phase and 3 hours later). The following solutions were injected: saline (0.9%), vehicle control solution (an identically processed and purified sample from bacteria containing the same plasmid but without the mouse *ob* gene), or OB protein [3 μg per mouse in 0.1 ml of saline (0.9%)—approximately 0.12 mg per kilogram of body weight per day for 50-g obese mice]. The dose of OB protein was based on results from an ascending-dose pilot IP study in *ob/ob* mice.

10. In this study, the total cumulative food intakes (expressed as grams per three mice) during the 10 days of treatment were 93 ± 3 g in the saline group, 111 ± 7 g in the vehicle control group, and 74 ± 2 g in the OB protein group. The total food intake in the OB protein group was significantly reduced compared with the saline (30%) and vehicle control (33%) groups [one-way analysis of variance (ANOVA), effect of treatment, $F(2,5) = 15.5$ ($P < 0.03$)]. In addition, the cumulative food intake in each treatment period in the OB protein group was significantly reduced compared with the vehicle control group [one-way ANOVA, effect of treatment: first treatment, $F(2,12) = 6.73$ ($P < 0.011$); second treatment, $F(5,59) = 4.17$ ($P < 0.003$)].
11. When similar *ob/ob* mice were totally fasted, the average weight loss was −6.8 ± 0.2 g after 4 days and −9.0 ± 0.2 g after 6 days of fasting.
12. DIO mice were produced by feeding AKR/J mice a high-fat diet [D. B. West, J. Waguespack, B. York, J. Goudey-Lefevre, A. R. Price, *Mamm. Genome* **5**, 546 (1994)]. Mice used were more than two standard deviations above the mean body weight of the chow-fed control mice.
13. Obese *ob/ob* and lean (+/?) mice were implanted with chronic jugular cannulas under pentobarbital anesthesia (80 mg per kilogram of body weight) [A. Mokhtarian, M.-J. Meile, P. C. Even, *Physiol. Behav.* **54**, 895 (1993)]. Methods used were as described in (*14*) except that mouse OB protein [3 μg in 0.1 ml of saline (0.9%)—approximately 0.06 mg/kg for obese mice] or an equal volume of vehicle control or saline (0.9%) solution was injected intravenously. The IV dose was based on half of the daily IP dose. Two separate preparations of mouse OB protein were used, and the results were combined.
14. We implanted single 27-gauge stainless steel chronic infusion cannulas into the lateral ventricle of the brain of pentobarbital-anesthetized (80 mg/kg) obese and lean mice using the following coordinates: −0.7 mm relative to bregma, 2 mm lateral of midline, and 2 mm down [T. J. Haley and W. G. McCormick, *Br. J. Pharmacol.* **12**, 12 (1957)]. The exteriorized cannula was secured on the skull with dental cement anchored with a jeweler screw. After a 16- to 18-hour overnight fast, experiments were conducted during the light phase. Each trial began with a 45-min acclimatization period followed by ICV injection of one of the following solutions: artificial CSF, vehicle control solution, or OB protein (1 μg per mouse, ~0.02 mg/kg for obese mice). Awake mice were lightly restrained, and a syringe fitted with a piece of precalibrated polyethylene tubing (PE20) was used to infuse 1 μl of the test solution followed by 1 μl of CSF to clear the cannula. Mice were then immediately replaced in the test cage with a preweighed petri dish containing a pellet of mouse chow. Trials were separated by at least 3 days. Successful cannula placement was verified by increased food intake after an ICV injection of 5 to 10 μg of neuropeptide Y [J. E. Morley, E. N. Hernandez, J. F. Flood, *Am. J. Physiol.* **253**, R516 (1987)]. Two separate preparations of mouse OB protein were used and the results were combined.
15. The corresponding percent changes in body weight were 4 ± 1%, 7 ± 1%, and −2 ± 1% in the CSF, vehicle control, and OB protein groups, respectively. Pre-injection body weights after the 16- to 18-hour fast were 40.0 ± 1.4 g, 35.8 ± 2.1 g, and 40.3 ± 1.5 g in the CSF, vehicle control, and OB protein groups, respectively. All values represent the mean ± SEM of groups of obese mice. The effect of OB protein treatment on body weight gain was significant ($P < 0.0001$) by one-way ANOVA without repeated measures. Effect of treatment, $F(2,26) = 30.67$ (*17*).
16. M. W. Schwartz *et al.*, *Endocr. Rev. 1992* **13**, 387 (1992); M. W. Schwartz *et al.*, *Endocr. Rev. 1994* **2**, 109 (1994).
17. Statistical differences in the means were tested with the Student's *t* test for unpaired or paired samples and one- and two-way ANOVA with repeated or nonrepeated measures as appropriate with $P < 0.05$ [G. W. Snedecor and W. G. Cochran, *Statistical Methods* (Iowa State University Press, Ames, IA, ed. 8, 1989)].
18. We thank G. Mackie, M. Renzetti, B. Simko, and M. Simpson (animal studies) and I. Fache, G. Plaetinck, J. Tavernier, T. Tuypens, J. Van der Heyden, and A. Verhee (expression and purification of recombinant mouse OB protein) for technical assistance and expertise; S. Ogawa for information about the ICV injection technique; D. Coleman for inspiration and encouragement; and M. Steinmetz for support, encouragement, and helpful comments.

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Induction of MHC Class I Genes in Neurons

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Whether neurons express major histocompatibility complex (MHC) class I genes has not been firmly established. The techniques of confocal laser microscopy, patch clamp electrophysiology, and reverse transcriptase–polymerase chain reaction were combined here to directly examine the inducibility of MHC class I genes in individual cultured rat hippocampal neurons. Transcription of MHC class I genes was very rare in neurons with spontaneous action potentials. In electrically silent neurons, transcription was noted, with expression of β_2-microglobulin under tighter control than in class I heavy chain molecules. Surface expression of class I molecules occurred only in electrically silent neurons treated with interferon γ. Immunosurveillance by cytotoxic T cells may be focused on functionally impaired neurons.

MHC class I heavy chain molecules are 45-kD integral membrane glycoproteins that assume their correct conformation after noncovalently binding β_2-microglobulin. This complex binds antigenic peptides for presentation to $CD8^+$ T cells (*1*). MHC class I molecules are expressed in most tissues, an exception being the healthy central nervous system (CNS). But the inability of CNS cells to express MHC class I products is by no means absolute. Glial components of the CNS can be readily induced to produce MHC determinants in vitro, or, under pathological conditions, in vivo. In contrast, MHC inducibility in normal neurons has not yet been demonstrated beyond doubt (*2*). The intricate association between CNS glia and neurons had made it impossible to resolve the issue by conventional morphology or molecular technology. Whether neurons are able to synthesize MHC class I products, and thus present antigen to T cells, is of clinical importance. In viral infections of the CNS, for example,

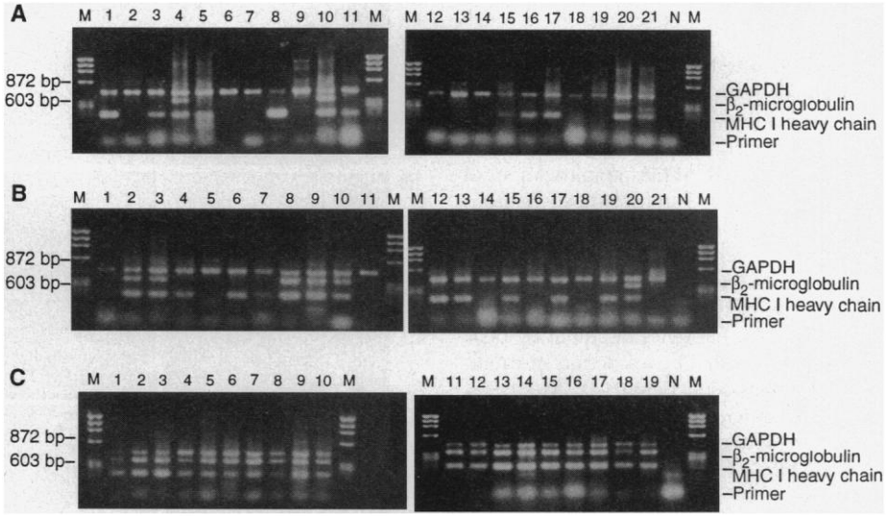

Fig. 1. RT-PCR analyses of MHC class I heavy chain, β_2-microglobulin, and GAPDH transcripts of (**A**) untreated, (**B**) IFN-γ–treated, and (**C**) IFN-γ– plus TTX-treated neurons. Only after treatment with IFN-γ plus TTX did all neurons express mRNA of β_2-microglobulin and MHC class I (*7, 10*). DNA molecular weight markers and PCR control reactions without the cDNA sample are shown in lanes M and N, respectively.

neurons lacking MHC molecules could serve as reservoirs for persistent viruses (3), while MHC-expressing neurons could be eliminated by virus-specific cytotoxic T cells.

To establish the conditions under which MHC class I genes are inducible in neurons, we analyzed the expression of mRNA for MHC class I heavy chain and β_2-microglobulin in single hippocampal neurons in different states of activity. Following the strategy of Lambolez *et al.* (4), we used whole-cell patch-clamp to identify and functionally characterize individual neurons. Negative pressure was then applied to the micropipette to extract minute samples of cytoplasm, which contains sufficient mRNA to assess by reverse transcriptase–polymerase chain reaction (RT-PCR) the gene repertoire currently being transcribed.

The cells investigated were morphologically and electrophysiologically differentiated, pyramidal-shaped neurons from hippocampal cultures of Lewis rats (5). Typically, after 10 to 14 days in culture, the hippocampal neurons responded to depolarizing current pulses with sodium-dependent fast action potentials (6). About half of these neurons showed spontaneous firing of action potentials, and we classified as "active" those neurons that spontaneously fired at least one action potential per minute; neurons without spontaneous action potentials within 5 to 10 min were classified as "silent."

The neuronal nature of the cells was ascertained in each case by their electrophysiological characteristics and was confirmed by detection of mRNA for microtubule-associated protein 2 (MAP2), a neuron-specific cytoskeletal protein. In contrast, amplification of complementary DNA for the astroglial marker gene glial fibrillary acidic protein (GFAP) was negative in all of these neurons but was unfailingly found in astrocyte-derived samples (7). The analysis of the extracellular solution from the cell culture after cytoplasms were harvested did not result in amplification of PCR products. With few exceptions, RT-PCR amplification of mRNA samples of patch-clamped neurons provided unequivocal signals for the household gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a control for quality and quantity of the cytoplasmic RNA sample (Fig. 1). Authenticity of amplified PCR products was verified by restriction site analyses and direct sequencing (8). Messenger RNA for β_2-microglobulin was detected in 1 out of 10 "active" and in 1 of 11 "silent" neurons. MHC class I heavy chain mRNA was expressed in 3 of 10 "active" and 8 of 11 "silent" neurons (Table 1). Only one of the

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Table 1. MHC class I heavy chain and β_2-microglobulin mRNA expression in single cells. "—" indicates no treatment.

Cell type	Bioelectric status	Treatment	Cells positive for mRNA for	
			β_2-microglobulin	MHC class I heavy chain
Neurons	Active	—	1/10 (10%)	3/10 (30%)
Neurons	Silent	—	1/11 (9%)	8/11 (73%)
Neurons	Active	IFN-γ	0/9 (0%)	3/9 (33%)
Neurons	Silent	IFN-γ	6/12 (50%)	11/12 (92%)
Neurons	Paralyzed	IFN-γ + TTX	19/19 (100%)	19/19 (100%)
Neurons	Paralyzed	IFN-γ + TTX + IFN-γ antibodies	1/7 (14%)	6/7 (86%)
Neurons	Paralyzed	TTX	1/11 (9%)	5/11 (46%)
Astrocytes	—	—	10/10 (100%)	10/10 (100%)

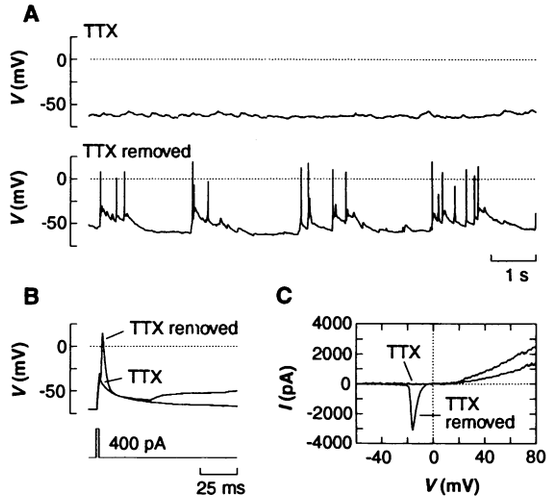

Fig. 2. Suppression of bioelectric activity of IFN-γ–treated hippocampal neurons by TTX. To monitor functional properties of neurons after treatment with IFN-γ (100 U/ml) and 1 μM TTX, membrane potentials (*V*) and ion currents (*I*) were recorded in the presence of TTX and after subsequent removal of TTX. (**A**) No spontaneous activity was observed in the presence of TTX (upper trace), whereas almost complete recovery was attained after TTX was washed out (lower trace). (**B**) Suprathreshold depolarizing currents (lower trace) failed to elicit action potentials in the presence, but not in the absence, of TTX (upper trace). (**C**) Sodium currents (downward deflections) were not observed in the presence of TTX but recovered after TTX removal, indicating that the lack of spontaneous and induced action potentials was due to blockade of sodium channels.

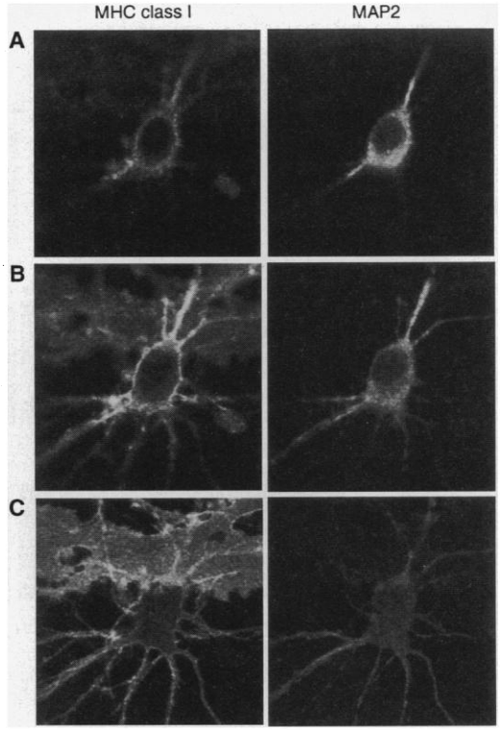

Fig. 3. Confocal laser microscopy of mixed hippocampal cell cultures treated with IFN-γ and TTX. Three sections of a single neuron are shown, after MHC class I surface staining (left) and subsequent intracellular staining of MAP2 (right). (**A** to **C**) MHC class I molecules are visible on the cell surface of the neuron and (C) on an astrocyte that forms part of the flat monolayer beneath the neuron (*11*).

active and one of the silent neurons expressed both transcripts simultaneously (Fig. 1A and Table 1). Hence, in the absence of spontaneous activity suppression of MHC class I gene control seems to be more relaxed, with class I heavy chain mRNA appearing more often than β_2-microglobulin.

The MHC class I expression patterns were even more distinct after treatment of neurons with interferon γ (IFN-γ), a proinflammatory cytokine known to induce MHC class I and β_2-microglobulin genes in most cell lineages. Treatment with IFN-γ (100 U/ml) for 72 hours did not affect the electrical activity of neurons, and among such treated cells only a minority contained mRNA for both chains, MHC class I and β_2-microglobulin (Fig. 1). None of the IFN-γ–treated neurons with spontaneous fast action potentials expressed β_2-microglobulin mRNA, and only 3 out of the 9 "active" neurons expressed MHC class I mRNA (Table 1). In contrast, 6 of 12 "silent" neurons expressed β_2-microglobulin and 11 of 12 expressed MHC class I heavy chain mRNA. All the neurons that expressed β_2-microglobulin and MHC class I were "silent" neurons without spontaneous action potential firing.

To test a possible correlation between functional status and MHC class I or β_2-microglobulin expression, we blocked the bioelectric activity of the neurons with the sodium channel blocker tetrodotoxin (TTX). Whole-cell recordings of neurons treated with IFN-γ in the presence of TTX confirmed the lack of spontaneous activity because of blockade of sodium currents (Fig. 2). TTX was removed immediately before patch-clamp analysis to facilitate electrophysiological identification of neurons. After removal of TTX, both spontaneous and evoked action potentials were observed. Although TTX treatment alone did not affect MHC class I expression, all neurons treated with TTX plus IFN-γ contained both β_2-microglobulin and MHC class I mRNA (Fig. 1 and Table 1). These observations confirmed that bioelectrically silent neurons are much more inducible for mRNA of MHC class I and β_2-microglobulin by IFN-γ than are neurons with spontaneous electrical activity.

Membrane expression of MHC class I proteins on living neurons was studied by immunofluorescence labeling with the monoclonal antibody OX-18, which binds rat class I (RT1.A) gene products and with a monoclonal antibody specific for the neuronal cytoskeleton protein MAP2. Confocal laser microscopy showed that some, but not all, of the hippocampal neurons treated with IFN-γ carried MHC class I molecules on their surface (Fig. 3). A total of 27 out of 78 neurons (35%) analyzed by confocal microscopy in four experiments were induced by IFN-γ to express MHC class I in the plasma membrane. This percentage of MHC class I–positive neurons is consistent with the fraction of silent neurons that contain transcripts for both β_2-microglobulin and MHC class I heavy chain. In the presence of TTX, virtually all neurons responded to IFN-γ induction. In 67 of the 72 neurons (93%) analyzed in four different sets, confocal microscopy identified MHC class I molecules on the outer cell membrane. TTX treatment significantly ($P < 0.01$; χ^2 test) increased the number of MHC class I–positive neurons.

Our study shows that neurons are capable of transcribing both MHC class I heavy chain and β_2-microglobulin genes; consequently, neurons can express MHC class I molecules on their surfaces. They therefore possess the basic requirements to interact with $CD8^+$ cytotoxic T cells. MHC class I expression, however, seems to be regulated by strict control mechanisms. It appears that positive signals, as given by IFN-γ, are required for membrane expression of MHC class I molecules, whereas negative signals, provided in the presence of bioelectric activity, seem to suppress MHC class I expression. There are various examples of neuronal gene expression being controlled by electrical membrane activity. For example, expression of cell adhesion molecules, or neurotrophic factors, has been positively correlated with neuronal membrane activity (9). In contrast to these cases, regulation of MHC class I gene expression seems to be under negative control.

The practical implication of our findings relates to immune surveillance and microbial infection of the CNS. Suppressed inducibility of MHC class I molecules in functionally active, fully intact neurons could contain neuronal damage by specific cytolytic T cells to levels compatible with those found under adequate brain function. In contrast, neurons with overt viral damage, with loss of bioelectric activity, would be susceptible to recognition by cytotoxic $CD8^+$ T cells. In these cases, T cell lysis would remove defunct cells that were serving solely as viral reservoirs.

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5. Hippocampal cell cultures were prepared from 18-day-old fetal Lewis rats, as described [G. A. Banker and W. M. Cowan, *Brain Res.* **126**, 397 (1977)]. Briefly, hippocampi were dissected and meninges removed. The trimmed tissue was dissociated by trituration through a fire-polished Pasteur pipette. Cells (2×10^5) were plated on 35-mm petri dishes that had been pretreated with poly-L-ornithine (0.5 mg/ml; Sigma) in 0.15 M boric acid for at least 6 hours. Cells were cultured in basal medium (Gibco) containing 10% fetal calf serum during the first week and in serum-free basal medium supplemented with bovine serum albumin (100 μg/ml), transferrin (100 μg/ml), insulin (5 μg/ml), progesterone (60 ng/ml), putrescine (16 ng/ml), sodium selenite (40 ng/ml), thyroxin (40 ng/ml), and triiodo-L-thyronine (30 ng/ml) (all from Sigma) during the second week. Thereafter, recombinant rat IFN-γ (100 U/ml; Hoffmann–La Roche), TTX (1 μM, Sigma), or rabbit IFN-γ antibodies (40 μg/ml; Hoffmann–La Roche) were added alone or in combinations to the cells for 72 hours as indicated in the figures and table.
6. Membrane potentials and ion currents were recorded from morphologically differentiated hippocampal pyramidal neurons in the whole-cell mode of the patch-clamp technique [O. P. Hamill, A. Marty, E. Neher, B. Sakmann, F. J. Sigworth, *Pfluegers Arch.* **391**, 85 (1981)] with an EPC9 amplifier (HEKA Elektronik, Lambrecht, Germany). Patch pipettes were pulled from borosilicate glass (Kimax) tubing with a resistance of 3 to 5 megohms and wide tip openings (2 to 3 μm) to allow reliable harvesting of cytoplasm at the end of recordings. To destroy RNA-degrading enzymes, we heated the pipette glass and AgCl wire to 220°C over 4 hours before use. The pipette-filling solution contained 135 mM KCl, 4 mM $MgCl_2$, 1 mM EGTA, and 5 mM Hepes (pH 7.3 to 7.4); the bath solution contained 135 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 5 mM KCl, and 5 mM Hepes (pH 7.3). Spontaneous action potentials were recorded in the current-clamp mode at −60 mV for at least 6 min. Additionally, action potentials were elicited from −60 mV by current pulses of 200 to 400 pA and 2.5 ms. Only cells responding to depolarizing currents with action potentials were considered as neurons and included in the present study. The membrane currents were recorded in voltage-clamp mode with the use of ramps (0.5 V/s) from −80 mV to +80 mV delivered from a holding potential of −80 mV to allow a semiquantitative estimation of sodium and potassium currents.
7. We harvested cytoplasm of neurons and astrocytes by applying negative pressure to a micropipette after electrophysiological recordings of the cells in whole-cell mode (*6*). Cell cytoplasm was used only for RT-PCR, when the pipette-membrane seal remained intact until the end of the harvesting. Thereafter, the tip of the pipette was broken in a test tube containing dithiothreitol (10 mM; BRL) and ribonuclease inhibitor (20 U; Promega). Random hexamer primers (1 μl; Boehringer Mannheim), deoxynucleotide triphosphates (dNTPs) (0.5 mM; Pharmacia), and Moloney murine leukemia virus reverse transcriptase (100 U; BRL) were added to the test tube. The resulting 10-μl mix was incubated for 1 hour at 37°C and stored at −80°C. To avoid false positive PCR amplification, we took special precautions as described [S. Kwok and R. Higuchi, *Nature* **339**, 237 (1989)]. Negative controls included samples of extracellular fluid and pipette solution. PCR amplification was controlled with a water sample instead of cDNA. Forward and reverse primers were each selected from two different exons with the program PRIMER (Whitehead Institute, Cambridge, MA). The respective primer sequences were for mouse GFAP [GenBank–European Molecular Biology Laboratory (EMBL) accession number K01347] [5′-AAGCTCCAAGATGAAACCAACCTGA-3′ (position 370, plus strand) and 5′-CCACGATGTTCCTCTTGAGGTGG-3′ (position 1117, negative strand)] and rat MAP2 (GenBank-EMBL accession number X51842) [5′-AGCCTGCAGCTCTGCCTTTAGC-3′ (position 415, plus strand) and 5′-AGGTCTGGCAGAGGTTGGTTAATGA-3′ (position 5053, negative strand)]. The PCR was performed in a volume of 50 μl containing 1 μl of the transcribed cDNA sample, dNTPs (0.2 mM; Pharmacia), 2.5 U of Ampli Taq (Perkin-Elmer/Cetus), and PCR buffer (Perkin-Elmer/Cetus). The cDNA was first denaturated at 95°C for 3 min, and primers (10 pmol) were added at 80°C (hot start). The PCR was performed by 45 cycles of the following regimen: 93°C, 1 min; 60°C, 1 min; 72°C, 1 min. Ten microliters of the amplified products was run along with the molecular weight marker (ΦX 174, Hae III–digested, Pharmacia; molecular weight, 1353, 1078, 872, and 603, shown on the left and right side of Fig. 1) on a 1.7% agarose gel stained with ethidium bromide.
8. The restriction sites (Apa I, Bgl II, and Bgl I) were confirmed in the PCR products for MHC class I, β_2-microglobulin, and GAPDH, respectively. Gel-pu-

rified PCR products (Quiagen) were sequenced with an automated sequence analyzer (Applied Biosystems). The sequences of the fragments for MHC class I (spanning positions 155 to 1138) and β_2-microglobulin (spanning positions 18 to 329) agreed with the published sequence of Lewis rat RT1.A^l and rat β_2-microglobulin, respectively.

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10. Primer sequences were for rat GAPDH (GenBank-EMBL accession number X02231) [5′-CCCACG-GCAAGTTCAACGG-3′ (position 220, plus strand); 5′-CTTTCCAGAGGGGCCATCCA-3′ (position 628, negative strand)]; for rat β_2-microglobulin (accession number Y00441) [5′-CGGTGACCGTGATCTTTCT-GGT-3′ (position 18, plus strand); 5′-GGTGACG-GTTTTGGGCTCCTT-3′ (position 329, negative strand)]; and for Lewis rat MHC class I (accession number L26224) [5 -TGCAGAGAGACTCAGGGC-CTACC-3′ (position 537, plus strand); 5′-TGC-CAGCTCAGGGAGATGTCA-3′ (position 714, negative strand) and 5′-TCGGCTACGTGGACCACACG-3′ (position 155, plus strand); 5′-TGCCAGCTCAGG-GAGATGTCA-3′ (position 714, negative strand) and 5′-TGCAGAGAGACTCAGGGCCTA- CC-3′ (position 537, plus strand); and 5′-GACTCTGGGTGTCACAG-GAGAGACC-3′ (position 1163, negative strand)]. MHC class I, β_2-microglobulin, and the constitutively expressed enzyme GAPDH were always amplified simultaneously. PCR products (10 μl) from multiplex PCR were digested at 37°C with the enzymes Bgl I, Bgl II, and Apa I (Boehringer Mannheim), which cut the PCR fragments of GAPDH, β_2-microglobulin, and MHC class I, respectively, at a single internal side.
11. Mixed hippocampal cell cultures were treated with IFN-γ (100 U/ml) and TTX for 72 hours; we labeled the cultures with monoclonal antibody OX-18 specific for MHC class I by adding antibodies 10 μg/ml; Serotec) to the culture for 30 min. Cells were washed with phosphate-buffered saline and fixed with 4% paraformaldehyde. After blocking with 2% goat serum and 2% bovine albumin, cells were visualized with fluorochrome Cy3–conjugated goat antibodies to mouse immunoglobulin (Dianova; 10 μg/ml). Cells permeabilized with 0.1% Triton X-100 were intracellularly stained with mouse monoclonal antibodies specific for MAP2 (Sigma; 10 μg/ml) followed by fluorochrome (dichlorotriazinyl) aminofluorescein-conjugated goat antibodies to mouse immunoglobulin (Dianova; 10 μg/ml). Twenty optical sections along the *z* axis were recorded by confocal laser scanning microscopy (Zeiss 410 and Leica). Baseline labeling levels were determined by parallel staining with irrelevant monoclonal antibodies (10 μg/ml) followed by fluorochrome Cy3–conjugated goat antibodies to mouse immunoglobulin.
12. We thank Ch. Czoppelt for technical assistance on the confocal laser microscopy, E. Hansert for statistical advice, M. Schwab for critically reading the manuscript, and G. Garotta (Hoffmann–La Roche) for rat IFN-γ and IFN-γ–specific antibodies. H.N. was holder of a scholarship from the Deutsche Forschungsgemeinschaft.

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Monitoring Release of Neurotrophic Activity in the Brains of Awake Rats

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Intracerebral microdialysis of awake rats was used to monitor the possible release of neurotrophic factors from brain cells in response to injury and excitation. Perfusates were tested with ganglia bioassays and enzyme immunoassay. Trophic activity was released after implantation of the microdialysis probe into the hippocampus but not into the striatum, as assessed by increased nerve fiber outgrowth from Remak's ganglion. Kainic acid treatment significantly increased the release of trophic activity from hippocampal sites. These findings suggest that the brain responds to mechanical injury as well as to certain excitatory stimuli by regional extracellular release of neurotrophic activity that is not identical to the actions of known neurotrophic factors.

It has been suggested that neurons in the brain may respond to different forms of stress by increased synthesis and release of neurotrophic or neuroprotective factors. However, there is no evidence that such stress-induced release to the extracellular compartment of the brain occurs in vivo. Although the presence of mRNA coding for three of the four known mammalian neurotrophins (*1*) [nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3)] has been demonstrated in neurons at the mRNA level by in situ hybridization (*2*), only two of the corresponding proteins, NGF and BDNF, have actually been demonstrated in vivo in the brain (*1*). In order to obtain information about the regional presence of neurotrophins in the brain, one possibility is to develop specific antibodies for immunohistochemical localization, a technique that has allowed cellular localization of BDNF protein in the brain (*3*). A second possibility is to use bioassays, taking advantage of the unique patterns of fiber growth responses seen, for instance, with different members of the neurotrophin family and with several different ganglia (*4*).

In the present study, we tested a new microdialysis probe with a membrane capable of dialyzing proteins the size of neurotrophins. We used this probe in vivo to monitor neurotrophic bioactivity in the hippocampus and striatum of rats and tested the effects of two different epileptogenic treatments. Released bioactivity was analyzed by addition of the perfusates to different ganglia and by enzyme immunoassay (EIA).

The dialysis probe was tested by in vitro dialysis of NGF solutions (*5*). NT-3 protein was measured with an EIA (*6*) that used a recently characterized antibody (*7*). The bioassay was done as described (*8*), using chick embryo spinal, sympathetic, ciliary, and nodose ganglia, as well as the unique Remak's autonomic ganglion from the dorsal mesorectum (*4*).

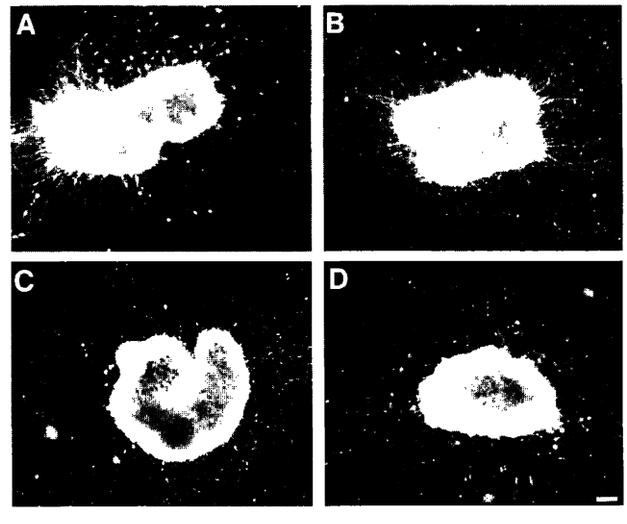

Fig. 1. Nerve fiber outgrowth from Remak's ganglion (**A** and **B**) and sympathetic ganglion (**C** and **D**) tested with hippocampal perfusates. After implantation of the microdialysis probe into the dorsal hippocampus (A and C), perfusates significantly stimulated nerve fiber outgrowth from Remak's ganglion (A) but not from the sympathetic ganglion (C). Kainic acid–induced seizures (B and D) markedly enhanced nerve fiber outgrowth from Remak's ganglion (B) but not from the sympathetic ganglion (D). Scale bar, 150 mm. (A) and (B) are examples of ganglia scored as positive; (C) and (D) are examples of ganglia scored as negative. There is a certain migration of cells from the explanted ganglia in (C) and (D), but no or almost no neurite extension.

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