ELSEVIER

MCN Molecular and Cellular Neuroscience

www.elsevier.com/locate/ymcne
Mol. Cell. Neurosci. 35 (2007) 24–37

Dynamic changes in CREB phosphorylation and neuroadaptive gene expression in area V1 of adult monkeys after monocular enucleation

Jasmin Lalonde* and Avi Chaudhuri

Department of Psychology, McGill University, 1205 Dr. Penfield Avenue, Montréal, Québec, Canada H3A 1B1

Received 10 September 2006; revised 24 January 2007; accepted 26 January 2007
Available online 6 February 2007

Our understanding of the molecular events that emerge after change in sensory input remains elusive, especially with regard to mature area V1. Here, we characterized P-CREB expression in area V1 of monkeys at multiple time-points after monocular enucleation (ME) to assess the possible contribution of CREB in visually deprived neocortex. Immunoblot assays and immunostainings showed that P-CREB is dynamically regulated in adult area V1, reaching a peak level between 5 and 30 days after ME, and becoming reduced at the 90-day post-ME time-point. This striking temporal increase in P-CREB level was paralleled by a concomitant increase of two CREB-regulated pro-survival effectors, namely Bcl-2 and Bcl-w. We present our results in the context of recent advances about adult visual neocortex and propose that ME induces a multifaceted CREB-mediated response that favors intrinsic stability of neurons and facilitates mature cortical networks to reorganize over a prolonged period.

Keywords: CREB; Bcl-2; Bcl-w; Monocular enucleation; Neuroplasticity; Neuroadaptation; Primary visual cortex; Primate

Introduction

Recent studies have revealed significant neuroplastic responses in adult visual cortex (area V1) after monocular deprivation (Sawtell et al., 2003; Frenkel and Bear, 2004; Lickey et al., 2004; He et al., 2006) and retinal lesions (Giannikopoulos and Eysel, 2006). The view that mature area V1 retains a degree of plasticity has motivated a search to better understand the molecular effectors that may be engaged after monocular enucleation (ME) and how these may compare between the critical period of development and adulthood.

One likely contributing effector of neuroplastic changes in mature area V1 is the cAMP response element-binding (CREB) protein (Obata et al., 1999; Pham et al., 2004; Suzuki et al., 2004). Phosphorylation of CREB (P-CREB) is a key regulatory event of gene transcription known to affect a range of neuronal functions, including cytoskeletal dynamics, synaptic plasticity, and cell survival (Lonze and Ginty, 2002). Furthermore, genome-wide search for DNA binding sites of CREB has revealed a diverse set of target genes in multiple ontological categories (Impey et al., 2004; Zhang et al., 2005). Thus, a sustained increase in area V1 of P-CREB subsequent to ME may represent the induction of a multifaceted CREB-dependent genomic response that guides neuroadaptive changes in adult visual neocortex.

A complementary issue to a putative increase in P-CREB expression concerns the downstream genes that may be regulated by CREB in response to ME. Despite the many possible genes that could be regulated by an increase in P-CREB in the mature visual cortex, one appealing category of downstream candidates is suggested by the molecular events that unfold after induced ischemia. These studies have shown that increased P-CREB levels in this situation enhance the expression of the pro-survival factor B-cell leukemia/lymphoma 2 (Bcl-2) (Tanaka et al., 2000; Meller et al., 2005). Furthermore, disruption of normal neuronal activity by ischemic stress has been shown to induce CREB phosphorylation and binding of the cofactor CREB-binding protein (CBP) to the promoter region of *bcl-2*. The resulting increase in Bcl-2 protein expression under metabolic stress has in turn been linked to the activation of the CaMK pathways and calcium influx (Mabuchi et al., 2001). These findings, in conjunction with the known reduction in inhibition in area V1 after prolonged removal of visual activity (Hendry and Jones, 1986; Hendry et al., 1990; Benevento et al., 1990; Morales et al., 2002), lead us to postulate that an increase in P-CREB level after prolonged ME may also be accompanied by enhanced expression of pro-survival factors of the Bcl-2 family. An increase of such effectors in this scenario may be important to prolong intrinsic neuronal stability and allow network restructuring in response to deafferentation and the resulting decrease in inhibitory activity.

We report here that area V1 of adult monkeys subjected to ME display elevated mRNA and protein expression of two pro-survival and CREB-responsive members of the Bcl-2 family (Bcl-2 and Bcl-w) in a highly specific spatial and temporal manner. This result is consistent with our finding of a striking increase in P-CREB expression after ME. This laminar increase in P-CREB level was especially marked in both subdivisions of cortical layer IVC of

* Corresponding author. Fax: +1 514 398 3255.
E-mail address: jasmin@ego.psych.mcgill.ca (J. Lalonde).
Available online on ScienceDirect (www.sciencedirect.com).

1044-7431/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.mcn.2007.01.015

adult monkeys 5 days after ME. We propose that partial removal of visual input by ME results in a complex cell-type- and layer-specific P-CREB-driven genomic response. One element of that response appears to involve the regulation of pro-survival members of the Bcl-2 family in specific cortical layers. We propose that the functions of these molecules may be important in providing intrinsic neuronal stability after prolonged sensory alteration in order to allow rearrangement of synaptic connections within the resistive extrinsic environment of the mature visual cortex.

Results

CREB and P-CREB immunoblot analysis in area V1 after ME

We first assessed CREB expression in total protein extracts of area V1 from both infant (25–38 days old) and adult monkeys over a range of time-points after ME. Average relative expression of CREB assessed by immunoblotting was unchanged in both developmental groups after ME (Fig. 1A). However, combined CREB relative expression for the normal, 1-day ME, and 5-day ME infant monkeys (95.7%±5.6%) was higher than CREB relative expression for the adult monkeys (71.3%±4.2%) at the same time-points (Fig. 1B). This result is consistent with a previous finding showing decreased expression of $CREB_{\alpha}$ and $CREB_{\Delta}$ in the dorsal lateral geniculate nucleus of mice during postnatal development (Pham et al., 2001). Total expression of CREB therefore appears to be developmentally regulated and remains unchanged in adult monkey visual cortex for at least 90 days after ME.

In contrast to total CREB expression, immunoblotting using an antiserum exclusive to P-CREB on total protein extracts of adult area V1 revealed a considerable increase in P-CREB signal as a result of ME. The largest difference was observed at 5 days after ME (65.6%) in comparison to basal P-CREB level (adult normal monkey). This difference remained relatively unchanged at the 30-day ME time-point (61.5%), but became reduced at 90 days after ME (Fig. 1C).

For infant monkeys, immunoblot detection of P-CREB in total protein extracts from area V1 revealed a more moderate increased expression at 5 days after ME (37.1%) when compared to normal infant. This result should be viewed in the context of a high level of constitutive P-CREB expression in normal infant area V1. Total protein extracts of normal infant monkeys at the height of the critical period displayed more P-CREB level (difference=36.7%) than extracts of normal adult monkeys (Fig. 1C). However, the difference in P-CREB expression between 5-day ME infant monkeys and 5-day ME adult monkeys was marginal (8.2%).

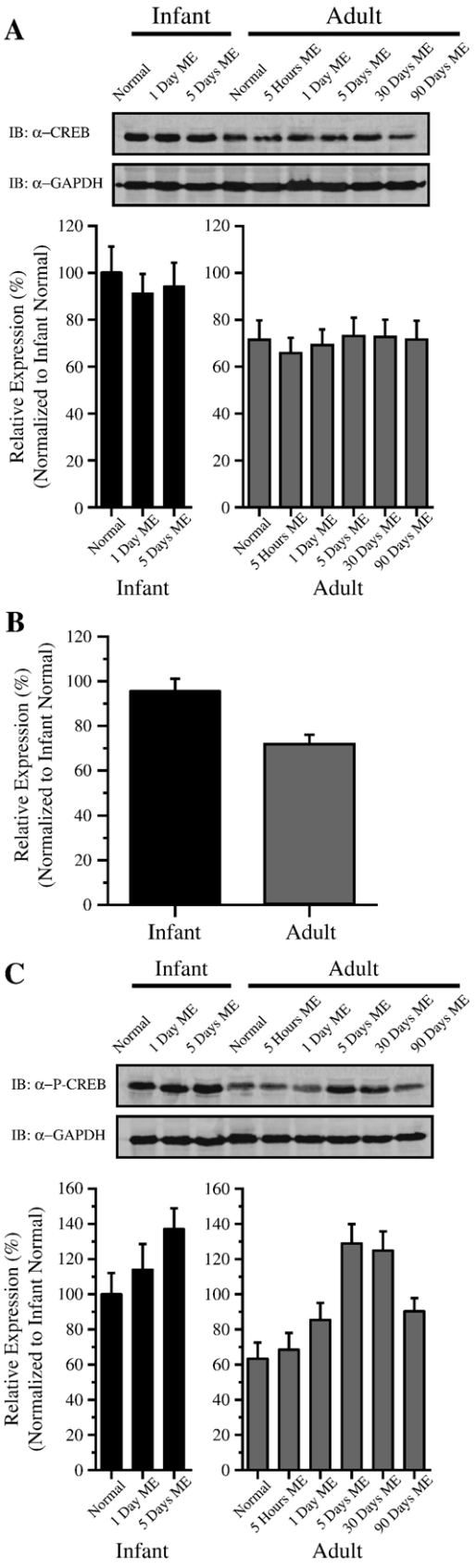

Fig. 1. Phosphorylation of CREB (P-CREB), but not total CREB expression, is dynamically increased after ME in monkey area V1. (A) Representative example of bands observed on CREB immunoblots for each time-point. Average CREB expression (n=8 immunoblots) in total protein extracts of infant and adult area V1 is similar between normal and enucleated animals for each developmental group. (B) Average CREB expression for normal, 1-day ME, and 5-day ME time-points is greater in infant than adult area V1. Infant, 95.7%±5.6%; Adult, 71.3%±4.2%. (C) Representative example of bands observed on P-CREB immunoblots for each time-point using area V1 total protein extracts. The average P-CREB level in unmanipulated animals is greater for infant than adult area V1 (difference=36.7%). However, average P-CREB level (n=8 immunoblots) is greatly increased after ME in adult in area V1 in comparison to the infant area V1. For all measures, data are expressed as mean±S.E.M.