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Comparison of the Electrophysiological Properties of Excitatory and Inhibitory Neurons in the Mouse Visual Cortex

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ABSTRACT

To understand the brain, it is essential to understand neuronal electrophysiological properties. The Allen Brain Atlas Data Portal contains extensive data recorded from neurons in the mouse visual cortex. Here, the electrophysiological properties of excitatory and inhibitory neurons were compared on the basis of the data provided. As is well known, most inhibitory neurons are characterized as fast-spiking neurons, and most excitatory neurons are characterized as regular-spiking neurons. Inhibitory interneurons show a broader range of electrophysiological properties. However, the characteristics of the two cell types overlap, as outliers are observed in both groups. Therefore, it is necessary to not only study the electrophysiological characteristics of individual neurons but also to consider morphological and molecular characteristics to understand neuronal function.

Key words : Allen Brain Atlas Data, Electrophysiological properties, Excitatory neuron, Inhibitory neuron, Visual cortex

Introduction

Signal processing of sensory information in the visual cortex depends on interactions between excitatory and inhibitory neurons. Investigating the electrophysiological properties of individual neurons is a key step in reconstructing the microcircuit of the visual cortex. However, the underlying mechanisms contributing to the electrophysiological properties of individual neurons are not entirely understood.

The most common neuron in the cerebral cortex is the excitatory neuron, also known as the pyramidal cell. These neurons are typically excited by specific sensory inputs. Thus, the main signaling pathway involves interaction among pyramidal cells. The GABAergic-inhibitory neurons, which account for 20% of cortical neurons, control the firing rate of excitatory neurons and thus, neuronal connectivity [1-3]. Distinguishable roles between excitatory and inhibitory neurons depend on the different membrane properties of each neuronal subgroup. Therefore, it is necessary to compare the membrane properties of these neurons.

Historically, excitatory and inhibitory neurons were distinguished using electrophysiological methods [4,5]. However, as molecular biology techniques developed, it became possible to distinguish the nature of different cell types using cell-specific markers. Inhibitory neurons release GABA or glycine, while excitatory neurons release neurotransmitters such as glutamate or aspartate. This difference results in different immunoreactivity. Additionally, during development, neurons undergo major morphological and electrophysiological changes, and the stage of neuronal development can be also identified by immunoreactivity [6]. Therefore, it may be necessary to re-examine the characteristics of cells according to molecular markers rather than through the electrophysiological and morphological criteria that have been used historically.

The Allen Brain Research Institute discloses a significant

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amount of electrophysiological data recorded in mice expressing fluorescent markers, using various Cre expression systems [7,8]. The Cre recombinase is expressed by a desired promoter, and results in the specific expression of a fluorescent protein in the target cell type. This method allows the fluorescent protein to function as a Cre reporter. Experiments can then be conducted targeting the cell type of interest. The available data allow for analysis of the characteristics of a great number of neurons. Analyzing this data allows for further investigation of the characteristics used to define each neuronal subgroup.

Here, electrophysiological data, from the Allen Brain Institute, on cortical neurons of the mouse visual cortex was collected and analyzed. Based on the Cre-reporter expression, the groups were divided into excitatory and inhibitory neurons. The membrane characteristics of these neuronal subtypes were then compared.

Methods

Data acquisition and analysis

The Allen Brain Atlas Data Portal (http://www.brain-map.org) is a rich repository integrating extensive gene expression, neuroanatomical, connectivity, electrophysiology and two-photon calcium imaging data [7,9-11]. This publicly accessible database is available for neuroscience research. It provides access to an application-programming interface for downloading and processing raw data. The Allen Cell Types Database (2015) contains electrophysiological and morphological data from single cells. These data were used in the current study.

The electrophysiology data were provided in the Neurodata Without Borders (NWB) file format, designed to store neurophysiological data and experimental metadata. NWB files can be extracted and analyzed using the Allen Software Development Kit, which is written in Python. The Allen Data Portal also provides the electrophysiological features of single neurons with metadata in the comma-separated values (CSV) file format. The metadata include the Cre line mice that was used, electrophysiological characteristics, and the Cre reporter expression of the recorded neuron. In this study, the data provided in the CSV file were used to analyze a large number of neurons, and the NWB files were used to confirm the responses of single neurons.

The data were used to distinguish excitatory neurons from inhibitory neurons in experiments using Cre reporter mice [12]. This is possible because different Cre reporters are expressed in excitatory and inhibitory neurons. The data from Cre reporter negative neurons were included in the Allen Data Portal, but these data were excluded from the current study.

Python 2.7 and the pandas library were used to acquire and process the data [13]. R 3.3 and the ggplot2 package were used for statistical analysis and box plotting [14,15].

Results

Data were obtained from 841 neurons, of which 615 cells were positive for the Cre reporter. After dropping the data with null value in electrophysiological properties, the remaining 490 neurons were analyzed. Based on the Cre reporter system (see Methods), 241 cells were determined to be excitatory neurons; 249 cells, inhibitory neurons.

1. Analysis of passive membrane properties

Before analyzing the excitatory characteristics of the cells, the passive membrane properties of the neurons were examined to investigate the neuronal behavior below action potential threshold. Inhibitory neurons had a higher resting membrane potential than excitatory neurons (Table 1). Although there was no difference in input resistance, inhibitory neurons had faster membrane time constants than excitatory neurons. According to the passive membrane model [16], the membrane time constants are proportional to the membrane resistance and membrane capacity (Eq 1).

$$\tau = R \times C \tag{1}$$

The letters τ, R and C indicate time constant, membrane resistance and capacitance, respectively. Thus, it can be inferred

Table 1. Summary of passive membrane properties of neurons in the mouse visual cortex

Passive membrane properties	Excitatory (n = 241)	Inhibitory (n = 249)	p Value
Resting membrane potential (mv)	−74.3 ± 4.7	−72.1 ± 5.2	< 0.001
Membrane constant (ms)	21.3 ± 6.7	14.4 ± 11.9	< 0.001
Input resistance (MOhm)	176.9 ± 58.2	177.8 ± 104.6	0.898

Table 2. Summary of single action potential properties of neurons in the mouse visual cortex

Single action potential properties	Excitatory (n = 241)	Inhibitory (n = 249)	p Value
Peak amplitude (mV)	38.0 ± 7.7	24.3 ± 9.1	< 0.001
Trough (mV)	−54.2 ± 3.2	−64.0 ± 4.7	< 0.001
Height (mV)	92.2 ± 7.9	88.3 ± 9.4	< 0.001
Ratio of upstroke and downstroke	3.4 ± 0.6	1.8 ± 0.7	< 0.001

Table 3. Summary of action potential train properties of neurons in the mouse visual cortex

Action potential train properties	Excitatory (N = 241)	Inhibitory (N = 249)	p Value
Adaptation index	0.06 ± 0.06	0.03 ± 0.08	< 0.001
Threshold (pA)	108.3 ± 54.3	181.4 ± 135.0	< 0.001
Inter spike interval (ms)	84.1 ± 32.2	42.3 ± 43.3	< 0.001
Firing rate	13.7 ± 6.4	47.2 ± 37.6	< 0.001
Time to peak (ms)	3.8 ± 1.9	7.4 ± 5.1	< 0.001

that the excitatory neurons are larger than the inhibitory neurons because the membrane capacity is proportional to the membrane surface area.

2. Analysis of single action potential properties

Action potentials are essential for signaling between neurons. The characteristics of action potentials depend on the neuronal density of voltage-gated sodium channels and voltage-gated potassium channels [17]. Excitatory neurons had a higher peak amplitude of single action potential than inhibitory neurons (Table 2). The ratio of upstroke velocity and downstroke velocity of single action potential was also higher in excitatory neurons than in inhibitory neurons. According to the Hodgkin-Huxley model [16], upstroke velocity and downstroke velocity depend on the conductance of sodium ions and potassium ions, respectively (Eq 2).

$$C\frac{dV}{dt} = g_{Na}m^3h(V_{Na}-V) + g_K n^4(V_K-V) + g_L(V_L-V) + I \quad (2)$$

The letters V, t, g and I indicate voltage, time, conductance density and current density, respectively. The letter m and h are the fraction of activation and inactivation gates of the sodium channel, respectively. The letter n represents the fraction of activation gates of the potassium channel. Thus, it can be assumed that the density of sodium channels is higher in excitatory neurons than in inhibitory neurons, or the density of potassium channels in excitatory neurons is lower in excitatory neurons than in inhibitory neurons. For the same reason, the trough after a single action potential was higher in excitatory neurons than in inhibitory neurons. The time to peak after ramp current injection was shorter in excitatory neurons. The height of a single action potential, calculated by the difference between the peak amplitude and trough, was higher in excitatory neurons. Taken together, these results suggest that the permeability of sodium channels in excitatory neurons is greater than that of inhibitory neurons.

3. Analysis of action potential train properties

Classically, excitatory neurons have been known as regular-spiking neurons; inhibitory neurons, fast-spiking neurons [4]. The action potential train response to sustained current injection was analyzed to identify the spiking pattern of each neuron (Table 3). The smaller threshold current of excitatory neurons can be explained using the same rationale that was described in the previous section. The firing rate was higher in inhibitory neurons, and the inter spike interval (ISI) was shorter in inhibitory neurons. The adaptation index was calculated using the following equation (Eq 3) [18].

$$\text{Adaptation index} = \frac{1}{N-1}\sum_{n=1}^{N-1}\frac{ISI_{n+1}-ISI_n}{ISI_{n+1}+ISI_n} \quad (3)$$

The adaptation index was greater for excitatory neurons, implying that the spike intervals in excitatory neurons increase owing to adaptation. This result reaffirms that inhibitory neurons are fast-spiking neurons.

The distribution of neuronal electrophysiological properties shows that the properties of the neurons in the group greatly overlap (Fig. 1). The overlapping distribution means that there is considerable heterogeneity among neurons within the group divided into the excitatory neurons and the inhibitory neurons.

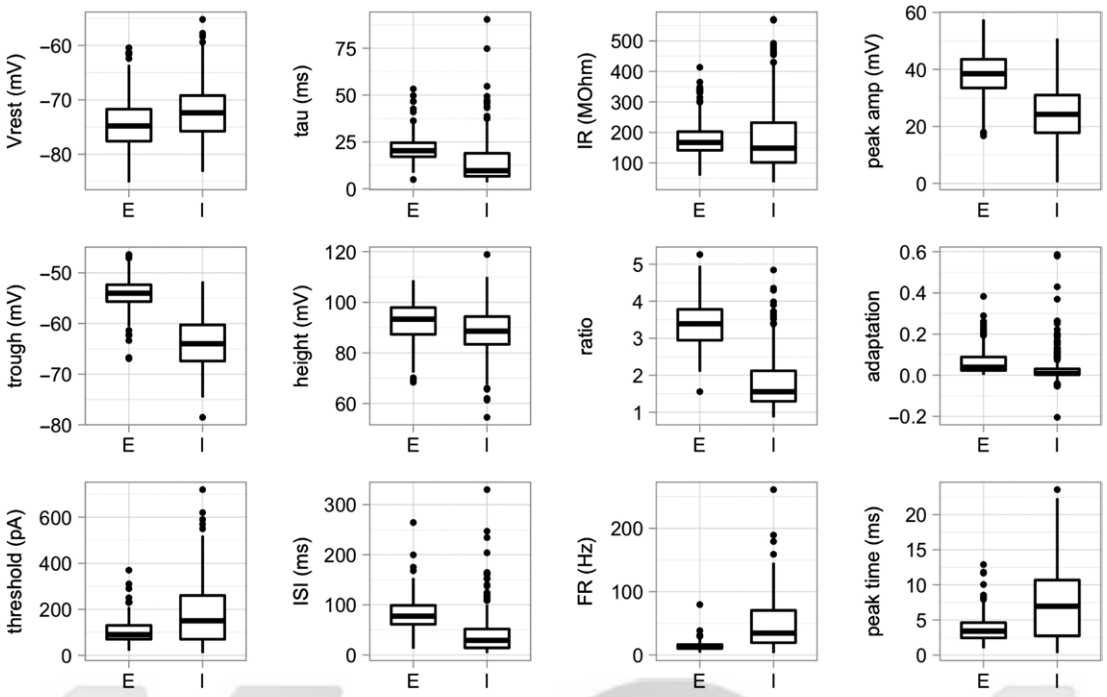

Fig. 1. Boxplot of electrophysiological properties of excitatory and inhibitory neurons. The vertical line extends from the smallest non-outlier to the largest non-outlier. The outlier data points are displayed as single dots. Boxes correspond to the 25 to 75 percentiles. The thick horizontal line in the box signifies the median of the data. ISI, inter stimulus interval; FR, firing rate; IR, input resistance; Vrest, resting membrane potential; peak amp, peak amplitude.

Despite the substantial heterogeneity, a significant amount of data analysis confirmed that there is a difference in the electrophysiological characteristics of excitatory and inhibitory neurons.

Discussion

This study examined the electrophysiological properties of neurons in the mouse visual cortex using data from the open source of the Allen Brain Atlas Data Portal. Similar to previous studies [3,4,19], differences were observed in the passive membrane characteristics and excitable membrane properties of excitatory and inhibitory neurons. In contrast, when considered at the single-neuron level, not all excitatory neurons were categorized as regular-spiking, and not all inhibitory neurons were categorized as fast-spiking. There were inhibitory neurons with a firing rate of less than 10 Hz, and excitatory neurons with a firing rate above 40 Hz (Fig. 1). The Allen Data Portal shares cell morphology and single-cell RNA-sequencing data along with electrophysiological data [20,21]. Therefore, it is necessary to study the morphological and molecular characteristics of neurons with atypical electrophysiological properties.

The larger variation in the electrophysiological properties of inhibitory interneurons observed in this study can be explained by the greater number of subgroups within this cell type than with excitatory neurons. Inhibitory neurons are known to show varied molecular and morphological characteristics depending on subgroup categorization [3,19]. The role of inhibitory neurons in the microcircuit is assumed to depend on subgroup categorization [2]. Therefore, further studies are needed to investigate the differences in electrophysiological properties between subgroups of inhibitory neurons.

Unlike electrophysiological experiments performed in a single laboratory, the aim of this study was to compare data from a large number of neurons. Nevertheless, there are limitations to this study. It is difficult to confirm that all excitatory neurons and inhibitory neurons are labeled in the Cre-mice line used by the Allen Brain Institute. Although only Cre-reporter-positive neurons were included in the study, Cre-reporter-negative neurons should be further investigated using single-cell RNA-sequencing data. Classifying neurons based on molecular characteristics obtained from RNA-sequencing data

would help determine neurons that are omitted from the Cre system [20].

In conclusion, the excitatory and inhibitory neurons analyzed in this study mostly have known electrophysiological characteristics known from previous studies. However, it is necessary to explore the diversity of electrophysiological characteristics within subgroups of inhibitory neurons. It is also necessary to investigate the characteristics of individual neurons with atypical electrophysiological properties.

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