alterations of both systems regulate body weight (*4*, *20*, *21*). A full understanding of the physiologic effects of the OB protein awaits further study, particularly identification of the OB receptor. Because a principle action of the OB protein is to make an animal thinner, we propose that this 16-kD protein be called leptin, derived from the Greek root leptós, meaning thin.

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13. Mice were individually caged in a pathogen-free environment and acclimated to a diet containing 35% (w/w) Laboratory Rodent Diet 5001 (PMP Feeds), 5.9% (w/w) tapioca pudding mix (General Foods), and 59.1% water, which has an energy content of 1.30 kcal/g. The diet was sterilized by autoclave and packed into 60-mm plastic dishes that were fixed to the tops of 10-mm petri dishes to recover the small amount of food spilled by the animal. The difference in weight of the food dish after each 24-hour period provided a measure of daily food consumption, after correction for evaporation. Weight loss due to evaporation was determined each day by placing food dishes in cages without mice in different areas of the animal room.
14. Each day the pair-fed animals were given the same number of calories that were consumed by the *ob* mice that received protein.
15. The carcass was oven-dried at 90°C for 4 to 7 days until weight was constant. The total body water was calculated as the difference between the two weights (minus blood drawn by cardiac puncture). The dried carcass was homogenized in a blender and duplicate 1-g aliquots were extracted with a soxhlet extraction apparatus containing a 3:1 mixture of chloroform:methanol. The extracted homogenate was dried overnight and reweighed to calculate body fat mass and lean body mass.
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Recombinant Mouse OB Protein: Evidence for a Peripheral Signal Linking Adiposity and Central Neural Networks

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The recent positional cloning of the mouse *ob* gene and its human homolog has provided the basis to investigate the potential role of the *ob* gene product in body weight regulation. A biologically active form of recombinant mouse OB protein was overexpressed and purified to near homogeneity from a bacterial expression system. Peripheral and central administration of microgram doses of OB protein reduced food intake and body weight of *ob/ob* and diet-induced obese mice but not in *db/db* obese mice. The behavioral effects after brain administration suggest that OB protein can act directly on neuronal networks that control feeding and energy balance.

The complex molecular mechanisms by which discrete ingestive behavior, continuous energy expenditure, and dynamic energy storage in adipose tissue are integrated remain unknown (*1*). However, several lines of evidence argue for circulating signals proportional to adipose tissue mass, possibly coming from adipose tissue, that act on the brain to regulate feeding behavior and energy balance (*1*–*3*). Obese *ob/ob* mice are a genetic model of profound, early onset obesity as a recessive trait (*4*), but the molecular basis of their obesity has eluded investigators (*2*, *3*, *5*). The recent cloning of the mouse *ob* gene and its human homolog, by means of positional cloning strategies, has shown that adipose tissue of *ob/ob* mice does not produce a

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mature *ob* gene product. The predicted amino acid sequence appears to have the features of a secreted protein (6).

We have overexpressed and purified to near homogeneity milligram quantities of a biologically active form of recombinant mouse OB protein from an *Escherichia coli* expression system (7). Obese *ob/ob* mice (8) were treated with 6 μg of mouse OB protein per mouse per day or appropriate control solutions twice a day by intraperitoneal (IP) injection for two 5-day periods separated by 2 days without treatment (9). The cumulative food intake over the two treatment periods was significantly reduced by 48 and 21%, respectively, in the group receiving OB protein compared with the control group receiving the vehicle solution (Table 1) (10). When treatment was withdrawn (days 6 and 7), the food intake of the OB protein group during these 2 days increased to amounts similar to, but not exceeding, that of control groups. No other behavioral or adverse effects were observed. The weight loss during the first treatment period in the OB protein group (percent change = −6.8 ± 1.3%; $P < 0.05$) was significantly greater than in the saline (−1.8 ± 0.4%) and vehicle control (−1.4 ± 0.7%) groups (Fig. 1) (11). The rapid weight gain observed in the OB protein group during the 2 days without treatment (Fig. 1, days 6 and 7) indicate that the effects of OB protein treatment are reversible. Stabilization of body weight at a reduced level when treatment was resumed indicated that, although the activity of OB protein was clearly reduced, its inhibitory effects on body energy balance of *ob/ob* mice were sustained during treatment. After the final treatment, mice that had been treated with OB protein returned to their pretreatment weight within 5 days.

When similar *ob/ob* mice received by IP injection 3 μg of OB protein per mouse per day for 5 days, food intake and body weight were significantly reduced, but the magnitudes of the reductions were less than those at the higher dose (Table 1). Together these studies show that the effects of OB protein on food intake and body weight were dose-related in *ob/ob* mice. Similar IP administration studies were also performed in diet-induced obese (DIO) mice (12) and obese *db/db* mice (*db* is the *diabetes* gene) (9). In DIO mice, OB protein reduced food intake and body weight in a dose-related manner (Table 1). However, when normal-weight control mice were treated with OB protein (12 μg per mouse per day), only a slight reduction in food intake was observed (Table 1). In *db/db* mice, administration of mouse OB protein (6 μg per mouse per day) had no effect on cumulative food intake or body weight (Table 1).

These experiments demonstrate that recombinant mouse OB protein is biologically active and has the expected dose-related activity to reduce the degree of obesity of *ob/ob* and DIO mice. However, in obese *db/db*

Table 1. Effect of repeated IP administration of mouse OB protein on food intake and body weight in obese and lean mice. The cumulative food intake and the change in body weight over the treatment period are given for mice receiving two identical IP injections each day of saline, vehicle control, or recombinant OB protein. Data are mean ± SEM. All experimental groups consisted of two cages of three mice per cage, and treatment periods were 5 days except when indicated. The treatment doses for OB protein are per mouse per day. Sa, saline; Vh, vehicle control.

Treatment and OB dose	Initial weight (g)	Cumulative food intake (grams per three mice)	Change in weight (g)
	14-week-old ob/ob *mice*		
Sa	50 ± 1	44.3 ± 1.9	−0.9 ± 0.2
Vh	49 ± 2	49.5 ± 10.2	−0.7 ± 0.4
OB 6 μg	48 ± 1	25.5 ± 1.2*	−3.3 ± 0.7*
	10-week-old ob/ob *mice*		
Vh	44 ± 2	42.7 ± 4.4	0.6 ± 0.4
OB 3 μg	47 ± 1	33.1 ± 2.1*	−2.4 ± 0.6*
	13-week-old DIO mice		
Sa	37 ± 1	36.0 ± 3.3	−1.9 ± 0.8
Vh	38 ± 1	43.9 ± 3.3	−0.6 ± 0.8
OB 6 μg	38 ± 1	35.5 ± 1.1*	−1.5 ± 0.6
	15-week-old DIO mice†‡		
Sa†§	37 ± 1	21.7 ± 5.6	−1.7 ± 0.9
Vh§	38 ± 1	26.7 ± 7.4	−1.8 ± 1.1
OB§ 30 μg	36 ± 1	13.2 ± 1.4*	−3.3 ± 0.7*
	15-week-old lean AKR/J mice†		
Vh	27 ± 1	28.7 ± 3.8	−1.4 ± 0.6
OB 12 μg	26 ± 1	24.2 ± 2.4	−1.3 ± 0.6
	8-week-old db/db *mice*		
Sa§	41 ± 1	53.1	−1.2 ± 0.2
Vh	39 ± 1	55.3 ± 0.8	−0.6 ± 0.2
OB 6 μg	41 ± 1	60.2 ± 7.2	−1.0 ± 0.1

*Significant difference between OB protein and vehicle control groups with $P < 0.05$ by ANOVA (17). †Three days of treatment. ‡One mouse per cage. §Three mice per group.

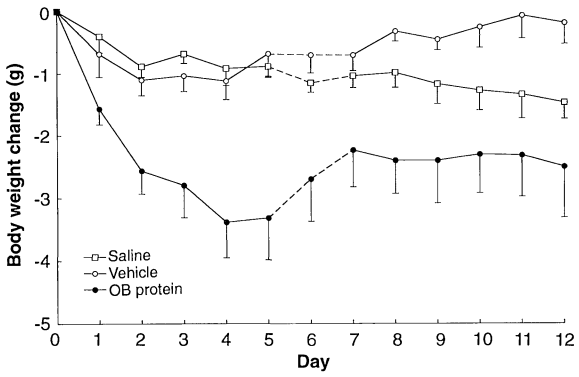

Fig. 1. Repeated IP injection of recombinant mouse OB protein for two 5-day periods reduced body weight in obese *ob/ob* mice. The graph shows the change in body weight from the pretreatment weight for obese *ob/ob* mice treated with two daily IP injections (0.1 ml each) of saline (open squares), vehicle control (open circles), or recombinant mouse OB protein (two injections of 3 μg per mouse per day; closed circles) (10). Values represent the mean ± SEM change in body weight in grams for groups of six mice for the two 5-day periods of treatment (solid lines) separated by 2 days without treatment (dashed lines). Pretreatment body weights and food intake during the first treatment period are given in Table 1. Body weight of the OB protein group is significantly different from the vehicle control group with $P < 0.05$ by two-way ANOVA with repeated measures (17) [effect of treatment, $F(2,215) = 74.2$ ($P < 0.0001$); effect of time, $F(11,215) = 0.97$ (not significant)].

Table 2. Effect of a single ICV administration of mouse OB protein on food intake and body weight gain in lean (+/?) and *db/db* mice. Cumulative 7-hour and 24-hour food intake and body weight gain in the 24 hours after overnight fasting and ICV injection of 1 μg of OB protein per mouse are given. Data are mean ± SEM. Body weight was measured before the ICV injection and 24 hours later. Mice were 6 to 7 weeks old.

Treatment	Initial weight (g)	Cumulative food intake (g)		Weight gain (g)
		7 hours	24 hours	
		Lean (+/?) mice		
Artificial CSF ($n = 5$)	18 ± 1	1.0 ± 0.1	4.2 ± 0.2	2.5 ± 0.7
Vehicle ($n = 6$)	18 ± 1	0.5 ± 0.1	3.6 ± 0.3	1.6 ± 0.3
OB ($n = 9$)	18 ± 1	0.3 ± 0.1*	2.3 ± 0.4*	0.5 ± 0.3*
		db/db *mice*		
Artificial CSF ($n = 9$)	29 ± 1	1.6 ± 0.2	6.6 ± 0.4	1.7 ± 0.1
Vehicle ($n = 10$)	28 ± 1	1.4 ± 0.1	6.3 ± 0.2	1.2 ± 0.2
OB ($n = 12$)	30 ± 1	1.2 ± 0.1	5.7 ± 0.5	1.1 ± 0.1

*Significant difference between OB protein and vehicle control groups with $P < 0.05$ by ANOVA (17).

mice, OB protein at a dose of 6 μg per mouse per day, which was effective in *ob/ob* mice, has no effect on food intake or body weight.

After mice were fasted overnight, single doses of recombinant OB protein (3 μg per mouse) or saline or vehicle control were administered by intravenous (IV) injection through chronically implanted jugular vein cannulas (*13*). In contrast to mice receiving saline or vehicle control, the food intake of the *ob/ob* mice injected with OB protein was significantly reduced and remained suppressed by 45% compared with mice treated with the vehicle control (Fig. 2A). No other behavioral or adverse effects were observed. However, body weight gain 24 hours after injection was not different. Similar results were obtained in lean littermate control mice (+/?) (*8*), but the magnitude of the reduction in food intake was attenuated and body weight gain was slightly, but significantly, reduced compared with the vehicle control group (Fig. 2B). Subgroups of mice in which the cannulas remained unblocked received injections of both recombinant OB protein and vehicle control in separate trials. The percent reduction of food intake after injection was greater in obese than in lean mice [46 ± 15% (n = 5) and 14 ± 8% (n = 6), respectively].

Single doses of recombinant mouse OB protein (1 μg per mouse) or appropriate control solutions were injected into the lateral ventricle in mice through chronically implanted intracerebroventricular (ICV) cannulas (*14*). During the first 30 min after ICV injection, most *ob/ob* mice ate after only a short delay. In contrast to mice receiving artificial cerebrospinal fluid (CSF) or vehicle control, the *ob/ob* mice injected with OB protein stopped eating after the first 30 min and most mice did not eat again during the remaining 6.5 hours of the experiment (Fig. 2C). In contrast to mice receiving CSF or vehicle control, mice treated with mouse OB protein did not regain any of the weight they had lost during the pre-injection overnight fast (body weight gain 24 hours after injection: CSF, 1.7 ± 0.4 g; vehicle control, 2.4 ± 0.3 g; and OB protein, −0.8 ± 0.3 g; P < 0.0001) (*15*). In studies in lean (+/?) mice, ICV injection of mouse OB protein also caused a reduction in food intake and attenuated the regain of body weight (Table 2). In contrast, ICV injection of OB protein did not reduce food intake or body weight gain in obese *db/db* mice (Table 2).

The demonstration that recombinant mouse OB protein can reduce food intake and body weight in *ob/ob* and DIO obese and lean mice provides further evidence for the hypothesis that a circulating protein-based signal, generated in adipose tissue, acts on central neuronal networks and plays an important role in the regulation of feeding behavior and energy balance (*2*, *5*). The duration of action of OB protein is longer than with many neuropeptides that modulate feeding and is reminiscent of that of centrally administered insulin (*16*).

The failure to observe a reduction in food intake and body weight after administration of mouse OB protein to obese *db/db* mice either peripherally or centrally, at doses that were effective in *ob/ob* mice, is consistent with the hypothesis that the genetic defect in *db/db* mice renders them unable to appropriately respond to OB protein, perhaps because of a defect in the OB protein receptor or the postreceptor signalling pathway (*2*, *5*, *6*).

The demonstration that mouse OB protein can alter feeding behavior and energy balance when placed directly in the lateral ventricle of the brain of obese *ob/ob* and lean (+/?) mice suggests that one or more brain areas are among the target sites for mouse OB protein. The identification of these brain areas will facilitate studies aimed at elucidating the neuronal pathways and networks and the underlying molecular mechanisms by which OB protein can influence feeding behavior and energy balance.

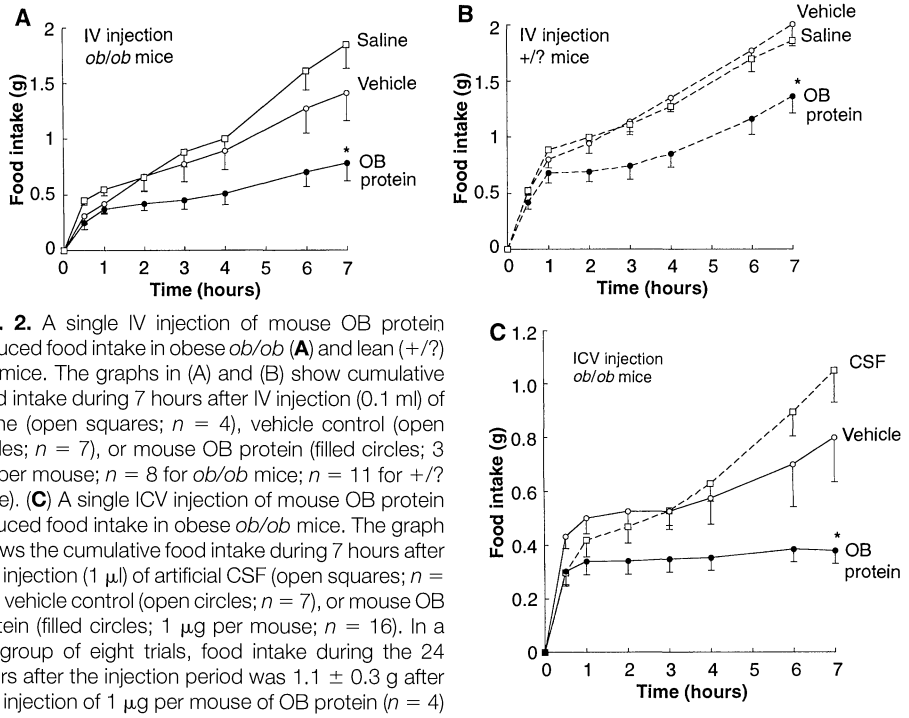

Fig. 2. A single IV injection of mouse OB protein reduced food intake in obese *ob/ob* (**A**) and lean (+/?) (**B**) mice. The graphs in (A) and (B) show cumulative food intake during 7 hours after IV injection (0.1 ml) of saline (open squares; n = 4), vehicle control (open circles; n = 7), or mouse OB protein (filled circles; 3 μg per mouse; n = 8 for *ob/ob* mice; n = 11 for +/? mice). (**C**) A single ICV injection of mouse OB protein reduced food intake in obese *ob/ob* mice. The graph shows the cumulative food intake during 7 hours after ICV injection (1 μl) of artificial CSF (open squares; n = 15), vehicle control (open circles; n = 7), or mouse OB protein (filled circles; 1 μg per mouse; n = 16). In a subgroup of eight trials, food intake during the 24 hours after the injection period was 1.1 ± 0.3 g after ICV injection of 1 μg per mouse of OB protein (n = 4) compared with 6.4 ± 0.2 g after no injection (n = 4). The asterisk indicates significant differences from the vehicle control group with P < 0.05 by two-way ANOVA without repeated measures (*17*). [For (A), effect of treatment, $F(2,14)$ = 10.67 (P < 0.0015); effect of time, $F(7,14)$ = 15.95 (P < 0.0001). For (B), effect of treatment, $F(2,14)$ = 15.65 (P < 0.003); effect of time, $F(7,14)$ = 55.1 (P < 0.0002). For (C), effect of treatment, $F(2,14)$ = 8.08 (P < 0.0046); effect of time, $F(7,14)$ = 9.65 (P < 0.002).]

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8. Male and female C57BL/6J *ob/ob* and +/?, C57BL/KsJ *db/db*, and AKR/J mice used in this study were obtained from the Jackson Laboratory, Bar Harbor, ME. Lean littermate control mice were not separated by genotype (either +/+ or +/*ob*) and are referred to as lean (+/?) mice. Mice (6 to 16 weeks of age) were housed in plastic cages under constant environmental conditions with a 12-hour dark:12-hour light cycle and had free access to mouse chow (except when fasted overnight before IV or ICV experiments) and water. All experimental procedures were performed according to protocols approved by the institutional Roche Animal Care and Use Committee.
9. Mice received two identical IP injections (0.1 ml each) each treatment day (at the beginning of the dark

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*).
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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)].
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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,

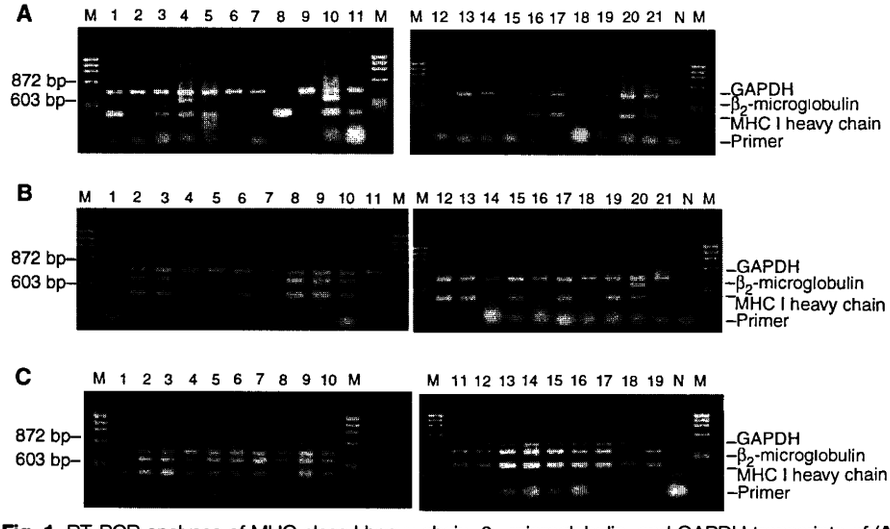

Fig. 1. RT-PCR analyses of MHC class I heavy chain, β_2-microglobulin, and GAPDH transcripts of (**A**) untreated, (**B**) IFN-γ–treated, and (**C**) IFN-γ– plus TTX-treated neurons. Only after treatment with IFN-γ plus TTX did all neurons express mRNA of β_2-microglobulin and MHC class I (*7, 10*).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