American Journal of Pathology, Vol. 148, No. 1, January 1996

Altered Neurofilament Expression Does Not Contribute to Lewy Body Formation

Catherine Bergeron,*† Connie Petrunka,* Luitgard Weyer,* and Michael S. Pollanen*

From the Centre for Research in Neurodegenerative Diseases and Department of Pathology, University of Toronto, and the Department of Pathology (Neuropathology),† The Toronto Hospital, Toronto, Ontario, Canada*

Lewy bodies (LBs) are cytoskeletal alterations found in several neurodegenerative disorders. Although neurofilaments are the main constituent of the LB, the precise mechanisms that underlie their formation remain speculative. To examine the pathogenesis of this inclusion, we measured the mRNA level of the low molecular weight neurofilament subunit in the nigral dopaminergic neurons of patients with LB disorders and neurologically normal controls. We found a small but significant decrease in the mean mRNA values in the LB group as compared with controls. However, a comparison of LB-bearing and non-LB-bearing neurons on the same section showed no significant difference between these two neuronal populations. We conclude that altered neurofilament expression is not a major contributory event in the pathogenesis of the LB. The decrease in neurofilament mRNA expression observed in the overall nigral dopaminergic neuronal population of LB disorders probably represents a nonspecific response to neuronal injury independent of LB formation. (Am J Pathol 1996, 148:267–272)

Several neurodegenerative diseases are characterized by extensive abnormalities in the neuronal cytoskeleton. A typical cytoskeletal inclusion body found in such disorders is the Lewy body (LB), an eosinophilic hyaline inclusion consistently observed in selectively vulnerable populations in idiopathic Parkinson's disease (PD) and diffuse LB disease (DLB). Although characteristic of LB disorders, they are increasingly recognized in amyotrophic lateral sclerosis[1] and Alzheimer's disease.[2] On this basis, the LB appears to be a major inclusion body that is formed in several neurodegenerative disorders.

Direct and indirect analysis of the LB has shown that its constitutive fibrils contain all three neurofilament (NF) subunits of molecular masses 68 kd (NF-L), 150 kd (NF-M), and 200 kd (NF-H) in both phosphorylated and nonphosphorylated forms.[3–8] The NF epitopes are spatially distributed with a predominance of NF-L or amino termini of NF subunits in the central portion of the inclusion, a phenomenon that suggests a possible segregation of proteolytic fragments.[9] The presence of ubiquitin in the LB also supports the concept of ongoing proteolysis.[5,10–12] Furthermore, the LB fibrils are detergent insoluble,[13,8] probably as a result of filament cross-linking. Athough neurofilaments likely accumulate in LBs as a result of post-translational modifications, increased NF synthesis may participate in LB formation. To further understand the pathogenesis of this inclusion, we therefore examined the expression of the core NF subunit (NF-L) in nigral dopaminergic neurons of individuals with LB disorders and normal controls.

Materials and Methods

Case Selection

Six cases of PD, four cases of DLB, and eight age-matched control subjects with no neurological disease were obtained from the archives of the Division of Neuropathology at the Toronto Hospital. The DLB cases were characterized by a widespread distribution of LBs in neocortex in addition to the subcortical pathological changes typical of PD. All cases with concomitant Alzheimer's disease were excluded from the study. Few cases displayed preamyloid

Supported by the Parkinson Foundation of Canada and the Medical Research Council of Canada.

Accepted for publication September 21, 1995.

Address reprint requests to Dr. C. Bergeron, Centre for Research in Neurodegenerative Diseases, 6 Queen's Park Crescent, Toronto, Ontario, Canada M5S 1A8.

Table 1. *Characteristics of the Cases Used for the Study*

Case	Diagnosis	PMI	Age	FFT
P1	DLB	7	73	35
P2	PD	5	73	24
P3	PD	12	76	12
P4	PD	7	90	16
P5	PD	17	74	15
P6	PD	8	76	37
P7	DLB	15	61	33
P8	DLB	5	79	26
P9	DLB	12	81	18
P10	PD	6	68	18
Mean ± SD		9 ± 4	75 ± 8	23.4 ± 9.0
C7	Pulmonary emboli	13	54	10
C8	Myocardial infarct	6	67	20
C9	Respiratory failure	9	67	10
C10	Myocardial infarct	16	83	10
C11	Myocardial infarct	9	72	17
C12	Sepsis	12	89	16
C13	Sepsis	10	73	16
C14	Myocardial infarct	7	75	22
Mean ± SD		10 ± 3	72 ± 10	15 ± 4.7*

PMI, post-mortem interval (hours); FFT, formalin fixation time (days).
*$P = 0.03$.

deposits (diffuse plaques) with no significant neuritic component. Neurofibrillary tangles were rare and confined to medial temporal structures. The cases were matched for age and postmortem interval (details provided in Table 1).

The brains were fixed in neutral buffered formalin for 10 to 37 days (mean ± SD = 20 ± 8 days) and selected blocks were embedded in paraffin. In each case, the midbrain at the level of the third cranial nerve nucleus was available for study.

In Situ *Hybridization*

Murine cDNA NF68 was provided by Dr. S. A. Lewis.[14] This 1.2-kb fragment codes for the second α-helical coil and carboxy tail region of the low molecular weight neurofilament subunit and has extensive homology with the human gene.[15] This probe is well characterized and has been used for *in situ* hybridization studies of Alzheimer's disease[16,17] and amyotrophic lateral sclerosis.[18] The cDNA was inserted in the plasmid vector BRLpT7/T13–19. The plasmid was linearized with *Sca*I (sense mRNA) and *Bam*HI (antisense mRNA) and transcribed *in vitro* using T3 and T7 polymerases with 25 μmol/L each of [^{3}H]UTP and [^{3}H]CTP following the protocol supplied by Bethesda Research Laboratories (Gaithersburg, MD). Transcripts were isolated by phenol extraction and repeated ethanol precipitations and reduced to a length of 50 to 150 bases by limited alkaline hydrolysis.[19] *In situ* hybridization was performed as previously described[17,18] with an exposure time of 56 days. Controls for probe specificity included hybridization with the sense strand and hybridization after RNAse treatment. The exclusive localization of the NF-L probe to nerve cells also provided confirmatory evidence of its specificity. For the first part of the study, all sections from PD, DLB, and control subjects were hybridized simultaneously to maintain constant experimental conditions. Six additional slides from each DLB and PD case were hybridized in three additional experiments to obtain sufficient numbers of LBs for statistical analysis.

Quantification

In each case, the substantia nigra pars compacta was divided in three equal regions (medial, middle, and lateral) to ensure uniform sampling throughout the structure. Only dopaminergic neurons were used for the study, identified by the presence of neuromelanin. Fifteen consecutive melanized neurons from each subregion were then randomly selected from low magnification photographs. Dopaminergic neurons without melanin-free cytoplasm in the plane of section were excluded from the analysis. Forty-five cells were examined in all controls and most LB cases (Table 1); in the other LB cases all available neurons were used for analysis. A LECO image analysis system (L2001, LECO Instruments, Montreal, PQ, Canada) was used to count autoradiographic grains, based on color detection. Briefly, after a neuron is identified and the color threshold optimized for grain detection, the image of the neuron is manually outlined using a mouse and cursor. A subroutine allows editing of the nuclear area. Through a series of Boolean operations the computer then automatically counts the grains within the outlined area. Further operations include a chord sizing step to eliminate features smaller than two pixels and five erosion cycles to resolve individual grains. Background determinations are performed on ten representative 1000-μm^2 areas of neuropil. Grain counts are expressed as a grain density (number of grains per square micron) after background correction. Because of color overlap between melanin and autoradiographic silver grains, we have modified our image analysis routine for pigmented neurons; after a pigmented neuron is selected, the melanin content is first outlined, as well as the cross-sectional area. The color threshold is then optimized for grain detection and, through a series of Boolean operations, the grain count is performed on the melanin-free cytoplasm as described above. Fifteen neurons from the adjacent red nucleus were also analyzed in each

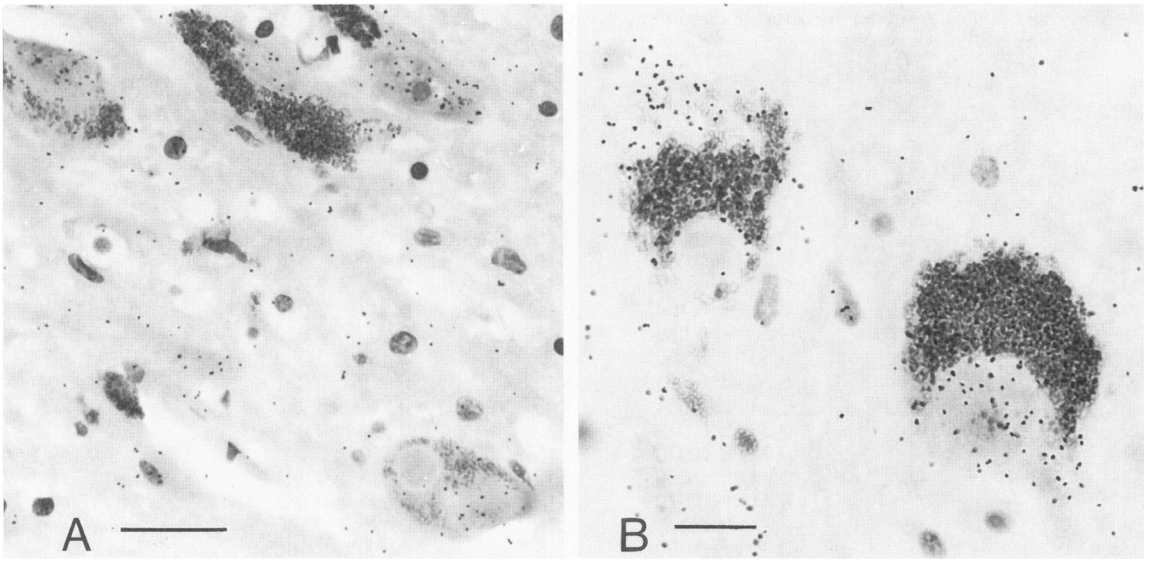

Figure 1. *NF-L mRNA hybridization grains in dopaminergic LB-bearing and non-LB-bearing neurons. The autoradiographic grains are present chiefly in the melanin-free cytoplasm. No grains are observed over the core of the LB, but a few grains can be seen over the halo of the LB.* **A:** *Case P8; scale bar, 20 μm.* **B:** *Case P5; scale bar, 10 μm. Hematoxylin and eosin counterstain.*

case to control for the RNA alterations that occur in human postmortem material as a result of agonal changes, postmortem interval, and fixation.[20,21] There was a good correlation between the mean values of both regions in each case (LB cases, r = 0.762, P = 0.017; controls, r = 0.787, P = 0.020) and we therefore normalized by dividing the mean NF-L mRNA value of the dopaminergic neurons in each case by that of the red nucleus and expressed the values as a ratio. The mean NF-L mRNA values of both groups were compared using the unpaired two-tailed t-test and the F-ratio testing of group variances. Regression analysis was performed to determine the relationship between NF-L mRNA levels and age, postmortem interval, and fixation time. The square root of each neuronal grain density after background correction was used for all statistical analyses.

In the second part of the study, we measured NF-L mRNA levels in all LB-bearing neurons from each case of PD and DLB. Each LB-bearing neuron was paired with an immediately adjacent non-LB-bearing neuron for analysis. The grain density was measured over the melanin-free cytoplasm excluding the LB. The mean value of both measurements was obtained for each case as well as the total LB pool and compared using the paired two-tailed t-test.

Results

NF-L mRNA hybridization grains were confined to the perikaryon and proximal portion of the neuronal processes (Figure 1). The grains were most abundant in the melanin-free cytoplasm; they were not present over the dense core of the LB but were occasionally observed in the surrounding corona (Figure 1). In four LB cases, marked neuronal loss was present and only a limited number of neurons were available for analysis (Table 2). NF-L mRNA levels were not significantly different in the dopaminergic neurons of the substantia nigra of control and DLB subjects. In the red nucleus, however, control cases exhibited a wider range of values with a small shift toward lower values (F-ratio testing of group variances 4.954, P = 0.0436; Figure 2). When the results were expressed as a ratio of the mean NF-L mRNA levels of dopaminergic neurons over red nucleus neurons to correct for the effect of RNA degradation (Figure 3), the NF-L ratios were slightly lower in the LB cases (P = 0.112) and a small shift toward lower values was again observed (F-ratio testing of group variances 7.742, P = 0.0119). Regression analysis showed no significant correlation between NF-L mRNA levels and age, postmortem interval, and fixation time.

A total of 60 pairs of neurons were analyzed from eight cases (Table 3). The mean NF-L mRNA level was slightly lower in LB-bearing neurons (paired t-test, two-tailed, P = 0.0591; Figure 4). The differences were very small and in case P5 the levels were actually slightly higher in the LB-bearing neurons. When all 60 pairs were pooled, NF-L mRNA levels were also slightly lower in the LB-bearing neurons (P = 0.100).

Table 2. *NF-L mRNA Levels in Neurons of the Substantia Nigra and Red Nucleus*

Case	S. Nigra n	S. Nigra NF-L mRNA*	Red Nucleus n	Red Nucleus NF-L mRNA	NF-L ratio†
P1	45	0.288 ± 0.067	15	0.438 ± 0.065	0.658
P2	40	0.339 ± 0.061	15	0.444 ± 0.073	0.763
P3	45	0.349 ± 0.070	15	0.476 ± 0.070	0.733
P4	45	0.404 ± 0.061	15	0.539 ± 0.065	0.75
P5	45	0.455 ± 0.073	15	0.634 ± 0.036	0.718
P7	45	0.272 ± 0.067	15	0.431 ± 0.072	0.63
P8	41	0.285 ± 0.057	15	0.344 ± 0.037	0.829
P9	18	0.332 ± 0.104	15	0.447 ± 0.077	0.743
P10	7	0.256 ± 0.085	15	0.503 ± 0.072	0.509
Mean ± SD		0.331 ± 0.065		0.473 ± 0.080‡	0.703 ± 0.093§
C7	45	0.366 ± 0.101	15	0.656 ± 0.072	0.558
C8	45	0.246 ± 0.064	15	0.211 ± 0.050	1.170
C9	45	0.335 ± 0.079	15	0.393 ± 0.046	0.851
C10	45	0.215 ± 0.076	15	0.208 ± 0.044	1.033
C11	45	0.347 ± 0.089	15	0.525 ± 0.081	0.660
C12	45	0.415 ± 0.072	15	0.531 ± 0.052	0.780
C13	45	0.371 ± 0.071	15	0.625 ± 0.050	0.593
C14	45	0.356 ± 0.050	15	0.290 ± 0.065	1.227
Mean ± SD		0.331 ± 0.067		0.429 ± 0.18	0.859 ± 0.259

*Mean mRNA levels expressed as grains per square micron.
†Ratio of mean substantia nigra/red nucleus NF-L mRNA levels for each case.
‡P = 0.044, F-ratio testing of group variances.
§P = 0.012, F-ratio testing of group variances.

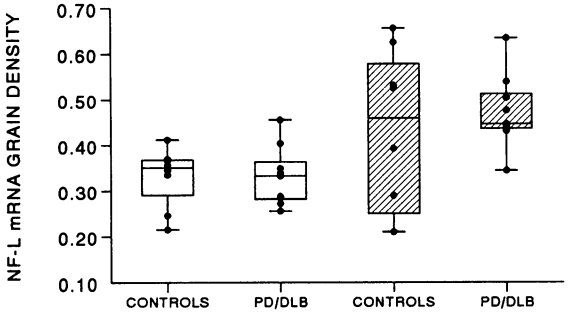

Figure 2. *Box plots of the mean NF-L mRNA grain density in the substantia nigra* (open bar) *and red nucleus* (hatched bar). *There is no significant difference between the two groups in the nigra. In the red nucleus, there is a greater scatter with a shift toward lower values* (F-*ratio testing of group variances,* P = *0.0436*).

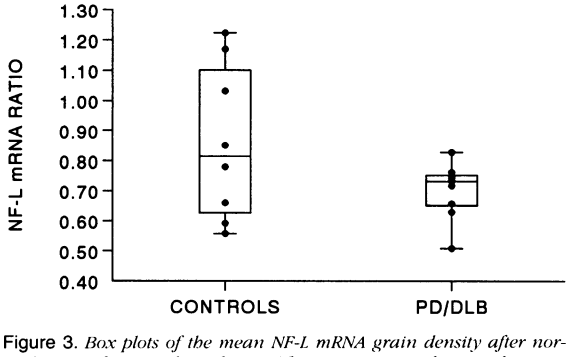

Figure 3. *Box plots of the mean NF-L mRNA grain density after normalization for RNA degradation. The NF-L mRNA values are lower in the Lewy body group, with a small shift toward lower values* (F-*ratio testing of group variances,* P = *0.0119*).

Discussion

We found a small but significant decrease in the mean NF-L mRNA levels of the overall nigral dopaminergic neuronal population in a group of patients with LB disorders when compared with age-matched controls. When we compared the NF-L mRNA levels in LB-bearing neurons to that of non-LB-bearing neurons in a direct paired study, however, we were unable to demonstrate a significant difference in NF-L mRNA levels between these two neuronal populations. These results suggest that NF expression is unlikely to represent an important step in the development of LB. The difference observed between the LB and control groups in the overall nigral dopaminergic neuronal population, which consists mostly of non-LB-bearing neurons, probably reflects a nonspecific response to neuronal injury independent of LB formation. Such a decrease in NF mRNA levels is seen in several pathological conditions such as axotomy,[22,23] aluminium intoxication,[24–26] Alzheimer's disease,[16,17,27,28] and amyotrophic lateral sclerosis.[18] NFs are important for the maintenance of axonal caliber and conduction velocity, but their absence is well tolerated, as seen in mutant quails[29] and transgenic mice expressing a neurofilament-β-galactosidase fusion protein.[30] Decreased NF-L mRNA levels in LB disorders may therefore represent a general response of the neuron to injury,[31] with down-regulation of the expression of certain proteins that are not essential to its survival, such as

Table 3. *NF-L mRNA Levels in Dopaminergic Neurons with and without LB*

Case	n	No LB	LB	Difference*
P1	11	0.290 ± 0.044	0.289 ± 0.053	0.001
P2	11	0.289 ± 0.074	0.287 ± 0.054	0.002
P3	7	0.298 ± 0.070	0.285 ± 0.098	0.013
P4	9	0.363 ± 0.070	0.312 ± 0.087	0.051
P5	8	0.444 ± 0.105	0.449 ± 0.065	−0.055
P6	3	0.340 ± 0.026	0.290 ± 0.049	0.050
P7	NA	NA	NA	NA
P8	8	0.313 ± 0.037	0.255 ± 0.038	0.056
P9	5	0.366 ± 0.060	0.365 ± 0.0835	0.001
P10	NA	NA	NA	NA
Mean ± SD		0.338 ± 0.053	0.317 ± 0.062†	0.021 ± 0.023
All LB	60	0.331 ± 0.083	0.312 ± 0.088	0.019 ± 0.088

*Difference in the grain density between non-LB-bearing neurons and LB-bearing neurons.
†$P = 0.059$, two-tailed paired *t*-test.
NA, not available.

NF. The down-regulation of tyrosine hydroxylase in PD further supports this concept.[32]

Hill et al[33] found decreased mRNA levels in both the low (NF-L) and high (NF-M) molecular weight subunits of the neurofilament in LB disorders; they report a greater decrease in the mean nigral dopaminergic neuronal NF-L mRNA levels between LB cases and controls than that observed in our study, a difference that probably reflects the inclusion in their study of cases of Alzheimer's disease, a disorder in which there is a marked decrease in NF-L mRNA levels.[16,17,27,28] In contrast to our results, Hill also found a significant decrease in NF-L and NF-H mRNA levels in LB-bearing neurons; this discrepancy is probably due to our different experimental approach, namely the use of a paired design to compare LB- and non-LB-bearing neurons on the same slide.

The findings of our study support the general conclusion that LBs are most likely formed as a result of complex post-translational alterations including abnormal accumulation, aggregation, aberrant phosphorylation, cross-linking, and proteolysis of NF subunits.[34] The decrease in NF-L mRNA expression observed in the overall nigral dopaminergic neuronal population of LB disorders probably represents a nonspecific response to neuronal injury unrelated to LB formation.

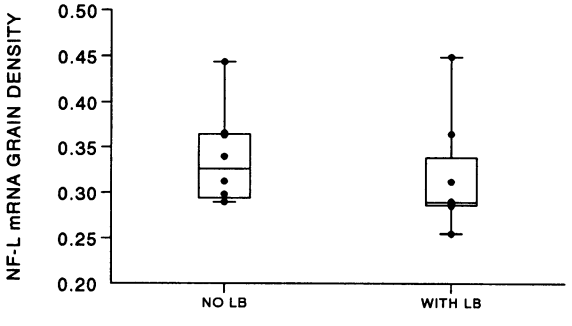

Figure 4. *Box plots of the mean NF-L mRNA grain density in LB-bearing and non-LB-bearing neurons in the dopaminergic nigral neurons of LB cases. The values are slightly lower in the LB-bearing group but fail to reach statistical significance. (Paired t-test, two tailed, P = 0.0591).*

References

1. Kato T, Katigiri T, Hirano A, Sasaki H, Arai S: Sporadic lower motor neuron disease with Lewy body-like inclusions: a new subgroup? Acta Neuropathol 1988, 76:208–211
2. Bergeron C, Pollanen M: Lewy bodies in Alzheimer disease: one or two diseases? Alzheimer Dis Assoc Disord 1989, 3:197–204
3. Goldman J, Yen S-H, Chiu F-C, Peress N: Lewy bodies of Parkinson's disease contain neurofilament antigens. Science 1983, 221:1082–1084
4. Forno L, Sternberger L, Sternberger N, Strefling A, Swanson K, Eng L: Reactions of Lewy bodies with antibodies to phosphorylated and non-phosphorylated neurofilaments. Neurosci Lett 1986, 64:253–258
5. Galloway P, Grundke-Iqbal I, Iqbal K, Perry G: Lewy bodies contain epitopes both shared and distinct from Alzheimer neurofibrillary tangles. J Neuropathol Exp Neurol 1988, 47:654–663
6. Schmidt ML, Murray J, Lee VM-Y, Hill WD, Wertkin A, Trojanowski JQ: Epitope map of neurofilament protein domains in cortical and peripheral nervous system Lewy bodies. Am J Pathol 1991, 139:53–65
7. Pollanen MS, Bergeron C, Weyer L: Detergent-insoluble cortical Lewy body fibrils share epitopes with neurofilament and τ. J Neurochem 1992, 58:1953–1956
8. Pollanen MS, Bergeron C, Weyer L: Deposition of detergent-resistant neurofilaments into Lewy body fibrils. Brain Res 1993, 603:121–124
9. Hill WD, Lee VM-Y, Hurtig HI, Murray JM, Trojanowski JQ: Epitopes located in spatially separate domains of each neurofilament subunit are present in Parkinson's

disease Lewy bodies. J Comp Neurol 1991, 309:150–160
10. Love S, Saitoh T, Quijada S, Cole G, Terry R: ALz-50, ubiquitin and tau immunoreactivity of neurofibrillary tangles, Pick bodies and Lewy bodies. J Neuropathol Exp Neurol 1988, 47:393–405
11. Bancher C, Lassmann H, Budka H, Jellinger K, Grudke-Iqbal I, Iqbal K, Wiche G, Seitelberger F, Wisniewski HM: An antigenic profile of Lewy bodies: immunocytochemical indication for protein phosphorylation and ubiquitination. J Neuropathol Exp Neurol 1989, 48:81–93
12. Lowe J, Blanchard A, Morrell K: Carboxy-terminal hydrolase (PGP 9.5) is selectively present in ubiquitinated inclusion bodies characteristic of human neurodegenerative disease. J Pathol 1990, 161:153–160
13. Galloway PG, Mulvihill P, Perry G: Filaments of Lewy bodies contain insoluble cytoskeletal elements. Am J Pathol 1992, 140:809–822
14. Lewis S, Cowan NJ: Genetics, evolution, and expression of the 68,000-mol-wt neurofilament protein: isolation of a cloned cDNA probe. J Cell Biol 1985, 100: 843–850
15. Julien J-P, Grosveld F, Yazdanbaksh K, Flavell D, Meijer D, Mushynski W: The structure of a human neurofilament gene (NF-L): a unique exon-intron organization in the intermediate filament gene family. Biochim Biophys Acta 1987, 909:10–20
16. Somerville MJ, Bergeron C, Yoong LK, McLachlan DR, Percy ME: Neuronal expression of the light chain neurofilament and superoxide dismutase-1 genes in temporal lobe of Alzheimer brain. Alzheimer's Disease: Basic Mechanisms, Diagnosis and Therapeutic Strategies. Edited by K Iqbal, D McLachlan, B Windblad, H Wisniewski. New York, John Wiley and Sons, 1991, pp 237–241
17. Somerville MJ, Percy ME, Bergeron C, Yoong LKK, Grima EA, McLachlan DRC: Localization and quantitation of 68 kDA neurofilament and superoxide dismutase-1 mRNA in Alzheimer brain. Mol Brain Res 1991, 9:1–8
18. Bergeron C, Beric-Maskarel K, Muntasser S, Weyer L, Somerville MJ, Percy ME: Neurofilament light and polyadenylated mRNA levels are decreased in amyotrophic lateral sclerosis motor neurons. J Neuropathol Exp Neurol 1994, 53:221–230
19. Pardue ML: *In situ* hybridization. Nucleic Acid Hybridization: A Practical Approach. Edited by BD Hames, SJ Higgins. Oxford, IRL Press, 1985, pp 179–202
20. Harrison PJ, Pearson RCA: *In situ* hybridization histochemistry and the study of gene expression in the human brain. Prog Neurobiol 1990, 34:271–312
21. Barton A, Pearson R, Najlerahim A, Harrison P: Pre- and postmortem influences on brain RNA. J Neurochem 1993, 61:1–11
22. Hoffman PN, Cleveland DW: Neurofilament and tubulin expression recapitulates the developmental program during axonal regeneration: induction of a specific β-tubulin isotype. Proc Natl Acad Sci USA 1988, 85: 4530–4533
23. Tetzlaff W, Alexander SH, Miller FD, Bisby MA: Response of facial and rubrospinal neurons to axotomy: changes in mRNA expression for cytoskeletal proteins and GAP-43. J Neurosci 1991, 11:2528–2544
24. Muma NA, Troncoso JC, Hoffman PN, Koo EH, Price DL: Aluminum neurotoxicity: altered expression of cytoskeletal genes. Mol Brain Res 1988, 3:115–122
25. Parhad IM, Krekoski CA, Mathew A, Tran PM: Neuronal gene expression in aluminum myelopathy. Cell Mol Neurobiol 1989, 9:123–138
26. Strong MJ, Mao K, Nerukar VR, Wakayama I, Yanagihara R, Garruto RM: Dose-dependent selective supression of light (NFL) and medium (NFM) but not heavy (NFH) molecular weight neurofilament mRNA levels in acute aluminum neurotoxicity. Mol Cell Neurosci 1994, 5:319–326
27. Guillemette JG, Wong L, McLachlan DRC, Lewis PN: Characterization of messenger RNA from the cerebral cortex of control and Alzheimer afflicted brain. J Neurochem 1987, 47:987–997
28. Clark AW, Krekoski CA, Parhad IM, Liston D, Julien J-P, Hoar DI: Altered expression of genes for amyloid and cytoskeletal proteins in Alzheimer cortex. Ann Neurol 1989, 25:331–339
29. Yamasaki H, Bennett GS, Itakura C, Mizutani M: Defective expression of neurofilament protein subunits in hereditary hypototrophic axonopathy of quail. Lab Invest 1992, 66:734–743
30. Eyer J, Peterson A: Neurofilament-deficient axons and perikaryal aggregates in viable transgenic mice expressing a neurofilament-β-galactosidase fusion protein. Neuron 1994, 12:389–405
31. Hoffman PH, Pollock SC, Striph GG: Altered gene expression after optic nerve transection: reduced neurofilament expression as a general response to axonal injury. Exp Neurol 1993, 119:32–36
32. Javoy-Agid F, Hirsch EC, Dumas S, Duyckaerts C, Mallet J, Agid Y: Decreased tyrosine hydroxylase messenger RNA in the surviving dopamine neurons of the substantia nigra in Parkinson's disease: an *in situ* hybridization study. Neuroscience 1990, 38:245–253
33. Hill WD, Arai M, Cohen JA, Trojanowski JQ: Neurofilament mRNA is reduced in Parkinson's disease substantia nigra pars compacta. J Comp Neurol 1993, 329:328–336
34. Pollanen MS, Dickson DW, Bergeron C: Pathology and biology of the Lewy body. J Neuropathol Exp Neurol 1993, 52:183–191