Molecular Neurobiology

ISSN0893-7648/94/9(1–3): 149–161/$6.60

Substantia Nigra Degeneration and Tyrosine Hydroxylase Depletion Caused by Excess S-Adenosylmethionine in the Rat Brain

Support for an Excess Methylation Hypothesis for Parkinsonism

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Abstract

The major symptoms of Parkinson's disease (PD) are tremors, hypokinesia, rigidity, and abnormal posture, caused by degeneration of dopamine (DA) neurons in the substantia nigra (SN) and deficiency of DA in the neostriatal dopaminergic terminals. Norepinephrine, serotonin, and melanin pigments are also decreased and cholinergic activity is increased. The cause of PD is unknown. Increased methylation reactions may play a role in the etiology of PD, because it has been observed recently that the CNS administration of S-adenosyl-L-methionine (SAM), the methyl donor, caused tremors, hypokinesia, and rigidity; symptoms that resemble those that occur in PD. Furthermore, many of the biochemical changes seen in PD resemble changes that could occur if SAM-dependent methylation reactions are increased in the brain, and interestingly, L-DOPA, the most effective drug used to treat PD, reacts avidly with SAM. So methylation may be important in PD; an idea that is of particular interest because methylation reactions increase in aging, the symptoms of PD are strikingly similar to the neurological and functional changes seen in advanced aging, and PD is age-related. For methylation to be regarded as important in PD it means that, along with its biochemical reactions and behavioral effects, increased methylation should also cause specific neuronal degeneration. To know this, the effects of an increase in methylation in the brain were studied by injecting SAM into the lateral ventricle of rats. The injection of SAM caused neuronal degeneration, noted by a loss of neurons, gliosis, and increased silver reactive fibers in the SN. The degeneration was accompanied with a decrease in SN tyrosine hydroxylase (TH) immunoreactivity, and degeneration of TH-containing fibers. At the injection site in the lateral ventricle it appears that SAM caused a weakening or dissolution of the intercellular substances; observed as a disruption of the ependymal cell layer and the adjacent caudate tissues. SAM may also cause brain atrophy; evidenced by the dilation of the cerebral ventricle. Most of the SAM-induced anatomical changes that were observed in the rat model are similar to the changes that occur in PD, which further support a role of SAM-dependent increased methylation in PD.

Index Entries: Parkinson's disease; methylation S-adenosyl-L-methionine; dopamine tyrosine hydroxylase; neuronal degeneration.

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Introduction

Parkinson's disease (PD) is a neurodegenerative disorder. Dopamine (DA)-containing cells located in the substantia nigra (SN) and with terminals in the neostriatum degenerate. The degeneration is associated with the disappearance of melanin pigments from the pars compacta of the SN *(1–3)*, and the depletion of DA from the neostriatum *(4,5)*. Tyrosine hydroxylase, the rate limiting enzyme for the synthesis of DA, is also depleted *(6)*. The ratio of the concentration of homovanillic acid (HVA) to that of DA (HVA/DA) increased in the neostriatum *(7)* and urine *(8)* in PD patients, showing that there is an increase in the turnover of DA in PD. The methylation step of DA metabolism seems to be particularly affected, because a substance with reactive properties similar to 3,4-dimethoxyphenylethylsmine (DIMPEA), the 3,4-dimethoxymetabolite of DA, was detected in the urine of PD patients *(9)*. Furthermore, the relative increased levels of HVA in PD could occur as a consequence of an increase in the methylation of DA, because HVA is formed primarily from the 3-methoxy-metabolite of DA *(10)*, 3-methoxy-4-hydroxy phenylethylamine (3-MT). Cerebral levels of serotonin (5-HT) *(11)* and norepinephrine (NE) *(12)* are also decreased, and the activity of acetylcholine (ACH) increased in the brain of PD patients.

The symptoms of PD are primarily related to the basal ganglia deficiency of DA and are expressed as slowness and awkwardness in executing skeletal muscles motor functions. There is difficulty in initiating movement, problems in maintaining movement, prolonged reaction time, flexed posture, deficiency in postural reflexes, muscular rigidity, and tremors. Treatments of PD are based mainly on managing the motor symptoms by replenishing the depleted DA, using L-DOPA, the precursor of DA, by stimulating DA receptors and by blocking muscarinic ACH receptors. The changes that cause the loss of DA cells in PD patients and the consequential symptoms are not known and therapy does not significantly change the progression of the disease.

Although the degeneration of the nigrostriatal dopaminergic cells is responsible for the major symptoms of PD and forms the basis for the rational therapy, it should be noted that all the symptoms seen in the PD patient are not related to the impairments of the nigrostriatal system *(13)*. There are depression, cognitive impairments, loss of libido, sleep disturbance, anorexia, orthostatic hypotension *(14,15)*, sweating *(16)*, and other autonomic dysfunctions *(17)* that cannot be explained by the changes in the nigrostriatal system. Instead those symptoms may be related to lesions observed in the locus ceruleus *(18,19)*, the hypothalamus *(20–22)* the dorsal nucleus of vagus *(23–26)*, several other brain regions *(27)*, and in the adrenal medulla *(28)*.

The multiregion degeneration in PD suggests that the process or factor that causes PD is not exclusively selective for the nigrostriatal system, but that the nigrostriatum may be more susceptible. The susceptibility may be related to the inherent physical and biochemical properties of the nigrostriatum and to other conditions that start early in life, for example, the endowment with a small population of SN DA neurons *(29)*.

The SN DA cells are suggested to be the most rapidly aging neurons in the brain *(30)*, and they decline in number as a function of aging *(31)*, the cause of which is unknown. Since normal motor functions will be compromised when the population of SN DA neurons is decreased to a critical value, it means that an individual endowed with a small population of SN DA neurons will be likely to experience impaired motor functions comparatively early. For that reason an endogenous factor or process that has a debilitating effect on SN DA neurons will preferentially victimize the individual with a small population of nigrostriatal dopaminergic neurons, and likely result in PD.

Increased methylation reactions may have an adverse accumulative effect on DA neurons, thus may be seen as a secondary process, precipitating PD in an individual endowed with a small population of SN DA neurons. The basis for the assumptions are:

1. SAM, the methyl donor, caused parkinsonian-types of behavioral and neurological changes *(32–35)*;
2. The process of methylation can cause similar biochemical changes to those seen in PD *(33,34)*;
3. Certain metabolites of methylation reactions are cytotoxic *(36,37)*;
4. There is a net age-related increase in methylation reactions *(38–44)*;
5. PD is age-related;
6. The afflictions of aging share many similarities with the symptoms of PD;
7. The nigrostriatum is enriched with substrates that avidly react with SAM and it is critically dependent on reaction products of methylation; and

8. L-DOPA, the most effective drug for PD, avidly reacts with SAM and can deplete its level.

The chemistry and physiology of SAM show marked similarity with the symptoms of PD. The depletion of DA, NE, and 5-HT seen in PD could be a result of the fact that all of these biogenic amines react avidly with SAM, the methyl donor. The increased ACH activity could occur because SAM is required to produce choline, the precursor of ACH, through the methylation of phosphatidylethanolamine to produce phosphatidylcholine, which hydrolyzes to form choline *(45)*. Furthermore, the increased HVA/DA and the presence of a DIMPEA-like substance in PD patients indicate that there is an increase in the methylation and turnover of DA, although a decrease in the metabolism of HVA may explain the relative increased levels. The SAM-dependent methylation of phospholipids *(46)* and other compounds *(37)* will produce cytotoxic and behaviorally active metabolites *(37)*. The chemical relevance of excess methylation to the symptoms of PD is also highlighted by the fact that L-DOPA, which is the most effective therapy for PD, avidly reacts with SAM to produce methylated L-DOPA in PD patients undergoing L-DOPA therapy *(47–50)*, and L-DOPA is also an effective depletor of SAM *(51)*. A more broad-based examination of the chemistry and physiology of methylation also suggests that methylation could play a role in PD. Regenerative processes and protein synthesis decline in the aged, which is a major predisposing condition for PD. The decline in regenerative processes and in protein synthesis are related to the decline in transcriptional and translational activities. In this connection methylation may be involved, because the methylation of DNA has been correlated with transcriptional silence of many genes *(52)* and unmethylation of DNA with gene transcription activity *(53)*.

Tremor and impaired motor functions occurred in rodents *(32–35)* following the injection of SAM into the lateral ventricle. The onset, intensity, and duration of the effects were dose-dependent and were antagonized by L-DOPA. SAM possibly decreased the motivation or capability to move, as well as the ability to continue moving once a movement is initiated *(34,35)*. Marked physiological similarities between SAM-induced impairments and the impairments seen in parkinsonism were noted *(33–35)*, and the SAM-induced motor aberrations in rats *(34)* showed many of the features reported for the subacute toxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) *(54,55)*, an agent used to produce animal models of parkinsonism.

SAM is the endogenous methyl donor and is a limiting factor in methylation reactions, therefore methylation reactions will be increased in the brain if the activity of SAM is increased. The nigrostriatum may serve as a focal point for SAM-dependent reactions because the nigrostriatum is enriched with biochemicals, e.g., the catechols and other biogenic amines that avidly react with SAM, and the function of the nigrosriatum is highly dependent on the products of methylation, for example, choline, which is the precursor of acetylcholine. To further determine whether an excess of methylation can precipitate conditions that resemble parkinsonism SAM was injected into the lateral ventricle of rats. Behavioral, biochemical, and neurological changes that show commonality with parkinsonism were studied. The SAM-induced changes in specific measures of behavior were reported in other communications *(34,35)*. This report will document the anatomical and histological degenerative changes and the depletion of tyrosine hydroxylase immunoreactivity that occur in the rat's brain following the administration of SAM into the lateral ventricle.

Materials and Methods

Animal Preparation

Sprague-Dawley male rats weighing 250–350 g (Harlam Labs, OH) were used in the experiments. The rats were acclimatized for about 1 wk in a colony room with 12-h light and 12-h dark cycle. Water and food were supplied ad libitum. Under chloral hydrate anesthesia (400 mg/kg) a stainless steel guide cannulae was stereotaxically placed for injection into the lateral ventricle of each rat. The cannulae was affixed with dental cement secured to the skull with two screws. The placement of the cannulae, with reference to bregma, was 1.4 mm lateral, 0.5 mm caudal, and the tip extended to the inner surface of the cranium, above the dura mater. A stainless steel rod was inserted into each cannulae to maintain its opening. The rats were allowed to recover for about 2–3 d. Injections were made in the right lateral ventricle 5 mm from the surface of the cranium, via a premeasured insertion cannulae, attached by polyethylene tubing (PE20) to a 10 µL Hamilton syringe. The injection in each rat was 2.5–5 µL of phosphate buffered saline (PBS) at pH 7.4,

for controls, or the toluenesulfonate salts of SAM prepared in 2.5–5 µL of PBS.

Histochemistry

Groups of rats were studied for the effects of SAM on brain histochemistry. These rats were reanesthetized with chloral hydrate (400 mg/kg) and transcardially perfused with cold PBS followed by 4% paraformaldehyde prepared in PBS. The brains were removed and placed in cold 15% sucrose, prepared in PBS, and kept at 4°C for about 24 h. The brains were then frozen in powdered dry ice and stored at –78°C. Fifteen to thirty micron sections were prepared in a cryostat and mounted on gelatin chrome-alum coated slides.

Tyrosine Hydroxylase Immunohistochemistry

A modified indirect immunohistochemical procedure *(56)* was used to determine the tyrosine hydroxylase (TH) immunoreactivity (IR). The slide mounted slices were preincubated in 0.3% Triton X-100 in PBS, pH 7.4, for three 5-min periods, then incubated in a similar buffer containing rabbit anti-TH serum, approx 1:1000 dilution and obtained from Pel-Freeze, or with non-TH-immune rabbit serum, as control, for about 24 h at 4°C. The slides were washed in 0.2% Triton X-100 PBS buffer for three 5-min periods and incubated in the buffer containing fluorescent labeled goat antirabbit serum, 1:300 dilution, in reduced light for 30 min. The slides were washed in Triton X-100 buffer for one 5-min period and in PBS for two 5-min periods. The sections were drained and coverslipped using Fluoromont, then viewed with an epifluorescent equipped microscope.

Nissl Staining

One set of the brain slices was stained with thionin or cresyl violet according to standard methods *(57)*. They were observed for degeneration and the morphological changes in nerve cell bodies and for the histological verification of the specific degeneration.

Silver Impregnation

Another set of slices was used for the silver impregnation of degenerating fibers. For these studies a modification of the Fink and Heimer (1966) *(58)* method was used. The slide mounted slices were rinsed in water for 5 min and soaked for 15 min in 0.05% potassium permanganate, then rinsed for 3 consecutive 3 min periods in distilled water, and bleached in a mixture of equal parts of 1% oxalic acid and 1% hydroquinone for 1 min. The slices were rinsed 3 times for 3 min each in distilled water, transferred to a solution of 1.5% silver nitrate and 0.5% lead nitrate for 45 min or longer, rinsed, and placed into a mixture of 0.25% sodium hydroxide and 0.25*M* ammonium hydroxide. The slices were rinsed again and placed into another solution of 1.5% silver nitrate for 45 min, drained and lightly rinsed (to obtain a light-dark staining), and placed into Nauta-Gygax reducing solution consisting of 8% ethyl alcohol, 0.25% formalin, and 0.025% citric acid for 1 min. They were then rinsed and transferred for 1 min in 0.5% sodium thiosulfate and rinsed 3 times for 2 min each. The slices were then dehydrated in graded alcohol and xylene, dried, rehydrated, counterstained with cresyl violet, and coverslipped with permount. The slices were microscopically examined and photographed.

Results

Rats that were sacrificed 1 d after receiving a single injection of SAM (1 µmol) showed no significant damage to the brain. At 4 d postinjection, the ependymal cell layer of the ipsilateral SAM-injected lateral ventricle showed disruption (Fig. 1C, arrows) and the cells of both caudate nuclei of the SAM-injected rats showed dense Nissl substances (Fig. 1B,C), as compared to the PBS control (Fig. 1A), however there was no apparent loss of neurons. The SAM-injected lateral ventricle was also enlarged as compared to the PBS injected rats. The substantia nigra, which contains the cell body of origin for the dopaminergic nerve endings in the caudate nucleus, did not show anatomical damage, as determined with cresyl violet staining following the single treatment.

Rats that received 4 consecutive daily injections of SAM (1 µmol/rat) or PBS and were sacrificed on d 6 after the last injection showed a disruption at the ventricular injection site (Fig. 2A,B). SAM caused a disruption of the adjacent caudate nucleus tissues, both at the site of injection (Fig. 2B) and caudal to the injection site (Fig. 2D), as compared to PBS (Fig. 2A,C). This disruption apparently is caused by a dissolution of the intercellular substances, because cellular loss was not evident. SAM also caused the dilation of the lateral ventricle (Fig. 2B,D), as compared to the PBS controls (Fig. 2A,C). This SAM-induced ventricular dilation is reminiscent of the dilation of the cerebral ventricles seen in

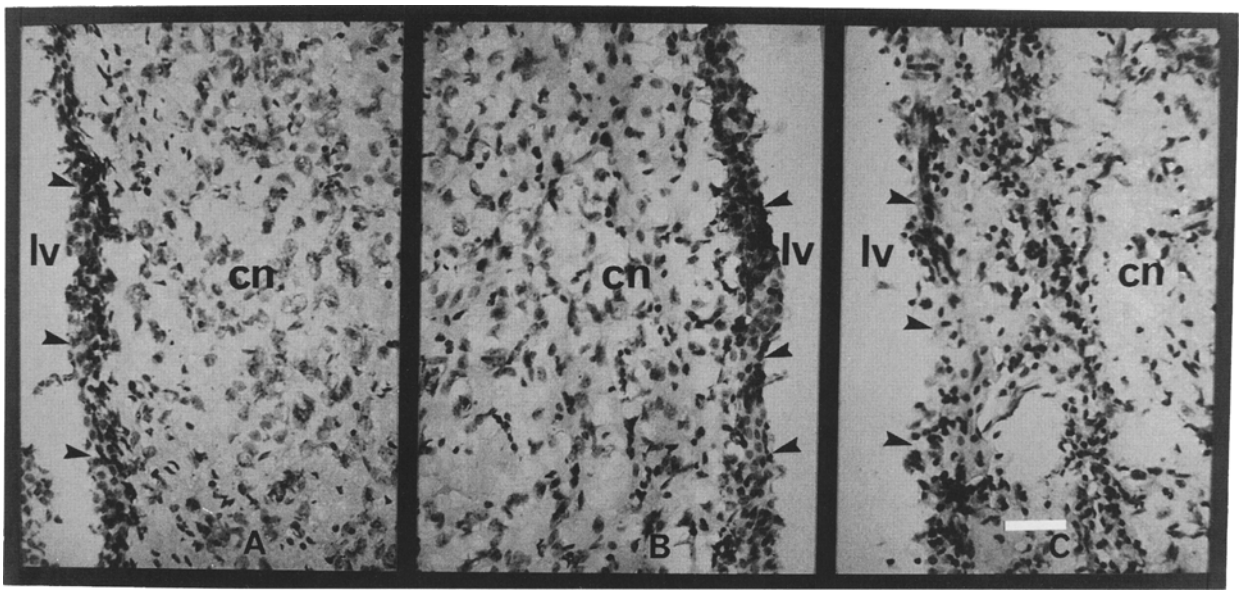

Fig. 1. Photomicrographs of the ventricular border of the caudate nucleus (cn) of rats. The disruption of the ventricular ependymal cell layer and caudate nucleus tissues is shown in **(C)**, as compared with the similar tissues in the PBS-injected rat **(A)**. Densed staining of Nissl substances are seen in the ipsilateral (C) and contralateral CN **(B)** of the SAM-injected rat, as compared to the PBS-injected rat (A). Bar = 50 μm.

PD victims and which is associated with cerebral atrophy.

Nissl staining showed degeneration in the substantia nigra (SN). The damage occurred predominantly at the level of, and rostral to the red nucleus (Figs. 3 and 4). The substantia nigra, especially the zona compacta region (C), shows a decrease in the population of neurons (Figs. 3D and 4D). The ipsilateral SN of the SAM-injected rats appears denuded and hypochromatic and contains an accumulation of phagocytic cells or showed gliosis, seen at the point of the arrows, in the pars reticulata (R) (Figs. 3D and 4D), as compared to the contralateral SN (Figs. 3C and 4C). At the level of the red nucleus the SN degeneration occurred both ipsilateral and contralateral, identified as a sparsity of cells in the SN of the SAM-injected rats (Figs. 3C,D), as compared to the PBS (Figs. 3A,B). In Fig. 5 a higher magnification of the dense accumulation of phagocytic cells or gliosis in the pars reticulata region of the ipsilateral SN of the SAM-injected rats is highlighted.

Shown in Fig. 6 are horizontal sections of the SN, highlighting the SAM-induced cytological changes at a higher magnification. The rats in this study were treated with 1 μmol/rat/d of SAM or with PBS for 3 consecutive days and sacrificed on d 4 following the last injection. Figure 6A shows a photomicrograph of the pars compacta region, taken from the side ipsilateral to the injected lateral ventricle of a PBS-treated rat, and Fig. 6B,C show the contralateral and ipsilateral pars compacta of a SAM-injected rat. A reduction in the cell population is shown in the SAM-injected rat (Fig. 6B,C), as compared to the PBS-treated rat (Fig. 6A). Note the marked reduction in the cell population in the ipsilateral pars compacta (Fig. 6C), as compared to the contralateral pars compacta of the SAM-injected rat (Fig. 6B) and the ipsilateral pars compacta of the PBS-treated rat (Fig. 6A). Several neurons in the substantia nigra of the SAM-injected rat (Fig. 6B,C) appear swollen and oval in shape when compared to the PBS-treated rat (Fig. 6A). Neurons in a degenerative fragmented state are also shown (Fig. 6B,C, arrow heads). Figure 6D–F show matching sections to A, B, and C, and highlight silver impregnation of degenerating fibers. A significant quantity of labeled fibers in both SNs of the SAM-injected rats is shown (Fig. 6E,F), as compared to the SN ipsilateral to the injected lateral ventricle of the PBS-injected rat (Fig. 6D).

TH-IR was also determined in rats treated once/d for 3 d and sacrificed on d 4. The intensity of TH-IR was strikingly reduced in the SNs of rats injected

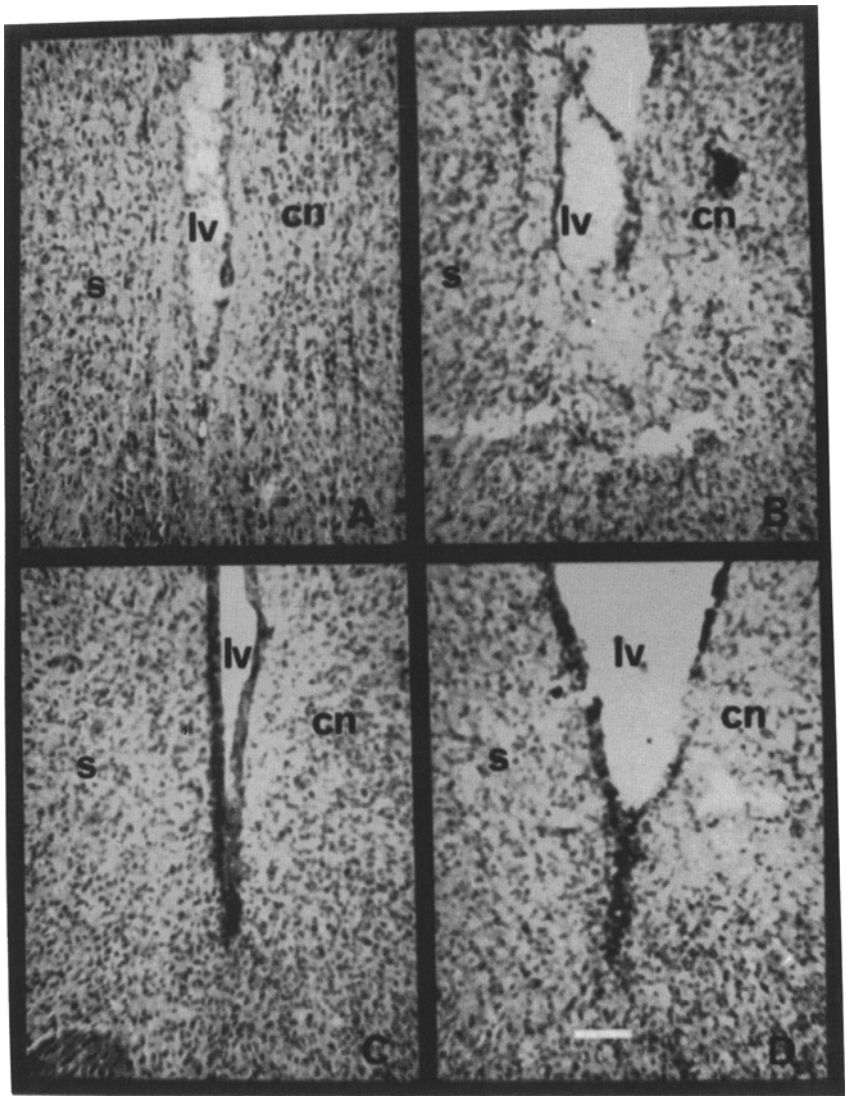

Fig. 2. Slices of the septum (s), lateral ventricle (lv) and caudate nucleus (cn) region of a rat brain. **(A)** and **(B)** represent slices prepared from the injection site and **(C)** and **(D)** slices caudal to the injection site. SAM caused disruption in the ependymal cell layer and cn (B) and (D) and dilation of the cerebral ventricle (D), as compared with PBS treated rats, lv = lateral ventricle, cn = caudate nucleus, s = septum. Bar = 125 μm.

unilaterally into the lateral ventricle with SAM (Fig. 7B,C), as compared to the injection with PBS (Fig. 7A). The decrease in the intensity of the TH-IR was more pronounced in the SN ipsilateral to the injection site (Fig. 7C), than in the contralateral side (Fig. 7B) of the SAM-treated animals, and both SNs showed reduced TH-IR than the intensity shown in the PBS controls (Fig. 7A). The photomicrographs also highlight the sparsity of cells in the SAM-injected SN, notably the ipsilateral side (Fig. 7C).

Brain slices of the caudate nucleus highlighting the cerebro-caudate border are shown in Fig. 7D–F. The injection of SAM did not cause a dramatic change in the intensity of the TH-IR in the caudate nucleus (Fig. 7D–F), however, there was a noticeable degeneration of fibers in the cerebral border of the sections from the SAM-injected rats. The degeneration appears as a disappearance of fine fiber from the adjacent cerebro-caudate border (ec), which is more noticeable on the ipsilateral side to the injection site (Fig. 7F). This suggests that degen-

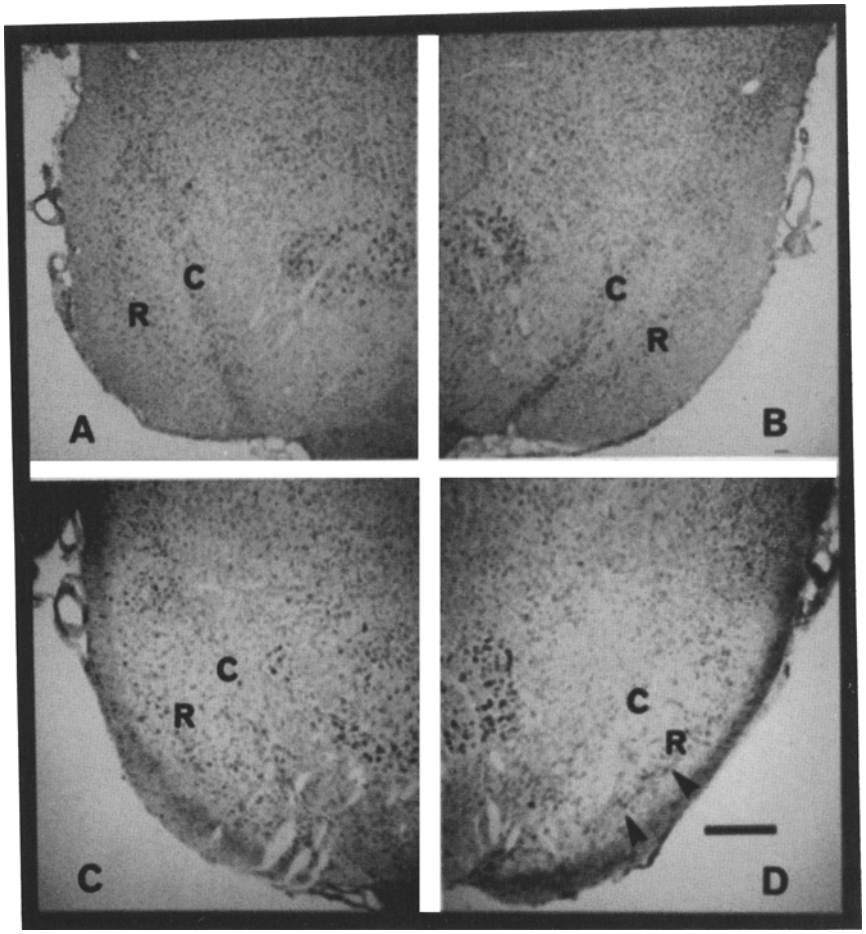

Fig. 3. Cross-sections of the substantia nigra (SN) region of rat brain at the level of the red nucleus. The contralateral and ipsilateral sections of a PBS-treated rat (**A** and **B**) and SAM-treated rat (**C** and **D**) are shown. Marked degeneration occurred in both SN of the SAM-injected rat, noted by loss of cells from the pars compacta (C) region of both SNs and gliosis in the pars reticulata (R) of the ipsilateral SN (D). The arrows identify the accumulation of phagocytic cells (gliosis). Bar = 500 μm.

eration of fibers in higher brain regions occurred following the injection of SAM.

Discussion

The results of this study show that the injection of SAM into the lateral ventricle of rats caused SN degeneration. The degeneration was most pronounced on the side ipsilateral to the injected lateral ventricle, evidenced by the disappearance of neurons and silver impregnation of fibers in the pars compacta and gliosis in the pars reticulata. The lesion consistently occurred at the level of the red nucleus, and may represent a somatotopically related effect. The SN lesion may be caused by the retrograde degeneration of axons beginning at dopaminergic nerve terminals in the caudate nucleus (CN), proximal to the injection site. The process may involve, first, the disruption of the protective ependymal cell layer at the border of the lateral ventricle and the CN. This disruption may cause cerebrospinal fluid constituents, including SAM, to gain access to the adjacent CN tissues. In the CN it is conceivable that SAM will react within the vicinity of the dopaminergic nerve terminals. It will methylate DA, depriving the DA receptors as well as the DA uptake system of DA, thus creating a dopaminolytic state, which may precipitate the DA-deficient movement disorders, like those reported previously *(32–35)*. Such an exposure of

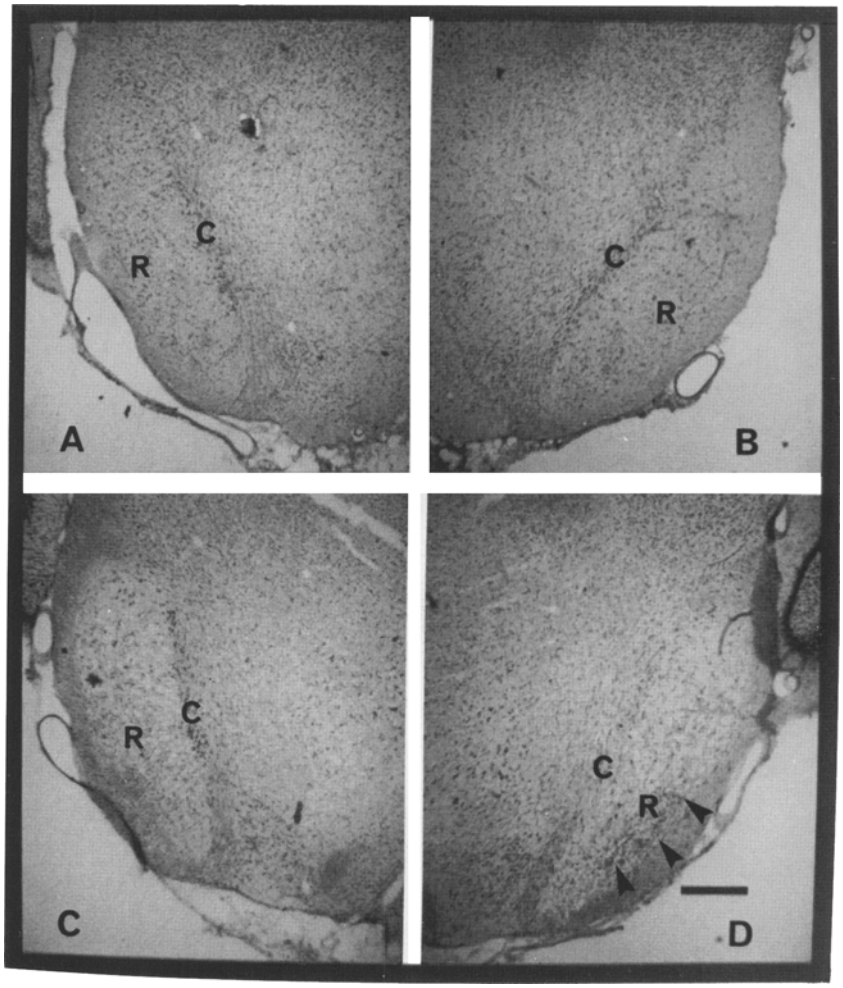

Fig. 4. Cross-sections of the substantia nigra (SN) region of rat brain, caudal to the red nucleus. The contralateral and ipsilateral sections of a PBS-treated rat (**A** and **B**) and SAM-treated rat (**C** and **D**) are shown. Degeneration occurred in the SN of the SAM-injected rat (D), noted by the patches of cell-loss in the pars compacta (C) region and gliosis (highlighted by arrows) in the pars reticulata (R), mainly of the ipsilateral SN (D). Bar = 500 μm.

the dopaminergic nerve endings will stimulate an increase in the turnover of DA, driven by the increased methylation/metabolic process, as well as the lack of DA on the presynaptic receptors that would normally stimulate the DA autoinhibitory release system. An increase in the methylation of DA will also increase HVA/DA and DIMPEA levels and will cause the depletion of DA. Similar biochemical changes are seen in PD *(7–9)*, and increased HVA/DA and depletion of DA following the injection of SAM have been observed *(59)*. SAM probably diffuses from the injected side to the contralateral CN where it may affect the DA nerve terminals and retrogradely cause contralateral SN degeneration. This suggests that the CN is a targeted site for the reaction of SAM and/or that the nigrostriatal region is particularly sensitive to the effects of SAM.

SAM Caused Ventricular Dilation

It also disrupted the ependymal cell layer and CN tissues, without evidence of causing cell loss. These effects may be caused by the dissolution of intercellular substances. Dilation of the cerebral ventricles has been reported to occur in PD *(62)*, and although it is clear that loss of dopaminergic cell terminals in the CN occurred in PD, changes in the structural integrity of the CN or neostriatum has not

Fig. 5. High magnification of the pars reticulata of a SAM-injected rat and highlight the accumulation of phagocytic cells (gliosis). Bar = 125 μm.

been reported to be a marker of PD. It would be of interest, therefore, to determine whether the protective ependymal cell layer is breached and fine intercellular substances damaged in the neostriatum of PD patients.

The depletion of TH-IR from the SN seems to parallel the extent of the damage seen, which suggests that the depletion of TH-IR may occur as a consequence of the degeneration. The mechanism for the neuronal lesion and TH-IR depletion that occurred following repeated injection of SAM may be caused by metabolic exhaustion of the DA producing and releasing mechanisms, secondary to the demand for replenishing the methylated DA. Excess SAM at the nerve endings may also increase the accumulation of toxic metabolites, for example, methylated catecholamines *(9)*, di-*N*-methylated betacarbolines *(37)*, homocysteine, phosphatidylcholine, and lysophosphatidylcholine. The methylation of phosphalipids *(46)* and proteins *(60,61)*, also, may cause the perturbation of nerve terminal membranes.

The TH-containing fibers in the CN are derived from cell bodies located in the SN. The SN showed marked degeneration following the injection of SAM, therefore it was surprising that the TH fibers in the CN did not show similar quantitative changes, but instead was slightly depleted only in the ipsilateral side following the injection of SAM. The sparing of the TH-IR in the CN suggests that the TH projections from the SN occurred in high density and in overlapping patterns, or TH may show a compensatory increase in synthesis and transport. The stability or the resistance of the CN TH may help to explain why PD symptoms are seen only when the SN DA cell population is substantially reduced; meaning that a small population of SN DA neurons is enough to maintain the spatial innervation of the CN. There were, however, noticeable degeneration of the dispersed TH-containing fibers seen along the frontal lobe caudate border on the ipsilateral side. If an excess of SAM is involved in PD, this may help to explain the association of cognitive impairments with PD *(63)*.

PD is an age-related disorder and shows many of the disabling characteristics that are seen in aging, so there may be some commonality between the factors that underlie PD and those that are involved in the aging process. One such factor may be an increase in SAM-dependent methylation. Incidentally, methylation is increased as a function of aging, noted by the increase in the utilization of SAM, the biological methyl donor *(38–42)*, increases in the activity of methionine-adenosyl transferase, the enzyme that synthesizes SAM *(43)*, as well as increases in the activities of various methyl transferases *(38–40,42)*. The increase in methylation reactions as a function of aging may account for the

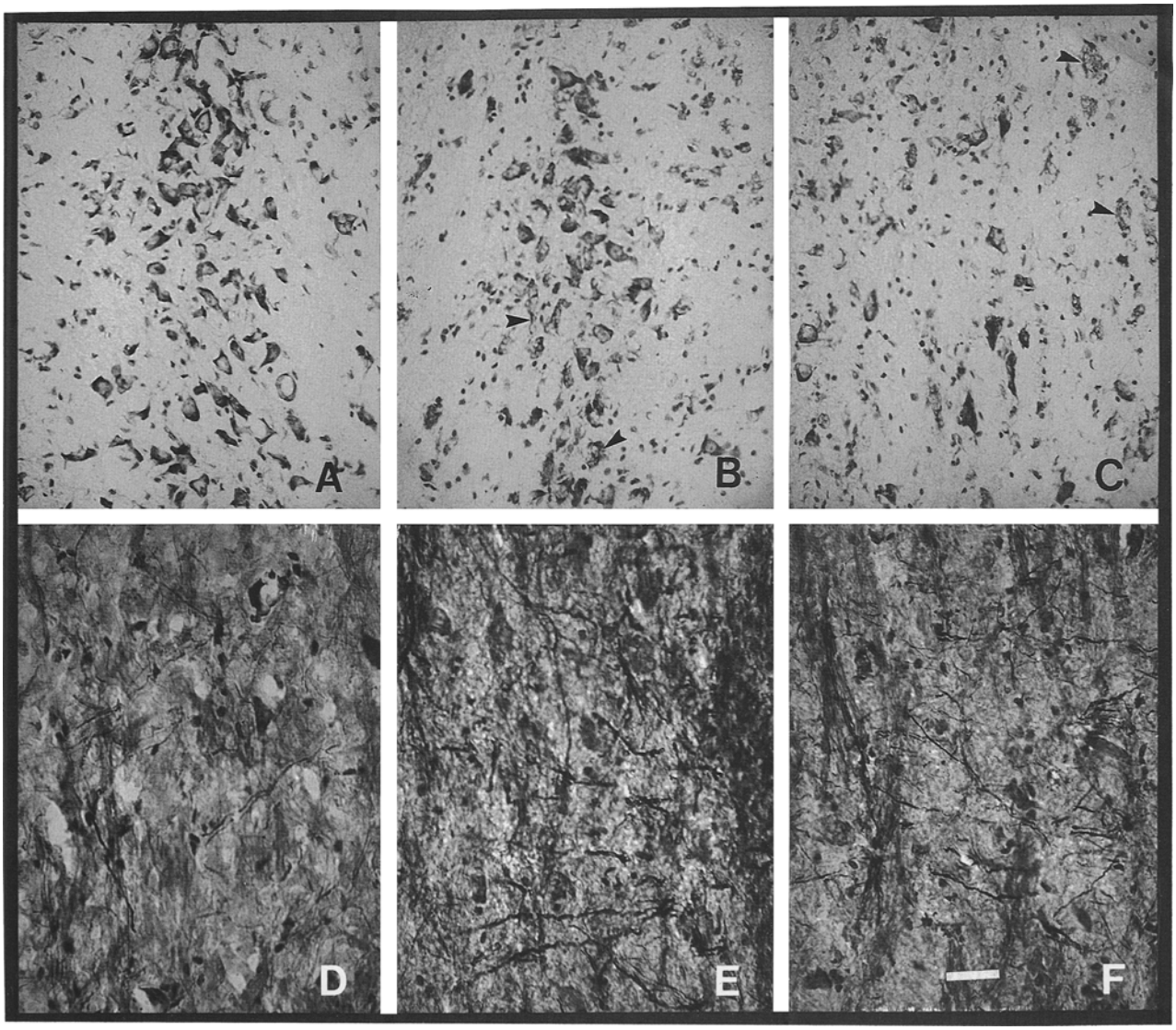

Fig. 6. Photomicrographs of a high magnification of horizontal sections of the pars compacta region of the substantia nigra of rats treated with PBS or with 1 μmol of SAM. The ipsilateral pars compacta of a PBS-injected rat (**A** and **D**), and the contralateral (**B** and **E**) and ipsilateral (**C** and **F**) pars compacta of a rat treated with SAM are shown. A, B, and C were Nissl stained. They highlight the loss of cells caused by SAM (B and C). The cell loss was more severe in the ipsilateral (C) as compared with the contralateral side (B) of the SAM-injected rat. Note also the degenerated cell fragments (arrows) in B and C. D, E, and F are matching sections to A, B, and C and show silver impregnation of degenerating fibers in the SAM-injected rat pars compacta (E and F), as compared to the PBS-injected rat pars compacta (D). Bar = 50 μm.

observed decreased levels of SAM *(39,41,42)* and increased levels of S-adenosylhomocysteine (SAH) and adenosine *(41)* in aged animals.

Increased methylation in aging and the proposed increase in PD *(33–35)* may be responsible for the fact that the markers of aging are strikingly similar to the symptoms of PD. In both conditions movement is slower, strides are shorter *(64)*, reaction time increases, posture is flexed, and facial expressions are blank and emotionally deficient. There are lesions in the SN and the locus ceruleus and DA, NE, and TH are depleted. There seems to be a preponderance of cholinergic activity, increased lipofuscin, and gliosis. The similarity between PD

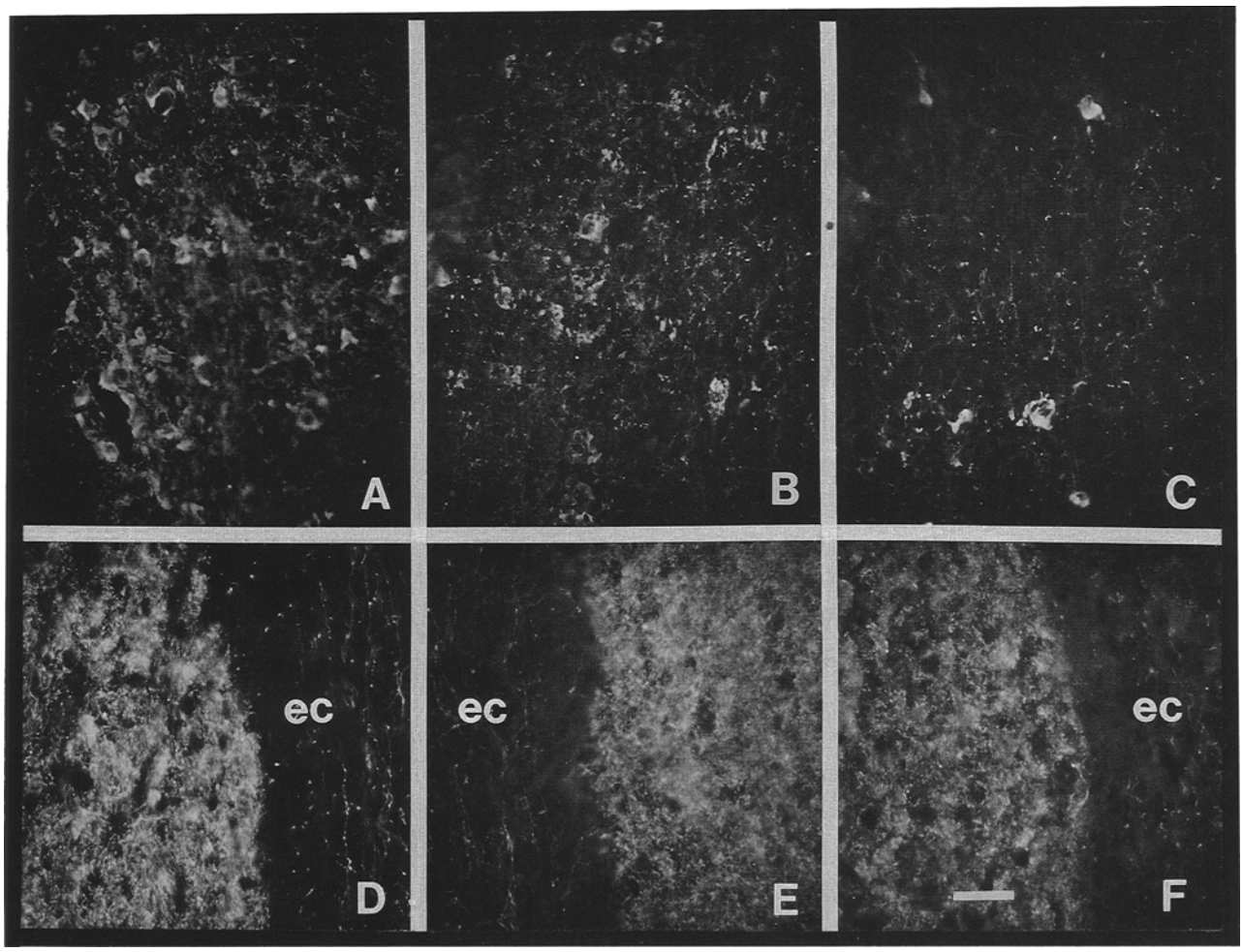

Fig. 7. Decrease in tyrosine hydroxylase immunoreactivity (TH-IR) in the pars compacta region of the substantia nigra (SN) of PBS or SAM-injected rats. Shown are the ipsilateral pars compacta **(A)** to the side of injection of a PBS-injected rat and the contralateral **(B)** and ipsilateral pars compacta of a SAM-injected rat. A reduction of TH-IR and TH immunoreactive cells in the pars compacta occurs following the injection of SAM (B and **C**), as compared with PBS (A). The loss of TH-IR and TH-IR-containing cells was severe in the ipsilateral side (C), as compared with the contralateral side (B) and with the pars compacta of a rat injected with PBS (A). **(D–F)** Photomicrographs representing rat caudate nucleus bordering the cerebrum (dark area). The sections matched A, B, and C. Moderate loss of TH-IR occurred in the caudate nucleus (F) ipsilateral to the SAM-injected lateral ventricle, and marked reduction of fine fibers occurred in the cerebrum region (ec) of the SAM-injected rats (E and F), with more severe reduction seen in the ipsilateral cerebrum (F). Bar = 50 μm.

and aging may support the description of PD as a premature rapid aging of the striatal DA system *(31)*, probably precipitated by an excess of SAM-dependent methylation reactions *(33)*, in individuals endowed with a small number of SN DA cells. Methylation may induce reactions that chronically debilitate the sensitive nigrostriatal motor controlling system, causing a gradual age-related decline in motor functions. The onset of parkinsonism will vary and will be dependent on whether, or when, the number of DA neurons decline below the minimum value necessary to maintain the negrostriatal motor functions.

Acknowledgments

The technical assistance of Reta Sharan is gratefully acknowledged. This work was supported by NIH RO1 NS28432 and NS31177 and RCMI RR03032.

References

1. Tretiakoff C. (1919) Thesis, Paris. (Cited in Schultz ref. *41*).
2. Foix C. and Nicolesco J. (1925) Masson Paris. (Cited in Jellinger K. [1986] Overview of morphological changes in Parkinson's disease. *Adv. Neurol.* **45,** 1–18).
3. Greenfield J. G. and Bosanquest F. D. (1953) *J. Neurol. Neurosurg. Psychiat.* **16,** 213.
4. Hornykiewicz O. (1966) *Pharmacol. Rev.* **18,** 925.
5. Poirier L. J. and Sourkes T. L. (1965) *Brain* **88,** 181.
6. Nagatsu T. (1990) *Adv. Neurol.* **53,** 207.
7. Yahr M. D. and Bering E. A. (1968) in *Parkinson's Disease: Present Status and Research Trends.* Columbia University Press, New York, p. 47.
8. Bernheimer H. and Hornykiewicz O. (1965) *Klin-ther. Wschr.* **42,** 711. (Cited in Schultz, ref. *41*).
9. Barbeau A. (1968) *Proc. Aust. Asso. Neurol.* **5,** 95.
10. Sharman D. F. (1976) in *Third Symposium on Parkinson's Disease* (Gillingham F. J. and Donaldson I. M. L., eds.), E. and L. Livingston, London, p 24.
11. Bernheimer H., Birkmayer W., and Hornykiewicz O. (1961) *Klin–ther. Wschr.* **39,** 1056. (cited in Schultz, ref. *41*).
12. Ehringer H. and Hornykiewicz O. (1960) *Klin-ther. Wschr.* **38,** 1236. (Cited in Schultz, ref. *41*).
13. Paulus W. and Jellinger K. (1991) *J. Neuropath. Exprl. Neurol.* **50,** 743.
14. Hoehn M. N., Crowlry T. J., and Rutledge C. O. (1976) *J. Neurol. Neurosurg. Psychiat.* **39,** 941.
15. Jovoy-Agid F. and Agrid Y. (1980) *Neurology* **30,** 1326.
16. Turkka J. T. and Myllyla V. V. (1987) *Eur. J. Neurol.* **26,** 1.
17. Ludin S. M., Steiger U. H., and Ludin H. P. (1987) *J. Neurol.* **235,** 10.
18. Shelby G. (1968) *J. Neurol Sci.* **6,** 517.
19. Alvord E. C. Jr., Forno L. S., Russke J. A., Kaufman R. J., Rhodes J. S., and Goetowski C. R. (1974) *Adv. Neurol.* **5,** 175.
20. Jager D. H. and Bethlem J. (1960) *J. Neurol. Neurosurg. Psychiat.* **6,** 283.
21. Ohama E. and Ikuta F. (1976) *Acta. Neuropathol. (Berl.)* **34,** 311.
22. Langston J. W. and Forno L. S. (1978) *Ann. Neurol.* **3,** 129.
23. Eadie M. J. (1963) *Brain* **86,** 781.
24. Vanderhaegen J. J., Poirier O., Steronon J. E. (1970) *Arch. Neurol.* **22,** 207.
25. Rajput A. H. and Rozdilsky B. (1970) *J. Neurol. Neurosurg. Psychiat.* **39,** 1092.
26. Forno L. S. and Norvill R. L. (1976) *Acta Neuropathol.* **34,** 183.
27. Jellinger R. (1986) *Adv. Nerurol.* **45,** 1.
28. Jager W. A. den (1969) *Arch. Neurol. (Chicago)* **21,** 615.
29. Ward C. D., Duvosin R. C., Ince S. E., Nutt J. D., Elridge R., and Calne D. B. (1983) *Neurology* **33,** 815.
30. Knoll J. (1988) *Mech. Aging and Dev.* **46,** 237.
31. McGree P. L., McGree E. G., and Suzeki J. S. (1977) *Arch. Neurol.* **34,** 33.
32. Charlton C. G. and Way E. L. (1978) *J. Pharm. Pharmac.* **30,** 819.
33. Charlton C. G. (1990) in *Basic Clinical and Therapeutic Aspects of Alzheimer's and Parkinson's Diseases,* vol. 1 (Nagatsu T. et al., eds.), Plenum, New York, pp. 333–339.
34. Charlton C. G. and Crowell B. Jr. (1992) *J. Pharm. Biochem. Beh.* **43,** 423.
35. Crowell B. G. Jr., Benson R., Shockley D., and Charlton C. G. (1993) *Beh. Neural. Bio.* **59,** 186.
36. Ernst A. M. (1962) *Nature (Lond.)* **193,** 178.
37. Collins M. A., Neafsey E. J., Matsubara K., Cobuzzi R. J. Jr., and Rollema H. (1992) *Brain Res.* **570,** 154.
38. Mays L. L. and Borek E. (1973) *Nature* **243,** 411.
39. Stramentinoli G., Gualano M., Catto E., and Algeri S. J. (1977) *J. Gerontol.* **32(4),** 392.
40. Tuomisto L. (1977) *J. Neurochem.* **28,** 271.
41. Gharib A., Sarda N., Chabannes B., Cronenberger L., and Pacheco H. (1982) *J. Neurochem.* **38(3),** 810.
42. Sellinger O. Z., Kramer C. M., Conger A., and Duboff G. S. (1988) in *Mechanism of Aging and Development* **43,** 161.
43. Volpe J. J. and Laster L. J. (1970) *J. Neurochem.* **17,** 413.
44. Baldessarini R. J. and Ropin I. J. (1966) *J. Neurochem.* **13,** 769.
45. Blusztajn J. R., Ziesel S. H., and Wurtman R. J. (1979) *Brain Res.* **179,** 319.
46. Hirata F. and Axelrod J. (1978) *Nature* **275(5677),** 219.
47. Muenter M. D., Sharpless N. S., and Tyce G. M. (1972) *Mayo Clin. Proc.* **47,** 389.
48. Feuerstein C., Tauche M., Serre F., Gavend M., Pellat J., and Perret J. (1977) *Acta. Neurol. Scand.* **56,** 79.
49. Mena M. A., Murados V., Brazen E., Reiriz J., and De Yebenes J. G. (1977) *Adv. Neurol.* **45,** 481.
50. Hardie R. J., Lees A. J., and Stern G. M. (1986) *Adv. Neurol.* **45,** 487.
51. Wurtman R. J., Rose C. M., and Matthyssee S. (1970) *Science* **169,** 395.
52. Mays-Hoopes L. (1985) in *Molecular Biology of Aging: Gene Stability and Gene Expression* (Sohal R. S., et al. eds.), Raven, New York, p. 49.
53. Albert B., Bray D., Lewis J., Raff M., Roberts R., and Watson J. D. (1989) *Molecular Biology of the Cell,* 2nd ed. Garland, New York.
54. Jenner P. J. and Marsden C. D. (1988) in *Parkinson's Disease and Movement Disorders* (Jankovic J. and Tolosa E., eds.), Urban and Schwarzenberg, Baltimore–Munich, pp. 37–48.
55. Schultz W. (1988) *Gen. Pharmac.* **19(2),** 153.

56. Coons A. H. (1958) in *General Cytochemical Methods* (Danielli J. F., ed.), Academic, New York, pp. 399–422.
57. Clark G. (1989) *Staining Procedures*, 4th ed., Williams and Wilkins, Baltimore, MD.
58. Fink R. P. and Heimer L. (1967) *Brain Res.* **4,** 369.
59. Crowell B. Jr. and Charlton C. G. (1992) *Neurosci. Abst.* **658,** 15.
60. Gagnon C., Axelrod J., and Brownstein M. J. (1978) *Life Sci.* **22,** 2155.
61. Diliberto E. J., Viveros O. H., and Axelrod J. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 4050.
62. Randal E. I. (1990) *Adv. Neurol.* **53,** 305.
63. Maybery H. S., Starkstein S. E., Sadzot B., Preziosi T., Andrezejewski P. L., Dannals R. F., Wagner H. N. Jr., and Robinson R. G. (1990) *Ann. Neurol.* **28,** 57.
64. Goldman J. and Cote L. (1991) in *Principle of Neural. J. Science*, 3rd ed. (Kendel E. R., Schwartz J. H., and Jessell T. M., eds.), Appleton and Lange, Norwalk, CT, pp. 974–983.