, 4	C, ?, III, 3	B, ß, II, 2	A, a, I, 1

F_i denotes the value of the i^{th} principal component. The profile rank is expressed by English capital letters A-G. The branch rank is expressed by Greek letters a-?. The contraction rank is expressed by Roman numerals I-VII. The symmetry rank is expressed by Arabic numerals 1-7. A, a, I and 1 represent the most prominent ranks.

DISCUSSION

In this study, 20 indices were selected to illustrate the morphological characteristics of neurons, and a neuronal morphological classification model was proposed based on the BP neural network to improve the feasibility and capacity of the classification method. Through PCA on the morphological data for neurons, a classification and nomenclature scheme suitable for all types of neurons was proposed. The mechanism can not only effectively recognize and classify neurons, but it can also distinguish between the same type of neuron from different organisms. However, it is important to consider the biophysical and functional characteristics of neurons as well in order to overcome the shortcomings of a method based only on morphological features-indeed, these have been included as components in larger bioinformatics models[30].

With time, dendrites can continue to grow and axons can grow additional terminals. As a result, neuronal morphological parameters will change as well. These morphological parameters are mainly represented by the principal components of F_1 and F_2. Some morphological parameters determined by the inherent properties of neurons change very little-these are mainly represented by the principal components of F_3 and F_4. Therefore, we can use morphological changes to study the growth characteristics of neurons.

Since the morphology of neurons is very complex, there are various features that characterize morphology from different perspectives. In this study, 20 morphological indices were selected to characterize neurons based on reference[31] and data from Neuronmorpho.org[27]. The

indices of a neuron can be appropriately described by morphological characteristics, including the number of stems, branch number, width, height, depth, diameter, length, etc. The original data used here for training were from the Neuromorpho.org website[27], the compneuro.org website[28], and the neuron research center of George Mason University[30, 32].

It is necessary to determine the principal components of classification features by correlation analysis of the original feature space to reduce the complexity of classification models. Based on these new features, an artificial neural network was then proposed based on the classification model using the morphological characteristics of neurons. In this model, we designed a multi-layer neural network, and then trained the network using known morphological data of neurons to obtain a nonlinear classifier. The classifier was then used to effectively conduct the morphological classification of neurons.

In conclusion, we obtained 7^4 (2 401) types of neurons in our classification model. After calculating the PCA values of a neuron, we determined its type based on the F_i (i = 1, 2, 3, 4). Thus there are four parts to the naming of the neuron type; which respectively denote profile information, branch information, contraction information, and symmetry information.

MATERIALS AND METHODS

Design

A study of neuron classification and nomenclature.

Time and setting

The experiment was performed at the School of Computer Science, National University of Defense Technology, China, from September to October 2010.

Materials

The neuron data were obtained from the following internet databases: Digital reconstructed neurons (http://neuromorpho.org/neuroMorpho/index.jsp); Neuronal morphology archive (http://www.compneuro.org/CDROM/nmorph/index/topindex_tn.html); and the LN database (http://krasnow.gmu.edu/L-Neuron/L-Neuron/database/index.html#Scorcioni)[27-29].

Methods

Classification model based on the PCA-BP neural network

It is important to select appropriate indices to describe neurons as precisely as possible. This study selected 20 parameters, and the definitions of these 20 morphological indices are provided in Table 4. Exact definitions and/or more information about these measurements are given in the L-Measure web site. Notably, each of these parameters is extracted from the whole neuron (axon plus dendrites). We used L-Measure to calculate these indices[33]. PCA transfers these 20 indices into a lower dimensionality reduction[34]. This method is frequently used to reduce the dimension of datasets and maintain features which make the greatest contribution to the variance. We selected the first m principal components, the corresponding eigenvalues of which are greater than 1. We then multiplied the coefficient vectors and the Z normalized data to obtain the principal component expressions (formula 1):

$$F_i = \sum_{j=1}^{20} C_{ij} ZB_j \quad (1)$$

F_i denotes the value of the i^{th} principal component. ZB_j denotes the Z normalized value of the j^{th} original index. C_{ij} denotes the coefficient of the i^{th} principal component and the j^{th} index.

Table 4 Definition of 20 morphological indices

Morphological index	Definition
S_{cell}	Somatic surface area
N_{stem}	Total number of trees
N_{bi}	Total number of bifurcations
N_{branch}	Total number of branches (biforcations plus terminations)
Neuronal width	95% of second principal component
Neuronal height	95% of first principal component
Neuronal depth	95% of third principal component
Diameter	Average branch diameter
Length	Total arborization length
Surface area (S)	Total arborization surface area
Volume (V)	Total internal volume of the arborization
$D_{euclidean}$	Maximum Euclidean (straight) distance from soma to tips
D_{path}	Maximum Path (along the tree) distance from soma to tips
O_{branch}	Maximum Branch order (number of bifurcations from soma to tips)
Contraction	Average Contraction (the ratio between Euclidean and path length calculated on each branch)
Broken degree Frag	Total number of reconstruction points
Asymmetric division PA	Average over all bifurcations of the absolute value of (n1-n2)/(n1+n2-2), where n1 and n2 are the numbers of tips in the two subtrees
Rall's Ratio	Average over all bifurcations of the sum of the diameters of the two daughters, elevated to 1.5, divided by the diameter of the parent, elevated to 1.5
BA_{local}	Average over all bifurcations of the angle between the first two daughter compartments
BA_{remote}	Average over all bifurcations of the angle between the following bifurcations or tips

In order to select principal components that mainly describe the morphology of neurons from 20 indices, we analyzed various representative neurons selected from those with different functions. SPSS was used to reduce the dimension of original morphological data and the four principal components were obtained. Results of the loading matrix of PCA showed that Soma surface, Number of Stems, Width, Height, Depth, Diameter, Length, Surface, Volume, Euclidean Distance, Path Distance, and Roll's Ratio have high load on the first principal component. Thus, the first principal component reflects the information of these 12 indices. Similarly, Bifurcation Number, Number of Branches,

Branch Order, and Fragmentation have high load on the second principal component. Contraction, Bifurcation Angle Local, and Bifurcation Angle Remote have high load on the third principal component. Partition Asymmetry has high load on the fourth principal component.

Establishment of the neuron classification model

BP neural networks were used to design a neuronal morphological classification model with the help of dimensionality reduction mentioned above. The BP neural network is a typical multi-layer feed forward neural network, consisting of an input layer, a hidden layer, and an output layer. It adopts S-type functions for activation. The BP neural network uses a back propagation algorithm to update the network connection weights in the training phase, and it recursively performs the following three steps[35]:

1. Forwarding inputs from the input layer to the output layer;
2. Back propagating the sensitivity from the output layer to the input layer;
3. Using a search algorithm to update connection weights and bias values.

In order to establish an appropriate neuron classification model, we first extracted the principal components from 20 morphological indices by PCA. The number of units in the input layer was determined by the number of principal components, and the number of units in the output layer was determined by the number of neuron categories. The number of units in the hidden layer was determined by the Kolmogorov theorem, which indicates that the number of units in hidden layers can be empirically calculated by (2) and (3):

$$N_h = \sqrt{N_{in} + N_{out} + 1} + h \quad (2)$$

$$N_{in} = \log_2 N_h \quad (3)$$

Where h is a constant between 1 and 10; N_h denotes the number of units in the hidden layer; N_{in} denotes the number of units in the input layer; N_{out} denotes the number of units in the output layer.

The BP neural network performs as a morphological classifier after completing structural design and training to meet performance requirements.

Character index determination

The results demonstrate that the four principal component features represent all the characteristics of neuronal morphology quite well. Therefore, we set the number of units in the input layer to 4 (N_{in} = 4). In addition, the spatial morphology of neurons was categorized into five types according to functionality: motor neurons, Purkinje neurons, pyramidal neurons, interneurons, and sensory neurons. The interneurons were further subdivided into bipolar, tripolar and multipolar interneurons. Therefore, we set the number of units in the output layer to 3 (N_{out} = 3). Table 5 shows the mappings from various neural network outputs to the corresponding types.

In summary, we designed a $(N_{in}) \times (2N_{in} + 1) \times N_{out}$ three-layer BP network, which has four input layer units, nine hidden layer units, and three output layer units. We adopted S-tangent functions as activation functions of the hidden layer, while S-logarithmic functions were used as activation functions of the output layer, because we confined the outputs to the interval [0, 1] to meet the classification requirements. Figure 3 illustrates the neural network. Furthermore, we set the performance of training error as 1×10^{-5}, and we selected the LM (Levenberg-Marquardt) search algorithm due to the high rate of training.

Table 5 Relationships between network outputs and neuronal morphology (the mappings from various neural network outputs to the corresponding types). For example, an output of $[0, 0, 1]^T$ indicates that the neuron is a motor neuron

Output	Neural morphology	Output	Neural morphology
$[0, 0, 0]^T$	Reserved	$[1, 0, 0]^T$	Bipolar interneuron
$[0, 0, 1]^T$	Moto neuron	$[1, 0, 1]^T$	Tripolar interneuron
$[0, 1, 0]^T$	Purkinje neuron	$[1, 1, 0]^T$	Multipolar interneuron
$[0, 1, 1]^T$	Pyramidal neuron	$[1, 1, 1]^T$	Sensory neuron

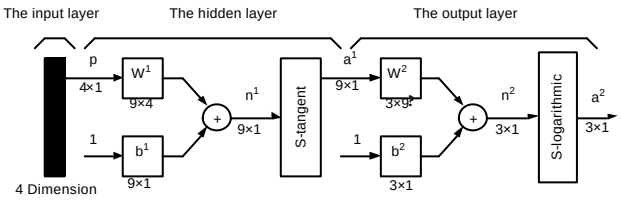

Figure 3 The designed back-propagating neural network.

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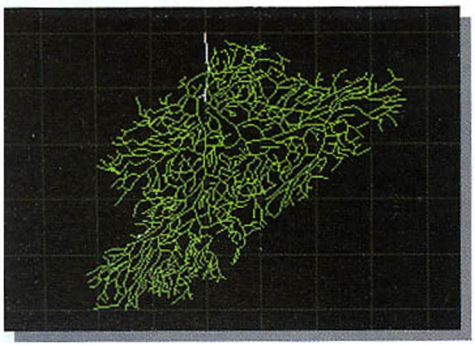
Cover Figure ——



Based on four principal components of neuronal morphology derived from principal component analysis, a nomenclature system for neurons has been obtained. The system can accurately distinguish between the same type of neurons from different species. This image depicts the morphological outline of guinea pig Purkinje neurons.

See pages 1925-1930.

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A proposal for the morphological classification and nomenclature of neurons

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