Experimental Neurology 306 (2018) 138–148

Contents lists available at ScienceDirect

Experimental Neurology

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

Research Paper

Neurosteroid allopregnanolone reduces ipsilateral visual cortex potentiation following unilateral optic nerve injury

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ARTICLE INFO

Keywords:
Optic nerve crush
Mouse visual cortex
Neurosteroids
$GABA_A$ receptor
Stimulus-selective response potentiation

ABSTRACT

In adult mice with unilateral optic nerve crush injury (ONC), we studied visual response plasticity in the visual cortex following stimulation with sinusoidal grating. We examined visually evoked potentials (VEP) in the primary visual cortex ipsilateral and contralateral to the crushed nerve. We found that unilateral ONC induces enhancement of visual response on the side ipsilateral to the injury that is evoked by visual stimulation to the intact eye. This enhancement was associated with supranormal spatial frequency thresholds in the intact eye when tested using optomotor response. To probe whether injury-induced disinhibition caused the potentiation, we treated animals with the neurosteroid allopregnanolone, a potent agonist of the $GABA_A$ receptor, one hour after crush and on post-injury days 3, 8, 13, and 18.

Allopregnanolone diminished enhancement of the VEP and this effect was associated with the upregulated synthesis of the δ-subunit of the $GABA_A$ receptor. Our study shows a new aspect of experience-dependent plasticity following unilateral ONC. This hyper-activity in the ipsilateral visual cortex is prevented by upregulation of GABA inhibition with allopregnanolone. Our findings suggest the therapeutic potential of allopregnanolone for modulation of plasticity in certain eye and brain disorders and a possible role for disinhibition in ipsilateral hyper-activity following unilateral ONC.

1. Introduction

There is now good evidence that the adult visual cortex can undergo experience-dependent structural and functional modifications (Karmarkar and Dan, 2006; Gilbert and Li, 2012; Cooke and Bear, 2014; Kaneko and Stryker, 2017). One of the most extensively studied forms of experience-dependent changes is the effect of monocular visual deprivation (MD) on ocular dominance (OD) plasticity (Hofer et al., 2006; Gavornik and Bear, 2014). While normal mice show strong contralateral eye dominance, deprivation of visual input from the contralateral eye leads to weakening of the response from the deprived contralateral eye and strengthening of the response from the open ipsilateral eye (Frenkel and Bear, 2004; Smith and Bear, 2010).

Removal of visual input from an eye to the brain is induced not only by deprivation, but also by direct injury to the eye or optic nerve (Tagawa et al., 2005; Syken et al., 2006; Datwani et al., 2009; Nys et al., 2015a; Nys et al., 2015b). Most studies focus on changes in the visual cortex contralateral to the injured/enucleated/deprived eye, but it is important to note that a loss of visual input or damage to one hemisphere can also induce modifications of the other hemisphere through cortico-cortical (Van Brussel et al., 2011; Vasconcelos et al., 2011) and callosal connections (Restani et al., 2009; Laing et al., 2015).

A well-characterized animal model of optic nerve injury – optic nerve crush (ONC) – produces an acute insult that leads to retrograde degeneration of the vast majority of retinal ganglion cells and induces substantial functional reorganization in the brain (Sabel, 1999; Kreutz et al., 2004; Macharadze et al., 2012). The brain reorganization that accompanies optic nerve injury is more clinically relevant (optic neuropathies, glaucoma, optic nerve trauma) than monocular deprivation models, but it is understudied.

Abbreviations: ALLO, allopregnanolone; DAPI, 4′,6-diamidino-2-phenylindole; GABA, gamma-Aminobutyric acid; GAD65, glutamate decarboxylase 65; LTD, long-term depression; LTP, long-term potentiation; MD, monocular deprivation; NMDA, *N*-methyl-D-aspartate; OD, ocular dominance; OKT, optokinetic tracking; ONC, optic nerve crush; PBS, phosphate buffered saline; SRP, stimulus-selective response potentiation; TBS, Tris-buffered saline; TNF-α, tumor necrosis factor alpha; V1, primary visual cortex; VEP, visual evoked potential; VGlut2, vesicular glutamate transporter 2

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https://doi.org/10.1016/j.expneurol.2018.05.005
Received 10 January 2018; Received in revised form 6 April 2018; Accepted 1 May 2018
Available online 02 May 2018
0014-4886/

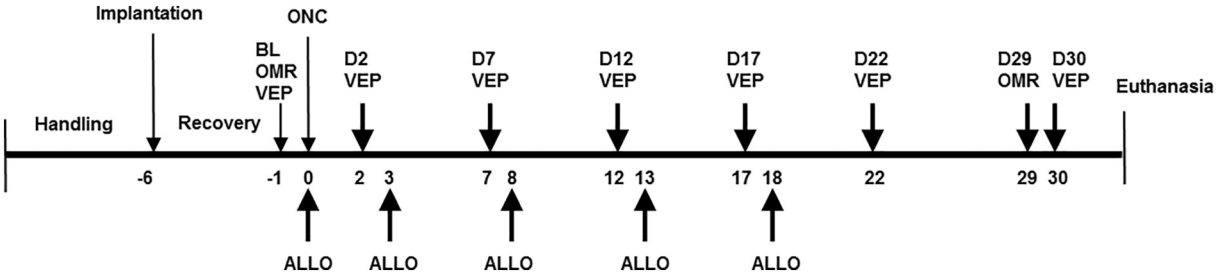

Fig. 1. Timeline for the experiment.

In this set of experiments we sought to determine how unilateral ONC injury affects both the contralateral and ipsilateral visual cortices of adult mice. We used a model of stimulus-selective response potentiation (SRP) to measure experience-dependent enhancement of cortical visually evoked potentials (VEP) on repeated presentation of a sinusoidal grating in a single orientation (Frenkel et al., 2006). We hypothesized that compared to MD, unilateral ONC will induce cortical enhancement of input from the intact eye, and consequently augmentation of cortical VEP ipsilateral to the intact eye (Sawtell et al., 2003; Frenkel et al., 2006). Since it was reported that OD plasticity is also associated with changes in visual behavior (Prusky et al., 2006; Iny et al., 2006), we measured the spatial frequency threshold following unilateral ONC with an optomotor test (Prusky et al., 2006; Tschetter et al., 2013).

Earlier studies on SRP and MD in adult rodents revealed that experience-dependent response enhancement in the visual cortex reflects strengthening of excitatory thalamo-cortical synaptic transmission in layer IV of the visual cortex (Frenkel et al., 2006; Coleman et al., 2010). To test whether post-ONC changes in VEP are also associated with increased excitatory transmission in thalamo-cortical synapses, we assessed expression of VGlut2, a vesicular glutamate transporter 2 specific to thalamo-cortical synapses (Nahmani and Erisir, 2005).

In contrast to the increased excitation observed after injury and in MD and SRP, cortical inhibition has been recently identified as a major determinant of normal sensory perception (Haider et al., 2013; Yazaki-Sugiyama et al., 2009; Smith and Bear, 2010; Chen et al., 2011; van Versendaal and Levelt, 2016). In this context, the role of GABA-active neurosteroids—positive modulators of $GABA_A$ receptors—in regulating inhibition has been established (Belelli and Lambert, 2005; Walker and Kullmann, 2012; Carver and Reddy, 2013). As an endogenous neurosteroid, allopregnanolone (ALLO) has been shown to increase inhibitory currents by activation of a wide range of $GABA_A$ receptors, particularly those containing the δ-subunit and considered responsible for tonic inhibition (Belelli and Lambert, 2005; Farrant and Nusser, 2005; Carver and Reddy, 2013; Reddy and Estes, 2016). ALLOhas also been shown to regulate expression and trafficking of the $GABA_A$ receptor subunits, leading to long-term inhibitory effects (Herd et al., 2007; Shen et al., 2005; Peng et al., 2009).

ALLO is one of the most potent positive modulators of cortical inhibition (Belelli and Lambert, 2005; Carver and Reddy, 2013; Crowley et al., 2016; Reddy and Estes, 2016) and has known neuroprotective properties (Djebaili et al., 2005; VanLandingham et al., 2006; Sayeed et al., 2009; Wang et al., 2010; Brinton, 2013; Irwin et al., 2014; Labombarda et al., 2013; Ishikawa et al., 2014; Guennoun et al., 2015). Given these properties, ALLO could be considered as a potential treatment for maladaptive cortical hyperexcitation. Pharmacological upregulation of $GABA_A$-mediated inhibition by ALLO can also be used for mechanistic dissection of post-injury plasticity resulting from increased excitation and decreased inhibition. We further hypothesized that ONC-induced potentiation of cortical VEP can be altered by ALLO upregulation of $GABA_A$-mediated inhibition. Reduction of potentiation by treatment with ALLO could suggest an involvement of $GABA_A$-mediated disinhibition as one of the possible mechanisms of injury-induced potentiation in the visual cortex.

2. Materials and methods

2.1. Animals

Young adult male C57BL/6 mice, 6 weeks of age, were obtained from the Jackson Laboratory (Bar Harbor, ME) and housed on a 12:12 h light:dark cycle with water and food access provided ad libitum. Procedures were approved by the Institutional Animal Care and Use Committee (Emory University protocol DAR-2003137-063018GN and Atlanta VA Medical Center protocol V008-13), and conformed to National Institutes of Health guidelines and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Experiments are reported here in compliance with the ARRIVE guidelines.

2.2. Experimental design

After a one-week acclimatization period to the laboratory environment with handling, the mice were randomly assigned to one of four groups ($n = 16$/group): Sham controls given vehicle (Sham/Veh), Sham controls given ALLO (Sham/ALLO), ONC given vehicle (ONC/Veh), ONC given ALLO (ONC/ALLO). Animals were implanted with electrodes over the primary visual cortex and allowed to recover for five days before baseline recording of VEP and an optomotor response (OMR) were performed. Mice were then subjected to ONC surgery or sham operation. Following ONC, VEP were recorded on post-injury days 2, 7, 12, 17, 22, and 30. OMR was assessed on post-injury day 29. Treatment with ALLO or vehicle was administered within one hour following ONC and on post-ONC days 3, 8, 13, and 18. At the end of the experiment, subgroups of mice in each experimental condition were euthanized for immunohistochemistry and western blot. The timeline for the experiment is presented in Fig. 1.

All parts of the study were performed blind and third-party concealment of treatments with individually uniquely coded vials was applied. The order of treatments was randomized by drawing vial code numbers from a hat without replacement using a randomized block design.

Animals were implanted with electrodes over the primary visual cortex, allowed to recover for five days, and baseline VEP and optomotor tresponse (OMR) were recorded. The animals were then subjected to ONC surgery or sham procedure. Following ONC, VEP were recorded on post-injury days 2, 7, 12, 17, 22, and 30. OMR was assessed on post-injury day 29. Treatment with ALLO or vehicle was administered one hour following ONC and on post-ONC days 3, 8, 13, and 18. At the end of the experiment, subgroups of mice in each experimental condition were killed for immunohistochemistry and western blot. Abbreviations: BL – baseline; OMR – optomotor response; VEP – visual evoked potentials, D2 – D30 – experimental days post ONC; ALLO – allopregnanolone treatment.