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RESEARCH ARTICLE

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Cellular hybridization for BDNF, *trk*B, and NGF mRNAs and BDNF-immunoreactivity in rat forebrain after pilocarpine-induced status epilepticus

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Abstract The messenger RNAs (mRNAs) for the neurotrophins, brain-derived neurotrophic factor (BDNF), and nerve growth factor (NGF), are upregulated during epileptic seizure activity, as visualized by in situ hybridization techniques. Neurotrophins might be protective against excitotoxic cell stress, and the upregulation during seizures might provide such cell protection. In this study, a high dose of pilocarpine (300 mg/kg) was used to induce long-lasting, limbic motor status epilepticus and a selective pattern of brain damage. The regulation of BDNF, *trk*B, and NGF mRNA was studied by in situ hybridization at 1, 3, 6, and 24 h after induction of limbic motor status epilepticus. BDNF immunoreactivity was examined with an anti-peptide antibody and the neuropathological process studied in parallel. BDNF mRNA increased in hippocampus, neocortex, piriform cortex, striatum, and thalamus with a maximum at 3–6 h. Hybridization levels increased earlier in the resistant granule and CA1 cells as compared to the vulnerable CA3 neurons. BDNF immunoreactivity was elevated in dentate gyrus at 3–6 h. *trk*B mRNA increased in the entire hippocampus. NGF mRNA in hippocampus appeared in dentate gyrus at 3–6 h and declined in hilar neurons at 6–24 h. Cell damage was found in the CA3 area, entire basal cortex, and layers II/III of neocortex. Endogenous neurotrophins are upregulated during status epilepticus caused by pilocarpine, which is related to the coupling between neuronal excitation and trophic factor expression. This upregulation of neurotrophic factors may serve endogenous protective effects; however, the excessive levels of neuronal hyperexcitation resulting from pilocarpine seizures lead to cell damage which cannot be prevented by endogenous neurotrophins.

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Introduction

The neurotrophins nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), NT-3, and NT-4 have attracted considerable interest as specific trophic factors that are essential for development and maintainance of neurons in the mammalian brain (Barde 1989; Thoenen 1991; Loughlin and Fallon 1993; Lindsay et al. 1994). NGF appears to be important in the function of cholinergic forebrain neurons in the adult brain (Longo et al. 1993). Similarly, trophic functions were proposed for BDNF as a paracrine, autocrine, or retrogradely transported factor in the forebrain (Lindsay 1993). The regulation of trophic factors has been primarily studied at the level of messenger RNA (mRNA), as it has been difficult to localize the low levels of BDNF protein expressed by neurons at basal levels. BDNF mRNA is upregulated in cultured neurons by the addition of different excitatory neurotransmitters (Zafra et al. 1990, 1991, 1992) and in hippocampus after injection of glutamate agonists (Berzaghi et al. 1993; Gwag and Springer 1993). Light exposure in dark-reared rats also increased BDNF mRNA levels in visual cortex (Castrén et al. 1992). There are several reports indicating an upregulation of NGF and/or BDNF mRNA in epileptic seizures (Gall and Isackson 1989; Zafra et al. 1990; Ballarín et al. 1991; Ernfors et al. 1991; Gall et al. 1991; Isackson et al. 1991; Dugich-Djordjevic et al. 1992; Rocamora et al. 1992; Gall 1993; Humpel et al. 1993; Wetmore et al. 1994). Increases in mRNA for NGF or BDNF have also been found after short global ischemia and hypoglycemia (Lindvall et al. 1992), spreading depression (Kokaia et al. 1993), cortical infarction (Comelli et al. 1992), in mechanical lesions (Ballarín et al. 1991), and in colchicine intoxication (Ceccatelli et al. 1991). Deafferentation of hippocampus decreases BDNF mRNA in some hippocampal

neurons (Beck et al. 1993; Berzaghi et al. 1993), whereas stimulation of septal afferents increases the hybridization signal in hippocampus (Boatell et al. 1992; Lindefors et al. 1992). In epileptic seizures as well as in ischemia and trauma, the release of excitatory amino acids and the excessive stimulation of glutamate receptors (excitotoxicity) play a major role in cell damage (Choi 1988; Meldrum 1991). The upregulation of neurotrophin mRNAs in several pathological conditions may indicate that trophic factors have a positive, protective function under conditions of cellular stress. Perhaps, an elevated level of neurotrophins is required to repair subtle neuronal changes during strong neuronal stimulation. In this case, exogenous application of specific factors might further help to ameliorate hyperexcitation-induced neuronal damage. Studies in cell culture systems have provided evidence for a protective role of trophic factors against metabolic stress (Cheng and Mattson 1991). NGF released from transplanted, genetically manipulated cells (Frim et al. 1993) or infused into a lesion area (Davies and Beardsall 1992) protected against local excitotoxic lesions in the rat brain. On the other hand, results of NGF application in global ischemia are so far contradictory (Shigeno et al. 1991; Beck et al. 1992; Yamamoto et al. 1992).

A critical question for a neuroprotective role is whether the increase in mRNA of a neurotrophin is translated into an increase in protein levels. An antibody raised to a peptide fragment of BDNF has been characterized recently (Wetmore et al. 1991, 1993), which was used to demonstrate an increase in BDNF immunoreactivity (BDNF-IR) that is thought to correlate with increased BDNF (protein) expression. Increases in BDNF-IR were found in several populations of hippocampal neurons after kainic acid-induced seizures (Wetmore et al. 1994) and in pentylenetetrazol (PTZ) kindling (Humpel et al. 1993). A recent study on electrical kindling could show a correlation of NGF mRNA and protein levels using enzymatic assays (Bengzon et al. 1992). These studies in seizures give an indication that the increase in mRNA may be translated into higher levels of trophic factor.

One precondition for a trophic response is the expression of the respective neurotrophin receptors by the target cells. BDNF is a ligand for the *trk*B receptor (Klein et al. 1990; Soppet et al. 1991; Squinto et al. 1991). Since receptors for *trk*B are widespread (Merlio et al. 1992), many neuronal systems might respond positively to BDNF increases, e.g., after retrograde transport or through paracrine or autocrine mechanisms. An additional role has been suggested for the low-affinity neurotrophin receptor (LANR; Rodriguez-Tebar et al. 1990). A recent study described increases in *trk*B mRNA in pathological conditions including seizures (Merlio et al. 1993), suggesting that the increase in BDNF mRNA is accompanied by an increase in the respective receptor. Although *trk*B is also activated by NT-3 and NT-4/5 (Berkemeier et al. 1991; Soppet et al. 1991; Squinto et al. 1991), these neurotrophins are either downregulated in seizures (NT-3; Rocamora et al. 1992) or occur at very low levels (NT4; Timmusk 1994).

In the present study, we used the high-dose pilocarpine model to induce status epilepticus and to look for the relationship between BDNF mRNA, BDNF-IR and *trk*B mRNA. NGF mRNA and LANR were also visualized. The high-dose pilocarpine model induces a long-lasting, strong status epilepticus that leads to cellular damage in several brain regions (Turski et al. 1983). This pattern has been studied in detail by immunohistochemical methods (Schmidt-Kastner et al. 1991; Schmidt-Kastner and Ingvar 1994). The major damage is found in neocortex, hilus and CA3 of hippocampus, and piriform cortex.

We sought to answer the following questions: (a) Does the increased expression of BDNF mRNA and BDNF-IR correlate with resistance or vulnerability of neurons to epileptic cell damage? (b) Do secondary decreases in BDNF mRNA or BDNF-IR and NGF mRNA relate to neuronal cell death? (c) Do changes in the neurotrophin receptor components (*trk*B, LANR) correlate with the responsiveness of affected neurons? And (d) do certain cell populations fail to upregulate BDNF mRNA, although this would be predicted on the basis of previous studies using seizures (Gall 1993; Humpel et al. 1993; Wetmore et al. 1994)? A summary of these results has already been presented (Schmidt-Kastner et al. 1993).

Materials and methods

Seizure experiments

Adult male Sprague-Dawley rats (body weight 200–350 g; B&K Laboratories, Sweden) were subjected to pilocarpine-induced status epilepticus (Turski et al. 1983; Schmidt-Kastner et al. 1991). Following premedication with 1 mg/kg *N*-methyl-scopolamine s.c. for 20 min, pilocarpine was given i.p. in a dose of 300 mg/kg. In all animals, the time of the pilocarpine injection was taken as the reference point. The clinical symptoms (Turski et al. 1983; Schmidt-Kastner et al. 1991) began with limbic seizures (LS) which lasted 15–30 min. Animals showed oral automatisms, head nodding, and stereotyped movements. Few animals developed only LS and a few motor seizures and then appeared normal after 2 h. In most animals, the phase of LS was followed by overt clonic motor activity of the forelimbs and rearing, which lasted for 6–12 h. This behavioral pattern was defined as limbic motor status epilepticus (LMSE). During the phase of motor activity the rectal temperature varied between 38 and 40°C, as measured in a separate set of animals (n=4). Minor stereotyped movements indicating residual epileptic motor activity persisted at 24 h. Animals that developed additionally generalized tonic-clonic seizures and related respiratory insufficiency were excluded from analysis.

The studies on neurotrophins were limited to the first 24 h, before severe damage was manifest in neuropathological stains. Groups of rats with a full response of LMSE after 300 mg/kg pilocarpine were studied at 1 (n=3), 3 (n=4), 6 (n=4), and 24 h (n=4) by in situ hybridization. Some animals that showed LS only after the same dose (n=1 at 1, 3, 6 h, each; and n=2 at 24 h) were included for comparison. Immunofluorescence studies with a peptide antibody against BDNF were performed after 3 h (n=3), 6 h (n=4), and 24 h (n=3) of survival in animals with LMSE. Control animals (n=12) had received a subcutaneous injection of *N*-methyl-scopolamine, which was required to block peripheral cholinergic receptors before application of pilocarpine (Turski et al. 1983). Also, controls were given an intraperitoneal injection of Ringer's

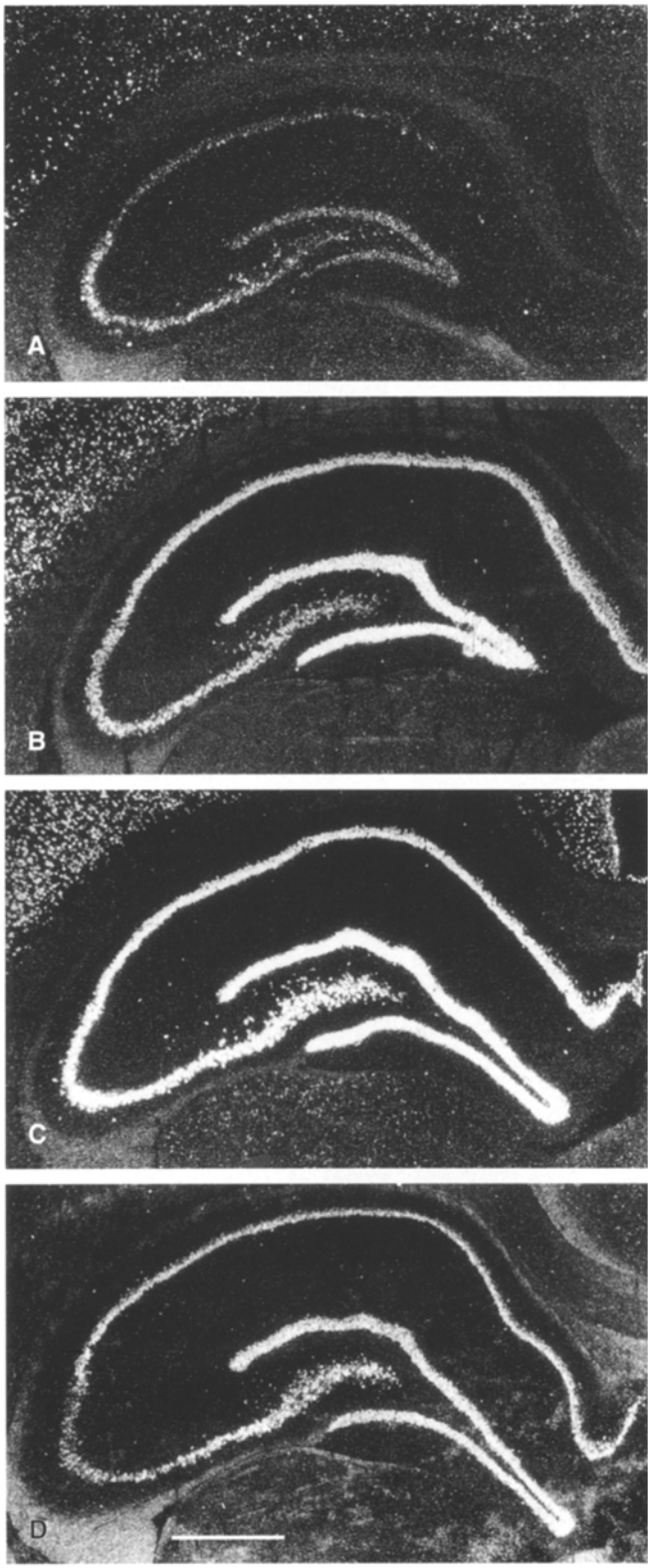

Fig. 1A–D In situ hybridization of brain-derived neurotrophic factor messenger RNA (mRNA) in dorsal hippocampus of rat. **A** Control. **B** Pilocarpine-seizures for 3 h. **C** Six hours of seizures. **D** At 24 h after induction of seizures, lateral CA3 shows a reduction of labeling in an area undergoing neuronal damage. *Scale bar* in **D** 1 mm for **A–D**

solution to control for any effects of the injection prodecure. Three normal animals were included for comparison.

At lower doses of pilocarpine, fewer animals develop the full syndrome of motor seizures (Turski et al. 1983). Therefore, another group of animals was injected with a lower dose of pilocarpine (250 mg/kg). Half of the animals injected with this lower dose showed only LS within 3 h of continuous observation. The others developed the full symptoms of LMSE. These rats with LS or LSME were studied at 3–4 h after injection (n=3 pairs).

In situ hybridization studies

Hybridization procedures for brain sections have been described previously (Wetmore et al. 1990; Humpel et al. 1993). Brains were removed following decapitation, frozen, and kept at –70°C until sectioning. Brains from control and experimental animals were mounted together on the same chucks, and hence sections received identical treatment in hybridization procedures. Sections were cut on a cryostat at 14 µm thickness in the frontal plane and thawed onto slides (ProbeOn; Fisher Biotech, USA).

Oligonucleotide probes complementary to the BDNF gene (Leibrock et al. 1989; nucleotide bp 250–298; 5′CTCCAGAGT CCCATGGGTCCGCACACCTGGGTAGGCCAAGCTGCCTTG 3′), to the *trk*B full-length receptor (Middlemas et al. 1991; bp 1879–1920; 5′CTGCGACTGCGTCAGCTCGGTGGGCGGGTT-ACCCTCTGCCAT 3′), NGF (Whittemore et al. 1988; bp 374–422; 5′AAGGGAATGCTGAAGTTTAGTCCAGTGGGCTTCAG-GGACAGAGTCTCC 3′), and the rat LANR (Radeke et al. 1987; bp 873–920; 5′GGCCACAAGGCCCACGACCACAGCAGCCA-GGATGGAGCAATAGACAGG 3′) were prepared (see also Wetmore et al. 1990, 1994; Humpel et al. 1993).

Antisense oligonucleotides were 3′end-labeled with [α-^{35}S] dATP using terminal deoxyribonucleotidyl transferase (NEN; DuPont, Scandinavia) and purified on Nensorb columns (NEN). The hybridizations were carried out on sections at 42°C overnight in a humidified chamber. About 0.1–0.15 ml of hybridization solution (50% formamide; 4×sodium chloride-sodium citrate buffer, SSC; 0.02% polyvinyl-pyrrolidone; 0.02% Ficoll; 0.02% bovine serum albumin; 10% dextrane sulfate; 0.5 mg/ml sheared salmon sperm DNA; 1% sarcosyl (*N*-lauroyl sarcosine); 0.02 M $NaPO_4$, pH 7.0; 50 mM dithiotreitol) containing 1×10^7 c.p.m./ml probe was applied. Sections were subsequently rinsed in 1×SCC, washed four times in 1×SSC for 15 min at 54°C, allowed to come to room temperature, then dehydrated through 70%, 90%, and 99% ethanol and air dried. Some sections were exposed to X-ray film for 2–4 weeks at –20°C. The other sections were dipped in Kodak NTB-2 photo emulsion (diluted 1:1 in water). The exposure time was 6–10 weeks at –20°C. Sections were then developed, fixed, lightly counterstained using cresyl violet (Nissl stain), dehydrated, and coverslipped. Dark- and light-field microscopy were used to evalute the material.

Immunofluorescence labeling for BDNF-IR and neuropathological changes

Animals were deeply anesthetized with pentobarbital (60 mg/kg) and perfused with 4% formaldehyde-0.4% picric acid. Cryostat sections were cut at 14 µm (n=3 at 3 h, n=4 at 6 h, n=3 at 24 h, n=5 sham controls). Sections were reacted with antiserum R1 diluted 1:500 in phosphate-buffered saline (PBS) containing 0.3% Triton X-100 overnight and bound antibody visualized by goat anti-rabbit IgG conjugated to FITC (1:50; 1–2 h). Negative controls were made by omission of the first antibody. The rabbit polyclonal antiserum R1 is directed against a peptide sequence specific for BDNF (Wetmore et al. 1991, 1993). In the following, we will refer to immunolabeling as "BDNF-IR," with the reservation that the antibody may also react with still-unknown members of the neurotrophin family or with proteins altered by the pathological processes.

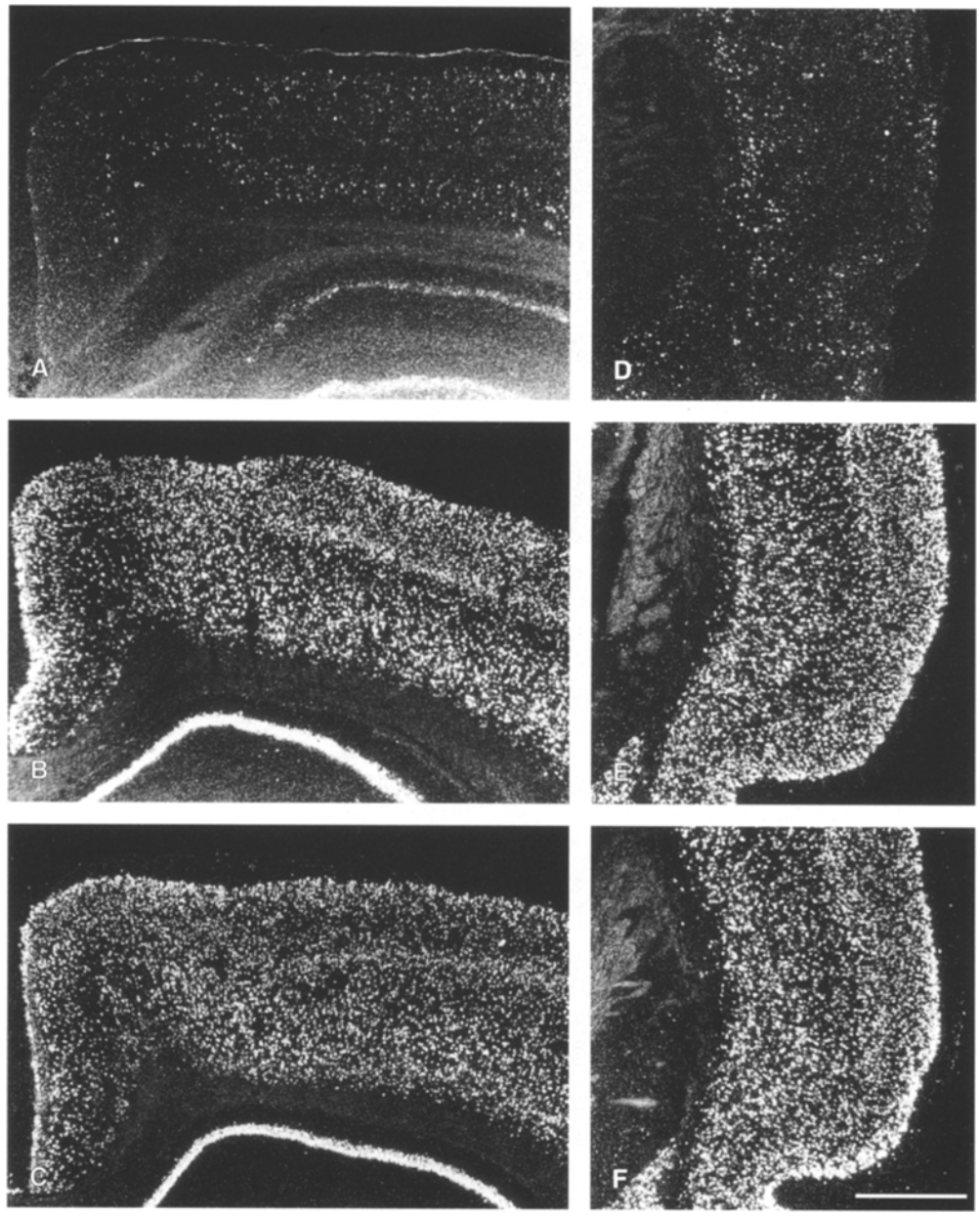

Fig. 2A–F In situ hybridization of brain-derived neurotrophic factor mRNA in neocortex. **A–C** Visual cortex (area 2) and retrosplenial cortex. **D–F** Parietotemporal cortex. **A,D** Control. **B,E** Three hours of pilocarpine seizures. **C,F** Six hours of pilocarpine seizures. An increase in hybridization signal is caused by pilocarpine seizures in layers II–VI. *Scale bar* in **F** 1 mm

Evaluation

In situ hybridization. Film autoradiograms were used for general orientation. Evaluation of in situ hybridization results was based on examination of emulsion-coated sections using a low-power dark-field condensor (Zeiss 445214) and conventional dark-field illumination at higher magnification. A section from a control or sham animal was available on each slide. The following structures were systematically screened in animals with limbic motor seizures: hippocampus, divided into dentate gyrus granule cells, CA3, and CA1; neocortex; and basal cortex (piriform and entorhinal cortex). A semiquantitative score system was used with: 0, no signals; 1, minor signals; 2, moderate signals; and 3, strong signals. The intensity was visually averaged over a given structure, and control hippocampus was taken as the reference. All available sections were evaluated and scores were calculated as means±SD for each structure at 1, 3, 6, and 24 h after injection (n=3–4 brains each; n=2–6 sections each). Stastical analysis was performed using ANOVA (P<0.05), and t-tests were adjusted for multiple comparisons (P<0.05). Other areas noted for qualitative evaluation of BDNF mRNA were hypothalamus, thalamus, amygdala, and striatum. *trk*B mRNA, LANR mRNA, and NGF mRNA were evaluated qualitatively.

Immunofluorescence for BDNF. Fluorescence intensity at the level of the neuronal cell bodies was estimated by a blinded observer using a grading system with fluorescence intensity scores from 0 to 4. Strong fluorescence over neurons of nucleus basalis (Wetmore et al. 1991) served as reference value 4. Immunolabeling was estimated in CA1, CA2, CA3, and granule cells of dentate gyrus in controls (n=5) and in experimental animals at 6 h (n=4) and 24 h (n=3) after pilocarpine injection. Cortical values were too variable for this analysis and were therefore omitted. Data are presented as means±SEM. Statistical analysis of score values was performed using t-tests under the assumption of a linear estimate of the visual scoring.

Neuropathology

The neuropathological effects of a high dose of pilocarpine have been described already (Turski et al. 1983; Schmidt-Kastner et al.

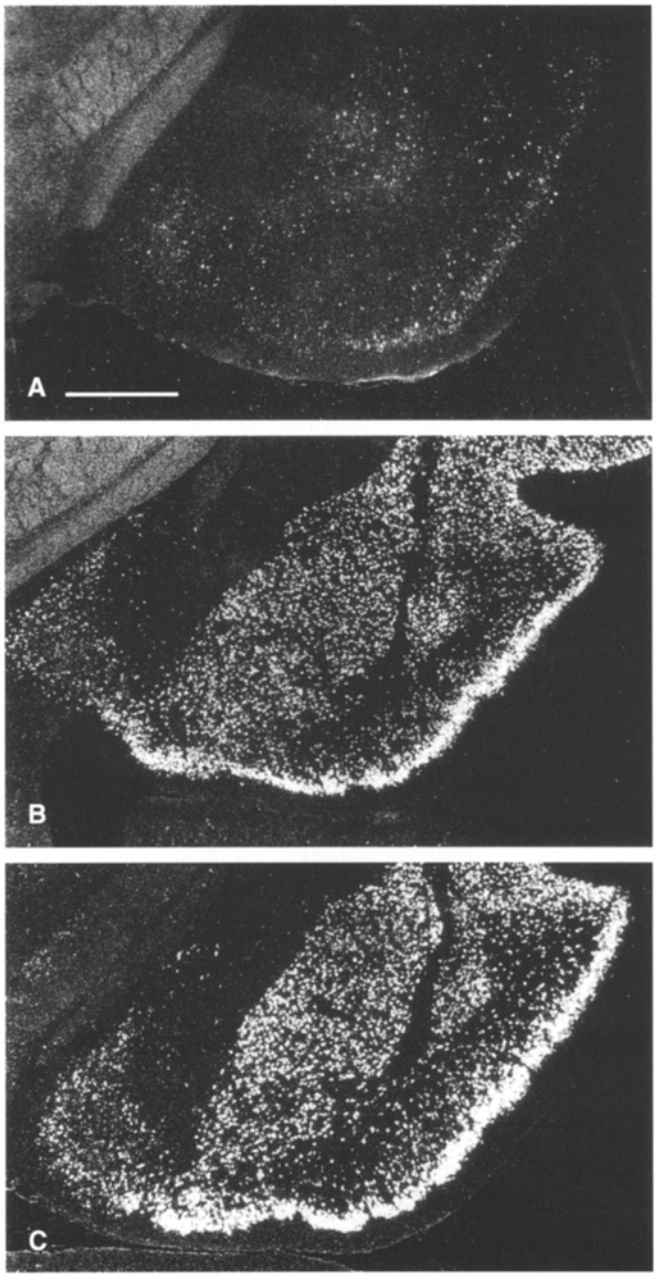

Fig. 3A–C In situ hybridization of brain-derived neurotrophic factor mRNA in basal cortical areas including piriform cortex and amygdaloid complex. **A** Control. **B** Three hours of pilocarpine seizures. **C** Six hours of pilocarpine seizures. *Scale bar* in **A** 1 mm

1991). Reference material from animals with 1 or 3 days' survival (n=4, each) from preceding studies was available (Schmidt-Kastner and Ingvar 1994). Here, unfixed or fixed cryostat sections parallel to the above material were Nissl-stained with cresyl violet and evaluated for overt neuronal cell damage such as severe shrinkage or loss of neurons.

Results

In situ hybridization of BDNF mRNA

Controls

The *N*-methylscopolamine-treated and Ringer-injected control rats did not show overt differences from three normal animals (not shown) for in situ hybridization of BDNF mRNA. BDNF mRNA was expressed at moderate levels in the granule cells of the dentate gyrus, in the CA3 pyramidal cell layer, and less densely in the CA1 sector (Fig. 1A). The hilus was devoid of labeling in the subgranular, central, and medial zones. Neocortex showed moderate cellular labeling in layers VI and II, and scattered labeling in other layers (Fig. 2A,D). Piriform cortex, endopiriform nucleus, and the basolateral nucleus of the amygdala had moderate labeling (Fig. 3A). Weak signals were noted in the ventral hypothalamus and medial thalamus; no hybridization occurred over the striatum. This pattern is in line with descriptions in the literature (Ernfors et al. 1990; Hofer et al.,1990; Phillips et al. 1990; Wetmore et al. 1990). The hybridization signals appeared to be entirely neuronal.

LMSE after 300 mg/kg pilocarpine

One hour. An increase in hybridization signal was seen in the dentate gyrus 1 h after 300 mg pilocarpine/kg, and the pyramidal cell layer of CA3–CA1 showed similar signals, indicating an increase in CA1. No changes occurred in the hilus. Slight increases were seen in the cortex cerebri.

Three hours. A marked increase in hybridization signal was seen in the dentate gyrus and along the entire pyramidal cell layer of CA3–CA1 (Fig. 1B) after 3 h. The hilus remained unchanged except for some scattered pyramidal cells close to the CA3 cell band. Increased hybridization was noted over granule cells (Fig. 4B), CA3 neurons (Fig. 4C) and CA1 neurons (Fig. 4D). In the entire cortex, an upregulation was noted (Fig. 2B,E), with specifically strong signals localized over neurons in layers VI–II (Fig. 4A). The piriform cortex and the basolateral nucleus of the amygdala were also strongly labeled (Fig. 3B). Increased hybridization also occurred over cells in the medial thalamus, ventral hypothalamus, and caudal parts of striatum in some animals.

Six hours. A very strong increase in silver grain density was seen over the granule cells of the dentate gyrus, and the pyramidal cell layer from CA3 to CA1 was characterized by strongly increased labeling (Fig. 1C) after 6 h. The entire cortical mantle was involved in a strong upregulation (Fig. 2C,F), and neurons in laminae VI–II were densely covered by many silver grains. Massive increases were noted in the piriform cortex-amygdala region (Fig. 3C). Some neurons in the ventral hypothalamus

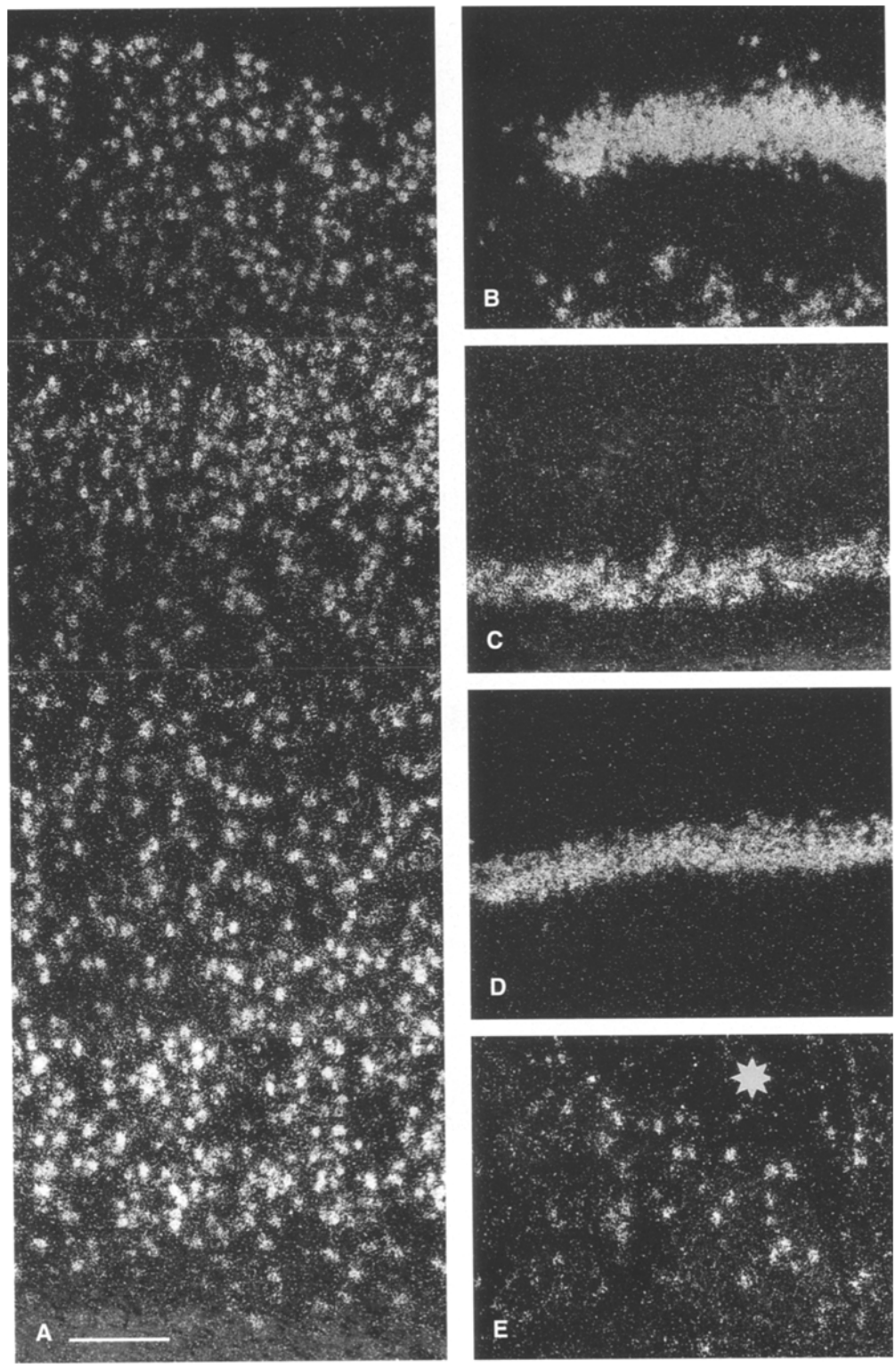

Fig. 4A–E Details of hybridization of brain-derived growth factor mRNA in cortical and hippocampal regions during pilocarpine seizures. **A** Neocortex, an increase in hybridization signal is found over layers II–VI at 3 h. **B** Granular cell layer of dentate gyrus at 3 h. **C** CA3 pyramidal region at 3 h. **D** CA1 pyramidal layer at 3 h. **E** Cells with increased signals surround a laminar lesion in the neocortex (*star*). *Scale bar* in **A** 190 μm for **A–E**

(Fig. 5G,H), medial thalamus (Fig. 5E,F), lateral thalamus (Fig. 5C,D), and subthalamic nucleus also showed increased hybridization signals. Cellular labeling was observed in posterior portions of striatum (Fig. 5A,B).

Twenty-four hours. The intensity of labeling in the dentate gyrus and CA3–CA1 had decreased after 24 h, but was still elevated compared with controls (Fig. 1D). In lateral parts of CA3, signals were lower than in medial parts, which may be related to cell death in the lateral area. In two animals, cortical labeling remained elevated in layer VI and II, while more irregular signals are seen in the middle layers. In two other cases, the layers IV–II of the parietal cortex showed tissue damage and hybridiza-

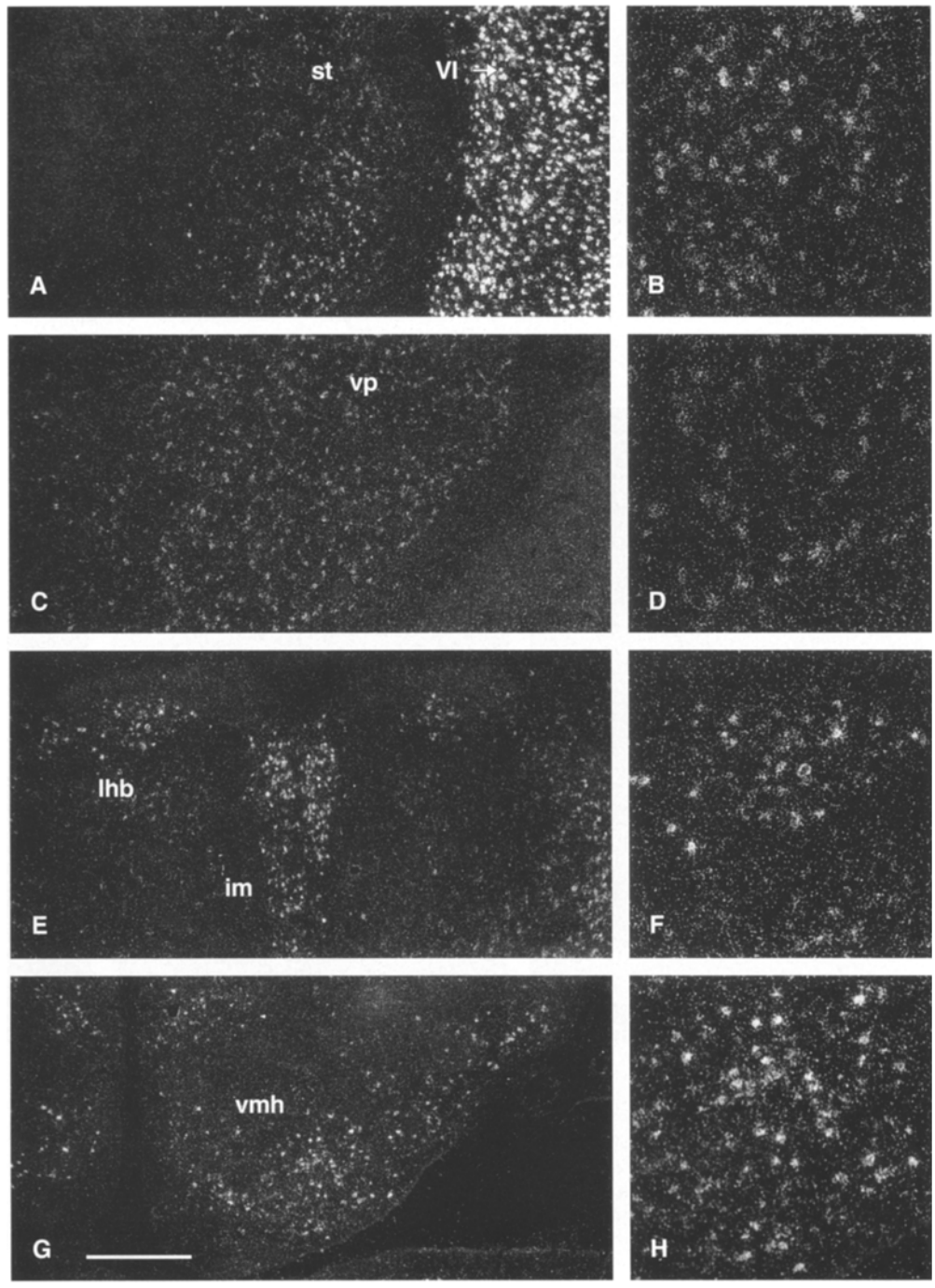

Fig. 5A–H Upregulation of brain-derived neurotrophic factor mRNA in several brain areas at 6 h of pilocarpine seizures. Paired overview and detailed view of labeled cells. **A** Posterior striatum (*st*) and layer VI of lateral neocortex. **B** Cellular labeling in striatum. **C** Lateral thalamus, ventral posterior nuclear complex (*vp*). **D** Cellular labeling. **E** Central thalamus with intermediodorsal thalamic nucleus (*im*) and lateral habenular nucleus (*lhb*). **F** Detailed view. **G** Ventral hypothalamus with labeling in ventromedial hypothalamic nucleus (*vmh*). **H** Detailed view. *Scale bar* in **G** 475 μm for **A,C,E,** and **G**; 190 μm for **B,D,F**, and **H**

tion signals were lost. Piriform cortex and amygdala showed tissue damage (infarction) leading to loss of specific signals. However, several neurons in the intact cortex close to these lesioned areas had maintained or increased labeling (Fig. 4E).

Quantitative analysis. The semiquantitative score values for the hippocampus and cortex are shown in Table 1. ANOVA was significant for all structures ($P<0.01$). Further analysis by *t*-test ($P<0.05$, adjusted for multiple comparisons) showed that significant increases occurred in granule cells, CA1, and neocortex at 1–24 h, in CA3 at 3, 6, and 24 h, and in basal cortex from 1 to 6 h (the 24-h time point was not evaluated owing to severe damage in this area).

LS after 300 mg/kg pilocarpine

One animal killed 1 h after 300 mg pilocarpine/kg showed an upregulation in the dentate gyrus. Another animal surviving 3 h also showed an increase in the dentate gyrus, and some enhanced hybridization in piriform cortex. The 6-h survival case showed a definite increase in

Table 1 Scores of signal intensity of hybridization signals for brain-derived neurotrophic factor messenger RNA in different areas and at different time points after induction of limbic motor status epilepticus by pilocarpine. Values are means±SD from three of four animals with two to six sections each (*N.D.* due to infarction-like lesions in basal cortex at 24 h, no score was determined). *DG* dentate gyrus granule cell layer, *neoctx.* neocortex, *basalctx.* basal cortex including piriform and entorhinal cortex.

	DG	CA3	CA1	Neoctx.	Basalctx.
Control	0.87±0.69	1.56±0.68	0.68±0.49	1.09±0.66	1.07±0.65
1 h	2.21±0.41*	1.45±0.82	1.48±0.84*	1.66±0.87*	1.59±0.87*
3 h	2.83±0.25*	2.69±0.48*	2.69±0.48*	2.33±1.05*	2.47±0.74*
6 h	3.0 ±0*	3.0 ±0*	3.0 ±0*	3.0 ±0*	3.0 ±0*
24 h	1.90±0.65*	2.17±0.45*	2.20±0.42*	2.0±0.51*	N.D.

Statistical analysis was performed by ANOVA in each structure over time and was significant for all structures at $P<0.01$. *t*-Tests were carried out by comparison with control group with $*P<0.05$ (corrected for multiple comparisons)

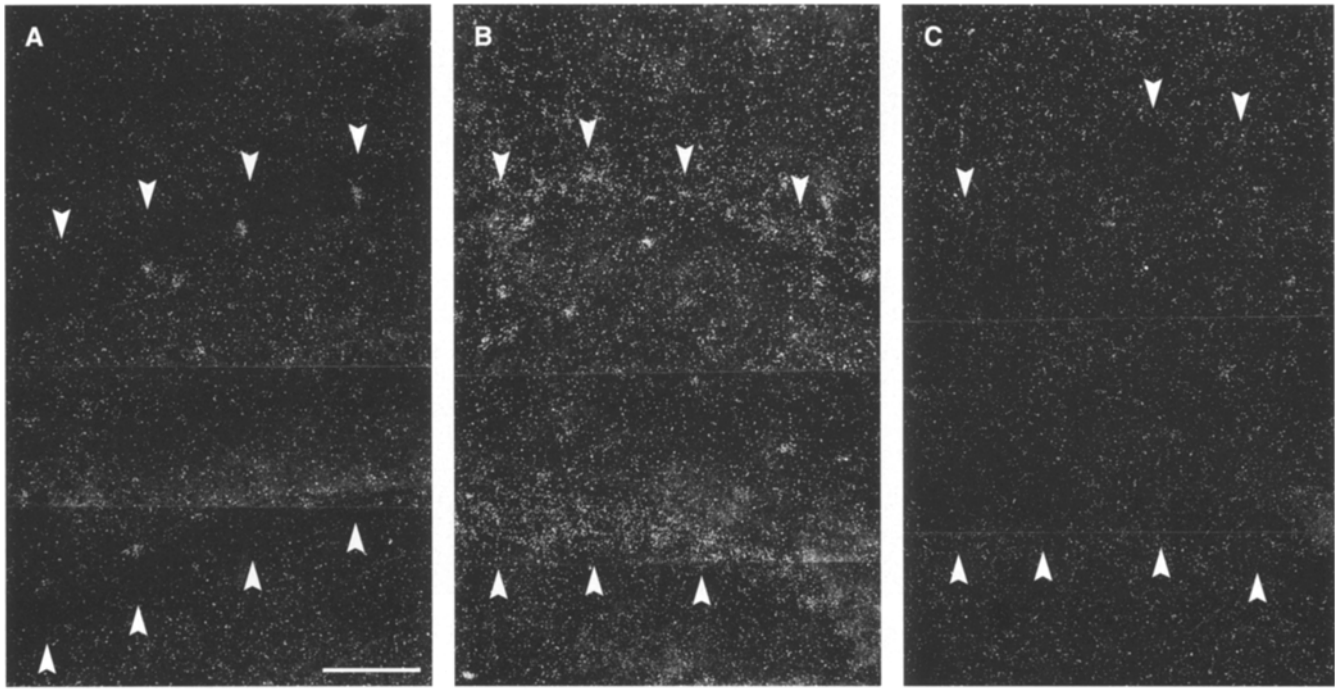

Fig. 6A–C Hybridization of nerve growth factor mRNA in hilus and dentate gyrus. The outer borders of the granule cell layer are indicated by *arrows*. **A** Control animal shows scattered cells in the hilus and no signals over the granule cells. **B** Pilocarpine seizures for 3 h, an increased labeling is found over the granule cell layer, and scattered neurons persist in hilus. **C** At 24 h after induction, labeling in the hilus has disappeared and granule cell labeling is absent. *Scale bar* 190 μm for **A–C**

piriform cortex and temporal neocortex. Animals killed after 24 h were not different from their controls.

Comparison of LS and LMSE after 250 mg/kg pilocarpine

The changes in BDNF mRNA varied according to seizure activity after 250 mg pilocarpine/kg. All animals with the full response of LMSE showed the same increase in BDNF mRNA in hippocampus and cortex at 3 h, as described above for the full dose (300 mg/kg). The posterior pole of striatum also revealed cellular labeling in these cases. In animals with LS only, there was an increase in silver grains overlying neurons in hippocampus, neocortex, piriform cortex, and amygdala, but hybridization signals were lower than in animals with LMSE (sections mounted on the same slide).

In situ hybridization of NGF mRNA

Controls. In hippocampus, scattered cells were labeled in the subgranular and medial parts of the hilus. Granule cells of the dentate gyrus were mostly negative (Fig. 6A). Other labeled cells were situated along the pyramidal cell layer of CA3–CA1, where they were often placed around the compact layer of pyramidal neurons. This anatomical distribution suggested that the cells with high NGF mRNA signals are a subclass of interneurons (Lauterborn et al. 1993). No specific signal was seen in neocortex, piriform cortex, or striatum.

One hour. There was a tendency for increases in grains overlying scattered neurons in hilus and CA3–CA1 after 1 h.

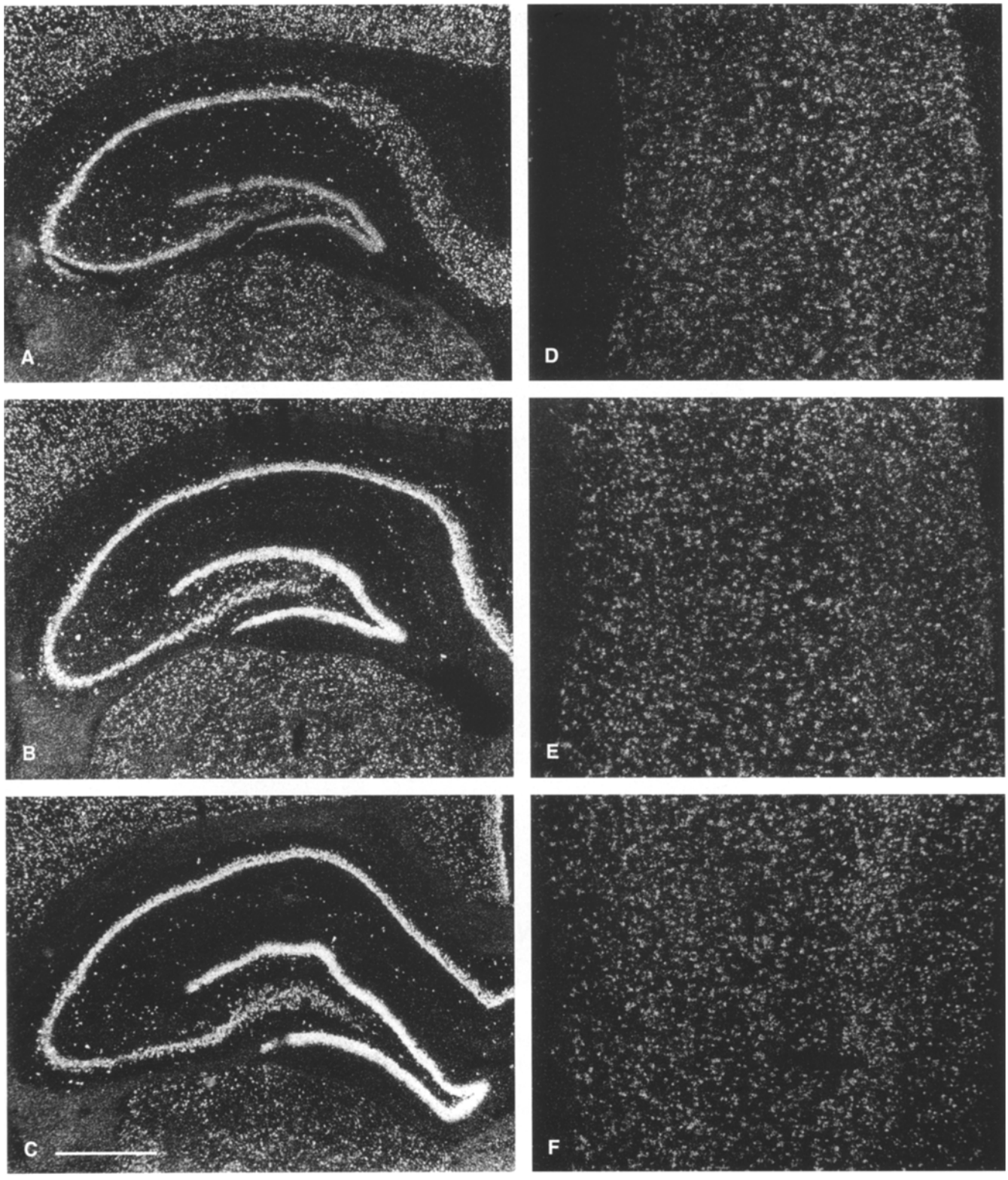

Fig. 7A–F In situ hybridization of *trk*B mRNA in hippocampus and parietal neocortex in controls (**A,D**) and at 3 h (**B,E**) and 6 h after induction of status epilepticus by pilocarpine. **A** Hippocampus of control, note that the medial parts of CA1 are merging into the subiculum. **B** Hippocampus at 3 h; note relative increase in signals in dentate gyrus and along the entire pyramidal cell band. **C** Hippocampus at 6 h; increased signals in granule cells and pyramidal cells. **D** Cortex of control case. **E** Cortex at 3 h. **F** Cortex at 6 h. There is no obvious change in signal intensity in cortex. *Scale bar* in **C** 950 μm for **A–C**; 475 μm for **D–F**

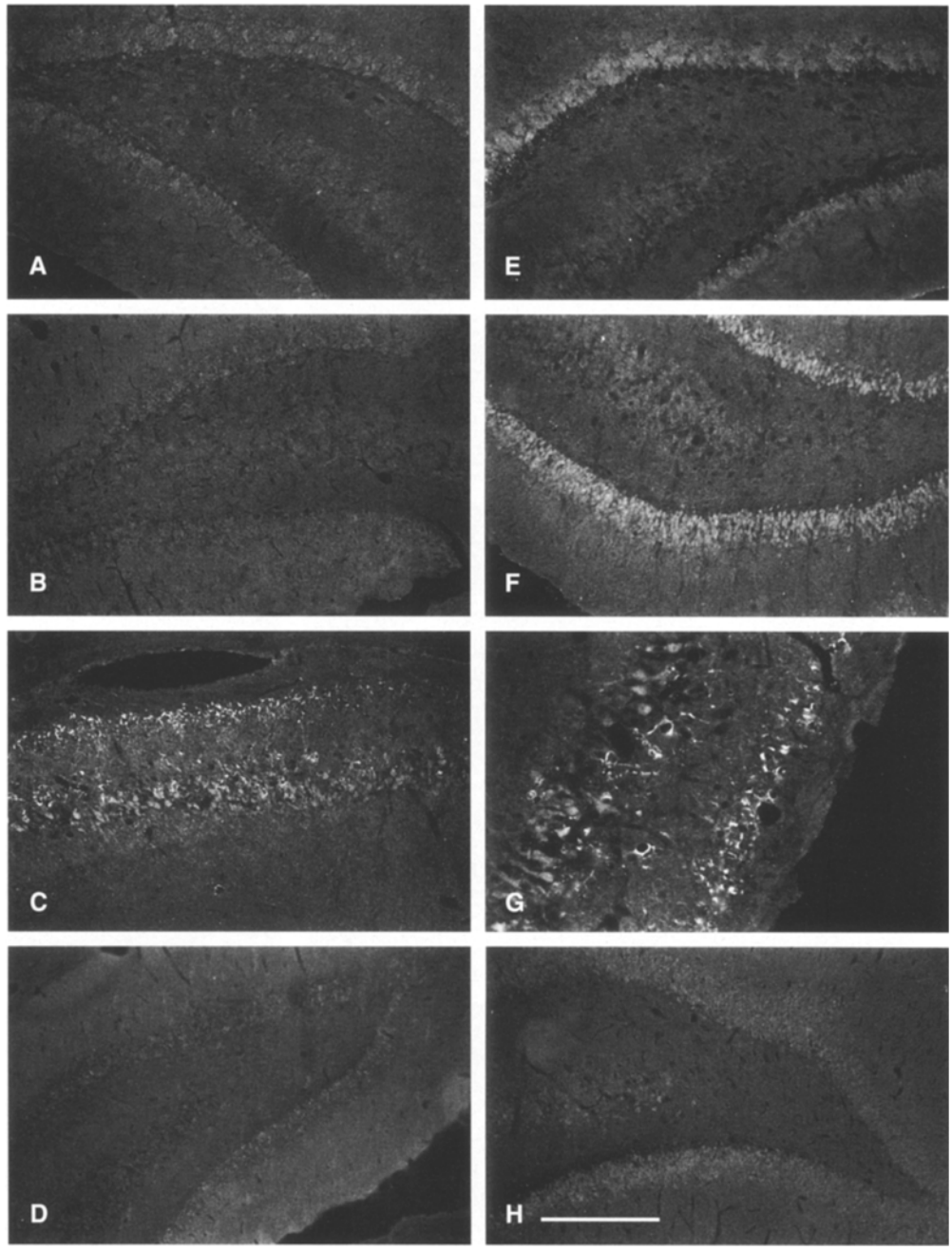

Fig. 8A–H Immunofluorescence labeling with anti-peptide antibody R1 in hippocampus of control animals and seizure cases. **A,E** Paired sections, dentate gyrus and hilus: control (**A**) and 3 h of seizures (**E**). Note increased labeling of granule cells. **B,F** Paired sections, dentate gyrus and hilus: control (**B**) and 6 h of seizures (**F**). Note increased labeling of granule cells. **C** CA2/CA3 region at 6 h of seizures with strong labeling of pyramidal cell layer and in stratum oriens. **G** Detailed view of stratum pyramidale and oriens with numerous strongly fluorescent processes. **D,H** Paired sections, granule cells: Control (**D**) and 24 h (**H**). *Scale bar* in **H** 285 μm in **A–F,H**; 142 μm in **G**

Three hours. The labeling in the seizure animals in hilar neurons and scattered neurons in the pyramidal cell layer appeared to be maintained or marginally increased after 3 h. Two animals had a positive signal overlying some portions of the dentate granular cell layer (Fig. 6B).

Six hours. Hilar cells were labeled in a scattered way, but the number and density of labeling had decreased after 6 h. Increased hybridization signals were seen over the granule cell layer in all cases.

Twenty-four hours. A patchy increase in signal was found over the granule cell layer in two cases after 24 h. In the others the signals had declined back to control values. However, the labeling of cells in the hilus had disappeared (Fig. 6C). The outer layers of the dorsal cortex showed some cellular signals, but these were barely above background. No increases occurred in areas known to have reactions of astroglial cells, e.g., hippocampus and cortex, suggesting that such glial elements do not express NGF mRNA.

In situ hybridization for *trk*B mRNA

Controls. The hybridization signal was widespread in the forebrain of controls. The granule cells, hilar cells, and pyramidal neurons as well as scattered cells in the den-

dritic layers were positive (Fig. 7A). Cells throughout all cortical layers were labeled, whereas the fiber tracts were negative (Fig. 7D). Positive hybridization was noted over other forebrain areas.

One hour. Animals had high signals over hippocampus, cortex, and striatum, but did not differ overtly from controls after 1 h.

Three hours. Hybridization over the granule cell layer of the dentate gyrus and in CA3–CA1 was increased (Fig. 7B) after 3 h, while neocortex did not differ from controls (Fig. 7E).

Six hours. The dentate gyrus granule cell layer and the CA3–CA1 sector revealed an increase in signal (Fig. 7C) after 6 h. The neocortex appeared to have normal hybridization (Fig. 7F).

Twenty-four hours. The signal overlying the dentate gyrus had returned to control values. Labeling in CA3–CA1 and neocortex was not overtly different from controls. Strong hybridization of some cells in and around the damaged tissue in the piriform cortex was noted, but no unspecific general increase was seen in this lesioned tissue.

In situ hybridization for LANR mRNA

Controls. Very strong hybridization signals were found over neurons along the medial pole of globus pallidus and along the internal capsule, i.e., in NGF-responsive cholinergic forebrain neurons of nucleus basalis, in controls. Another prominent group was seen in the lateral hypothalamus. The dendritic layers of hippocampus had some spurious groups of grains, whereas the granular and pyramidal layers were nearly devoid of labeling. No signals were seen in cortex or striatum.

Seizure animals. At all survival times, the labeling in nucleus basalis and lateral hypothalamus was fully maintained, and an increase could not be identified. Cells were not labeled in the granule cell layer and pyramidal cell layer. At 24 h, there was slightly more diffuse labeling in the lateral CA3 area. No cellular signals were seen in infarcted basal cortex, indicating that there is no unspecific interaction between oligonucleotide probes and damaged tissue.

BDNF immunoreactivity

Controls. A consistent pattern of labeling was obtained in controls, whereas the intensity of immunoreaction was variable (for detailed descriptions, see Wetmore et al. 1991, 1993, 1994). In hippocampus, the granule cells had distinct labeling, whereas few neurons in the hilus were positive (Fig. 8 A,B,D). Neurons in CA3 had low levels of immunoreactivity in cell bodies and faint labeling in stratum lucidum (mossy fiber layer). In the CA2 region strong positivity of cell bodies and faint dendritic labeling was observed. Pyramidal cells in CA1 revealed only some positive staining. In neocortex, scattered neurons were faintly positive and there were labelled pyramidal cells in the piriform cortex. Minor labeling occurred within striatum. No specific staining occurred after omission of the primary antibody.

Three hours. The granule cells were slightly smaller and more strongly fluorescent, and empty spaces appeared between the cells (Fig. 8E) after 3 h. Also, a band of light background staining separated the granule cells from the hilus, which appeared swollen. The reactivity remained low in CA3, while the CA2 sector was more strongly labeled. A small band of tiny dots was found in mid-dorsal levels in stratum oriens of CA2. Area CA1 was only moderately stained. No reactivity occurred in neurons in the dendritic layers. No systematic changes occurred in cortex or striatum.

Six hours. There was an evident difference in signals between the granule cells and the adjacent subgranular portions of the hilus (Fig. 8F). The granule cells were strongly immunoreactive, and the signals were clearly increased as compared to controls. A diffuse immunoreactivity was seen around the CA3 pyramidal cell layer within the hilus. In the lateral CA3 region, there was only moderate labeling of cell bodies in the pyramidal cell layer and low reactivity in the mossy fiber layer (stratum lucidum). The CA2 cells were strongly labeled. The CA1 neurons showed slightly more labeling than controls, but this increase might only be apparent owing to compression, since empty spaces were seen between the somata. Intensely fluorescent structures were seen in outer stratum pyramidale and stratum oriens of CA2 (Fig. 8C,G). Few immunoreactive grains occurred also in stratum oriens and lacunosum-moleculare of CA3 or in stratum radiatum of CA1. These structures might be small dendritic elements which revealed more BDNF or a modified protein which cross-reacted with the present antibodies. Alternatively, degenerating axons and terminals were labeled which originated from other brain regions (e.g., contralateral CA3, entorhinal cortex). The labeling in cortex and striatum did not change in a systematic manner.

Twenty-four hours. Labeling of the granule cells was not much different from controls (Fig. 8H), and immunoreactions in hilus and medial CA3, CA2, and CA1 were low after 24 h. Some defects in BDNF labeling in the pyramidal cell layer of CA3 correlated with the cell damage seen by Nissl stains. In basal cortical areas, the infarcted region revealed decreased labeling, whereas intact pyramidal cells in layer II surrounding this lesion showed enhanced immunoreactivity. No systematic changes occurred in neocortex or striatum.

Evaluation of scores. The changes of immunofluorescence scores are listed in Table 2. The higher score val-

Table 2 Scores of immunfluorescence intensity in different hippocampal regions of controls and seizure animals. Values are means± SEM (*CA1, CA2, CA3* areas of the hippocampus, *DG* dentate gyrus granule cells)

		CA1	CA2	CA3	DG
Control	n=5	0.7 ± 0.6	1.4 ± 0.5	0.8 ± 0.6	1.1 ± 0.5
6 h	n=4	1.3 ± 0.8	2.8 ± 0.9	0.5 ± 0.5	2.1 ± 0.4*
24 h	n=3	0.7 ± 0.3	2.7± 0.4	0.3 ± 0.3	1.1 ± 0.5

t-Tests failed to show significant differences among seizure animals and controls, but there was a trend toward increased labeling in the dentate gryus at 6 h. *P<0.1

ues of dentate gyrus and CA2 reflect the qualitative observations in controls (see Wetmore et al. 1994). There was a tendency toward increased labeling in CA2 at 6 and 24 h, which was related to formation of grains around the pyramidal layer. A marginal increase was found in the CA1 region. A stronger increase was found in the dentate gyrus at 6 h, although this difference failed to reach significance (t=1.47, df=7, P<0.1). The (nonsignificant) decline of values in CA3 reflects the neuronal loss in this region.

Neuropathology

Animals with LMSE studied at 3 or 6 h after induction showed compression and shrinkage of cell bodies in hippocampal cell layers. At 24 h, some neuronal damage was noted in the hilus and in lateral parts of the CA3. The granule cells of the dentate gyrus and most of CA1 and CA2 were intact. Laminar lesions were noted in layers II–III of neocortex. Infarction developed in the piriform/entorhinal cortex-amygdala region from 6–24 h onward, which was visible as loss of tissue integrity. The section material from the previous study (Schmidt-Kastner and Ingvar 1994, 1996) was used as reference, and it showed similar neuronal lesions in hippocampus, cortex, and basal cortex at 24 h under optimal conditions of fixation.

Discussion

The present study shows that LMSE induced by pilocarpine leads to an increase in BDNF mRNA in hippocampus, cortex, thalamus, and striatum. Alterations of BDNF-IR were noted in the hippocampus. Hybridization for *trk*B mRNA increased transiently in hippocampus. NGF mRNA increased in the dentate gyrus and decreased in the hilus, while no changes of LANR mRNA were seen. In the following, aspects of neurotrophin localization, seizure-induced increases in BDNF mRNA, and protein changes will be compared with the pathological pattern of cell damage. Since changes in BDNF mRNA were widespread throughout the forebrain, whereas NGF mRNA increased only in the dentate gyrus, the following discussion is focused on BDNF.

In the present study on pilocarpine-induced seizures, BDNF mRNA increased in all hippocampal regions, the entire neocortex, piriform cortex, and parts of the amygdala after high-dose pilocarpine and LMSE. The changes described here by section hybridization and emulsion autoradiography for pilocarpine-induced LMSE in adult rats are similar to those described in northern blots and in situ hybridization using film autoradiograms of very young and adult rats given pilocarpine (Berzaghi et al. 1993). Another study showed increased cellular hybridization for BDNF mRNA over cortical areas in the same model (Castrén et al. 1993). This finding is in line with observations on most other models of generalized seizures (Gall 1993). The changes also resembled alterations seen in kainic acid-induced status epilepticus (Ballarín et al. 1991; Gall et al. 1991; Dugich-Djordjevic et al. 1992; Wetmore et al. 1994). Differences in the time course of changes reflect different methods to elicit generalized seizures. Interestingly, the increase in hybridization intensity at 3 h was the same in the main group injected with 300 mg/kg pilocarpine, as in the animals injected with 250 mg/kg, provided that the animals had developed LMSE. Animals with LS and few motor seizures alone showed an increase in BDNF mRNA in various areas. Seizure activity may progress in limbic circuits and lead to metabolic stimulation in the absence of sustained motor seizures. However, the hybridization signals were much lower in animals with limbic seizures as compared to animals with the full pattern of LMSE, as was seen in the experiments with 250 mg/kg of pilocarpine. Thus, minor seizures can also elevate the BDNF mRNA signals (Gall 1993). This interpretation is in line with the finding that pilocarpine increased BDNF mRNA in very young rats, although animals did not show overt seizure activity (Berzaghi et al. 1993). Levels of BDNF mRNA decreased in hippocampus and cortex at 24 h as compared to the 6-h time point. There was some evident decline of BDNF mRNA in the lateral CA3 region that had neuronal damage, but signals also declined in CA1 and dentate gyrus, which were intact. This suggests that the decrease in seizure activity was mainly responsible for the decrease in BDNF mRNA. There was no unspecific increase in labeling in overtly damaged tissue for any of the oligonucleotide probes used here, which indicates specificity of the changes reported here.

A comparison of the different seizure models suggests that enhanced excitation per se is relevant for the upregulation of BDNF mRNA, and not the chemical agent or electrical stimulus used to trigger the seizure process. Pilocarpine initially stimulates central muscarinic choliner-

gic receptors and then several glutamatergic pathways are activated (Turski et al. 1983; Clifford et al. 1987). Glutamate is released in hippocampus in the early stage of pilocarpine seizures (Millan et al. 1993). BDNF expression appears to be coupled to neuronal excitation and depolarization through glutamate receptors (Zafra et al. 1990, 1991, 1992). A recent analysis of the promoters in the BDNF gene suggests a complex network of regulation by excitatory amino acid receptors (Metsis et al. 1993). Blockade of non-NMDA receptors prior to kainic acid application prevented seizures and increases in BDNF mRNA (Wetmore et al. 1994). Direct injection of NMDA into the ventricle leads to an increase in BDNF mRNA (Berzaghi et al. 1993; Gwag and Springer 1993). Thus, both NMDA and non-NMDA type of glutamate receptors are involved in the regulation of BDNF expression, and the stimulation of several types of glutamate receptors would be expected as seizures propagate abnormal electrical activity through the forebrain. The changes in BDNF mRNA most probably reflected enhanced gene expression (Gall 1993), although a stabilization of existing mRNAs cannot be excluded. However, there was no generalized increase in mRNA levels, as LANR mRNA remained stable. Increase in BDNF mRNA might be a regulatory response to acute changes in intra- or extracellular concentrations of BDNF in seizures. It was speculated that BDNF might be released from intracellular stores in strong neuronal excitation and seizures (Wetmore et al. 1994). Enhanced resynthesis of BDNF by increasing BDNF mRNA would then indicate a feedback control mechanism.

The anti-peptide antibody to BDNF has been extensively characterized (Wetmore et al. 1991, 1993). Generally, there was a good correlation between the presence of BDNF mRNA (with and without stimulation), and BDNF-IR. In hippocampus, control rats had staining in the granule cells, some labeling in CA3, higher levels in CA2, and, again, moderate levels in CA1. The granule cells had the highest hybridization signals, moderate signals were seen in CA3 and low signals in CA1. In the neocortex of controls, BDNF-IR was high in layers VI and II, and these layers have high BDNF mRNA signals in controls. The dorsal endopiriform nucleus had strong BDNF-IR and high mRNA signals. This comparison confirms the specificity of BDNF peptide-antibodies for the study of the perfusion-fixed rat brain (Wetmore et al. 1991, 1993). In fact, preliminary data for another BDNF antibody showed a considerable overlap in labeled structures (Yan et al. 1994).

Changes in BDNF mRNA can now be compared with those of BDNF-IR. The region with the strongest upregulation of BDNF mRNA was the dentate gyrus, which also showed enhanced immunoreactivity for BDNF. Both the CA3 and CA1 regions showed increases in BDNF mRNA at all time points during and after seizures. Immunoreactivity for BDNF increased slightly (but not significantly) in the CA1 regions, but not in CA3. BDNF mRNA was increased in all layers of neocortex, whereas BDNF-IR did not show systematic changes. The piriform cortex showed an early and substantial upregulation of BDNF mRNA. An increase in BDNF mRNA and immunoreactivity in the neurons was seen at the immediate border of such cortical infarcts, which may reflect a reaction of intact neurons. Similarly, upregulations of BDNF mRNA have been observed at the border of ischemic infarcts (Comelli et al. 1992). Work has indicated an increase in BDNF-IR in other areas and with another BDNF-antibody after kainic acid seizures (Yan et al. 1994). We did not see such changes in a preceding study on kainic acid seizures (Wetmore et al. 1994), nor in the present investigation of pilocarpine-induced seizures. Furthermore, data on measurements using the enzyme-linked immunosorbent assay technique confirmed an increase in BDNF protein after seizures (Nawa et al. 1995). Thus, there is now converging evidence that BDNF protein may increase after seizures, although differences in the spatial and temporal aspects remain to be clarified. These might be due to differences in the epitopes detected by different anti-peptide antibodies. BDNF might also be released from cells (Wetmore et al. 1994), and this pool might escape from immunohistochemical analysis, while showing up in measurements in tissue samples.

The above comparison suggests that there is no straight correlation between the enhanced expression of BDNF mRNA and BDNF protein. Similar observations were made previously in PTZ-induced seizures (Humpel et al. 1993) and in kainic acid-induced seizures (Wetmore et al. 1994). There are several explanations for this observation. First, enhanced synthesis of the protein may not be detectable within cell bodies, because BDNF might be released into the extracellular space (Wetmore et al. 1994). Second, protein synthesis was blocked in vulnerable neuronal populations during seizures (Kiessling et al. 1984). Thus, only certain neurons such as granule cells may be capable of using increased BDNF mRNA levels for enhanced synthesis of the neurotrophin protein. Third, although perhaps less likely, the immunohistochemical changes might have been caused by alterations of preexisting BDNF molecules. Moreover, compression of these neurons by swelling of the neuropil might also induce local increases in immunoreactivity. Clearly, while in situ hybridization is able to precisely locate possible sites of synthesis of a given protein, our results emphasize that levels of mRNA and indeed changes of mRNA levels cannot be directly correlated to intracellular levels of the corresponding proteins. For this purpose it is necessary also to monitor protein levels more directly using immunohistochemistry.

Granule cells were undamaged, so that one could propose a protective role for BDNF, especially since both mRNA and immunoreactivity increased. However, at the same time one could argue that resistant cells per se have a higher capacity to increase mRNA and protein synthesis. Neurons in the lateral CA3 area were damaged, whereas CA1 neurons were intact within the present observation period (Schmidt-Kastner and Ingvar 1996). A significant increase in BDNF mRNA signals occurred in

CA1 at 1 h, whereas increases in CA3 were seen only at 3 h. Theoretically, this difference could be important for the vulnerability of CA3 neurons. In neocortex, neurons in layers II/III were damaged, whereas neurons in other layers survived. Upregulations of BDNF mRNA occurred in all layers and at early time points. Consequently, there was no close correlation between changes of intracellular BDNF mRNA or protein levels and resistance to cell damage. Thus, the upregulation of BDNF mRNA appears to occur in most neuronal groups which express this factor under baseline conditions, and it is not a specific response of neurons undergoing damage. Obviously, a neurotrophin cannot be expected to counteract all aspects of pathophysiological dysfunction in pharmacologically provoked seizures. Changes in energy metabolism, ionic disturbances, cytotoxic edema formation, and glial swelling may proceed independently (Ingvar et al. 1987; Meldrum 1991). Such changes may not be amenable to regulation by neurotrophins. Trophic factors may also play a role in the long-term effects of seizures, e.g., in sprouting of mossy fibers in the dentate gyrus (Humpel et al. 1993). On the other hand, BDNF was recently shown to enhance calcium influx in cultured hippocampal neurons (Berninger et al. 1993). Excitotoxic mechanisms of neuronal damage in seizures involve the uncontrolled influx of calcium ions (Meldrum 1991), and thus an additional stimulation of calcium fluxes by BDNF could be detrimental. However, there was no correlation between damage of neurons and upregulation of BDNF mRNA in the present experiments.

An interesting observation was that the anti-peptide antibody showed small grains with BDNF-IR in stratum oriens of CA2 as well as in distal stratum radiatum of CA1. These grains might be due to storage of BDNF within processes of pyramidal cells when the release was altered or synthesis enhanced. Alternatively, the changes may have occurred in terminal branchings of afferent axons when uptake or transport were altered. Such change could also be due to incipient cell death of neurons in contralateral CA3 or entorhinal cortex. However, a cross-reaction with other proteins cannot be excluded.

The question is whether BDNF, if produced and released in enhanced amounts, would be effective on the respective receptor systems. LANR might be required for some aspects of BDNF activity (Rodriguez-Tebar et al. 1990; Hantzopoulos et al. 1994). If LANR mRNA was to be expressed at low levels even within hippocampal neurons, then seizures apparently could not boost the signal to detectable levels. The mRNA expression for *trk*B was enhanced in the dentate gyrus as well as in the CA3 to CA1 region. The increases in *trk*B signals during pilocarpine-seizures suggested that regions with an upregulation of BDNF also increased the expression of the respective *trk*B receptors. Similar increases in *trk*B mRNA were found in other seizure models (Humpel et al. 1993; Merlio et al. 1993). The parallel change in gene expression for BDNF and its receptor suggests a regulated response which enables enhanced paracrine or autocrine effects of BDNF. However, it is also possible that *trk*B message was upregulated as a response to decreased extracellular levels of BDNF. Theoretically, NT-3 and NT-4 also act on *trk*B receptors (Soppet et al. 1991; Squinto et al. 1991). Downregulation of NT-3 in several seizure models (Rocamora et al. 1992; Gall 1993) might lead to a feedback regulation of *trk*B mRNA. The role of NT-4 is presently unclear.

NGF mRNA increased in the granule cells of the dentate gyrus, but not visibly in other regions. An increase in NGF mRNA was also reported in hippocampus of very young rats injected with pilocarpine (Berzaghi et al. 1993). The modest response of the NGF system in pilocarpine-induced seizures suggests that this trophic factor is unlikely to be involved in an endogenous protection. NGF mRNA increased in cortex cerebri in other models, and a second increase in NGF might occur at later stages (Gall 1993; Lauterborn et al. 1994). NGF mRNA decreased in the hilus of the dentate gyrus at 6 h after pilocarpine seizures and was severely reduced at 24 h. A recent study showed a colocalization of NGF mRNA and glutamic acid decarboxylase, a marker for GABAergic neurons (Lauterborn et al. 1993). The change of NGF mRNA, then, most likely reflected the cell death of hilar neurons (Schmidt-Kastner and Ingvar 1996).

Further studies on subpopulations of neurons in the hilus and their expression of neurotrophin expression have been performed recently, and preliminary data suggest absence of BDNF protein from several hilar neurons outside the CA3 cell band (R. Schmidt-Kastner, C. Wetmore, L. Olson, unpublished work). The persistence of NGF mRNA in the CA3 and CA1 sector then reflected the resistance of interneurons. Similarly, the increase in NGF levels after ischemia (Shozuhara et al. 1992) could be related to the preservation of interneurons. The relative resistance of hippocampal interneurons should be studied in more detail, since trophic support for NGF-dependent septal cholinergic neurons might continue even if the hippocampus is afflicted by extensive neuronal damage to pyramidal cells.

The striatum is involved in the spread of seizure activity in the pilocarpine-model (Turski et al. 1989) and may undergo minor cell damage (Schmidt-Kastner et al. 1991). Control striatum does not express detectable levels of BDNF mRNA (Lindsay et al. 1994). Positive hybridization over cells became visible at 3–6 h of pilocarpine seizures. This finding documents at the cellular level that neurons in striatum are able to produce BDNF when stimulated by seizures. A recent study using RNase protection analysis found BDNF mRNA expression in striatum after kainic acid (Timmusk 1994). These observations open the possibility that BDNF may be a target-derived trophic factor for dopaminergic neurons in substantia nigra. Further studies on BDNF in striatum are indicated.

In summary, pilocarpine-induced seizures led to an increase in BDNF mRNA in hippocampus, cortex, thalamus, and striatum. *trk*B mRNA increased in hippocampus. This finding suggests a role for strong neuronal excitation in the regulation of BDNF and its receptor in pilo-

carpine seizures. The functional significance of an increased BDNF mRNA remains unclear. The delayed increase in BDNF mRNA in the vulnerable CA3 region as compared to the earlier increase in the more resistant granule cells and CA1 pyramidal cells points to a direct protective effect of BDNF against neuronal damage caused by excitotoxic levels of stimulation in seizures. If such self-protection exists, however, it can only be incomplete, since many neurons are indeed lost, probably due to the manifold pathophysiological events in status epilepticus. A protective effect of BDNF or other trophic factors must be tested by direct application of the factors into vulnerable brain areas. Interestingly, in this respect, a recent study could show that the pretreatment with basic fibroblast growth factor protected hippocampal neurons against cell death induced by kainic acid seizures (Liu et al. 1993). We conclude that pilocarpine-induced seizures are associated with marked regional and temporal changes of BDNF mRNA, BDNF protein, and *trk*B mRNA.

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