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Effect of NCAM on aged-related deterioration in vision

Margaret Po-shan Luke [a], Terry L. LeVatte [a], Amanda M. O'Reilly [a], Benjamin J. Smith [b], François Tremblay [c,d], Richard E. Brown [e], David B. Clarke [a,f,g,h,*]

[a] Department of Medical Neuroscience, Life Science Research Institute, Dalhousie University, Halifax, Nova Scotia, Canada
[b] Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada
[c] Department of Physiology and Biophysics, Dalhousie University, Halifax, Nova Scotia, Canada
[d] Department of Ophthalmology and Visual Sciences, Dalhousie University, Halifax, Nova Scotia, Canada
[e] Department of Psychology and Neuroscience, Life Science Centre, Dalhousie University, Halifax, Nova Scotia, Canada
[f] Department of Surgery (Neurosurgery), Life Science Research Institute, Dalhousie University, Halifax, Nova Scotia, Canada
[g] Department of Medicine (Endocrinology), Life Science Research Institute, Dalhousie University, Halifax, Nova Scotia, Canada
[h] Department of Ophthalmology & Visual Sciences, Life Science Research Institute, Dalhousie University, Halifax, Nova Scotia, Canada

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ABSTRACT

The neural cell adhesion molecule (NCAM) is involved in developmental processes and age-associated cognitive decline; however, little is known concerning the effects of NCAM in the visual system during aging. Using anatomical, electrophysiological, and behavioral assays, we analyzed age-related changes in visual function of NCAM deficient (−/−) and wild-type mice. Anatomical analyses indicated that aging NCAM −/− mice had fewer retinal ganglion cells, thinner retinas, and fewer photoreceptor cell layers than age-matched controls. Electroretinogram testing of retinal function in young adult NCAM −/− mice showed a 2-fold increase in a- and b-wave amplitude compared with wild-type mice, but the retinal activity dropped dramatically to control levels when the animals reached 10 months. In behavioral tasks, NCAM −/− mice had no visual pattern discrimination ability and showed premature loss of vision as they aged. Together, these findings demonstrate that NCAM plays significant roles in the adult visual system in establishing normal retinal anatomy, physiology and function, and in maintaining vision during aging.

1. Introduction

As we age, our vision deteriorates due to the progressive decline in visual acuity, decreased contrast sensitivity, and an increase in the dark adaptation threshold (Salvi et al., 2006). These progressive changes are associated with changes in lens flexibility, altered retinal electrophysiological properties, a reduction in the number of neurons, and regression of dendritic arbors in the retina (Freund et al., 2011; Samuel et al., 2011). Aging may be accompanied by a number of degenerative diseases of the eye, including glaucoma, cataracts, diabetic retinopathy, and macular degeneration, all of which can lead to irreversible blindness. Although the underlying causes remain unclear, the decline of visual function in aging is associated with both genetic and environmental factors (Hogg et al., 2009).

The development of the visual system involves a temporal sequence of neurochemical signals which regulate synaptic connections and those processes are modulated by visual experience (Belanger and Di Cristo, 2011; Chen et al., 2012). The neural cell adhesion molecule (NCAM) is involved in many stages of the development of the mouse visual system (Bartsch et al., 1990; Clandinin and Feldheim, 2009; Missaire and Hindges, 2015). Little is known, however, concerning the role of NCAM during age-related disorders of visual functions.

NCAM is a glycoprotein of the immunoglobulin superfamily, containing 2 major transmembrane isoforms (180 and 140 kDa) and a smaller glycophosphatidyl inositol–linked isoform (120 kDa) (Soroka et al., 2008). All major NCAM isoforms can be modified by polysialic acid (PSA), which is widely expressed during central nervous system development. NCAM is the most abundant PSA carrier in mammals (Nelson et al., 1995), and the removal of NCAM abolishes almost all the PSA in the nervous system (Finne et al., 1983). In the retina, the highly sialylated form of NCAM is expressed throughout all retinal layers during development (Bartsch et al., 1990). However, in adult mice, it is located exclusively on astrocytes and Müller glial cells of the retina and on

* Corresponding author at: Departments of Surgery (Neurosurgery), Medical Neuroscience, Medicine (Endocrinology) and Ophthalmology & Visual Sciences, Dalhousie University, Life Science Research Institute, 1348 Summer Street, Halifax, NS, Canada, B3H 4R2. Tel.: +1 (902) 494 6021; fax: 902-494-1212.
E-mail address: d.clarke@dal.ca (D.B. Clarke).

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astrocytes of the optic nerve (ON) (Bartsch et al., 1990). Although PSA-NCAM is not expressed in adult retinal ganglion cells (RGCs), it has been demonstrated that Müller glia processes ensheath most retinal neurons; therefore, PSA-NCAM resides in close proximity to neurons, including RGCs (Bartsch et al., 1990; Stone et al., 1995). In the mouse visual cortex, PSA and NCAM are highly expressed during development; however, the expression of PSA declines in response to visual system sensory input shortly after eye opening (Di Cristo et al., 2007).

There are a number of age-related changes in neural circuitry underlying vision in the mouse (Samuel et al., 2011). NCAM has been shown to play an important role in age-related cognitive impairments (Bisaz et al., 2013), but no research has examined the role of NCAM in age-related visual dysfunction. The purpose of the current work was to investigate the effects of NCAM in the visual system during aging. We used anatomical, functional, and behavioral analyses to determine how NCAM deficiency in mice affects age-related changes in visual function. We have found that, in aging wild-type (WT) animals, PSA levels are lower in the retina but higher in the visual cortex, and both regions exhibit differential expression of NCAM isoforms. Despite the fact that young adult NCAM −/− mice have more RGCs, the total number of ON axons remains the same as in WT mice. In the absence of NCAM, retinal activity increases in young adult mice; however, as the knockout animals age, there is a dramatic drop in retinal activity amplitude and thinning of the retinas. These NCAM-dependent changes are associated with significant functional consequences: impaired pattern discrimination ability in adulthood and premature loss of visual acuity as the animals age.

2. Materials and methods

2.1. Subjects

Homozygous NCAM −/− mice and their WT littermates (n = 10 mice of mixed sex for each of the 2, 10, 18, and 24 months groups) on a C57BL/6J background were obtained by crossing heterozygous male and female animals (generously provided by Dr. Victor Rafuse), and genotypes of mice were determined by polymerase chain reaction using primers and conditions as indicated previously (Cremer et al., 1994). Animals were bred in-house and were cared for according to the Guide and Use of Experimental Animals of the Canadian Council on Animal Care. The experimental protocol (number 13–081) was approved by the Dalhousie Committee on Animal Care.

2.2. Immunohistochemistry

Animals were anesthetized and transcardially perfused with chilled 4% paraformaldehyde. The eyes were removed, post-fixed overnight at 4 °C, and cryoprotected in 30% sucrose. Retinal sections (16 μm) were prepared by orienting the eyes in optimal cutting temperture compound (Tissue-Tek, Miles Laboratories) and cutting with a cryostat (Leica CM1850) along the temporal-nasal orientation of the eye. Coronal brain sections through the visual cortex (25 μm) were also sliced with a cryostat. The sections were immunoreacted with anti-NCAM (MAB310, Millipore; 1:100) and anti-PSA-NCAM (MAB5324, Millipore; 1:500) antibodies. Immunohistochemistry was performed as previously described (Murphy et al., 2009).

2.3. Immunoblot

Ten retinas and visual cortex per group were used in the 2-, 10-, and 18-month-old WT samples but only 5 were collected for the 24-month-old animals. Anti-NCAM (MAB310, Millipore; 1:500), anti-PSA-NCAM (MAB5324, Millipore; 1:1000), and anti-actin (A2066, Sigma; 1:5000) antibodies were used to determine protein expression by standard sodium dodecyl sulfate–polyacrylamide gel electrophoresis immunoblotting (Murphy et al., 2007).

2.4. Morphometric analysis

To determine the thickness of 2- and 18-month-old retinas, retinal sections (16 μm) were prepared as indicated previously. To ensure consistency in counting cells, only retinal sections that included the ON stump were selected. The sections were stained with nuclear (Hoechst; 1: 50,000, Sigma) and synaptic (Bassoon; 1: 500, Enzo Life Sciences) markers. Five animals were examined per group and retinal images (n = 5 section/mouse retina) were taken using fluorescence microscopy (Zeiss LSM510 META). Thicknesses of the whole retinas, and each of their relative layers, were measured 300 μm (central), 1100 μm (middle), and 1900 μm (peripheral) from the edge of the ON head using ImageJ. The number of rows of photoreceptor cells in the outer nuclear layer (ONL) was counted on the same retinal images using the method of Smith et al., (2008).

2.5. RGC labeling and counting

Using the retinal sections (n = 5/group) prepared previously, we labeled RGCs using an anti-Neuronal Nuclei (NeuN) (1:500; Millipore) antibody and an anti-choline acetyltransferase (1:400; Millipore) antibody, a method shown to be effective for RGC quantification in young adult and senescent animals (Buckingham et al., 2008). RGCs were counted in the ganglion cell layer (GCL) of the whole retinal cross sections using the method of Smith et al., (2008).

2.6. Transmission electron microscopy analysis

For transmission electron microscopy analysis, the ONs were prepared as previously described (Murphy et al., 2007). The images of 100–130 nm ON sections were obtained under an electron microscope (EM300; Philips). For RGC axon counting, whole ON cross sections were photographed (~20–30 micrographs) and counted manually using the method of Murphy et al., (2007).

2.7. Electroretinogram recording

Mice were anesthetized using a mixture of ketamine (100 mg/kg), acepromazine (0.62 mg/kg), and xylazine (15 mg/kg), placed into a stereotaxic frame, and their pupils were dilated with 0.5% cyclopentolate HCl drops (Alcon, Fort-Worth, TX, USA). They were kept at a constant body temperature (37 °C) by a heated pad with rectal temperature feedback. An active electrode (Dawson-Trick-Litzkow-plus microconductive fiber, Diagnosys, Littleton, MA, USA) was placed on the mouse's corneal surface and hydrated with 2.5% hydroxypropyl methylcellulose solution. A reference electrode was inserted at the base of the nose and a ground electrode was placed on the tail (Platinum subdermal electrodes, Grass Instruments, Quincy, MA, USA). Signals recorded from the corneal electrode were first collected and amplified 10,000-fold by a differential amplifier with a bandwidth of 3–1000 Hz (P511, Grass Instruments), then digitized into 300 sample points at a rate of 1000 Hz by an A/D instrument converter (GW Instruments, Summerville, SC, USA) (Smith et al., 2013).

After overnight dark adaptation, animals were exposed to a series of strobe flash visual stimuli generated by a PS3 photic-stimulator (Grass Instruments) with increasing intensity