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Research report

Effects of a 14-day period of hindpaw sensory restriction on mRNA and protein levels of NGF and BDNF in the hindpaw primary somatosensory cortex

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Abstract

Neurotrophins have been reported to play an important role in neuronal plasticity and to be regulated by neuronal activity and/or neurotransmitters. Recently, we have shown that hindpaw sensory restriction induces a cortical reorganisation in the hindpaw primary somatosensory cortex, and that acetylcholine plays a significant role in this process. Sensory restriction was obtained by hindlimb suspension for 14 days. In this study, we examined the effects of a long period of hindpaw sensory restriction on the NGF and BDNF mRNA and protein expressions in the hindpaw somatosensory cortex. mRNA and protein levels were assessed by RT–PCR and ELISA, respectively. First, we found that NGF and BDNF mRNA relative levels increased after hindpaw sensory restriction. Second, the level of NGF protein increased, whereas that of BDNF remained unchanged. This differential response of NGF and BDNF proteins to sensory restriction suggested different levels of gene regulation, i.e., at pretranslational or posttranslational states. Moreover, inasmuch as our results differ from other models of sensory restriction (dark rearing, whisker removal, etc.), we hypothesized that the regulation of neurotrophin expression is dependent on the type and duration of the sensory restriction. In conclusion, we argue that neuronal plasticity induced by hindpaw sensory restriction requires neurotrophin expression.

Theme: Neural basis of behavior
Topic: Neural plasticity

Keywords: Neuronal plasticity; Adult rat; Hindlimb suspension; ELISA; RT–PCR

1. Introduction

Numerous studies have shown that the adult cortex is likely to undergo plastic changes (see Ref. [3] for review). This high degree of plasticity is often observed in response to peripheral lesions. However, cortical plasticity has also been reported to be use-dependent. For instance, we have demonstrated in previous studies that a long-term (2 weeks) hindpaw sensory restriction by hindlimb suspension in the adult rat induced a reorganisation of the cortical map [29] and a higher activation of cortical cells to peripheral stimulation [12,28]. These changes may be the result of a change in the inhibition/excitation balance inasmuch as data obtained after hindlimb suspension suggest a down-regulation of GABAergic function [10,12]. Moreover, studies performed on several models of sensory restriction have emphasized the role of acetylcholine in cortical plasticity [11,22,45]. It is also well established that neurotrophins play central roles in the plasticity of the adult central nervous system [33,54]. First, neurotrophins and their receptors are expressed in areas of the brain that undergo plasticity, in particular hippocampus and neocortex [15,18,30,51,59]. Second, neurotrophins regulate both synaptic transmission and neuronal growth. At central synapses, neurotrophins can

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have acute effects on synaptic function and lead to enhancement of excitatory transmission [5,23] and/or suppression of inhibitory transmission (see Refs. [26,35] for review). Third, in the cortex, neurotrophins are synthesized in an activity-dependent manner by neurons. Indeed, mRNA levels of both nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) are rapidly and strongly up-regulated in the neocortex by activation of glutamate receptors [61], GABA receptor antagonist application [17] or epileptiform activity [16,40]. The expression of neurotrophins is also induced by physiological stimuli in vivo, such as exposure to light stimulation in the rat [6] and mechanical stimulation of whisker in mice [47] and rats [34,40]. On the other hand, a down-regulation of BDNF mRNA levels is observed after a reduction in electrical activity by GABAergic receptor stimulation [17,61] or by one to several weeks of light deprivation in the adult rat visual cortex [2,6]. However, light deprivation has no effect on NGF mRNA level [6,49].

Most experiments described above concerned strong nonphysiological stimuli such as chronic depolarization and neurotransmitter application in dissociated cultures or brain slices. Moreover, the changes in mRNA and protein expression levels were generally established in the very short term (minutes to hours) following the modification of neuronal activity and were transient. Few studies concerning long-lasting regulation of these levels have been carried out, and these were mainly focused on the visual cortex during development. Whether changes could be observed after a long period (14 days) of sensory restriction in the primary somatosensory cortex (SmI) of adult rats remains to be determined. Thus, the aim of the present study was to test the effects of a long-lasting decrease in afferent flow on the expression of neurotrophins in the adult somatosensory cortex. We hypothesize that a 14-day period of sensory restriction could induce modifications in NGF and BDNF expression.

2. Materials and methods

2.1. Animals

Forty-nine young adult male Wistar rats (250–300 g, Iffa-credo, France) were divided into two groups: C (control, n=23) and S-R (hindpaw sensory restricted, n=26). The animals were housed under temperature- and light-controlled conditions (23 °C, 12-h light/12-h dark cycle). All procedures described below were approved by both the Agricultural and Forest Ministry and the National Education Ministry (veterinary service of health and animal protection, authorization 59-00980).

2.2. Hindpaw sensory restriction

Hindpaw sensory restriction was obtained for 14 days using the hindlimb unloading model [38]. Briefly, the tail of each rat was cleaned, dried and wrapped in antiallergenic adhesive plaster. This cast was secured to an overhead swivel that permitted 360° rotation and allowed the rats to walk freely on their forelimbs and have free access to food and water. The rats were unloaded by the tail at ~30° head-down angle to avoid a contact of the hindlimbs with the ground. A 14-day experimental period was chosen inasmuch as, in a previous electrophysiological study [29], it has been shown that this period induced an important reorganization of the primary somatosensory cortex.

2.3. Hindpaw somatosensory cortex removal

The animals were killed by decapitation after a light isoflurane anesthesia. The head was placed quickly in a stereotaxic frame and was surrounded with ice. A craniotomy was performed to expose the somatosensory cortex. The dura mater was incised and resected. A plastic cylinder (1 mm inner diameter) mounted on a syringe was used to remove a column of hindpaw SmI cortex by aspiration at the stereotaxic coordinates anterior -1 and lateral 3 mm with Bregma as bone reference point. These coordinates correspond to the functional center of the hindpaw cortical map in C and S-R rats [4]. The hindpaw SmI cortex was immediately frozen and stored at −80 °C for later use in RT–PCR and ELISA analyses. The total duration of the hindpaw SmI cortex removal did not exceed 4 min.

2.4. Determination of NGF and BDNF mRNA levels

2.4.1. RNA extraction

We used a RT–PCR procedure to evaluate the NGF and BDNF mRNA levels [42]. Total RNA was extracted from hindpaw SmI tissue by use of RNeasy Mini kit (Qiagen), which permitted to extract total RNA from a very small amount of tissue (<30 mg). Briefly, according to the manufacturer's instructions, the samples were lysed and homogenized in the presence of a highly denaturing guanidine isothiocynate-containing buffer which immediately inactivated RNases to ensure isolation of intact RNA. Ethanol was added to provide appropriate binding conditions, and the sample was then applied to an RNeasy minispin column where total RNA bound to the membrane and contaminants were efficiently washed away. Finally, total RNA was eluted in 30 μl diethylpyrocarbonate-treated water (DEPC). Total RNA concentration (μg/μl) was assessed spectrophotometrically. Before the RT reaction, RNA samples were treated by DNAse I (Invitrogen) for 1 h at 25 °C.

2.4.2. Reverse transcription (RT)

One microgram of total RNA stock was reverse transcribed in a 20-μl total volume reaction using the following assay mixture: RT buffer (50 mM Tris–HCl, pH 8.5, 8 mM $MgCl_2$ and 30 mM KCl), 20 units avian myoblastosis virus RT, 40 units RNase inhibitor (Roche), 2.5 μM primer