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Dendrite plasticity in the lateral geniculate nucleus in primate glaucoma

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ABSTRACT

Neural degeneration in glaucoma involves retinal ganglion cells and neurons of their major target, the lateral geniculate nucleus (LGN). Dendrites of relay LGN neurons projecting to the visual cortex were studied by immunocytochemical and quantitative Sholl analysis in combination with confocal microscopy and 3D-morphometry. In non-human adult primate glaucoma, relay LGN neurons showed reduced dendrite complexity and length, and these changes were modified by NMDA receptor blockade. Dendrite plasticity of LGN relay neurons in adult primate glaucoma has implications for potential disease modification by treatment interventions.

1. Introduction

Glaucoma is a major cause of irreversible blindness worldwide, characterized by retinal ganglion cell (RGC) loss (Weinreb & Khaw, 2004). Degeneration of retinal ganglion cells spreads trans synaptically to their main target, the lateral geniculate nucleus (LGN) of the brain in human glaucoma (Gupta, Ang, Noel de Tilly, Bidaisee, & Yucel, 2006; Gupta et al., 2009). Previous studies in experimental primate glaucoma, show degenerative changes in the LGN including neuron loss (Weber, Chen, Hubbard, & Kaufman, 2000; Yucel, Zhang, Gupta, Kaufman, & Weinreb, 2000), neuron atrophy (Weber et al., 2000; Yucel, Zhang, Weinreb, Kaufman, & Gupta, 2001), reactive gliosis (Sasaoka et al., 2008), and microglial activation (Imamura et al., 2009). Neuron loss and atrophy increase with increasing RGC loss as do metabolic changes observed in the visual cortex in primate glaucoma. (Yucel, Zhang, Weinreb, Kaufman, & Gupta, 2003; Yucel et al., 2000, 2001).

Dendrites are fine processes emerging from the cell body of neurons that form elaborate dendritic arbors supporting post-synaptic contact elements (Johnston & Narayanan, 2008). Disturbances to dendrite branching can disrupt neural network organization and lead to neural dysfunction, as in human neurological disorders including Alzheimer's disease (Moolman, Vitolo, Vonsattel, & Shelanski, 2004). Their fine structure is maintained by microtubules, and among the associated proteins, microtubule-associated protein-2 (MAP2) is enriched in dendrites (Cassimeris & Spittle, 2001; Ichihara, Kitazawa, Iguchi, Hotani, & Itoh, 2001) playing an important role in dendrite morphology and branching (Dehmelt & Halpain, 2005; Harada, Teng, Takei, Oguchi, & Hirokawa, 2002; Matus, 1994). MAP2 has been used as a marker to assess dendrites in neurodegenerative diseases (Goedert, Crowther, & Garner, 1991; Kaufmann, Naidu, & Budden, 1995; Kwei, Jiang, & Haddad, 1993; Matesic & Lin, 1994).

In glaucoma, dendrites in the primate retina undergo atrophy (Morgan, Uchida, & Caprioli, 2000; Weber, Kaufman, & Hubbard, 1998). A study in which LGN interneurons confined to the LGN and relay neurons projecting to the visual cortex were not discriminated, showed dendrite changes in primate glaucoma (Gupta et al., 2007). However, detailed dendrite evaluation of the LGN relay neurons under conditions of glaucoma at various stages of disease and following pharmacological treatment has not been previously investigated. Memantine is an NMDA glutamate open-channel blocker approved as a neuroprotective agent for the treatment of cognitive impairment in Alzheimer's disease (Reisberg et al., 2003). The purpose of this work is to identify and quantify dendrite characteristics of LGN relay neurons in a primate model of glaucoma to determine whether they are altered, and if so, whether these changes are modifiable by memantine (Bormann, 1989).

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2. Materials and methods

2.1. Subjects

All experiments were performed following the guidelines of the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research.

Six LGNs from six normal adult cynomolgus monkeys (*Macaca fascicularis*) from the University of Wisconsin, Madison were used as controls. They were perfused intracardially with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Intraocular pressure (IOP) measurements were performed *in vivo* before sacrifice with a pneumatonometer (Digilab, Norwell, MA) under light sedation (intramuscular injection of 5 mg/kg of ketamine hydrochloride) and topical anesthesia (5% proparacaine hydrochloride) (Yucel et al., 2000). The mean IOP ranged from 12.3 to 21.0 mm Hg and the maximum IOP ranged from 13.0 to 26.0 mm Hg. There was significant difference in mean IOP ($P < 0.001$) or maximum IOP ($P < 0.001$) between the vehicle-treated glaucoma group and the normal group.

Experimental glaucoma was induced in adult monkeys (*Macaca fascicularis*) by laser scarification of the trabecular meshwork (Hare et al., 2004). The duration of IOP elevation was 14 months. Three animals out of 18 were excluded from the present study due to either suboptimal fixation or unidentifiable LGN layers. Two groups of monkeys were evaluated and compared: (1) seven vehicle-treated (VT) monkeys with glaucoma and (2) eight memantine-treated (MT) monkeys with glaucoma who received a daily oral dose of 4 mg/kg of memantine. In both the VT and MT glaucoma groups, the treatment was continued during 14 months until sacrifice (Hare et al., 2004). There was no significant difference in mean IOP ($P = 0.43$) or maximum IOP ($P = 0.73$) between the VT and MT glaucoma groups (Yucel, Gupta, Zhang, Mizisin, Kalichman, & Weinreb, 2006). Previously published optic nerve counts of these animals showed no significant difference in mean percent optic nerve fiber loss between the VT and the MT glaucoma groups (Yucel, Gupta et al., 2006).

2.2. Tissue processing

Perfusion fixation under deep general anesthesia and tissue processing and 40 μm-thick serial sections were performed (Yucel et al., 2000). Every seventh section was mounted onto a glass slide and stained with cresyl violet. Care was taken to use the same tissue processing procedures for all monkey brains. Sections containing left LGN with six layers were randomly selected for each of the normal control animals and each of the VT and MT glaucoma animals.

2.3. Double immunofluorescence labeling of MAP2 and parvalbumin

LGN sections were double-labeled with a monoclonal antibody against MAP2 (Clone HM-2, Sigma, St. Louis, MO), and a polyclonal antibody against parvalbumin (PV28, Swant, Bellinzona, Switzerland), a calcium-binding protein, a specific marker for relay LGN neurons (Johnson & Casagrande, 1995). The tyramide signal amplification (TSA) kit (Invitrogen Inc., ON, Canada) was used to enhance immunostaining. Sections were washed with phosphate buffered saline (1 × PBS, 3 × 10 min). All subsequent washes were performed with 1 × PBS, pH 7.35 (3 × 5 min). Sections were incubated with 0.2% Triton-X (Sigma, St. Louis, MO) in PBS (2 × 5 min). After PBS wash, endogenous peroxidase activity was quenched by 3% H_2O_2 (2 × 15 min). Sections were washed with PBS and blocked in 1% blocking reagent (TSA kit). Incubation in parvalbumin [1:500] was extended to 2 h at room temperature, overnight at 4 °C and then left at room temperature for another 2 h. Sections were washed with PBS and incubated in anti Rabbit-HRP [1:100] (TSA kit). After PBS wash, sections were incubated in Tyramide-Alexa Fluor-488 diluted in Amplification Buffer [1:100] (2 × 10 min). Sections were washed with PBS, quenched with 3% H_2O_2 (2 × 15 min), and blocked in 1% blocking reagent. Incubation in MAP2 [1:100] was extended to 2 h at room temperature, overnight at 4 °C and then left at room temperature for another 2 h. Sections were washed with PBS and incubated in anti Mouse-HRP [1:100] (TSA kit). After being washed again in PBS, sections were incubated in Tyramide-Alexa Fluor-555 [1:100] (2 × 10 min) diluted in Amplification buffer (TSA kit). Sections were washed again with PBS, mounted with antifade PVA-DABCO onto Vectabond (Vector Laboratories, Burlingame, CA) coated slides and cover-slipped. Immunostaining experiments for all three groups were performed simultaneously with same reagents. Negative controls were obtained by omitting the primary antibodies.

2.4. Morphological assessment

Immunofluorescence-labeled sections were viewed using an Olympus bright field BX51 upright microscope with a color digital camera (Microfire, Optronics Inc. Goleta, CA) and a computer monitor. The LGN layers were identified as layers 1–6 from ventral to dorsal. The ventral layers 1 and 2 are magnocellular layers, while the remaining dorsal layers 3–6 are parvocellular layers. Layers 1, 4 and 6 of the left LGN are connected to the glaucomatous right eye while layers 2, 3 and 5 are connected to the non-glaucomatous left eye. We analyzed MAP2-immunoreactive dendrites of parvalbumin-positive relay neurons projecting to the primary visual cortex (Johnson & Casagrande, 1995), located in left LGN magnocellular layer 1 and parvocellular layer 6 connected to the glaucomatous right eye.

2.5. Quantitative analysis

2.5.1. Confocal image acquisition

Sections from MT and VT glaucoma groups, and normal controls were examined using LaserSharp 2000 software (Biorad Cell Science Division, Hernelhempstead, UK) with a digital camera on an upright confocal laser microscope (Biorad Radiance, Bronx, NY on Nikon E800 Upright Microscope, Melville, NY) in a masked fashion. Five sampling sites of 500 μm × 500 μm within each magnocellular layer 1 and parvocellular layer 6 were selected. Within each sampling site, one stack of images was taken under red (CY3 for MAP2) and green (FITC for parvalbumin) channels separately with a 60× oil-immersion objective at zoom of 1.5× and 512 × 512 pixels by LaserSharp2000 program. Serial stack images of 18 μm depth and 0.5 μm step size were collected using the same camera settings for power, gain, iris aperture size, and offset to maintain consistent settings for comparison. All images were obtained using a Kalman filter with three passes.

2.5.2. Automated tracing and 3D reconstruction

Stacks of images were imported (Neurolucida; MicroBrightField, Inc., Colchester, VT) and neurons were traced with an automated tracing software (AutoNeuron, MicroBrightField Inc., Colchester, VT). Tracing of MAP2-immunoreactive dendrites (CY3 channel) and parvalbumin-immunostained cell bodies (FITC channel) of stacks of confocal images was performed separately. Two modes of the automated tracing software were used to trace neurons: interactive mode for MAP2-immunostained dendrites, and automatic mode for parvalbumin-immunostained cell bodies. Configuration parameters used for tracing for each neuron were: (1) maximum process diameter, 1.5 μm, (2) ignore somas smaller than, 2.0 μm, (3) soma detector sensitivity, 55 and 65 for layers 1 and 6, respectively, and (4) interactive mode.