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Study of laser uncaging induced morphological alteration of rat cortical neurites using atomic force microscopy

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HIGHLIGHTS

- A platform for combining laser uncaging stimulation with atomic force microscopy scan is set up for neuroscience researches.
- Uncaging of glutamate at low frequency induces a calcium-related cortical neurite collapse.
- The platform has the advantages of super resolution imaging, highly localized stimulation and convenient platform building.

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ABSTRACT

Activity-dependent structural remodeling is an important aspect of neuronal plasticity. In the previous researches, neuronal structure variations resulting from external interventions were detected by the imaging instruments such as the fluorescence microscopy, the scanning/transmission electron microscopy (SEM/TEM) and the laser confocal microscopy. In this article, a new platform which combined the photochemical stimulation with atomic force microscopy (AFM) was set up to detect the activity-dependent structural remodeling. In the experiments, the cortical neurites on the glass coverslips were stimulated by locally uncaged glutamate under the ultraviolet (UV) laser pulses, and a calcium-related structural collapse of neurites (about 250 nm height decrease) was observed by an AFM. This was the first attempt to combine the laser uncaging with AFM in living cell researches. With the advantages of highly localized stimulation (<5 μm), super resolution imaging (<3.8 nm), and convenient platform building, this system was suitable for the quantitative observation of the neuron mechanical property variations and morphological alterations modified by neural activities under different photochemical stimulations, which would be helpful for studying physiological and pathological mechanisms of structural and functional changes induced by the biomolecule acting.

1. Introduction

Neuronal plasticity has drawn more and more attention of the neurobiologists. Both neuronal structure and function alterations can be induced by neuronal activities during the neuronal plasticity process. It was reported in some early studies (Shinoda et al., 2010) that multiple induction of long-term depression (LTD) caused a persistent reduction in the amount of dendritic synapses of developing hippocampal neurons. Whereas, long-term potentiation (LTP) induction (Geinisman, 2000) elicited the formation of synapses between activated axon terminals and newly emerging dendritic spines. Additionally, strong synaptic activation that triggered LTP (Maletic-Savatic et al., 1999) caused an *N*-methly-D-aspartate (NMDA) receptor-dependent growth of spines and filopodia. Neural activity (Malenka, 2002) could modify the behavior of neural circuits by modifying the strength or efficacy of synaptic transmission, modulating the excitability properties of individual neurons, eliciting the growth of new synaptic connections and pruning away the existing ones.

Abbreviations: SEM, scanning electron microscopy; TEM, transmission electron microscopy; AFM, atomic force microscopy/atomic force microscope; LTP, long-term potentiation; LTD, long-term depression; NMDA, *N*-methly-D-aspartate; MEA, micro-electrode array; UV, ultraviolet; PBS, phosphate buffer saline.

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Activity-dependent structural remodeling of neurons is a cellular basis for learning and memory (Matsuzaki et al., 2004) and many researchers have been focusing on the neuronal structure variations induced by exogenous stimulations. Neuronal activities could be elicited by drug administration, sensory stimulus and electrical stimulation, and the corresponding structural variations could be detected by the fluorescence microscopy, the laser confocal microscopy and SEM/TEM. For example, Halpain et al. (1998) stimulated the cultured hippocampal neurons with NMDA and observed the fluorescently labeled neurons with a light microscopy. But as the diffusion of the drug molecules in the medium was not well controlled, therefore, the stimulation could not be precisely positioned in a small region. Okamoto et al. (2004) delivered electric stimulations to the hippocampal pyramidal dendrites via a glass electrode, but it was difficult to comprehend the response of neurons to the electrical stimuli as well as to control the spatial extent of the stimulations (Joucla and Yvert, 2012). Cheetham et al. (2014) imaged synaptically connected pyramidal neurons with a confocal microscopy after altering the sensory experience of rats by whisker trimming. The effects of the sensory stimulation on individual neurons in the central nervous system could not be well explained. Additionally, the resolution of the laser confocal microscopy in *z*-direction was several hundred nanometers, which seemed insufficient for precise quantification of the minute structural changes such as spine shrinkage. Relatively low resolution was also the problem existing in another research with two-photon imaging (Matsuzaki et al., 2004). Deitch and Rubel (1989) investigated the changes of the dendritic ultrastructure during the rapid dendrite atrophy process with the TEM. The SEM was adopted by Korobova and Svitkina (2010) to study the mechanism of dendritic spine morphogenesis. The SEM and TEM investigated the morphological characters of cells with the superior resolution of several nanometers. But the necessary metallic coating of samples led to cell's death and membrane shrinkage, which made SEM/TEM unsuitable for the real-time cell observations.

In brief, although the methods mentioned above are very helpful to explore the mechanisms of neuronal plasticity, these poorly controlled stimulation manners, with a relatively low imaging resolution of the optical microscopy or the limitation of the SEM/TEM for living cells, are less powerful for the quantitative investigations of the dynamic neuronal structure alterations.

Owing to its superior imaging resolution, AFM has been widely used in biological researches (Drake et al., 1989; Radmacher et al., 1992) to obtain the quantitative height information of the biological molecules such as amino acids, DNA (Bustamante et al., 1992; Hansma et al., 1992), and proteins (Karrasch et al., 1994; Gonçalves et al., 2006; Pfreundschuh et al., 2014) and to form 3D images without complicated sample preparation. AFM has also been used for investigating cell morphological characteristics (Espenel et al., 2008; Le Grimellec et al., 1998; Martin et al., 2013; Parpura et al., 1993; Putman et al., 1994; Zanger, 1998) and cell mechanical properties (Franz et al., 2007; Mathur et al., 2001; Puech et al., 2005; Radmacher et al., 1996; Shi et al., 2011; Smith et al., 2005; Sun et al., 2005; Thie et al., 1998).

Moreover, AFM has been combined with other scientific equipments to provide more specific detections. Some meaningful results had been obtained by combining AFM with the classic optical microscope (Ponce et al., 2010; Vesenka et al., 1995) and the confocal fluorescence microscope (Kassies et al., 2005; McNally et al., 2005), respectively. Pamir et al. (2008) combined patch-clamp with AFM for mechanical probing of living cells. Likewise, the microelectrode array (MEA) had been integrated with AFM for living cell researches (Cogollo et al., 2011; Shenai et al., 2004).

On the other hand, as a promising neural stimulation technique, laser uncaging has been used as an effective stimulation method to control chemical delivery with high temporal and spatial resolution for investigations of the cellular physiological activities. First, photolyzable compounds (Ellis-Davies, 2007; Mayer and Heckel, 2006) were synthesised by adding the light-active groups to the biomolecules. The synthesised compounds were biologically inactive. The corresponding photolyzable compounds of many biomolecules such as neurotransmitters, nucleotides, calcium, nucleic acids and polypeptides had been synthesised. For example, the caged glutamate had been widely used in neurobiological researches (Matsuzaki et al., 2001; Molnar and Nadler, 1999; Wang et al., 2000). Then, after absorbing specific photon energies, the chemical bonds between the photosensitive groups and biomolecules were cleaved and the active biomolecules released participated in physiological activities. The amount of uncaged active biomolecules depends on the laser intensity, excitation volume and photolyzable compound concentration. By uncaging, the biomolecules can be delivered within microsecond timescales and the uncaging region can be precisely confined to a small area.

Normally, the photolysis of caged biomolecules is implemented by a classic single-photon laser system or two-photon excitation system with better penetration ability and precise position controlling (Brown et al., 1999). A simple and less expensive solution is to build an optical fiber laser delivery device. The laser uncaging technique had been combined with electrophysiological recording tools such as the MEA (Ghezzi et al., 2008) and patch-clamp (Kandler et al., 1998; Kantevari et al., 2009; Nikolenko et al., 2011; Shoham et al., 2005) to investigate the physiological activities.

To get the highly localized stimulation and super resolution imaging in neuroscience researches, in this study, the platform combining laser uncaging with AFM was set up. Morphological alterations of cultured cortical neurites induced by the local glutamate release were investigated, where the 375 nm UV laser was employed for uncaging. It was found that laser uncaging (frequency 1 Hz, pulse width 50 ms, pulse train duration 3 min) resulted in a remarkable neurite collapse. The spatial resolution of the stimulation was about 5 μm and the imaging resolution was about 3.73 ± 1.04 nm. These results suggest that this platform would be suitable for the quantitative observation of the neuronal mechanical property variations and morphological alterations modified by neural activities under different photochemical stimulations, which would be helpful to study mechanisms of structural and functional changes induced by the biomolecule acting.

2. Materials & methods

2.1. Cell culture

The cortical neurons were obtained from P1 Sprague-Dawley rats (provided by Laboratory Animal Center of Zhejiang Province, Hangzhou, China). The procedures in this study were approved by the Animal Care and Use Committee of Zhejiang University. The cell culture method was similar to what had been described in some other articles (Beaudoin et al., 2012; Xu et al., 2012). The cells were dissociated from the brain tissues to obtain a homogenous cell suspension. 14 mm coverslips were treated with piranha solution (36 ml of 98% H_2SO_4 added to 60 ml 30% H_2O_2) for 6 h. The coverslips were taken out from the solution and rinsed five times in the deionized water. Then, they were autoclaved and coated with 0.01% poly-l-lysine (molecular weight 70,000–150,000) overnight. The cells were seeded on the coverslips in a Petri dish at the concentration of 5×10^5 cells/ml. 24 h after cell plating, the culture medium was replaced by a new one, which consisted of Neurobasal (Gibco), 1% penicillin-streptomycin (Invitrogen), 200 mg/l L-glutamine (Invitrogen) and 20 ml/l B27 (Gibco). The culture was fed with the medium containing Neurobasal every three days. The temperature and humidity of the cultivation were kept constant