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Light regulates expression of brain-derived neurotrophic factor mRNA in rat visual cortex

(nerve growth factor/*trkB*/tetrodotoxin)

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ABSTRACT **Specific sensory input has profound transient and long-lasting effects on the function of corresponding sensory cortical areas both during development and in adulthood. To study whether neurotrophic factors might play a role in such processes, we investigated the effects of light on the nerve growth factor and brain-derived neurotrophic factor (BDNF) mRNA levels in rat visual cortex. Keeping adult rats in the dark or preventing normal activity of retinal ganglion cells by intraocular injection of tetrodotoxin significantly decreased the levels of BDNF mRNA in the visual cortex but not in other cortical areas. Exposure to light after a period in darkness rapidly restored the mRNA to control levels. These alterations in visual input had no effect on nerve growth factor mRNA. The mRNA of *trkB*, the putative signal-transducing receptor unit for BDNF, was also decreased in darkness, although less than BDNF mRNA. BDNF mRNA levels increased in the visual cortex of newborn rats after eye-opening. This increase is retarded, although not completely abolished, by rearing the pups in darkness. Thus, the levels of BDNF mRNA are rapidly regulated by sensory input during development and in adulthood. BDNF may therefore play an important role in formation and in activity-dependent modulation of specific connections in the visual cortex.**

Visual input has profound influences on neuronal connectivity in the visual cortex. During a critical period of development, receptive fields in the visual cortex are determined in a use-dependent competition between the two eyes (1, 2). Although the neuronal connections in adult visual cortex were previously thought to be fairly stable, recent experiments indicate that alterations in sensory input have rapid and marked effects on functional and structural connectivity in the adult sensory cortex (3, 4). The activation of *N*-methyl-D-aspartate (NMDA)-type glutamate receptors is thought to play an essential role in these use-dependent changes (5). However, the mechanisms through which NMDA receptor activation leads to alterations in the neuronal connectivity are poorly understood.

Nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), two members of the neurotrophin gene family, are predominantly synthesized by neurons in the central nervous system (6–17). Neuronal activity has been shown to regulate the mRNAs for NGF and BDNF in the hippocampus (18–20). The basal expression of these two neurotrophins in hippocampal neurons seems to be predominantly determined by the balance between glutamatergic and GABAergic transmitter systems (19). The rapid and subtle activity-dependent regulation of synthesis of these two neurotrophins suggests that, in addition to supporting the survival and regulation of specific neuronal properties, these neurotrophins may also act as mediators of functional and structural changes in neuronal connectivity. We have investigated here whether changes in visual input regulate the expression of NGF and BDNF mRNA in the visual cortex during development and in adulthood. Our results demonstrate that light regulates the expression of BDNF mRNA in the visual cortex both in adults and during development, whereas there were no detectable changes in NGF mRNA levels.

MATERIALS AND METHODS

Adult male Wistar rats (170–200 g) were used in all experiments. Control rats were housed under normal animal-house illumination with a 12-h light/dark cycle. They were killed in the early afternoon. The effect of visual input on BDNF mRNA levels was investigated by keeping rats in total darkness for 7 days. Rats in the dark group were sacrificed quickly by cervical dislocation, and the rest were exposed to normal animal-house illumination for the times indicated in Fig. 4. Tetrodotoxin (TTX; 0.15 mM; 5 μl) was injected into the right eye of the rats under ether anesthesia, and rats were killed after 6 h. For the developmental study, six pups were sacrificed at the age of 7 days (before eye-opening) and littermates were allowed to grow with the mother either in total darkness or in normal animal-house illumination (see above) until the age of 14 (when the eyes had just opened) or 20 days. In all cases, primary visual cortex from both hemispheres was rapidly dissected (21) and pooled, except in the TTX experiment, where the right side served as a control for the left side. Samples were rapidly frozen and stored at −80°C until RNA extraction.

Total RNA was isolated (22), separated on a 1.3% agarose gel, and transferred to a Hybond-N nylon membrane (Amersham). The filters were prehybridized for 2 h at 65°C in 50% formamide/3× SSC (1× SSC = 150 mM NaCl/15 mM sodium citrate)/5 mM Na_2EDTA/0.5% SDS/5× Denhardt's solution/250 μg of denatured salmon sperm DNA per ml/50 mM phosphate buffer, pH 7.0. They were then hybridized overnight at 65°C in the same buffer together with ^{32}P-labeled complementary RNA (cRNA) probes for BDNF, NGF, *trkB*, 18S rRNA, or β-actin (18, 23). Filters were washed twice for 10 min in 2× SSC/0.1% SDS at room temperature and for 15 min in 0.2× SSC/0.1% SDS at 72°C and exposed to x-ray film. The amount of RNA was estimated with a laser scanning device (LKB). The two transcripts of BDNF were regulated in a similar manner and the upper transcript was used for quantitative evaluation. Our probe for *trkB* was prepared from a clone that corresponds to the extracellular domain and thus recognizes all the splice forms of *trkB* (24, 25). The

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Abbreviations: BDNF, brain-derived neurotrophic factor; NGF, nerve growth factor; NMDA, *N*-methyl-D-aspartate; LGN, lateral geniculate nucleus; TTX, tetrodotoxin; cRNA, complementary RNA.

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quantitative determination refers to the highest transcript [9 kilobases (kb)], which represents the tyrosine kinase-containing form (25), although the 7.5-kb band, which represents the truncated form without the tyrosine kinase domain (25), was similarly regulated. The quantitative data are normalized to the amount of β-actin mRNA or 18S rRNA (for the developmental study) on the same lane.

In situ hybridization was performed in 12-μm-thick frozen sections, which were postfixed in 4% buffered paraformaldehyde, treated with acetic anhydride, and hybridized overnight at 42°C in a buffer containing 50% formamide, 4× SSC, 50 mM phosphate buffer (pH 7.0), 1% lauryl sarkosine, 1× Denhardt's solution, 250 μg of yeast tRNA per ml, 0.5 mg of denatured salmon sperm DNA per ml, 10% dextran sulfate, and 100 mM dithiothreitol. The probe was a single-stranded ^{35}S-labeled cDNA probe transcribed from BDNF sense cRNA with reverse transcriptase and random priming to a specific activity of 2–3 × 10^9 cpm/μg (26). The sections were washed under increasing stringency up to 0.5× SSC at 60°C, dehydrated, and exposed to Kodak NTB-3 autoradiographic emulsion for 12 weeks. Control probe transcribed from antisense BDNF cRNA showed only background hybridization (results not shown).

RESULTS

Rats kept in total darkness for 1 week had only ≈50% of BDNF mRNA in their visual cortex as compared to the control rats, which had been kept in normal animal-house illumination with a 12-h light/dark cycle (Fig. 1). The exposure to light after 7 days in darkness restored BDNF mRNA to control levels within 1 h and it remained at this level thereafter (Fig. 2*a*). In contrast, corresponding changes in illumination had no effect on the NGF mRNA levels in the visual cortex (Fig. 2*b*). The mRNA for *trkB* receptor tyrosine kinase, a putative signal transducing unit of the BDNF receptor (27–29), was also influenced by light in a similar manner, although the changes were smaller than those for

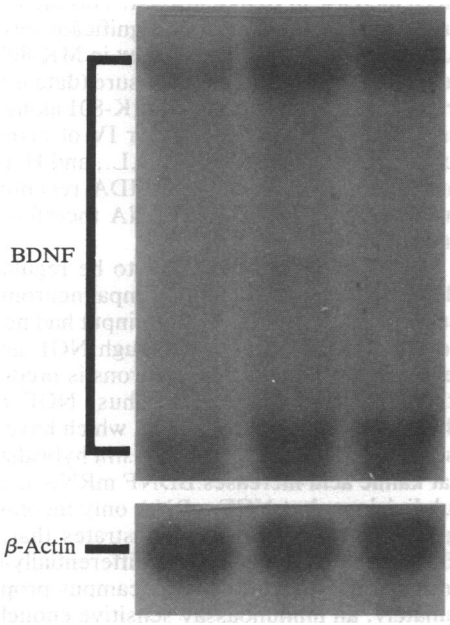

FIG. 1. Effect of light on BDNF mRNA levels in rat visual cortex. RNA was extracted from the visual cortex from rats kept in total darkness for 7 days (left lane), from rats exposed to normal animal-house illumination for 3 h after 7 days in darkness (middle lane), or from control rats (right lane). The two upper bands represent the two transcripts of BDNF, and the lower band represents the same lanes hybridized with β-actin.

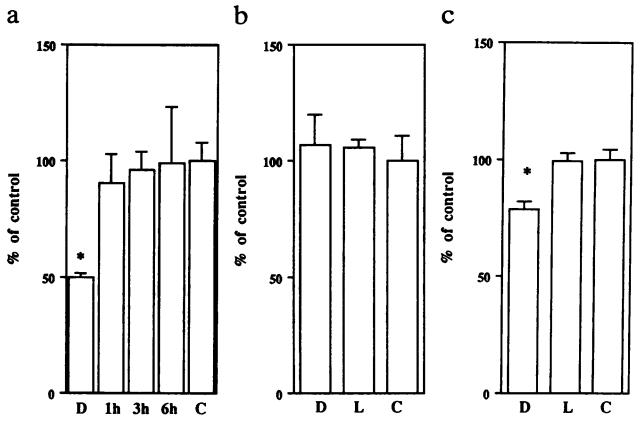

FIG. 2. Effect of light on levels of BDNF (*a*), NGF (*b*), and *trkB* (*c*) mRNA in the visual cortex. (*a*) Rats were kept in darkness for 7 days (D) or exposed to light for 1 h, 3 h, or 6 h after 7 days in darkness. Control (C) rats were kept in animal-house illumination in a 12-h light/dark cycle. (*b* and *c*) After 7 days in the dark (D), rats were exposed to light for 3 h (L). Controls (C) were treated as in *a*. Total RNA was extracted from the visual cortex and hybridized. Means ± SEM; *, $P < 0.05$ (Student's *t* test).

BDNF (Fig. 2*c*). BDNF mRNA levels in the frontal cortex and hippocampus were not significantly influenced by either dark or light exposure, demonstrating that the observed changes are specific for the visual cortex (data not shown).

In situ hybridization demonstrated that BDNF mRNA is expressed in individual neurons in layers II–III and VI in adult rat visual cortex (Fig. 3 *Left*). There were very few, if any, BDNF mRNA-positive neurons in layer IV, which receives direct input from the lateral geniculate nucleus (LGN). The morphological appearance of the BDNF mRNA-positive cells suggests that they are neurons. However, it has not yet been possible to combine *in situ* hybridization at this level of sensitivity with other histochemical methods for a further characterization of these neurons. In agreement with the results obtained with the Northern blots, rats kept in darkness had a markedly reduced number of BDNF mRNA-positive neurons in both superficial and deep cortical layers (Fig. 3 *Right*).

To study whether the effect of light is mediated by neuronal activity of the retinocortical pathway, we injected TTX into the right eye and measured BDNF mRNA in the ipsilateral and contralateral visual cortex. In rats, the majority of fibers

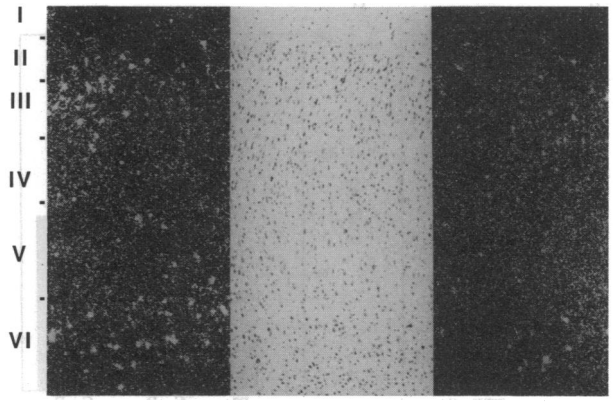

FIG. 3. *In situ* hybridization with ^{35}S-labeled probe for BDNF in the primary visual cortex from a control rat (*Left*) and from a rat kept in darkness for 7 days (*Right*), photographed under dark-field illumination. (*Middle*) Bright-field photograph of *Right*. Roman numerals denote cortical layers. Note that, in the control cortex, BDNF mRNA-positive cells are concentrated in layers II–III and VI. (×100.)

originating from retinal ganglion cells cross at the optic chiasm and project via the LGN to layer IV of the contralateral primary visual cortex (30). Thus, the input to the visual cortex in one hemisphere originates predominantly from the contralateral eye. Intravitreal injection of saline had no effect on the BDNF mRNA levels in the visual cortex (data not shown). However, 6 h after injection of TTX, BDNF mRNA had decreased significantly in the left visual cortex as compared to the right side (Fig. 4*a*). This suggests that the activity of the retinocortical pathway determines the level of BDNF mRNA in the visual cortex. *In situ* hybridization did not reveal BDNF mRNA-positive neurons in the LGN (data not shown).

Alterations in the visual input during development have powerful and permanent effects on visual function. This raises the question of whether expression of BDNF mRNA is dependent on the visual input during development as well as in adulthood. To answer this question, we reared rats in darkness and determined the levels of BDNF mRNA in the developing visual cortex. BDNF mRNA significantly increased between postnatal day 7 (when the eyes are still closed) and 14, when the eyes have just opened; a further increase was observed on day 20 (Fig. 4*b*). Rearing pups in darkness retarded, but did not completely abolish, the increase in expression of BDNF mRNA in the visual cortex. Thus, at both postnatal days 14 and 20, rats reared in darkness had significantly less cortical BDNF mRNA than did corresponding control rats. The expression of NGF mRNA in the visual cortex at this time was too low to be reliably quantified.

DISCUSSION

The results presented here demonstrate that physiological neuronal activity regulates expression of the neurotrophic factor BDNF. BDNF mRNA was significantly decreased when the visual input was impaired by keeping rats in the dark in developing as well as mature visual cortex. The decrease in cortical BDNF mRNA after an intraocular injection of TTX demonstrates that the effect of light is mediated through the activity of the retinocortical pathway. BDNF mRNA is increased in the visual cortex concomitantly with eye-opening, suggesting that BDNF may play an important role in the use-dependent formation of connections that takes place in the visual cortex at that time. Interestingly, none of these treatments influenced the levels of NGF mRNA, suggesting that these two neurotrophins may have different functions in the visual cortex.

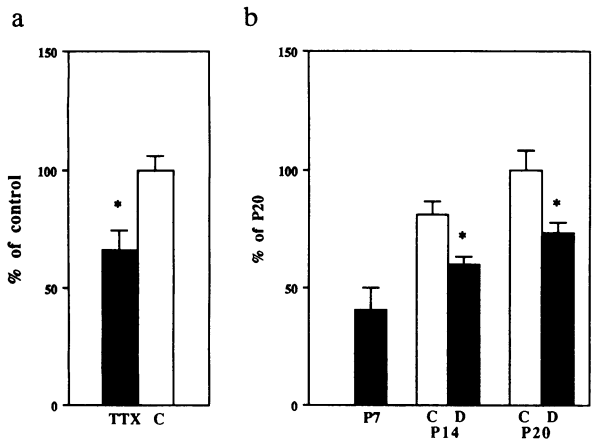

FIG. 4. (*a*) TTX was injected into the right eye and BDNF mRNA was measured 6 h later in the visual cortex of left (TTX) and right (C) hemispheres. (*b*) BDNF mRNA in the visual cortex of 7-day-old rats (P7) and 14- or 20-day-old rats reared in normal illumination (P7, P14 C, P20 C) or in darkness (P14 D, P20 D). Means ± SEM; *, $P < 0.05$ (Student's *t* test).

Few substances have been shown to be regulated by visual input in the visual cortex. Monocular deprivation in monkeys reduces the amount of cytochrome oxidase (31), calcium/calmodulin-dependent protein kinase (32), γ-aminobutyric acid, and glutamate dehydrogenase (33) in the ocular dominance columns corresponding to the deprived eye. These effects are usually seen only after a relatively long time. It is interesting to note that neuronal activity regulates BDNF mRNA levels in hippocampal neurons through activation of the calcium/calmodulin-dependent protein kinase (43). It should, however, be kept in mind that most of the data concerning the visual cortex have been obtained from animals with binocular vision, such as cats and monkeys, and the significance of the species differences has not yet been clarified.

An immediate early gene *zif-268* (NGFI-A, Krox-24, egr-1) is regulated by light in the adult rat visual cortex (34). *zif-268* decreases in darkness and produces a transient peak above the control level shortly after light exposure (34). BDNF, however, does not show a peak of expression after light exposure but increases within 1 h to a steady control level. *In situ* hybridization indicates that *zif-268* is predominantly expressed and regulated in layer IV (34), which receives input from the neurons in the LGN. BDNF mRNA, however, is mainly confined to layers II–III and VI.

What might be the molecular mechanisms that mediate the activity-dependent alterations in BDNF mRNA in the visual cortex? NMDA-type glutamate receptors seem to play a critical role in use-dependent modulation of the connectivity in the visual cortex (5, 35). In hippocampal cells, NMDA receptors regulate the basal level of BDNF mRNA and MK-801, a noncompetitive NMDA-receptor antagonist, reduces BDNF mRNA levels in rat hippocampus (19). We therefore tested whether the blockade of NMDA receptors by an injection of MK-801 (0.2 mg/kg i.p., under a dim red light, which did not increase BDNF mRNA) would prevent the light-induced increase in BDNF mRNA. This did not seem to be the case, because there was no significant difference in BDNF mRNA levels in the visual cortex in MK-801-injected and saline-injected rats after light exposure (data not shown). However, we recently observed that MK-801 alone paradoxically increases BDNF mRNA in layer IV of many cortical areas, including visual cortex (E.C., D.L., and H.T., unpublished data). The involvement of NMDA receptors in the light-induced increase of BDNF mRNA therefore requires further investigation.

BDNF as well as NGF are known to be regulated in an activity-dependent manner in hippocampal neurons (18–20). In the visual cortex, however, sensory input had no effect on the low levels of NGF mRNA. Although NGF and BDNF mRNA regulation in hippocampal neurons is predominantly similar, there are also differences. Thus, NGF mRNA is increased by glucocorticoid hormones, which have no effect on expression of BDNF mRNA (36). *In situ* hybridization has shown that kainic acid increases BDNF mRNA in all hippocampal subdivisions, but NGF mRNA only increases in the dentate gyrus (8, 14–17). This demonstrates that, in some respect, BDNF and NGF mRNA are differentially regulated in the pyramidal neurons in the hippocampus proper.

Unfortunately, an immunoassay sensitive enough to measure levels of BDNF protein in the visual cortex is not yet available. There is therefore no direct evidence as to whether alterations in BDNF mRNA levels are also reflected in the alterations of BDNF protein. We have shown, however, that a decrease in NGF mRNA in hippocampus after the administration of NMDA receptor antagonist MK-801 rapidly decreases NGF protein levels in both hippocampus and septum,

where NGF is retrogradely transported after being released in the hippocampus (19). This indicates that release as well as production of NGF may be regulated in an activity-dependent manner. Whether this is also true for BDNF remains to be demonstrated.

The *trkB* gene is expressed in at least two different splice forms in a tissue-specific manner (24, 25). One of the splice products is a functional receptor with a tyrosine kinase domain, and the other form encodes a truncated receptor without this domain (24, 25). We analyzed expression of the two highest transcripts (9 and 7.5 kb), which encode a functional receptor and a truncated receptor, respectively (25). Both transcripts were decreased in the visual cortex of rats kept in the dark, although to a lesser extent than those of BDNF mRNA. Our results suggest that either the same factors that regulate expression of BDNF mRNA concomitantly influence the expression of *trkB*, or that *trkB* is regulated by BDNF. Preliminary results indicate that although *trkB* mRNA returned to the control level 3 h after exposure to light, it seemed to be lower than the control level after 1 h, indicating that recovery from the dark takes place at a slower pace for *trkB* than for BDNF mRNA.

What could be the physiological function of BDNF in the visual cortex? The visual cortex of binocular animals is organized into ocular dominance columns during development (1). The formation of these columns takes place through an NMDA receptor-mediated and use-dependent competition between the afferents from the eyes during the critical period of development (2). It has been proposed that afferent fibers compete for a neurotrophic factor, the production of which would be activity dependent (37). NGF, when injected in relatively large quantities directly into the visual cortex, has recently been reported to interfere with formation of the ocular dominance columns in the cat primary visual cortex (37). The physiological role of NGF in the formation of ocular dominance columns is, however, complicated by the fact that the expression of *trk*, the signal-transducing receptor unit for NGF (38, 39), has not been reported to be expressed by cortical neurons but seems to be confined to the septum (40). Our data indicate that, at least in rats, the expression of NGF in the visual cortex is quite low and it seems to be independent of visual input. There is evidence to suggest that high concentrations of NGF may act as an agonist at the functional BDNF high-affinity receptor (41). As the BDNF receptor *trkB* is widespread in neocortex (40, 42), and BDNF mRNA is regulated by visual input both during development and in adulthood, it seems reasonable to suggest that BDNF, rather than NGF, would be the neurotrophin that plays a role in formation of the ocular dominance columns.

Recent experiments suggest that adult sensory cortex may have more plasticity than was previously expected (3, 4). Neurons whose receptive fields have been destroyed can acquire new receptive fields over a considerable distance and, at least in the visual cortex, this effect is remarkably rapid (3, 4). The continued regulation of BDNF mRNA by visual input in the adult visual cortex suggests that BDNF may play an important role in the plasticity of the visual cortex in the rat both during development and in adulthood.

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