Neuroscience Research xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Neuroscience Research

journal homepage: www.elsevier.com/locate/neures

Review article

Merging advanced technologies with classical methods to uncover dendritic spine dynamics: A hot spot of synaptic plasticity

Panchanan Maiti[a,*], Jayeeta Manna[b], Michael P. McDonald[a,c]

[a] Department of Neurology, University of Tennessee Health Science Center, Memphis, TN 38163, USA
[b] Department of Physiology, University of Tennessee Health Science Center, Memphis, TN 38163, USA
[c] Department of Anatomy & Neurobiology, University of Tennessee Health Science Center, Memphis, TN 38163, USA

ARTICLE INFO

Article history:
Received 26 September 2014
Received in revised form 17 February 2015
Accepted 19 February 2015
Available online xxx

Keywords:
Dendritic spine
Synaptic plasticity
Golgi stain
Fluorescent labeling
Protein engineering
Super resolved optical microscopes

ABSTRACT

The structure of dendritic spines determines synaptic efficacy, a plastic process that mediates information processing in the vertebrate nervous system. Aberrant spine morphology, including alterations in shape, size, and number, are common in different brain diseases. Because of this, accurate and unbiased characterization of dendritic spine structure is vital to our ability to explore and understand their involvement in neuronal development, synaptic plasticity, and synaptic failure in neurological diseases. Investigators have attempted to elucidate the precise structure and function of dendritic spines for more than a hundred years, but their fundamental role in synaptic plasticity and neurological diseases remains elusive. Limitations and ambiguities in imaging techniques have exacerbated the challenges of acquiring accurate information about spines and spine features. However, recent advancements in molecular biology, protein engineering, immuno-labeling techniques, and the use of super-resolution nano-microscopy along with powerful image analysis software have provided a better understanding of dendritic spine architecture. Here we describe the pros and cons of the classical staining techniques used to study spine morphology, and the alteration of dendritic spines in various neuropathological conditions. Finally, we highlight recent advances in super-resolved nanoscale microscopy, and their potentials and pitfalls when used to explore dendritic spine dynamics.

Contents

1. Introduction 00
2. Why is it important to study dendritic spines? 00
3. Classical techniques used to explore dendritic spine morphology 00
   3.1. Golgi stain 00
   3.2. Golgi-Cox staining 00
   3.3. Modified Golgi-Cox/rapid Golgi stains 00
4. Fluorescent labeling of neurons and dendritic spines 00
   4.1. Use of lipophilic dye 00
   4.2. Transfection methodology and protein engineering 00

Abbreviations: 3-D, three-dimensional; EM, electron microscopy; CLSM, confocal laser scanning microscope; CCD, charged couple device; TPM, two-photon microscope; MPLSM, multi-photon laser scanning microscopy; sptPALM, single particle tracking photo activated localization microscopy; uPAINT, universal point accumulation imaging in nanoscale topography; STED, stimulated emission depletion; STORM, stochastic optical reconstruction microscopy; DH-QPM, digital holographic quantitative phase microscopy; AD, Alzheimer's disease; PD, Parkinson's disease; HD, Huntington's diseases; GFP, green fluorescent protein; PSD, postsynaptic density; shRNA, short hair pin ribonucleic acid; YFP, yellow fluorescent protein; TEM, transmission electron microscope; ER, endoplasmic reticulum; sER, smooth ER; GABA, gamma amino butyric acid; IP3, inositol 1,4,5-triphosphate; STORM, stochastic optical reconstruction microscopy; PALM, photo activated localization microscopy; FPALM, fluorescence photo-activation localization microscopy; PAINT, point accumulation for imaging in nanoscale topography; SIM, structured illumination microscopy.

* Corresponding author at: Department of Neurology, University of Tennessee Health Science Center, 855 Monroe Avenue, Johnson Building, Memphis, TN 38163, USA. Tel.: +1 9012462649.
E-mail addresses: panchananm@gmail.com (P. Maiti), manna.jayeeta15@gmail.com (J. Manna), mike@tennessee.edu (M.P. McDonald).

http://dx.doi.org/10.1016/j.neures.2015.02.007
0168-0102/

Please cite this article in press as: Maiti, P., et al., Merging advanced technologies with classical methods to uncover dendritic spine dynamics: A hot spot of synaptic plasticity. Neurosci. Res. (2015), http://dx.doi.org/10.1016/j.neures.2015.02.007

5. Advanced microscopic tools to elucidate dendritic spine architecture 00
5.1. Ultra structure of dendritic spine by electron microscopy 00
5.2. High-resolution optical imaging to elucidate dendritic spine structure 00
5.2.1. Confocal laser scanning microscope (CLSM) 00
5.2.2. Two/multi photon microscopes 00
5.2.3. Stimulated emission depletion (STED) microscope 00
5.2.4. Super resolved single fluorphore microscopes (STORM, PALM, FPALM, PAINT) 00
5.2.5. Fiber-optic endomicroscopy 00
6. Conclusion 00
Author's contribution 00
Conflict of interest 00
Acknowledgements 00
References 00

1. Introduction

Almost a hundred billion neurons and an estimated hundred trillion (10^{14}) synapses make the human brain the most complex structure known (Williams and Herrup, 1988; Nimchinsky et al., 2004). These neurons are involved in maintenance of basic brain functions as well as learning, memory, and higher-order thought processes. Maintaining healthy synaptic structure is therefore critical to the preservation of normal brain functions. Importantly, all higher-order neuronal communications are mediated by dendritic spines – specialized knob-like structures protruding from dendritic shafts (Hering and Sheng, 2001; Nimchinsky et al., 2002; Bourne and Harris, 2007). They are considered specialized, semi-autonomous postsynaptic compartments on which most excitatory synapses (over 95% in vertebrate brain) impinge (Hering and Sheng, 2001; Nimchinsky et al., 2002; Bourne and Harris, 2007). The dendritic spines are of multiple shapes and sizes, with diverse functions depending on type and activity of the neurons (Jones and Powell, 1969; Harris et al., 1992; Hering and Sheng, 2001; Nimchinsky et al., 2002; Bourne and Harris, 2007). Most importantly, spine morphology determines the strength and stability of the synapse, and can be significantly altered in neurodevelopmental and neurodegenerative diseases (Fiala et al., 2002; van Spronsen and Hoogenraad, 2010). Experimental evidence suggests that abnormal spine morphology is a principal cause of synaptic dysfunction in a number of neurological and neuropsychiatric disorders (Fiala et al., 2002; Bredesen et al., 2006; Rubinsztein, 2006). However, in order to understand the role of dendritic spines in synaptic plasticity and disease, it is first vital to characterize them accurately – not only their numbers, but also their three-dimensional (3D) structure (Calabrese et al., 2006; Kasai et al., 2010). Because of diffraction limitations and lack of spatial resolution in light microscopy, the dynamics and nanoscale structure of spine necks and distributions of spine proteins remained unexplored for many years. Recently, new technologies have facilitated significant advances in our understanding of the basic structure and function of dendritic spines. Notably, the development of super-resolution fluorescence microscopes has enabled capture of nanoscale-level spine structures in living neurons non-invasively. However, a great deal still needs to be done, particularly with respect to dendritic spine morphology and its role in impaired synaptic plasticity in neurological disorders. In this review we will discuss how classical methods and novel approaches can be used in a complimentary fashion to discover the detailed structures and functions of dendritic spines.

2. Why is it important to study dendritic spines?

There are several reasons to study the structure, function, genesis, and loss of dendritic spines. In addition to developmental changes, it is important to explore spine dynamics when the brain is under stress or in an injury or disease state because structural abnormalities in dendritic spines are thought to underlie symptomatology in many neuropathological states (Fiala et al., 2002). Changes associated with impaired cognitive function include loss or decrease in spine number or density, reduced size, increased number of immature spines and varicosities, distortion of spine shape, and enhanced ectopic spine formation (Table 1; Fiala et al., 2002; Penzes et al., 2011). For example, a reduction in spine size ad reduced dendritic length has been reported in the striatum of schizophrenics and the motor cortex of infants with Down's syndrome (Roberts et al., 1996; Marin-Padilla, 1972). In contrast to reductions in spine density, increased spine density has been observed during brain development in such conditions as phenylketonuria, fragile-X syndrome, and exposure to an enriched environment (Table 1; Huttenlocher and Dabholkar, 1997; Lacey, 1985; Irwin et al., 2001; Globus et al., 1973; Berman et al., 1996). Formation of varicosities is another morphological change common following brain injury, which might be due to abnormal organization of microtubules or actin polymerization. Swelling of dendritic trunks can also produce varicosities, as observed in progressive neurodegenerative disorders such as Pick's disease, frontal lobe dementia, and motor neuron disease (Sotrel et al., 1993; Hogan et al., 1987; Ferrer et al., 1990, 1991). Ectopic spines can be observed during early development in olivopontocerebellar atrophy, fetal alcohol syndrome, Menkes disease, epilepsy, and in cats with GM2 gangliosidosis (Ferrer et al., 1988; Mohamed et al., 1987; Hinton et al., 1991; Walkley et al., 1990).

In addition to alterations in spine size and number, ultrastructural intra-spine changes, including cytoplasmic densification, hypertrophy of organelles or spine volume, and formation of aberrant synapse-like connections have all been linked with

Table 1
Dendritic spine pathology in neurological diseases.

Spine pathology	Occurrences
Decreased spine density	Deafferentation, agenesis, mental retardation, malnutrition, poisoning, alcohol abuse, epilepsy, spongiform encephalopathies, Alzheimer's disease, and others
Increased spine density	Some types of deafferentation, environmental enrichment, Fragile-X syndrome, sudden infant death syndrome, stimulatory drug use
Reduction in spine size	Sensory deprivation, schizophrenia, Down syndrome
Distortion of spine shape	Deafferentation, agenesis, mental retardation, malnutrition, poisoning, alcohol abuse, epilepsy, spongiform encephalopathies
Varicosity formation	Acute excitotoxicity, traumatic injury and edema, epilepsy, hypoxia/ischemia
Ectopic spines	Olivopontocerebellar atrophy, Menkes disease, metabolic storage diseases

Reprinted with permission from Fiala et al. (2002).

Please cite this article in press as: Maiti, P., et al., Merging advanced technologies with classical methods to uncover dendritic spine dynamics: A hot spot of synaptic plasticity. Neurosci. Res. (2015), http://dx.doi.org/10.1016/j.neures.2015.02.007