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DIFFERENTIAL REGULATION OF SYNAPSIN PHOSPHORYLATION BY MONOCULAR DEPRIVATION IN JUVENILES AND ADULTS

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Abstract—The rodent visual cortex retains significant ocular dominance plasticity beyond the traditional postnatal critical period. However, the intracellular mechanisms that underlie the cortical response to monocular deprivation are predicted to be different in juveniles and adults. Here we show monocular deprivation in adult, but not juvenile rats, induced an increase in the phosphorylation of the prominent presynaptic effecter protein synapsin at two key sites known to regulate synapsin function. Monocular deprivation in adults induced an increase in synapsin phosphorylation at the PKA consensus site (site 1) and the CaMKII consensus site (site 3) in the visual cortex ipsilateral to the deprived eye, which is dominated by non-deprived eye input. The increase in synapsin phosphorylation was observed in total cortical homogenate, but not synaptoneurosomes, suggesting that the pool of synapsin targeted by monocular deprivation in adults does not co-fractionate with excitatory synapses. Phosphorylation of sites 1 and 3 stimulates the release of synaptic vesicles from a reserve pool and increases in the probability of evoked neurotransmitter release, which may contribute to the strengthening of the non-deprived input characteristic of ocular dominance plasticity in adults. © 2010 IBRO. Published by Elsevier Ltd. All rights reserved.

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The shift in ocular dominance induced by brief monocular deprivation is a sensitive assay of the level of synaptic plasticity available to synapses in the binocular region of the primary visual cortex. Ocular dominance plasticity is robust in juveniles (Wiesel and Hubel, 1963) and was previously thought to be confined to an early postnatal critical period ending near puberty. However, accumulating evidence demonstrates that the rodent visual cortex retains significant ocular dominance plasticity beyond the traditional critical period. It remains to be determined if the intracellular signaling cascades linking monocular deprivation to a shift in ocular dominance are the same in the juvenile and adult cortex.

In juveniles, two temporally distinct changes in thalamocortical synaptic transmission are observed following monocular deprivation: a rapid decrease in the strength of synapses serving the deprived eye, followed by a slower increase in strength of synapses serving the non-deprived eye (Frenkel and Bear, 2004). The rapid decrease in deprived eye synaptic strength is similar to the long-term depression (LTD) of excitatory synaptic responses induced by low frequency stimulation (LFS). Depression of inputs serving the deprived eye and LFS-LTD are both activity-dependent and require NMDAR activation. In addition, monocular deprivation in juveniles occludes subsequent LFS-LTD in layer IV (Heynen et al., 2003). Over the course of development, the ability to express deprivation-induced depression of the thalamo–cortical projections decreases (Fox and Wong, 2005), with a time course similar to the developmental loss of LFS-LTD in layer IV (Dudek and Friedlander, 1996; Jiang et al., 2007). The slower increase in the strength of synapses serving the non-deprived eye following prolonged monocular deprivation in juveniles shares many characteristics with the long-term potentiation (LTP) of synaptic responses induced by high frequency stimulation (HFS). The potentiation of inputs serving the non-deprived eye and HFS-LTP are both activity-dependent and require activation of NMDARs (Sawtell et al., 2003).

The response to monocular deprivation in adults differs in many ways from the response in juveniles. In adult rodents, the response to monocular deprivation includes an increase in the physiological response to stimulation of the non-deprived eye, an increase in the spatial acuity of the non-deprived eye and an expansion of cortical territory representing the non-deprived eye (Sawtell et al., 2003; Pham et al., 2004; Tagawa et al., 2005; Prusky et al., 2006; Fisher et al., 2007; Sato and Stryker, 2008; Lehmann and Löwel, 2008). The duration of monocular deprivation required to reveal a maximal ocular dominance shift in adults is longer than what is required in juveniles. Other factors influencing ocular dominance plasticity in adults include the age at initiation of monocular deprivation and the history of visual experience prior to the monocular deprivation (He et al., 2006, 2007; Hofer et al., 2006, 2009).

Pharmacological and transgenic manipulations implicate the involvement of several second-messenger pathways and subsequent activation of intracellular protein kinases as mediators of ocular dominance plasticity in juveniles. Ocular interest is the role of activity-dependent protein kinases, which can rapidly translate a change in visual experience to changes in synaptic function. For example, inhibition of the cAMP-dependent

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Abbreviations: AMPA, α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate; cAMP, cyclic AMP; HFS, high frequency stimulation; LFS, low frequency stimulation; LTD, long-term depression; LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartic acid; PKA, protein kinase A.

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protein kinase PKA activity blocks the ocular dominance shift observed in response to monocular deprivation in juvenile rodents and cats (Beaver et al., 2001; Fischer et al., 2004; Rao et al., 2004) while activation of PKA promotes the strengthening of the non-deprived input following monocular deprivation in adult cats (Imamura et al., 1999). Similarly, transgenic mice expressing a mutant form of the calcium–calmodulin-dependent kinase CaMKII that inhibits calcium-independent activity have a significant deficit in ocular dominance plasticity (Taha et al., 2002). Examination of the role of activity-dependent protein phosphorylation in ocular dominance plasticity has focused primarily on postsynaptic targets, consistent with the significant contribution of the postsynaptic specialization to the maintenance of long-term changes in synaptic strength. For example dephosphorylation of a PKA-consensus site (serine 845) on the GluR1 subunit of the AMPAR is observed in the visual cortex contralateral to the deprived eye following brief monocular deprivation in juveniles, but not adults (Heynen et al., 2003).

One of the primary targets of activity-dependent protein kinases is the presynaptic vesicle-associated phosphoprotein synapsin (Leenders and Sheng, 2005). Activity-dependent regulation of synapsin phosphorylation/dephosphorylation has been implicated in synaptogenesis, synaptic plasticity, neurotransmitter release, and aging (Greengard et al., 1993; Hilfiker et al., 2005; Corradi et al., 2008). Three genes encode the family of synapsin proteins (synapsin I, II and III). The mRNA for synapsin I and II are alternatively spliced to produce two isoforms, synapsin Ia and b and synapsin IIa and b (Evergren et al., 2007). All synapsin isoforms share a consensus sequence for phosphorylation by PKA at the amino terminus called site 1 (serine 9 in synapsin Ia/b, serine 10 in synapsin IIa/b). In addition, synapsin 1a and Ib share a consensus sequence for phosphorylation by CaMKII at the carboxy terminus called site 3 (serine 603). Phosphorylation at sites 1 and 3 reduces the affinity of synapsins for actin and the lipid bilayer of synaptic vesicles (Hosaka et al., 1999; Stefani et al., 1997), and thereby initiates the disassociation of synapsin from synaptic vesicles. Activity-dependent phosphorylation of synapsins has been implicated in the plasticity of activity-dependent regulation of neurotransmitter release, including an increase in synaptic vesicle exocytosis in response to synaptic stimulation (Fiumara et al., 2007; Hilfiker et al., 2005; Menenon et al., 2006). To characterize the role of synapsin and synapsin phosphorylation in ocular dominance plasticity, we determined the distribution of synapsin isoforms in the juvenile and adult visual cortex, as well as the regulation of synapsin phosphorylation following monocular deprivation in juvenile and adult rats.

EXPERIMENTAL METHODS

Male and female pigmented Long Evans rats were raised on site with 12 h light: 12 h dark per day, with food and water available *ad libitum*. Animal care and use conformed to the guidelines of the US Department of Health and Human Services and have been approved by the University of Maryland Institutional Animal Care and Use Committee.

Monocular deprivation

Juveniles (P28) or adults (P70-90) were anesthetized with ketamine/xylazine (50 mg/10 mg/kg i.p.). The margins of the upper and lower lids of one eye were trimmed and sutured together. The animals were then sacrificed immediately (sham operated controls) or returned to a normally lighted environment for 3 days of monocular deprivation. If any sign of suture opening or infection was observed, animals were excluded from further analysis.

Quantitative immunoblots

Subjects were anesthetized with sodium pentobarbital (75 mg/kg i.p.), and the binocular region of the primary visual cortex was dissected in dissection buffer (2.6 mM KCl, 1.23 mM NaH_2PO_4, 26 mM $NaCO_3$, 212.7 mM sucrose, 10 mM dextrose, 0.5 mM $CaCl_2$, 1 mM $MgCl_2$, 100 μM kynurenic acid, pH 7.2, saturated with O_2/CO_2). Synaptoneurosomes were prepared from visual cortex as described (Hollingsworth et al., 1985; Quinlan et al., 1999; Heynen et al., 2000). Equal concentrations of synaptoneurosome or homogenate protein, determined using the BCA assay (Pierce, Rockford, IL, USA), were resolved on 7.5% polyacrylamide gels, transferred to nitrocellulose and probed with anti-phospho-synapsin antibody (site 1, 1:700, rabbit polyclonal, Cell Signaling, Danvers, MA, USA; site 3, 1:500 rabbit polyclonal, Santa Cruz Biotechnology, Santa Cruz, CA, USA) then stripped and reprobed with anti-synapsin antibody (1:500, rabbit polyclonal, Cell Signaling) in Tris buffered saline, pH 7.3 containing 1% milk and 0.1% Triton X-100, followed by incubation in appropriate secondary antibody coupled to horseradish peroxidase (1:3500, Amersham, Piscataway, NJ, USA). Visualization of immunoreactive bands was induced by enhanced chemiluminescence (Amersham ECL) captured on autoradiography film (Amersham Hyperfilm). Each immunoblot was exposed to autoradiography film multiple times to insure we were operating within the linear range of the assay. Digital images, produced by densitometric scans of autoradiographs on a ScanJet 7400C (Hewlet Packard, Palo Alto, CA, USA), were quantified using NIH Image 1.60 software. All values were normalized to a standard run on the same gel, to allow comparisons across gels. In Figs. 1, 2, the data are normalized to actin as a gel-loading control. In Figs. 3–6, normalization to a gel-loading control was not necessary as the data are expressed as the ratio of sequential probes (phosphorylated synapsin/total synapsin) of the same gel. Quantification was performed blind to the experimental condition.

RESULTS

To ask if synapsin expression and phosphorylation was regulated over the course of cortical development, we compared the level of synapsins and phoshorylated synapsins in the visual cortex of juvenile (postnatal day 28) and adult (postnatal day 70–90) pigmented Long Evans rats. All synapsin isoforms share a consensus sequence for phosphorylation at the amino terminus called site 1 (serine 9 in synapsin Ia/b, serine 10 in synapsin IIa/b). Site 1 phosphorylation is targeted *in vivo* by protein kinase A PKA, which reduces the affinity of synapsin for synaptic vesicles and actin. Homogenate prepared from the primary visual cortex of normal-reared (12 h light: 12 dark/day) juveniles and adults were subjected to quantitative immunoblotting with an antibody that recognizes each isoform of synapsin only when phosphorylated on site 1, (phospho-