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Neuronal activity regulates the developmental expression and subcellular localization of cortical BDNF mRNA isoforms in vivo

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Activity-dependent changes in BDNF expression have been implicated in developmental plasticity. Although its expression is widespread in visual cortex, developmental regulation of its different transcripts by visual experience has not been investigated. Here, we investigated the cellular expression of different BDNF transcripts in rat visual cortex during postnatal development. We found that transcripts I and II are expressed only in adults but III and IV are expressed from early postnatal stage. Total BDNF mRNA is expressed throughout the age groups. Transcripts III and IV show a differential intracellular localization, while former was detected only in cell bodies, latter is present both in cell bodies and dendritic processes. Inhibition of visual activity decreases the levels of exons, with exon IV transcript almost disappearing from dendrites. In vitro experiments also confirmed the above results, indicating activity-dependent regulation of different BDNF promoters with specific temporal and cellular patterns of expression in developing visual cortex.

Introduction

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family and is involved in neuronal survival and differentiation. Beyond its classical neurotrophic role, there are evidences suggesting that BDNF plays an important role in activity-dependent plasticity and development of the visual cortex. BDNF is produced by cortical pyramidal neurons only, while its high affinity receptor TrkB, is present on both pyramidal neurons and interneurons (Cellerino et al., 1996; Gorba and Wahle, 1999). BDNF mRNA and the protein are regulated during postnatal development in the primary visual cortex and are modulated by visual experience (Bozzi et al., 1995; Capsoni et al., 1999a,b; Castren et al., 1992; Schoups et al., 1995). BDNF modulates the maturation of GABAergic cortical circuitry (Huang et al., 1999), the morphology of neurons (McAllister et al., 1995), and synaptic plasticity processes (Akaneya et al., 1997; Jiang et al., 2003; Kinoshita et al., 1999; Sermasi et al., 1999, 2000). In addition, BDNF has been shown to inhibit ocular dominance column formation (Cabelli et al., 1995).

The rat BDNF gene consists of one 3′ exon (exon V) encoding for the BDNF protein and for the 3′ untranslated (3′UTR) tail and four different 5′ exons (exons I, II, III and IV) (Timmusk et al., 1993). These 5′ exons are linked each to a different promoter that directs the expression of BDNF in a tissue specific way. Alternative promoter usage, differential splicing, and the use of two different polyadenylation sites within each of the four transcription units generate eight different BDNF mRNAs (Ohara et al., 1992). Recent evidences indicate that there could be more than eight splicing forms (Zuccato et al., 2001). Therefore, probes that bind to the exon V of the BDNF gene, which contains the coding sequence, are able to recognize all the different splicing forms. Previous studies have shown that these promoters are differentially expressed in different brain regions (Timmusk et al., 1994) and that the promoters of exons I and II are neuron-specific, while the others (III and IV) are also active in non-neural tissue (Nakayama et al., 1994). Interestingly, different injury-promoting agents induce different promoters (Kokaia et al., 1994) possibly through activation of a different set of receptors (Metsis et al., 1993). It has also been shown that motor activity, hormones, and circadian rhythms differentially regulate the transcription of

Abbreviations: BDNF, brain-derived neurotrophic factor; DAB, diaminobenzidine; DEPC, diethyl pyrocarbonate; DIG, digoxigenin; KA, Kainic acid; KCl, potassium chloride; MAP2, microtubule associated protein; MDDL, maximal distance of dendritic labeling; mRNA, messenger ribonucleic acid; PBS, phosphate buffered saline; PFA, paraformaldehyde; RMV, relative migration value; RT-PCR, reverse transcription polymerase chain reaction; SSCT, saline sodium citrate Tween-20; TTX, Tetrodotoxin.

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different BDNF isoforms (Koibuchi et al., 1999; Lauterborn et al., 1998; Oliff et al., 1998; Russo-Neustadt et al., 2000). Although BDNF isoform expression has been extensively studied in many brain regions, data in primary visual cortex are still lacking.

More recently, the attention has been attracted by translocation of BDNF mRNA towards neuronal processes. The first report on the translocation of BDNF mRNAs in the dendrites was shown in cultured hippocampal neurons (Tongiorgi et al., 1997). It was also reported that BDNF dendritic localization extended to the distal dendrites on depolarization. Some other mRNAs localized in dendrites of neuronal cells are MAP2, Tau, β-actin, CaMKII-α, and Arc (Burgin et al., 1990; Garner et al., 1988; Kleiman et al., 1994; Kosik et al., 1989; Link et al., 1995; Lyford et al., 1995). Since protein synthesis can occur in neuronal dendrites, the displacement of BDNF mRNA together with its activity-dependence towards distal dendrites may represent an important aspect related to activity-dependent synaptic plasticity and maturation of neuronal circuitry in the visual cortex. Indeed, we have previously shown that in rats reared in dark, BDNF mRNA disappears from the apical dendrites of cortical neurons and reappears in this compartment when animals are re-exposed to light (Capsoni et al., 1999a,b).

Since it is known that BDNF plays a fundamental role in activity-dependent plasticity and development, the aims of this study were (1) to investigate the expression and subcellular localization of five BDNF isoforms in the visual cortex during the postnatal development; and (2) to know how visual/electrical activity regulates the expression and subcellular localization of the different BDNF transcript in ex vivo visual cortex and in primary cultures of cortical neurons.

By the aid of reverse transcription polymerase chain reaction (RT-PCR) and non-radioactive in situ hybridization, we were able to characterize and quantify the cellular expression of different BDNF transcripts. Our analysis was carried out at different stages of visual circuitry development (P13, P23, P40, and P90) and in cultures of cortical neurons. The role of activity was further assessed using Tetrodotoxin (TTX) injection into the eyes of one set of animals to inhibit activity and intra-peritoneal injection of kainic acid to increase the activity. We found that BDNF transcripts are present in rat visual cortex with a specific temporal and cellular pattern of expression during postnatal development and that their expression levels are modulated by activity. Another important result of this study is that the subcellular localization of different BDNF transcripts is modulated by neuronal activity and is characteristic for each BDNF transcript.

Results

Developmental expression of different BDNF transcripts in visual cortex

A semi-quantitative analysis of the developmental expression of different BDNF transcripts in the visual cortex was carried out by RT-PCR using primers specific for exons I, II, III, IV, or a pair of primers for the coding region of exon V that amplified all BDNF transcripts. Expression levels of BDNF exons I and II were very low in visual cortex and were found only at late stages of postnatal development. Exon I was detected from P40, when a faint band was visible (Fig. 1A), increasing from P40 to P90 (the increase in the band intensity was around 17%, Fig. 1B). The exon II primers produced two specific bands with size 230 and 309 bp, which are designated as II short and II long, respectively. These, as described before (Zuccato et al., 2001), denote two splice variants of exon II. We observed that exon II long is more abundant than the exon II short and that these two splicing variants are differently regulated during development. The exon II short shows an up-regulation by 10% from P40 to P90, whereas the II long fragment was maintained at the same level (Fig. 1B). Exon III was present at all investigated postnatal ages. The transcript was at a very low level of expression at P13 (Fig. 1A), with a significant increase ($P < 0.001$) of around 40% by P40. After P40, exon III increases twofold and thereafter maintained a stable level (Figs. 1A, B). The exon IV expression levels at the different developmental stages followed the same curve of Exon III. There was very faint expression at P13 followed by a rapid increase of expression levels from P23 to P40 (Fig. 1A).

The exon V, representing all isoforms, was expressed in all age groups used in this study (Fig. 1A). BDNF exon V showed a significant increase ($P < 0.001$) in expression levels from P13 onwards reaching a plateau at P40 (Fig. 1B).

BDNF isoforms have a different expression pattern and subcellular localization during postnatal development

We have used a high sensitive non-isotopic in situ hybridization to study and compare the cellular expression pattern of the five different transcripts of BDNF at different postnatal ages. BDNF exon I transcript was detected only at P90 and no staining was visible at earlier ages. The staining was weak even at P90, being more intense in cortical layer 4 (Figs. 2A, B, C). In all stained cells, the staining was restricted to the cell soma.

BDNF exon II transcripts were detected from P40 onwards with a riboprobe that recognizes both the short and long isoforms of exon II. The staining at P40 was very faint (data not shown). At P90 (Figs. 2D, E, F), stained cells are distributed throughout the different cortical layers and both the cell bodies and proximal dendrites appeared labeled. The exon III transcript became detectable already at the earliest stage of development tested and showed increased staining from P23 onward (Figs. 2G, H), especially in the cortical layer 4. At P40, that is, when the critical period was almost over and the maturation process of cortical neurons was nearly accomplished (Figs. 2I, J), exon III transcript was heavily expressed throughout all cortical layers and a similar pattern was still present at P90 (Figs. 2K, L). At the subcellular level, the staining was restricted only in the cell body (Fig. 4J).

Exon IV transcript also started to be expressed from an early developmental stage (P13) (Fig. 3A) in all cortical layers with a low staining intensity. The labeling progressively increased through P23 (Figs. 3C, D) until P40 (Figs. 3E, F), with an expression pattern similar to that of exon III transcript. Many different cell types were stained throughout all visual cortical layers. It was clear that the transcript IV was expressed in the cell soma and dendrites (Figs. 3G, H). Sense control showed no staining (Fig. 3B).

As we saw not much dendritic staining in the P13 animals in our in situ hybridization experiments, we hypothesized that this could be due to an immature state of the dendrites at this stage. We then carried out an immunohistochemistry for MAP2, which could be an indicator for dendritic maturation, and found that the dendrites were well developed at P23 and later stages of development. In P13 we found lesser dendritic staining for MAP2 (data not shown).