Vision Research 114 (2015) 68–78

Contents lists available at ScienceDirect

Vision Research

journal homepage: www.elsevier.com/locate/visres

Experience-dependent central vision deficits: Neurobiology and visual acuity

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ARTICLE INFO

Article history:
Received 3 September 2014
Received in revised form 6 January 2015
Available online 7 February 2015

Keywords:
Amblyopia
Binocular
AMPAR
Acuity
Monocular deprivation
Central vision

ABSTRACT

Abnormal visual experience during childhood often leads to amblyopia, with strong links to binocular dysfunction that can include poor acuity in both eyes, especially in central vision. In animal models of amblyopia, the non-deprived eye is often considered normal and what limits binocular acuity. This leaves open the question whether monocular deprivation (MD) induces binocular dysfunction similar to what is found in amblyopia. In previous studies of MD cats, we found a loss of excitatory receptors restricted to the central visual field representation in visual cortex (V1), including both eyes' columns. This led us to ask two questions about the effects of MD: how quickly are receptors lost in V1? and is there an impact on binocular acuity? We found that just a few hours of MD caused a rapid loss of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor proteins across all of V1. But after a few days of MD, there was recovery in the visual periphery, leaving a loss of AMPA receptors only in the central region of V1. We reared animals with early MD followed by a long period of binocular vision and found binocular acuity deficits that were greatest in the central visual field. Our results suggest that the greater binocular acuity deficits in the central visual field are driven in part by the long-term loss of AMPA receptors in the central region of V1.

1. Introduction

Early visual experience shapes the maturation of circuits in visual cortex and development of visual perception. Abnormal experience during the critical period by depriving one eye of vision (monocular deprivation, MD) causes a loss of responsiveness in visual cortex (V1) to the deprived eye (Wiesel & Hubel, 1965a), poor acuity through that eye (Dews & Wiesel, 1970), and various binocular dysfunctions (Blake & Hirsch, 1975; Blake, Crawford, & Hirsch, 1974). These changes are known as ocular dominance plasticity (Hubel, Wiesel, & LeVay, 1977) and just a few weeks of MD early in life can cause long-term loss of vision (Dews & Wiesel, 1970). Often the visual deficits are restricted to the deprived eye, however, several studies of children with amblyopia have found contrast sensitivity and other vision deficits in both eyes (Birch, 2013; Chatzistefanou et al., 2005; Leguire, Rogers, & Bremer, 1990; Simons, 2005), especially for vision in the fovea (Agervi, Nilsson, & Martin, 2010).

Animal studies using MD typically describe changes in V1 effecting the deprived eye. But two recent studies have shown that the initial impact of MD on V1 is a loss of responsiveness in binocular excitatory neurons (Hengen et al., 2013; Kuhlman et al., 2013) followed by a transient reduction in activation of inhibitory interneurons (parvalbumin-positive, PV+) affecting responsiveness of both eyes (Kuhlman et al., 2013). These studies show that the early phase of experience-dependent plasticity in V1 includes a loss of binocular responses.

PV+ inhibitory interneurons are an integral part of the neural circuitry that mediates experience-dependent plasticity in V1 (Hensch, 2014). The input to PV+ neurons is dominated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors that contain GluA2/3 subunits (Kooijmans et al., 2014). Furthermore, both rapid and long-term plasticity caused by MD involve changes in AMPARs and their trafficking proteins (Heynen et al., 2003; Lambo & Turrigiano, 2013; Yashiro et al., 2009; Yoon et al., 2009). Dynamic movement of AMPARs in and out of the postsynaptic membrane by trafficking proteins,

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http://dx.doi.org/10.1016/j.visres.2015.01.021
0042-6989/

including PICK-1 and Ube3A, are important mechanisms regulating experience-dependent synaptic plasticity (Colledge et al., 2003; Greer et al., 2010; Lee & Kirkwood, 2011; Sheng & Hyoung Lee, 2003; Yashiro et al., 2009). AMPARs also mediate the main feedforward stimulus driven response of neurons in V1 (Self et al., 2012). Monocular deprivation causes a loss of feedforward input to V1 neurons from the deprived eye (Ma, Li, & Tao, 2013) and in normal animals, the feedforward responses set up spatial tuning properties of V1 neurons (Lamme, Supèr, & Spekreijse, 1998). Thus, AMPA receptors sit at a nexus connecting ocular dominance plasticity and feedforward tuning of V1 neurons that ultimately underlies visual acuity.

Previously, we studied the effect of MD in cats on expression of synaptic receptors in V1 and found a loss of AMPARs restricted to the region of V1 representing the center of vision (Beston, Jones, & Murphy, 2010). Furthermore, anatomical investigation of another glutamate receptor (NMDA) found a central loss that included both eyes' ocular dominance columns (Murphy, Duffy, & Jones, 2004). These results show that MD drives regional, as well as ocular dominance column plasticity changes in V1. Furthermore, since the loss of receptors affects both eyes' columns in the central region of V1 it raises the possibility that MD might affect binocular acuity.

Our earlier studies did not address how quickly AMPAR proteins are lost in the center of vision, or if the regional loss affects the vision of both eyes. We addressed these questions by studying the effect of various lengths of MD on expression of AMPARs (GluA2) and trafficking proteins (PICK-1 and Ube3A) in different regions of V1, and on long-term changes in binocular visual acuity.

2. Materials and methods

2.1. Animals and rearing conditions

To determine the effect of brief monocular deprivation (range 6 h–7 days) on AMPAR expression in V1 we used 5 animals, and to study changes in visual acuity after early monocular deprivation we used 17 animals. All experimental procedures were in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision research, and the Code of Ethics of the World Medical Association (Declaration of Helsinki), and approved by the McMaster University Animal Research Ethics Board. The animals used to examine rapid changes in AMPAR protein expression in V1 were studied at 5 weeks of age which is the peak of the critical period for ocular dominance plasticity in the cat (Olson & Freeman, 1980) and received either no deprivation, 6 h, 1 day, 4 days, or 7 days of monocular deprivation. Acuity measurements were made on animals reared with either normal vision ($n = 3$), or monocular deprivation for different durations during the critical period to create mild (deprivation from 4–6 weeks of age, $n = 8$), moderate (deprivation from eye opening to 5 weeks of age, $n = 3$), or more severe vision loss (deprivation from eye opening to 6 weeks of age, $n = 3$). After deprivation these animals were given about 3 months of binocular visual experience during which visual acuity was measured daily with both eyes open.

Monocular deprivation was done by suturing the eyelids closed with 5-0 vicryl using aseptic surgical techniques, gaseous anesthetic (isoflurane, 1.5–5%, in oxygen) for induction and maintenance of anesthesia, and following procedures that have been described previously (Murphy & Mitchell, 1987). The sutured eyes were checked daily to ensure that the lid margins remained closed. At the end of deprivation the closed eye was re-opened by carefully parting the fused lid margins using aseptic surgical techniques.

2.2. Measurement and analysis of AMPAR subunit and receptor trafficking proteins – GluA2, PICK, Ube3A

2.2.1. Tissue collection

Animals were euthanized with Euthanol (165 mg/kg), and perfused transcardially with cold 0.1 M phosphate buffered saline (PBS) (4 °C; 80–100 ml/min). The brain was removed from the skull and immersed in ice cold PBS. A series of tissue samples (approx. 2×2 mm) were taken from V1 as described previously (Beston, Jones, & Murphy, 2010). For each cat, we used samples from V1 representing the center of vision (<5°, $n = 2$)), the visual periphery (~10–50°, $n = 2$–3), and the monocular field of vision (>60°, $n = 1$) region of V1 (Tusa, Palmer, & Rosenquist, 1978). Each cortical tissue sample was rapidly frozen on dry ice and stored at −80 °C. All tissue samples were taken from visual cortex contralateral to the deprived eye.

2.2.2. Tissue sample preparation

The tissue sample was suspended in cold homogenization buffer (1 ml buffer: 50 mg tissue, 0.5 mM DTT, 1 mM EDTA, 2 mM EGTA, 10 mM HEPES, 10 mg/L leupeptin, 100 nM microcystin, 0.1 mM PMSF, 50 mg/L soybean trypsin inhibitor) and homogenized using a glass–glass Dounce tissue homogenizer. Homogenized samples were suspended in 10% SDS, heated for 10 min, and then stored at −80 °C. Protein concentrations were determined using bicinchoninic acid (BCA) assay guidelines (Pierce, Rockford, IL) and samples were diluted to a standard concentration of 1 μg protein/ml.

2.2.3. Immunoblotting

The samples (25 μg) were separated on polyacrylamide gels (SDS–PAGE) and transferred to polyvinylidene difluoride membranes (PVDF-FL) (Millipore, Billerica, MA). Each sample was run three times. Blots were pre-incubated in blocking buffer (Odyssey Blocking Buffer 1:1 with PBS) for 1 h (Li-cor Biosciences; Lincoln, NE), then incubated in primary antibody overnight at 4 °C using the following concentrations: GluA2, 1:2000 (Invitrogen, Carlsbad, CA), Ube3A (E6AP), 1:2000 (Bethyl Laboratories, Montgomery, TX), PICK-1, 1:200 (NeuroMab, Davis, CA), β-Tubulin; 1:4000 (Imgenex, San Diego, CA). The blots were washed with PBS containing 0.05% Tween (Sigma, St. Louis, MO) (PBS-T) (3×10 min), incubated (1 h, room temperature) with the appropriate IRDye labeled secondary antibody (Anti-Mouse, 1:8000, Anti-Rabbit, 1:10,000) (Li-cor Biosciences; Lincoln, NE), and washed in PBS-T (3×10 min). The bands were visualized using the Odyssey scanner (Li-cor Biosciences; Lincoln, NE) then the blots were stripped and reprobed so that each blot was probed with each of the antibodies (Blot Restore Membrane Rejuvenation kit, Chemicon International, Temecula, CA).

2.2.4. Band analysis

To analyze the bands we scanned the blots (Odyssey Infrared Scanner) and quantified the bands using densitometry (Licor Odyssey Software version 3.0; Li-cor Biosciences; Lincoln, NE). Density profiles were determined by performing a subtraction of the background, integrating the pixel intensity across the area of the band, and dividing the intensity by the width of the band to control for variations in band size. β-tubulin normalization was used as the loading control, and for each sample, expression of the synaptic proteins was divided by β-tubulin expression. We verified that β-tubulin expression did not vary across conditions (all p values were n.s.) and thus was an appropriate loading control (Fig. 1). A control sample, made by combining a small amount from each sample, was run on each gel so the density of each sample was quantified relative to the control (sample density/control density).