P300 amplitude, we assume that the P300 manifests the activation of some information processing activity that is invoked by the appearance of task-relevant events, its amplitude inversely related to its expectancy. It has been suggested that this "subroutine" is involved in updating, or revising, the model of the environment maintained in working memory (*16*). The resources on which this updating activity depends seem to be limited in their availability, and, when deployed in the service of one task, their availability to be of service to other tasks is reduced.

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References and Notes

1. D. Kahneman, *Attention and Effort* (Prentice Hall, Englewood Cliffs, N.J., 1973).
2. D. Navon and D. Gopher, *Psychol. Rev.* **86**, 214 (1979).
3. D. A. Norman and D. G. Bobrow, *Cognit. Psychol.* **7**, 44 (1975).
4. C. D. Wickens, in *Attention and Performance*, R. Nickerson and R. Pew, Eds. (Erlbaum, Englewood Cliffs, N.J., 1980).
5. G. D. Ogden, J. W. Levine, E. J. Eisner, *Human Factors* **21**, 529 (1979).
6. For example, J. B. Isreal, C. D. Wickens, G. L. Chesney, E. Donchin, *ibid.* **22**, 211 (1980).
7. E. Donchin, W. Ritter, W. C. McCallum, in *Event-Related Brain Potentials in Man*, E. Callaway, P. Tueting, S. H. Koslow, Eds. (Academic Press, New York, 1978).
8. Some investigators [for example, E. Spelke, W. Hirst, O. Neisser, *Cognition* **4**, 215 (1976)] have questioned the extent to which resources truly are "fixed," in quantity, or whether in fact these may "expand" with increases in task difficulty (*1*). As long as it is assumed that any expansion in supply is insufficient to compensate entirely for increased demands, we argue that the concept of reciprocity of primary and secondary task resources remains valid—secondary task resources will be less available as primary task demand increases.
9. Assuming the framework that resources are multidimensional (*2, 4*), we have chosen manipulations of tracking difficulty that impose demands on the same perceptual-cognitive resources that are assumed to underlie the processes manifest in P300 elicited by either auditory or visual stimuli (*6*). Hence, increasing the order of tracking control requires perception of higher derivatives of the error to maintain effective performance [C. D. Wickens *et al.*, *Proceedings of the 17th Annual NASA Conference on Manual Control*, NASA Technical Manual, 1981). Decreasing input predictability similarly demands greater perceptual anticipation.
10. The electroencephalogram (EEG) was recorded from three midline sites (Fz, Cz, and Pz according to the 10-20 system) and referred to linked mastoids. The vertical electro-oculogram (EOG) was recorded by placing one electrode below and one above the left eye. Two ground electrodes were positioned on the left side of the forehead. Electrode impedances did not exceed 5 kohm/cm. The EEG and EOG were amplified with amplifiers (Van Gogh model 50000) (time constant, 10 seconds; upper half-amplitude, 35 Hz; roll-off, 3 dB per octave). Both EEG and EOG were sampled for 1280 msec, beginning 100 msec before stimulus onset. The data were digitized every 10 msec. The ERP's were filtered off-line (−3 dB at 6.29 Hz and 0 dB at 14.29 Hz) before statistical analysis.
11. Subjects participated in two experimental sessions. The first consisted of 30 blocks of practice trials, 10 with first-order control dynamics and 20 with second-order control dynamics. In session 2, subjects performed two practice blocks (first- and second-order) before participating under experimental conditions. The 30 experimental blocks were composed of two replications of the 15 conditions (four difficulty manipulations by three secondary tasks and three single-task tracking blocks). The order of presentation of the conditions was counterbalanced across subjects.
12. The root-mean-square error means for the three difficulty conditions (1P, 1U, and 2U) were 137, 208, and 249 [$F(2, 22) = 289.7$, $P < 0.0001$; paired contrasts between adjacent levels, $P < 0.01$ in both cases]. The interaction between experimental condition and primary task difficulty was also statistically significant [$F(6, 66) = 9.5$, $P < 0.001$].
13. The means for the subjective difficulty ratings in the count-only, 1P, 1U, and 2U conditions were 1.46, 3.07, 3.54, and 4.71 [$F(3, 33) = 99.2$, $P < 0.0001$].
14. The data bases submitted to the PCA's were composed of 288 average waveforms (12 subjects by four primary task difficulty manipulations by two stimuli by three electrodes) containing 128 (1.28-second) points each. Five components were derived from each PCA. The component scores obtained from the PCA's were analyzed in repeated-measures analyses of variance. All analyses were repeated after the single trial waveforms within each condition had been adjusted for differences in latency. This was accomplished to ensure that lower amplitude of the averaged waveform in certain conditions did not result from increased variability in latency. In all cases the analyses on the latency-adjusted waveforms showed the same statistical effects as on the unadjusted waveforms. Hence, we believe our effects are the result of true P300 amplitude changes and not changes in latency variability.
15. The reason P300 did not decline with tracking difficulty in the visual flash condition cannot be stated with certainty, although lack of a difficulty effect on secondary task visual P300's is consistent with the results of another unpublished study in our laboratory. We propose two hypotheses. (i) Tracking error is higher in the flash than in the auditory probe condition. Given the competition for the two tasks for visual input, subjects may have biased their allocation of resources toward the probes to a greater extent when the probes were visual than when they were auditory. (ii) The visual flash condition may be placed in the middle of an ordered continuum defining the degree of separation between primary and secondary task stimuli. Like the step probes, the visual flash probes share the common visual modality and spatial location; but like the auditory probes, they are independent events from stimuli in the tracking task. Since the step probes produce increasing amplitude with primary task difficulty and the auditory probes a decreasing amplitude, the visual flash probes, in the middle of this continuum, might reflect the compromise of these two trends, that is, no effect.
16. E. Donchin, *Psychophysiology* **18**, 493 (1981).
17. This research was supported by the Air Force Office of Scientific Research under contract F49620-79-C-0233 with Dr. Alfred Fregley as technical monitor.

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Lewy Bodies of Parkinson's Disease Contain Neurofilament Antigens

Abstract. *The Lewy body, a histological hallmark of Parkinson's disease, is a filamentous inclusion residing most prominently in pigmented neurons of the brainstem. Immunocytochemical reactions of Lewy bodies were examined with antisera to several filamentous proteins of the nervous system and positive reactions were found with those against neurofilaments. An abnormal organization of the neuronal cytoskeleton may be a pathological feature of Parkinson's disease.*

Parkinson's disease (PD) is a progressive, neurodegenerative disorder characterized by dysfunction in tone, movement, and posture. Decreased dopamine levels in the nigrostriatal pathway have been observed in PD (*1*). Since the dopamine deficit is accompanied by a loss of neurons in the substantia nigra (*2*), it is likely that neurotransmitter losses are secondary to neuronal death. It is therefore important to identify abnormalities of neuronal form and function in addition to neurotransmitter decreases. In this report we present evidence for a marked disorganization of the neuronal cytoskeletal system in neurons of PD by demonstrating that the Lewy body, a histological hallmark of idiopathic PD, contains neurofilament antigens.

Lewy bodies are intraneuronal, cytoplasmic inclusions that were originally described in neurons of the substantia innominata and dorsal motor nucleus of the vagus (*3*). They have since been found in neurons of the substantia nigra and other brainstem nuclei (particularly those that are aminergic) and in the hypothalamus, spinal cord, sympathetic ganglia, and, rarely, the cerebral cortex (*2, 4*). Although Lewy bodies have been recognized for many years by neurologists and neuropathologists, nothing has been known about their molecular composition, save that histological reactions have implied a proteinaceous nature (*5*). Since they represent an abnormality that accompanies neuronal loss in a major neurodegenerative disease, the identification of their components may help to illuminate the details of abnormal nerve cell metabolism.

Ultrastructural studies have demonstrated that Lewy bodies are composed of filamentous structures. At the periphery the filaments emerge radially, often admixed with granular or vesicular material. The denser core is composed of apparently random, tightly packed aggregations of filaments, vesicular profiles, and poorly resolved granular material (*6, 7*). The diameter of the filaments has been given as 7 to 8 nm (*6, 7*), a size commensurate with that of intermediate filaments, which are polymeric organelles composed of a class of acidic proteins found in a wide variety of cell types (*8*). The intermediate filaments of neurons, neurofilaments, seem to be pecu-

liar to neurons, however (*9*), and are composed of three major proteins with molecular weights around 210,000, 160,000, and 70,000. These proteins appear to be separate gene products but share structural features and antigenic sites (*10*). Given the availability of antisera to several central nervous system (CNS) filamentous proteins and the use of immunocytochemical techniques, we examined the reactions of Lewy bodies with a series of antisera against CNS filament proteins. Of all those tested, only those specific for neurofilament proteins gave positive results.

Using a peroxidase-antiperoxidase technique (PAP) (*11*), we performed immunocytochemistry on paraffin sections of brains from four patients with idiopathic PD and from one patient with PD and dementia whose cerebral cortex also contained Lewy bodies. We used four different rabbit antisera to neurofilament (NF) polypeptides: antiserum 1, raised against rat NF protein of 210,000 daltons; antiserum 2, raised against rat NF protein of 70,000 daltons (the antigens used in raising antisera 1 and 2 had been isolated in electrophoretically pure form from rat CNS by column chromatography) (*12*); antiserum 3, raised against human NF protein of 210,000 daltons, the antigen having been cut out of polyacrylamide gels after sodium dodecyl sulfate–gel electrophoresis (*13*); and antiserum 4, raised against reassembled bovine NF's (*14*). By immunocytochemistry on tissue sections all antisera reacted with round inclusions in the cell bodies of nigral and cortical neurons, the morphology of which appeared to be typical of Lewy bodies (Fig. 1). Many inclusions displayed homogeneous staining, while others showed accentuation of the periphery.

To demonstrate conclusively that the immunocytochemical reaction recognized Lewy bodies, we stained sections with hematoxylin and eosin to show Lewy bodies by their characteristic eosinophilia and photographed a number of inclusions. The slides were then decolorized and treated with NF antiserum, and the bound immunoglobulin was detected by the PAP method. Thus, the same neuron could be viewed after staining by the two techniques. All Lewy bodies identified by their eosinophilia also reacted with NF antiserum (Fig. 1); conversely, all NF-positive profiles in neuronal cell bodies were sites of Lewy bodies. The specificity of the reaction was tested by adsorbing the antiserum to the 210,000-dalton NF protein and the antiserum to the 70,000-dalton NF protein with the column-purified 210,000- and 70,000-dalton polypeptides, respectively. Prior adsorption prevented subsequent PAP reaction (Fig. 1). That Lewy bodies were indeed present on the sections stained with the adsorbed antisera was confirmed by subsequent staining with hematoxylin and eosin.

In addition to testing the NF antisera, we tested a rabbit antiserum against chicken gizzard actin that reacts with actin in the mammalian CNS (*15*) and a rabbit antiserum raised against a two-cycle purified CNS microtubule fraction (2XMT) that reacts with the neurofibril-

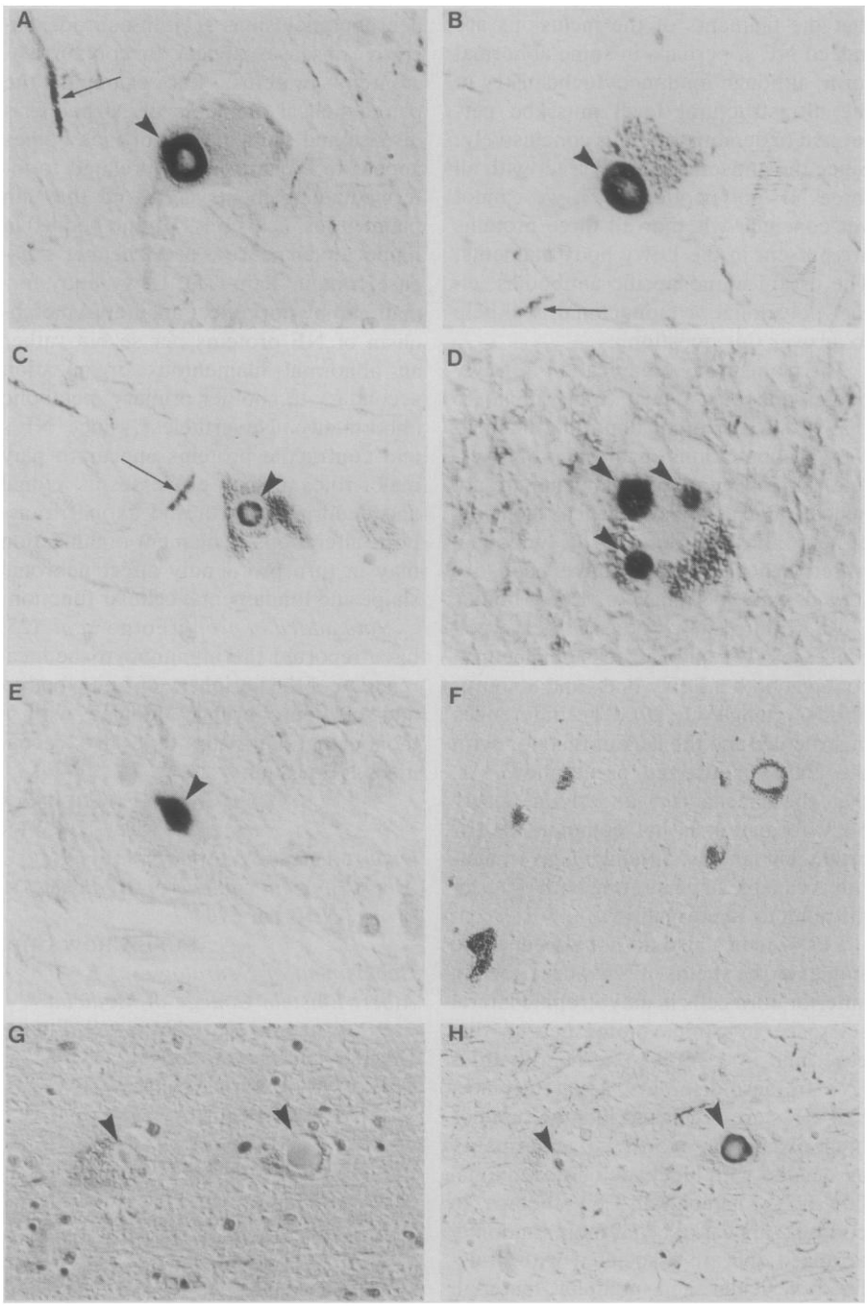

Fig. 1. Reaction of the rounded, cytoplasmic Lewy bodies in pigmented nigral neurons (A to D) and a cortical neuron (E) with antiserum 1 (arrowheads). Binding of antibody (1:250 dilution of antiserum) was visualized by subsequent reaction with swine antiserum to rabbit immunoglobulin G, rabbit PAP, and diaminobenzidine (*13, 15, 16*). In several Lewy bodies the core remains unstained (A to C), while other inclusions stain more diffusely (D and E). The PAP reaction of NF antisera with axons (arrows in A to C) is a normal finding. Magnifications: (A) to (C), ×530; (D) and (E), ×750. (F) Section of substantia nigra after reaction with antiserum 1 (1:250 dilution) adsorbed with the 210,000 NF protein (10 μg/ml). Neither Lewy bodies nor axons show reaction product. The dark, granular material in the nigral neurons in (A) to (D) and (F) is neuromelanin, which did not react with any of the antisera (×200). (G) Section of substantia nigra stained by hematoxylin and eosin, showing two Lewy bodies (arrowheads) (×350). (H) PAP reaction of the same neurons shown in (G) after destaining and restaining with the NF antiserum, demonstrating that the two Lewy bodies (arrowheads) react with the antiserum (×350).

lary tangles of Alzheimer's disease but does not behave like an antiserum to tubulin (*16*). Neither the actin nor the 2XMT antiserum reacted with Lewy bodies. Nonimmune rabbit serum did not react with sections.

Our immunocytochemical observations demonstrate a specific molecular constituent in Lewy bodies. It is likely that the filaments of the inclusions are indeed NF's, perhaps in some abnormal form, although immunocytochemistry at the ultrastructural level must be performed to demonstrate this conclusively. Since the antisera we used react with all three NF polypeptides (*17*), we cannot yet conclude whether all three proteins are present in the Lewy body filaments. The use of monospecific antibodies, either polyclonal or monoclonal, will help to answer this question.

The filamentous organization in Lewy bodies does not appear to be similar to that of several other abnormal filamentous accumulations in the nervous system. Thus, Lewy body filaments are not helical, like the paired helical filaments of neurofibrillary tangles (*18*). Individual paired helical filaments have been observed at the periphery of Lewy bodies (*7*) or within Lewy bodies in nerve cell processes (*19*), and an occasional neuron contains both a Lewy body and a neurofibrillary tangle (*7, 20*). The differences in structure and the lack of reaction with the 2XMT antiserum argue, however, that the mechanism underlying Lewy body formation is not common to that producing tangles, although a given neuron is capable of generating both types of filamentous abnormalities.

Lewy bodies also do not appear to be similar to the skeins of NF's described in anterior horn cells in amyotrophic lateral sclerosis, in canine motor neuron disease, and in several toxic neuropathies (*21*). In these disorders, apparently normal NF's are organized in large bundles in perikarya or axons, an abnormality associated with blockade of slow axonal flow (*22*). Filaments in Lewy bodies, in contrast, appear to be far more randomly arranged and are associated with ill-defined vesicular and granular material. Structures similar to the Lewy body have been described, however, in neurons of patients with familial and Guamanian forms of amyotrophic lateral sclerosis (*19, 23*), indicating that Lewy body formation may be a pathological feature of degenerative motor neuron disease.

Our observations indicate that a marked disorganization of NF proteins is a pathological feature of PD. It is of interest that several of the most common neuronal inclusions seen in neurodegenerative diseases appear to contain filamentous proteins. For example, the paired helical filaments of Alzheimer's disease and the filaments of Pick bodies appear to be antigenically related to intermediate filaments (*24*), and the thin filamentous inclusions (Hirano bodies) in hippocampal neurons of Alzheimer's disease contain actin (*15*). Lewy body formation may not reflect an altered metabolism of NF proteins per se but rather an abnormal filamentous organization secondary to another primary metabolic abnormality. Nevertheless, since NF's and contractile proteins appear to play major roles in such processes as axonal and dendritic growth and axonal transport, alterations in filament organization may in turn profoundly affect neuronal shape and fundamental cellular function.

Note added in proof: Forno *et al.* (*25*) have reported the immunocytochemical reaction of the periphery of Lewy bodies and of neurofibrillary tangles with a monoclonal antibody that also recognizes NF protein.

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References and Notes

1. O. Hornykiewicz, in *Recent Advances in Parkinson's Disease*, F. H. McDowell and C. H. Markham, Eds. (Davis, Philadelphia, 1971), p. 33.
2. J. G. Greenfield and F. D. Boxanquet, *J. Neurol. Neurosurg. Psychiatry* **16**, 213 (1953).
3. F. H. Lewy, in *Handbuch der Neurologie*, M. Lawandowsky, Ed. (Springer, Berlin, 1912), p. 920.
4. L. E. Lipkin, *Am. J. Pathol.* **35**, 1117 (1959); W. A. Den Hartog Jager and J. Bethlem, *J. Neurol. Neurosurg. Psychiatry* **23**, 283 (1960); H. Okazaki, L. E. Lipkin, S. M. Aronson, *J. Neuropathol. Exp. Neurol.* **20**, 237 (1961); L. S. Forno, *J. Am. Geriatr. Soc.* **17**, 557 (1969); E. Ohama and F. Ikuta, *Acta Neuropathol.* **34**, 311 (1976).
5. J. Bethlem and W. A. Den Hartog Jager, *J. Neurol. Neurosurg. Psychiatry* **23**, 74 (1960); M. R. Issidorises, C. Mytilineou, W. O. Whetsell, Jr., M.D. Yahr, *Arch. Neurol.* **356**, 633 (1978).
6. P. E. Duffy and V. M. Tennyson, *J. Neuropathol. Exp. Neurol.* **24**, 398 (1965); S. Roy and L. Wolman, *J. Pathol.* **99**, 39 (1969); L. S. Forno and R. Norville, *Acta Neuropathol.* **34**, 183 (1976).
7. M. Tomonaga, *Acta Neuropathol.* **53**, 165 (1981).
8. E. Lazarides, *Nature (London)* **283**, 249 (1980).
9. W. T. Norton and J. E. Goldman, in *Proteins of the Nervous System*, R. A. Bradshaw and D. M. Schneider, Eds. (Raven, New York, 1980), p. 301.
10. R. H. K. Liem, S.-H. Yen, M. L. Shelanski, *J. Cell Biol.* **79**, 637 (1978); F.-C. Chiu and W. T. Norton, *J. Neurochem.* **34**, 1149 (1980); H. Czosneck, D. Soifer, H. M. Wisniewski, *J. Cell Biol.* **85**, 726 (1980); L. Autilio-Gambetti, M. E. Velasco, J. Sipple, P. Gambetti, *J. Neurochem.* **37**, 1260 (1981).
11. L. Sternberger, *Immunocytochemistry* (Wiley, New York, ed. 2, 1979).
12. F.-C. Chiu and W. T. Norton, *J. Neurochem.* **39**, 1252 (1982).
13. S.-H. Yen and K. L. Fields, *J. Cell Biol.* **88**, 115 (1981).
14. R. K. H. Liem, *J. Neurochem.* **38**, 142 (1982).
15. J. E. Goldman, *J. Neuropathol. Exp. Neurol.* **42**, 146 (1983).
16. S.-H. Yen, F. Gaskin, R. D. Terry, *Am. J. Pathol.* **104**, 77 (1981).
17. Antisera 1 to 3, although raised against purified single NF components, react with all three NF proteins, as judged by specific precipitin reactions during rocket immunoelectrophoresis (F.-C. Chiu and S.-H. Yen, unpublished observations). Antiserum 4 was raised to a mixture of all three NF components (*14*).
18. M. Kidd, *Nature (London)* **197**, 192 (1963); H. M. Wisniewski, H. K. Narang, R. D. Terry, *J. Neurol. Sci.* **27**, 173 (1976).
19. L. S. Forno, P. J. Barbour, R. Norville, *Arch. Neurol.* **35**, 818 (1978).
20. A. Hirano, N. Malamud, T. S. Elizan, L. T. Kurland, *ibid.* **15**, 35 (1966).
21. S. Carpenter, *Neurology* **18**, 841 (1968); L. C. Cork *et al.*, *J. Neuropathol. Exp. Neurol.* **38**, 209 (1979); A. W. Clark, J. W. Griffin, D. L. Price, *ibid.* **39**, 42 (1980); J. Griffin, L. C. Cork, J. C. Troncoso, D. L. Price, in *Human Motor Neuron Diseases*, L. P. Rowland, Ed. (Raven, New York, 1982), p. 419; A. Hirano, *ibid.*, p. 75.
22. J. W. Griffin, P. N. Hoffman, A. W. Clark, P. T. Carroll, D. L. Price, *Science* **202**, 633 (1978).
23. A. Hirano, L. T. Kurland, G. P. Sayre, *Arch. Neurol.* **16**, 232 (1967); K. Takahashi, H. Nakamura, E. Okada, *ibid.* **27**, 292 (1972).
24. P. Gambetti, G. Shecket, B. Ghetti, A. Hirano, D. Dahl, *J. Neuropathol. Exp. Neurol.* **42**, 69 (1983); B. H. Anderton *et al.*, *Nature (London)* **298**, 84 (1982).
25. L. S. Forno, A. N. Strefling, L. A. Sternberger, N. H. Sternberger, L. F. Eng, paper presented at the Annual Meeting of the American Association of Neuropathologists, St. Louis (1983).
26. We thank R. H. K. Liem for one of the NF antisera, A. Hirano, W. T. Norton, and R. D. Terry for comments on the manuscript, and S. Geier, A. Crowe, and P. Garcia for technical assistance. Supported by PHS grants NS 17125, AG 01136, and NS 02476, PHS Teacher-Investigator Award NS 00524 (J.E.G.), and PHS Research Career Development Award AG 00028 (S.-H. Yen).

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