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Cell size-dependent Nogo-A expression in layer V pyramidal neurons of the rat primary somatosensory cortex

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Abstract

Nogo-A mRNA and protein are present in the perikarya of neurons in both the intact and injured CNS. The present study focused primarily on Nogo-A protein expression in the primary somatosensory cortex of the adult rat. Coronal brain sections were probed with double-immunofluorescent labeling against Nogo-A together with NeuN, RBPC, or MAP-2 for confocal imaging. The sizes of the cell somata in pyramidal neurons and the thicknesses of neurites were measured on the captured confocal images. Nogo-A was expressed in larger pyramidal neurons and thicker neurites in layer V, but not in smaller pyramidal neurons and thinner neurites. Considering the morphological properties and the cell soma size reported in previous studies together with the present data, Nogo-A-positive neurons of layer V appear to be intrinsically bursting neurons that project axons to the subcortical regions. This suggests that intraneuronal Nogo-A may play roles in neurite growth and axonal regeneration of the corticofugal neurons, but not of columnar intrinsic neurons, in layer V of the S1 barrel cortex. Additionally, this study demonstrates a novel result, which is that layer V pyramidal neurons of the S1 barrel cortex exhibit a pattern of cell size-dependent intraneuronal Nogo-A expression.

Keywords: Nogo-A; Primary somatosensory cortex; Layer V; Pyramidal neuron; Neurite; Expression pattern

Nogo-A is a myelin-associated neurite outgrowth inhibitor involved both in the failure of axonal regeneration and in the restriction of compensatory plasticity following lesions in the adult mammalian CNS [2,5]. Nogo-A is expressed mainly by oligodendrocytes, but nogo-A mRNA expression in neurons has been also reported [5], and many studies have demonstrated the cellular distribution and the expression patterns of nogo-A mRNA and Nogo-A protein in neurons in the intact and injured CNS of the adult and fetal humans and rodents. In the intact CNS, nogo-A mRNA is expressed by several neuronal types, including spinal motor neurons, dorsal root ganglia, sympathetic neurons, retinal ganglion cells, and neocortical, hippocampal, and Purkinje neurons [6,7,8,10]. Nogo-A protein is also strongly expressed by motor neurons, dorsal root ganglia, sympathetic neurons, retinal ganglion cells, and many Purkinje cells, but it is weak in some neocortical neurons [7,8,13].

Nogo-A associates primarily with the endoplasmic reticulum membrane [5]. A recent study demonstrated that Nogo-A is present in the neuronal perikaryon, on the plasma membrane, and in the nucleus [9]. Intraneuronal Nogo-A immunoreactivity is the densest in the cytoplasm and much less dense in the nucleus [8,9]. Most neurons in the CNS that express Nogo-A protein in their perikarya show only weak expression in their axons [7,8]. This is remarkably distinct from PNS neurons, in which axons in peripheral nerves exhibit strong Nogo-A immunoreactivity in both their perikarya and axons [8]. Depending on the subcellular localization of Nogo-A, several potential functions of Nogo-A in neurons are indicated. Nogo-A might act as a multifunctional signaling molecule involved in such diverse functions as neurite outgrowth, cell survival or death decisions, and synaptic plasticity [4,7]. Nogo-A might also have an intracellular function in neurons beyond inhibitory activity on neurite growth and axonal regeneration [7,13]. However, the intraneuronal functions of Nogo-A are still not clear.

In in situ hybridization and immunohistochemical studies on Nogo-A in neocortical neurons of the rat brain, nogo-A mRNA was easily detectable in neuronal perikarya [6,10], especially

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in projection neurons [8], but Nogo-A protein expression was weak [8]. Neurons in layer V of the cortex strongly expressed nogo-A mRNA during the early postnatal period [7,10]. In the neocortex of the adult rat, neurons in layers II/III, V and VI exhibited strong nogo-A mRNA expression, but neurons in layer IV exhibited weaker expression [7].

The present study focused mainly on elucidation of Nogo-A protein expression in the primary somatosensory (S1) cortex of the adult rat by the use of immuno-fluorescence confocal imaging. The data demonstrate a pattern of neuron size-dependent Nogo-A expression in layer V pyramidal neurons of the S1 barrel cortex.

Male Sprague–Dawley rats, 12 weeks old, were used in this experiment. The animals were housed in plastic cages at constant temperature and humidity. The rats were provided with food and water ad libitum and housed under a natural light–dark cycle. The animals were sacrificed with an overdose of pentobarbital and were then perfused transcardially with 0.05 M phosphate-buffered saline (PBS) containing 4% paraformaldehyde in a 0.2 M phosphate buffer at pH 7.4. The brains were removed and were postfixed in the same perfusing solution overnight at 4 °C and were then transferred into a 30% sucrose solution for cryoprotection for at least 2 days. From the brain region between 5.20 and 7.20 mm of the interaural line, serial coronal sections of 20 μm thickness were made using a freezing microtome (Leica, 2800N, Germany). Every fifth section was stained with Nissl's method to identify the location of the S1 barrel cortex, the succeeding sections were stained by the free-floating 3,3′-diaminobenzidine (DAB) reaction, and all other sections were stained by double-immunofluorescent labeling for confocal imaging. For immunohistochemistry, briefly, the free-floating sections were rinsed with 0.05 M PBS and incubated for 15 min in 1% hydrogen peroxide PBS at room temperature, and the sections were incubated overnight at 4 °C with the primary antibody (1:200, anti-Nogo-A, sc-25660, Santa Cruz, USA). The sections were then incubated with biotinylated anti-rabbit secondary antibody (1:200, Vector Laboratories, USA) for 50 min at room temperature, after which the avidin–biotin complex (ABC, 1:200, Vector Laboratories, USA) method was carried out with peroxidase coupling in a mixture containing DAB (Sigma, USA) and 0.03% H_2O_2 for 2–5 min. The double-immunofluorescent labeling was performed using primary antibody against Nogo-A (1:200, sc-25660, Santa Cruz, USA) together with antibody against NeuN (1:100, Chemicon, USA), RBPC (anti-rat brain pyramidal cell antigen, 1:1000, Swant, Switzerland), or MAP-2 (1:200, Sigma, USA). The fluorescent secondary antibodies used were anti-rabbit Cy2 and anti-mouse Cy3 (Jackson ImmunoResearch, USA). Images of the fluorescently labeled coronal brain sections were captured using confocal laser-scanning microscopy (Carl Zeiss, LSM 510 META, Germany). The colocalizations of Nogo-A expression (Cy2, green wave) in neurons and neurites with NeuN, RBPC, and MAP-2 expression (Cy3, red wave) were observed on separated and merged digital images. The sizes of the cell somata of the pyramidal neurons and the thicknesses of the neurites were measured on the captured images with LSM Image Examiner (Ver. 3.2.0, Zeiss, Germany) software.

A representative oligodendrocyte and a classical pyramidal neuron from layer V of the S1 cortex, stained with DAB against the Nogo-A antibody, are shown in Fig. 1. As expected, the oligodendrocyte exhibited strong immuno-reactivity against Nogo-A in the cell soma and in the radially extended neurites (Fig. 1A). The neurons in all layers of the S1 barrel cortex exhibited observable levels of Nogo-A expression (figures not shown), consistent with previous reports. In particular, the pyramidal neurons in layer V exhibited distinct immuno-reactivity against the Nogo-A antibody in the neuronal cytoplasm, in the apical and basal dendrites, and even in the axon (Fig. 1B).

In order to reconfirm that Nogo-A is expressed in the neuronal cytoplasm and in neurites, double-immunofluorescent labeling was performed against Nogo-A and against NeuN as a neuronal marker, RBPC as a pyramidal neuron marker [16], or MAP-2 as a neurite marker, and labeling was visualized by confocal imaging of neighboring cortex sections. As shown in the merged section of Nogo-A and NeuN in Fig. 2, all Nogo-A-(+) pyramidal neurons were also NeuN-(+), but not all NeuN-(+) neurons were positive for Nogo-A. As shown in the images for Nogo-A and RBPC in Fig. 2, an oligodendrocyte, which was not labeled with

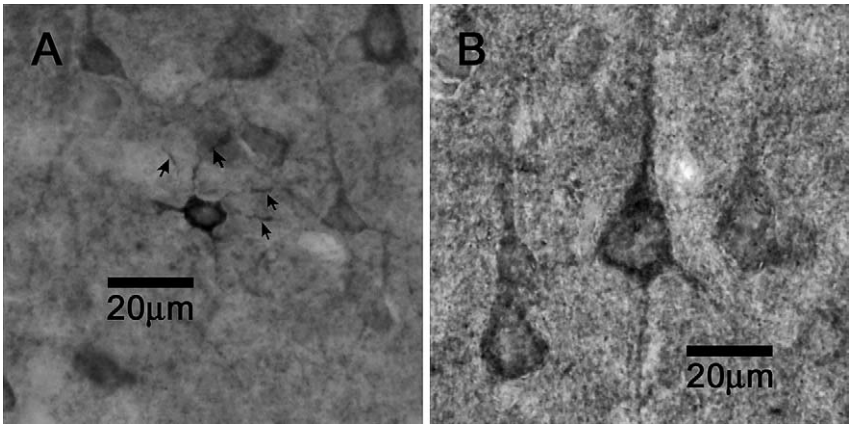

Fig. 1. Representative oligodendrocyte and pyramidal neuron from layer V of the S1 cortex stained with DAB immunohistochemistry against Nogo-A. (A) The oligodendrocyte exhibits strong immuno-reactivity against Nogo-A in the cell soma and in the radially extended neurites. (B) The pyramidal neuron in layer V expresses precise immuno-reactivity against Nogo-A in the neuronal cytoplasm, in the apical and basal dendrites, and in the axon.