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专家述评

编者按:大脑的基本功能是处理并传输来自外周的感觉刺激,从而实现感知周围世界的功能。这项功能是由在大脑中广泛分布的神经元来履行的。因此,神经元如何处理感觉信息已成为神经科学家冥思苦想的核心问题,然而其具体机制尤其是感觉输入的突触作用还未得到深入研究。此篇论文分析总结树突组织处理感觉输入信息的模式,就目前实验研究的离体和在体方面取得的成就进行梳理,并对双光子成像技术以及未来此领域的研究方向进行展望。



皮层感觉信息处理之成像研究
——皮层神经元感觉信息输入的树突模式

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[**摘要**] 神经元由胞体、树突和轴突 3 部分组成。其中树突接收其他神经元轴突通过电信号刺激释放出的神经递质,从而产生信息输入。因此,研究感觉输入的关键是了解感觉信息处理过程中树突接收信息的特点和规律。突触输入中存在多种树突分布和组织处理模式,在离体情况下,目前已提出几种树突组织处理模式:①1 个神经元只接受 1 种特异性的信息传入;②突触传入信息整合沿单个树突分布;③具有同一特征的信息传入聚集在同一树突部分;④传入信息的整合分布于整个树突上。在在体情况下,科学家通过双光子成像技术提出突触信息传入的椒盐型树突组织模式,也就是说,具有相同方向的信息输入分布于整个树突树上,整个树突树作为"运算单元"接收各种感觉信号。换句话说,树突上的树突棘接收不同感觉信号的空间分布是非常不均匀的,即使是同一树突上的相邻树突棘也可以接收和编码不同的感觉信息。在未来,我们需要继续发展超速双光子显微成像技术,以便验证在更深皮层中 (>100~300 μm) 甚至皮层下的大脑区域是否也存在此类椒盐型组织模式? 此外,还需要探索更多的基本问题,例如,信息传入的复杂性是如何决定神经元的传出信号的? 树突输入信息组织处理在清醒动物体内是怎样的?

[**关键词**] 感觉信息传入;皮层神经元;树突棘;在体双光子显微成像
[**中图法分类号**] R338.13 [**文献标志码**] A

Watching synapses during sensory information processing in the cortex
——Dendritic pattern of sensory inputs in cortical neurons

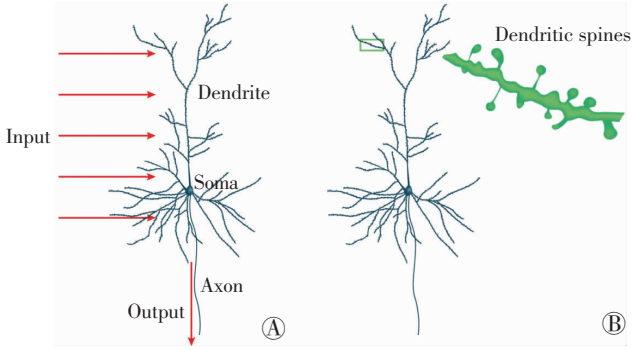
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The basic function of brain is to process and transmit sensory stimuli from the environment, which allows human beings and animals to make sense of the world. Neurons widely distributed in the brain are required for achieving this function. Therefore, how the neurons work for processing sensory information is a central question that has fascinated neuroscientists for decades. In the mammalian brain, the cerebral cortex, located in the outer layer of cerebrum, contains specific areas being considered as higher terminals that receive and process sensory information, which are called sensory areas. Until now, while much attention has been paid to understanding how sensory information is processed in the cortex, the underlying cortical circuitry mechanisms and particularly the synaptic rules for the organization of sensory inputs are just superficially touched. In general, a neuron in the cortex consists of three compartments: dendrites, soma and axon. Neurons capture information from other neural cells by dendrites, and subsequently send the in-

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formation to others by axonal terminals (Fig. 1A). The first step during this process is to receive information inputs by dendritic spines, small membranous protrusions distributed in the entire dendrites (Fig. 1B), from axons coming from other neurons by means of neurotransmitter release triggered by electrical signals. From the morphological point of view, neurons were considered as "mysterious butterflies of the soul" by Santiago Ramon Cajal, and recently the dendrites of neurons were referred as "wings of these butterflies"[1]. From the functional aspect, a neuron can be just considered as a miniature input-output device. It is one of the central challenges in the neuroscience field to understand the working rules at the input site in this device, namely, the dendritic organization of synaptic inputs during sensory processing.



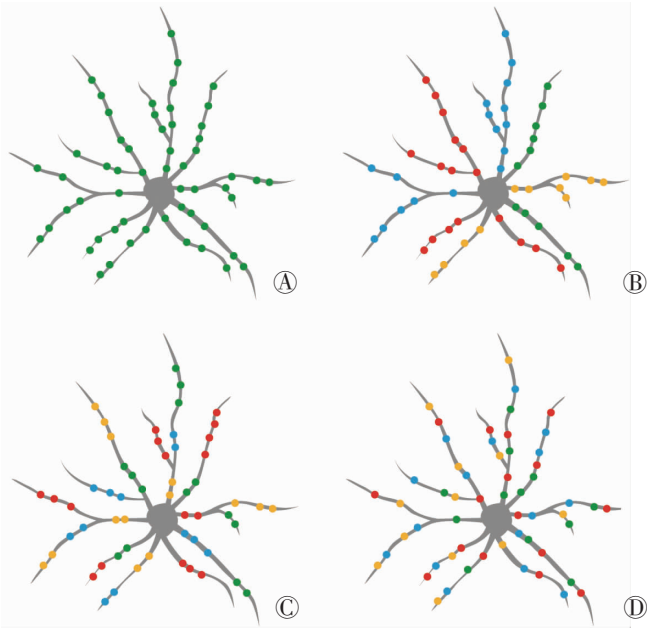
A: Neurons receive information inputs by dendrites and send out information by axons. B: Spines are distributed over the dendritic tree

Fig 1 Different compartments of a cortical neuron

Multiple working models of dendritic organization of synaptic inputs

Spines cover the entire dendrites of many neurons, and have been widely accepted as major sites for receiving synaptic inputs that contain specific features of information[2]. The integrative properties of inputs by dendrites are determined by multiple factors, including dendritic morphology, active conductances, excitation-inhibition balance, and most importantly spatio-temporal arrangement of synaptic inputs[3-4].

In recent years, tremendous efforts have been made to understand the dendritic arrangement of synaptic inputs. Based on a number of experimental studies performed in brain slice preparations, several working models of how dendrites organize feature-specific synaptic inputs have been proposed: (1) All inputs to a neuron are specific for a single feature (Fig. 2A)[5]; (2) Integration of synaptic inputs are distributed along individual dendrites (Fig. 2B)[6-7]; (3) Inputs with shared features are clustered on the same dendritic branch (Fig. 2C)[8]; (4) Integration of inputs is distributed throughout the entire dendritic tree (Fig. 2D)[9]. These inconsistent models may be caused by non-physiological conditions of *in vitro* preparation (e.g. disconnected network, or temperature, or other factors), or due to the artificial synaptic stimulations used (e.g. focal electrical stimulation with electrode, or uncaged glutamate). Therefore, what the truth in the real life is, namely how dendrites organize sensory inputs, needs to be investigated under *in vivo* conditions.



A: All inputs to a neuron are specific for a single feature; B: Integration of synaptic inputs distributed along individual dendrites; C: Inputs with shared features clustered on the same dendritic branch; D: Integration of inputs distributed throughout the entire dendritic tree

Fig 2 Different working models of dendritic pattern of feature specific synaptic inputs

Development of *in vivo* imaging approach for dissection of dendritic organization of sensory inputs

Understanding how dendrites receive and organize sensory inputs requires a proper approach that is suitable for stable recordings in living brain. In theory, two techniques can be probably used for detecting individual synaptic inputs in the dendrites: (1) dendritic electrophysiological recordings, such as patch-clamp recording[10] or intracellular sharp electrode recordings[11]. The most of experiments with these recordings so far have been done in slice preparations or single cells. Although people have

already stated *in vivo* recording with electrode in such fine structures[11], the limited number of recording sites makes it impossible to map multiple inputs in a large area. (2) Instead, the most effective way for analyzing dendritic signals is to image the dynamics of intracellular calcium concentration[12] with two-photon microscopy. Two-photon imaging allows us to look into the brain through the highly scattering tissue. Indeed, the dendritic calcium imaging of action potential-related signals in cortical neurons *in vivo* was done for the first time more than ten years ago[13]. In addition, imaging method is able to provide large field of view for mapping multiple input sites in the dendritic field. Therefore, dendritic calcium imaging by two-photon microscopy, together with somatic whole-cell patch-clamp recordings (Fig. 3), is suitable for us to study the spatio-temporal arrangement of sensory inputs *in vivo*.

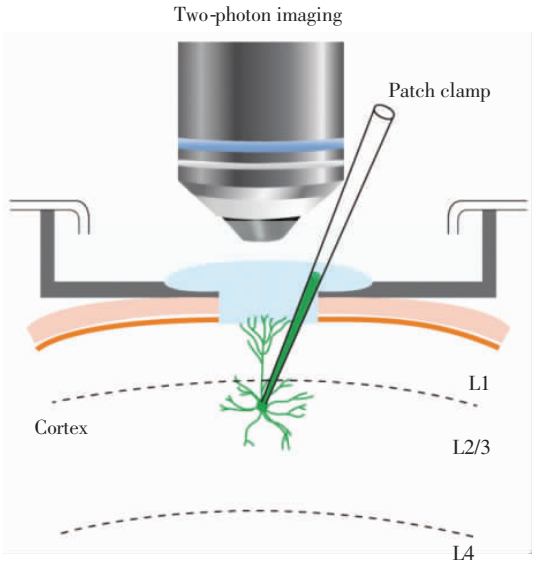


Fig 3 Schematic illustration of two-photon calcium imaging with whole-cell patch clamp recording in cortical neurons *in vivo*. Note that the patch electrode is filled with a calcium dye

The landmark work in which two-photon imaging technique was used to detect sensory-evoked signals in the dendrites *in vivo* was published in 2010[14]. In this work done in layer 2/3 neurons of mouse visual cortex, an intracellular electrode was used to fill single cortical neurons with a Ca^{2+}-sensitive indicator, and two-photon microscopy was used to image the Ca^{2+} signals evoked by visual stimuli with different orientations. The observed rapid and localized signals with this approach were distributed throughout the dendritic field and probably represented sensory input sites in the dendrites. While this was the first time to detect sensory-evoked input signals in mammalian neuronal dendrites *in vivo*, an important question still remained regarding the precise nature of the sensory inputs to cortical neurons, namely, whether these sensory inputs represent individual synapses or rather small clusters of synapses on a dendritic branch[15]. This question was soon addressed just one year later by the development of a new variant of two-photon microscopy that was capable of detecting calcium signals from individual spines of cortical neurons *in vivo*[16]. In this study, sound-evoked calcium signals were recorded in the spines and dendrites of mouse layer 2/3 neurons of auditory cortex. The stable recording of signals from such fine structures *in vivo* relied on the development of a new method named low-power temporal oversampling (LOTOS) that was achieved by using the ultra-high speed two-photon imaging device (sampling rate: 1 000 frames/s). LOTOS procedure helps to increase the yield in fluorescent signal and to reduce phototoxic damage. In line with previous *in vitro* studies[17-18], the auditory-evoked spine calcium responses were mostly compartmentalized in spines. In addition, calcium imaging allows to record multiple synaptic sites at the same time and thereby to map functionally the individual synaptic inputs of specific sensory stimulation *in vivo*[12]. This LOTOS-based spine calcium imaging was recently applied to also understand dendritic coding of sensory inputs in mouse vibrissal cortex[19].

Salt-and-pepper like pattern of dendritic organization of synaptic inputs

As mentioned above, different working models of dendritic organization of sensory inputs have been emerged based on the studies performed *in vitro*. Two-photon dendritic calcium imaging, particularly LOTOS-based spine calcium imaging enabled us to directly explore this fundamental issue *in vivo* with sensory stimulation. The experiments were first performed at dendritic levels in layer 2/3 neurons of mouse visual cortex[14]. One might expect that inputs with similar orientations would be clustered onto the same dendrite, which could be necessary for the dendrite performing superlinear summation of synaptic inputs[20]. Interestingly, the findings *in vivo* suggested no evidence for such a dendritic computational rule. Instead, we report that the inputs of similar orientation preference are dispersed throughout the whole dendritic

field. Therefore, the "computational unit" for sensory information in this type of neurons might be the whole dendritic tree, rather than individual dendritic branch[21].

This salt-and-pepper like distribution of sensory inputs that have similar features was further confirmed in layer 2/3 pyramidal neurons of mouse auditory cortex by imaging calcium signals in individual spines[16]. This experiment involving the LOTOS procedure demonstrated that synaptic inputs to the same dendrite were found to be highly heterogeneous, namely even neighboring spines on the same dendrite were able to be tuned to different stimuli. This heterogeneity of synaptic inputs at dendritic level probably represents the diversity of response properties of neurons in local cortical networks as described recently[22-23]. This distributed pattern of synaptic inputs would argue for the existence of linear integration in the dendrites of this type of cortical neurons[2, 9].

Future directions

At present, due to the technical constraints, our knowledge about the dendritic organization of sensory inputs in cortical neurons is largely restricted to neurons that are located near the cortical surface, i. e. at a depth of 100-300 μm[14-16, 19]. Future development of two-photon microscopy may help to answer whether this salt-and-pepper like organization also exists in deeper cortical layers or even in subcortical brain regions. In addition, further studies should also be performed to understand what happens in different cell types and in different species.

A neuron can receive thousands of synaptic inputs that contain different features of information. Another open question is how the complex mixture of inputs would determine the response behavior of a neuron, i. e., the selectivity for stimulus features. Therefore, one of the next directions should focus on the dissection of input-output relationship during sensory stimulation.

Finally, the current information of dendritic arrangement of sensory inputs was acquired from anesthetized animals. Whether the dendritic maps of inputs in awake animals is the same also needs to be explored.

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