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Counting Neurons: Comparing Invasive and Noninvasive Techniques

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ABSTRACT

This article discusses the various techniques—categorized into invasive and noninvasive—used in neuroscience research for counting neurons and estimating their density. Invasive techniques involve physically removing and staining brain tissue to count neurons, while noninvasive techniques allow for the examination of brain structure and function in live animals or humans. Examples of invasive techniques include stereology, optical fractionator, manual cell counting, confocal microscopy, and electron microscopy. Examples of noninvasive techniques include magnetic resonance imaging (MRI), electroencephalography (EEG), and functional near-infrared spectroscopy (fNIRS). The advantages and disadvantages of each technique are summarized in Table 1. Recent advancements in noninvasive NIR spectroscopic technique to estimate neuron density are highlighted. Future research and technological advancement, especially in noninvasive techniques, could lead to a better understanding of neural circuits, their function, and our daily lives.

Keywords: Neurons; Non-Invasive; Invasive; Neurodegenerative Disease; Optical; NIR

Abbreviations: NIR-OS: NIR-Optical Spectroscopic; FNIRS: Functional Near-Infrared Spectroscopy; EED: Electroencephalography; MRI: Magnetic Resonance Imaging

Introduction

The past 30 years have seen significant advancements in cell and neuron counting, greatly expanding our understanding of neural circuits and their function. With continued research and technological development, it is likely that even greater insights will be gained in the coming years. In neuroscience research, counting neurons provides vital information about the structure and function of the brain. Techniques for counting neurons are divided into two categories: invasive and noninvasive. Invasive techniques involve physically removing and, if necessary, staining brain tissue from an animal or human brain to identify and count the neurons. This provides accurate neuron counts but is destructive and can only be used on deceased animals or humans or when tissues are removed by biopsy. There are several methods used to count neurons or to estimate their density. One such method is stereology, which involves counting a representative sample of neurons in a defined area and

extrapolating this number to estimate the total number of neurons in the region of interest [1,2]. Optical fractionator is a method that involves counting neurons in a series of systematically sampled tissue sections [3,4]. The optical fractionator method is highly reliable and can be used to estimate the total number of neurons in a given tissue volume. It involves counting neurons in a series of systematically sampled tissue sections using a combination of light microscopy and computer-assisted image analysis. This method can also be used to estimate the size, distribution, and spatial arrangement of individual neurons.

Manual cell counting methods involve visually identifying and counting neurons under a microscope [5]. This can be done in brain slices or in whole brains that have been cleared using different techniques. Confocal microscopy is a technique that is often used in neuroscience research to visualize and count neurons in tissue sections [6-8]. It uses a laser light source to scan the sample and

create high-resolution, 3D images of the tissue. Finally, electron microscopy is a high-resolution imaging technique that can be used to visualize the ultrastructure of individual neurons and synapses, and to estimate the number of neurons in a given region of the brain [9-11]. Conversely, noninvasive techniques do not require the removal of brain tissue and can be performed on live animals or humans. These techniques offer a way to examine brain structure and function *in vivo*. Magnetic resonance imaging (MRI) uses a powerful magnet and radio waves to create detailed images of the brain [12,13]. MRI can be used to study brain structure and function, and to investigate disease or injury-associated brain changes. Another non-invasive technique

is electroencephalography (EEG), in which electrodes attached to the scalp are used to record electrical activity in the brain [14,15]. In addition to studying brain function and activity, EEG has been used to investigate a range of neurological and psychiatric disorders. Finally, functional near-infrared spectroscopy (fNIRS) is a non-invasive technique that uses light to measure changes in blood flow and oxygenation in the brain, which can be used to study brain function and activity [16,17]. These non-invasive techniques offer a potent means to study the brain without the need for invasive procedures, allowing for safer and more ethical research on live animals or humans.

Table 1: Summary of the advantages and disadvantages of

- A) Invasive techniques and
- B) Non-invasive techniques to investigate neuronal activities.

Technique	Advantages	Disadvantages
A) Invasive Techniques		
Stereology	Unbiased estimation of neuron density, applicable to most brain regions	Requires extensive training, time-consuming, and costly
Optical fractionator	Accurate estimation of neuron density, applicable to most brain regions	Requires extensive training, time-consuming, and costly
Manual cell counting	Simple and straightforward, applicable to most brain regions	Prone to observer bias, time-consuming, and labor-intensive
Confocal microscopy	High-resolution imaging of individual neurons and subcellular structures	Limited to small regions of tissue, may require tissue destruction, and can be time-consuming
Electron microscopy	High-resolution imaging of individual neurons and synapses, useful for studying ultrastructural details	Requires tissue destruction, costly, and time- consuming
B) Non-invasive Techniques		
MRI	Non-invasive, allows for imaging of brain structure and function <i>in vivo</i>	Relatively low spatial resolution compared to other techniques, expensive equipment, and can be time-consuming
EEG	Non-invasive, allows for the measurement of brain activity in real-time	Limited spatial resolution, prone to artifacts, and may require specialized equipment
fNIRS	Non-invasive, allows for the measurement of changes in blood flow and oxygenation in the brain	Low spatial resolution and can be prone to artifacts, may require specialized equipment
NIR-OS	Potentially non-invasive, simple, cheap, and fast	New technique: more work needs to be done

Note: summarizes the advantages and disadvantages of the aforementioned techniques.

Discussion

It is critical to note that these non-invasive techniques cannot estimate the number of neurons in the human brain. However, these non-invasive techniques provide indirect measures of neuronal activity and brain function rather than directly estimating the number of neurons. For the first time, we recently estimated neuron density using NIR optical spectroscopic (NIR-OS) through noninvasive techniques that may provide valuable information about brain activity and connectivity [18]. Using Beer's law and the Mie model,

the density of neurons in the examined gray matter tissue sample was estimated as roughly 40,000 neurons/mg [18]. The estimated depth of penetration in the cerebral cortex at 800 nm is approximately 3.8 mm [18]. However, using longer and more suitable wavelengths could achieve deeper brain penetration [19]. The NIR-OS technique has potential advantages of being potentially non-invasive, simple, cheap, and fast, but as a newly developed technique, more work needs to be done to check its accuracy in estimating the number of neurons in a localized region.

Conclusion

Briefly, invasive techniques provide a reliable neuron count, but can only be performed on deceased animals or humans. Conversely, noninvasive techniques can be performed on live subjects and have the potential to count neurons. We must focus more on noninvasive optical spectroscopic and imaging techniques to count neurons. Using these methods, we will be able to assess the health status of the desired brain region fast, affordably, and safely in the near future. We believe that the present technique will have great potential in estimating the density of neurons noninvasively and could benefit the healthcare field.

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Conflict of Interest

The author has no conflict of interest relevant to this study to declare.

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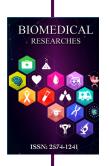
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