

ORIGINAL ARTICLES

Alterations in Glutathione Levels in Parkinson's Disease and Other Neurodegenerative Disorders Affecting Basal Ganglia

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Reduced glutathione (GSH) and oxidized glutathione (GSSG) levels were measured in various brain areas (substantia nigra, putamen, caudate nucleus, globus pallidus, and cerebral cortex) from patients dying with Parkinson's disease, progressive supranuclear palsy, multiple-system atrophy, and Huntington's disease and from control subjects with no neuropathological changes in substantia nigra. GSH levels were reduced in substantia nigra in Parkinson's disease patients (40% compared to control subjects) and GSSG levels were marginally (29%) but insignificantly elevated; there were no changes in other brain areas. The only significant change in multiple-system atrophy was an increase of GSH (196%) coupled with a reduction of GSSG (50%) in the globus pallidus. The only change in progressive supranuclear palsy was a reduced level of GSH in the caudate nucleus (51%). The only change in Huntington's disease was a reduction of GSSG in the caudate nucleus (50%). Despite profound nigral cell loss in the substantia nigra in Parkinson's disease, multiple-system atrophy, and progressive supranuclear palsy, the level of GSH in the substantia nigra was significantly reduced only in Parkinson's disease. This suggests that the change in GSH in Parkinson's disease is not solely due to nigral cell death, or entirely explained by drug therapy, for multiple-system atrophy patients were also treated with levodopa. The altered GSH/GSSG ratio in the substantia nigra in Parkinson's disease is consistent with the concept of oxidative stress as a major component in the pathogenesis of nigral cell death in Parkinson's disease.

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The process underlying dopaminergic cell loss in the substantia nigra (SN) in patients with Parkinson's disease (PD) is unknown. Recent evidence from post-mortem studies indicates that the induction of oxidative stress may play a role. Thus, in the SN of patients dying with PD there are increased levels of total iron [1, 2], decreased ferritin levels [3], increased lipid peroxidation [4, 5], and a reduced activity of complex I of the mitochondrial respiratory chain [6]. Free radical toxicity normally is prevented by a range of antioxidant vitamins and protective enzymes. Most of these appear to be intact in the SN in PD. Thus, levels of catalase and glutathione peroxidase are normal or moderately reduced [7-9] while superoxide dismutase activity may be increased [10]. Normal levels of ascorbic acid and α -tocopherol are found in the SN in PD [3, 11].

Another important component protecting against oxidative stress is reduced glutathione (GSH), which may be depleted in PD. Glutathione plays a major role in the removal of peroxides, so preventing the formation of more damaging oxygen radicals, such as the hydroxyl radical. Normally a high ratio of reduced GSH to oxidized glutathione (GSSG) is maintained [12]. Glutathione is also important for the maintenance of α -tocopherol and ascorbic acid in the reduced state [13]. A disappearance of total glutathione and GSH content in the SN of patients dying with PD was first reported by Perry and colleagues [14]. However, these findings were criticized [15] because of the high proportion of GSSG reported in control subjects and because of the total absence of GSH in PD patients. Nevertheless, the concept of altered glutathione levels

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in PD is supported by a more recent report by Riederer and colleagues [2] who showed total glutathione levels to be reduced by 50% in the SN. It remains unclear whether a reduction in GSH levels does occur in SN, whether this is specific to PD, or whether it occurs in other neurodegenerative disorders affecting the SN. It is also unknown whether any change in glutathione in SN in PD occurs as a result of levodopa treatment.

Consequently, we have analyzed GSH and GSSG levels in a range of brain areas in patients with PD and in patients with other degenerative disorders, namely multiple-system atrophy (MSA), progressive supranuclear palsy (PSP), and Huntington's disease (HD), and compared them to levels in normal individuals.

Materials and Methods

Brain tissue from patients dying with PD, PSP, or MSA and from control subjects dying of nonneurological disorders was obtained from the Brain Bank of INSERM U289, Hôpital de la Salpêtrière, Paris, and the Parkinson's Disease Society Brain Bank, London. Tissue from patients dying with HD was supplied by Dr Gavin Reynolds, Department of Biomedical Science, University of Sheffield.

Tissue Preparation

In London, at autopsy, brains were removed and divided midsagittally. One-half of the brain was immediately frozen at -20°C , transported on Cardice to the Brain Bank, and frozen at -70°C until dissection. The other half of the brain was placed in 10% formal saline solution for at least 6 weeks prior to neuropathological examination. In Paris, less than 2 hours after autopsy, the brainstem was first separated from the rest of the brain. Subsequently, one hemisphere of the brain was stored at -70°C until dissection, while the other half was used for neuropathological examination.

The SN (total or zona compacta), putamen, caudate nucleus, globus pallidus, and cerebral cortex (Brodmann area 10) were dissected from frozen brain according to the technique described by Dexter and coauthors [4]. Samples from each brain area were stored at -70°C until the time of bio-

chemical analysis. Brain samples from patients with neurological disease were assayed at the same time as samples from control subjects, which were matched as much as possible for age and postmortem delay. For some subjects, insufficient material was available for assays to be undertaken in all regions of the individual brains. The numbers of samples studied from each brain area in each group are shown in Figures 3 through 6. Whether total SN or zona compacta alone was utilized is stated in the legends of the individual figures.

Different control brains were used for comparison with brains representing each of the neurodegenerative diseases studied. This was undertaken to ensure appropriate age matching and that postmortem handling, storage, and dissection were as similar as possible in each group. This was particularly important when brain material came from different brain banks and every effort was made to obtain matched control samples from the same source of brain material.

Parkinson's Disease Group

Brain tissue was obtained from 13 control subjects with no known history of psychiatric or neurological disorder and 16 PD patients (Table 1). Histological diagnosis of PD was confirmed in all the tissues utilized by the severe neuronal loss in the SN pars compacta and the presence of Lewy bodies in surviving cells. The control subjects and PD patients were closely matched for age and postmortem delay (time between death and removal of brain). The caudate dopamine content was significantly lower in the PD patients ($1.02 \pm 0.25 \mu\text{g/gm}$) compared to the control subjects ($2.84 \pm 0.40 \mu\text{g/gm}$; $p < 0.05$, Student's *t* test).

Multiple-System Atrophy Group

Brain tissue was obtained from 10 control subjects with no known history of psychiatric or neurological disorder and 7 MSA subjects (Table 1). Pathological diagnosis of MSA was confirmed by the presence of striatonigral degeneration with marked gliosis and cell loss in the SN, caudate nucleus, and putamen. In addition, there were pathological changes that varied in severity involving the olivopontocerebellar system and preganglionic autonomic nuclei. Lewy bodies were not observed in any brain area from MSA patients. Control subjects and MSA patients were closely matched for age and postmortem delay.

Table 1. Characteristics of Control Subjects and Parkinson's Disease (PD) and Multiple-System Atrophy (MSA) Patients^a

Patient Details	Control (n = 13)	PD (n = 16)	Control (n = 10)	MSA (n = 7)
Age (yr)	73.5 \pm 2.9	77.1 \pm 1.3	68.3 \pm 5.7	63.4 \pm 3.0
Sex				
Female	1	4	1	2
Male	12	12	9	5
Age at onset of disease (yr)		66.8 \pm 2.5 (59–73)		56.4 \pm 2.8 (47–72)
Duration of disease (yr)		12.4 \pm 3.1 (5–26)		7.0 \pm 0.9 (3–10)
Levodopa dosage level at time of death		470 \pm 130 mg/day (100–1,000 mg)		618 \pm 116 mg/day (330–1,100 mg)
Time between death and removal of brain (hr)	15.6 \pm 2.2	13.4 \pm 1.9	14.2 \pm 1.5	17.0 \pm 3.6

^aValues are expressed as mean \pm standard error of mean. Ranges of values are indicated in parentheses.

Table 2. Characteristics of Control Subjects and Progressive Supranuclear Palsy (PSP) and Huntington's Disease (HD) Patients^a

Patient Details	Control (n = 15)	PSP (n = 11)	Control (n = 10)	HD (n = 10)
Age (yr)	80.3 ± 1.9	70.0 ± 2.2 ^b	50.1 ± 4.7	52.9 ± 3.5
Sex				
Female	9	6	1	3
Male	5	5	9	7
Duration of disorder (yr)		NA		9.4 ± 1.9
Time between death and removal of brain (hr)	9.4 ± 1.8	11.8 ± 1.9	36.0 ± 7.3	50.7 ± 10.7 ^b

^aValues are represented as mean ± standard error of mean.

^b*p* < 0.05 as compared to control subjects, Student's *t* test.

NA = not available.

Progressive Supranuclear Palsy Group

Brain tissue was obtained from 15 control subjects with no known history of psychiatric or neurological disorder and 11 PSP patients (Table 2). Pathological diagnosis of PSP was confirmed by nerve cell loss in the SN, globus pallidus, and brainstem nuclei, including the locus ceruleus and superior colliculus, and the presence of globose neurofibrillary tangles in surviving neurons in the pallidum, subthalamic nucleus, midbrain, and dentate nucleus. Control subjects and PSP patients were closely matched for postmortem delay, but the mean age of PSP patients was significantly lower than that of control subjects.

Huntington's Disease Group

Brain tissue was obtained from 10 control subjects with no known history of psychiatric or neurological disorder and 10 HD patients (Table 2). Morphological diagnosis of HD was confirmed by macroscopic atrophy with gliosis and marked neuronal loss in the caudate nucleus and putamen. The time between death and removal of the brain was longer for the HD patients than the control subjects.

Measurement of Glutathione Levels

Brain tissue was homogenized in 6 volumes ice-cold 0.4 M perchloric acid containing 0.1 mM diethylenetriaminepentaacetic acid using a microsonic probe. The samples were centrifuged at 4,000 rpm, 4°C for 15 minutes, and the supernatant was analyzed for GSSG and GSH content immediately after homogenization.

Measurement of Oxidized Glutathione

GSSG was measured by the enzymatic recycling procedure described by Tietze [16] and Slivka and coauthors [15].

An aliquot (0.15 ml) of the tissue supernatant was added to 1.35 ml of 11 mM *N*-ethylmaleimide (NEM) in 100 mM potassium phosphate buffer containing 5 mM ethylenediaminetetraacetic acid, pH 7.5. After a 20-minute incubation period at room temperature, the reaction mixture was passed through Sep-Pak C-18 cartridges (Millipore, Waters Associates, Watford, United Kingdom) to remove unreacted NEM. Preliminary experiments showed 99.6% (n = 6) of the unreacted NEM was retained by the cartridges.

Spectrophotometric assays were performed using 1.5 ml of eluate to which 0.4 mM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), 0.17 mM NADPH, and 16 µg/ml of GSSG reduc-

tase were added. The final assay volume was 2.0 ml. The reaction was initiated by the addition of GSSG reductase.

The rate of reduction of DTNB to 5-thio-2-nitrobenzoate (TNB) was measured at 412 nm using a Shimadzu double-beam spectrophotometer at ambient temperature for 5 minutes. A standard curve was constructed using known amounts of synthetic GSSG (10–100 ng). The standard curves to GSSG were linear (*r* = 0.994) over this concentration range.

Measurement of Reduced Glutathione

GSH was measured using a minor modification of the method described by Reed and coauthors [17]. An aliquot (0.17 ml) of tissue supernatant was added to the internal standard (1 mM cysteic acid) and 0.88 M iodoacetic acid. Excess sodium hydrogen carbonate was added to precipitate sodium perchlorate. The samples were incubated at room temperature in the dark for an hour. Subsequently, 0.5 ml of an alcoholic solution of 1.5% (vol/vol) 2,4-dinitrofluorobenzene was added and the samples were incubated for a further 4 hours. Then diethyl ether (1.0 ml) was added to the samples, which were shaken and then centrifuged at 2,000 rpm, room temperature, for 20 minutes. The aqueous phase containing derivatized glutathione was separated and analyzed by high-performance liquid chromatography (HPLC).

Aliquots (10 µL) were injected onto a Spherisorb S-5 amino CDS column (25 × 4.6 mm, Phase Separations) and eluted with an ammonium acetate gradient in glacial acetic acid, methanol, and water (pH 5.05; flow rate 1.00–1.25 ml/min at 2,700 psi). GSH levels were measured using a Waters ultraviolet detector (model 441) at a wavelength of 365 nm. Chromatographic peaks were integrated by a Waters 645 data module. GSH derivatives were quantified in relation to the internal standard (cysteic acid).

Statistics

GSH and GSSG levels in each brain region from control subjects and patients with PD, MSA, PSP, or HD were compared using a 1-tailed two-tailed Student's *t* test.

Results

Glutathione Levels in Control Subjects

A high GSH/GSSG ratio (mean, 318:1; range, 226–800:1) was observed in control subjects from all the groups examined for the present study. There was no

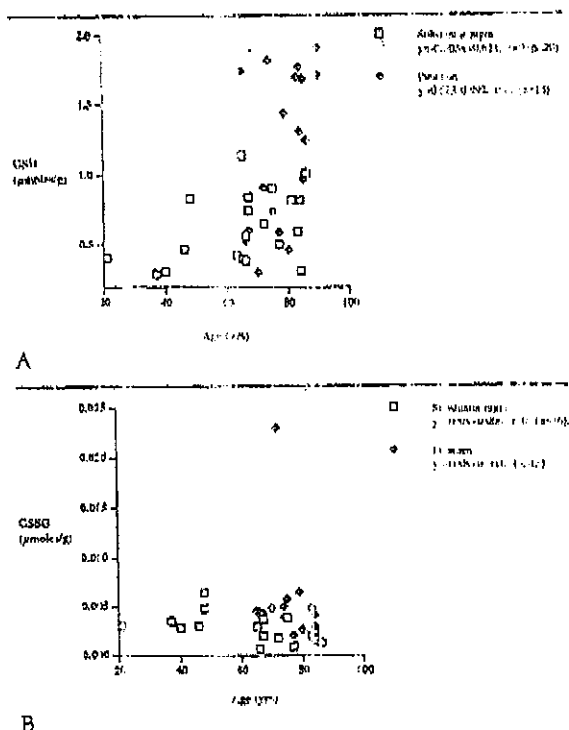


Fig 1. Linear regression analysis for (A) reduced glutathione (GSH) levels ($\mu\text{mol/gm wet weight}$) with age; and (B) oxidized glutathione (GSSG) levels ($\mu\text{mol/gm wet weight}$) with age in the total substantia nigra and putamen from control subjects. There was no correlation between glutathione levels and patient age (regression coefficient < 0.01 ; $n = 13-20$).

obvious correlation between age and GSH or GSSG levels in the SN and putamen of control subjects (Fig 1). Similarly, there was no correlation between post-mortem delay (time between death and removal of brain) and GSH (Fig 2A) or GSSG (Fig 2B) content in the SN and putamen.

Glutathione Levels in Parkinson's Disease

There was a significant reduction in GSH in the SN (40%) from all PD patients compared to control subjects (PD: $n = 16$, $0.45 \pm 0.03 \mu\text{mol/gm wet weight}$; control: $n = 13$, 0.75 ± 0.46 ; $p < 0.01$). This applied to both the SN pars compacta and to the total SN (Fig 3A). GSH levels in the cerebral cortex and putamen were somewhat decreased compared to control values, but these changes did not reach statistical significance. No alterations in GSH occurred with other brain areas studied.

In contrast, GSSG levels (Fig 3B) were not altered in PD patients compared to control subjects. The GSSG content in the SN from all PD patients was insignificantly increased by 29% (PD: $n = 13$, $0.0031 \pm 0.0004 \mu\text{mol/gm wet weight}$; control: $n = 12$, 0.0024 ± 0.0004 , $p = 0.19$, not significant [NS]).

Glutathione Levels in Multiple-System Atrophy

The GSH content of the lateral globus pallidus from MSA patients was approximately double (196%) that found in control subjects (Fig 4A). There was a corresponding reduction (60%) of the GSSG content in the lateral globus pallidus in MSA patients (Fig 4B). There were no significant changes in GSH or GSSG levels in other brain areas examined.

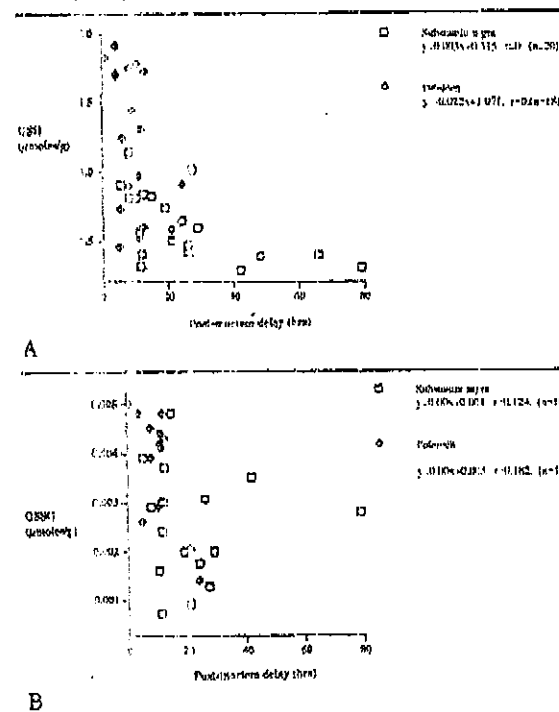
Glutathione Levels in Progressive Supranuclear Palsy

GSH levels in PSP patients were reduced in the caudate nucleus (51%) (Fig 5A). There were no alterations in GSH content in the cerebral cortex, putamen, or SN. GSSG content in PSP patients was not altered in any of the brain areas examined (Fig 5B). (There was not sufficient nigral tissue to measure GSSG levels.)

Glutathione Levels in Huntington's Disease

The GSH content in cerebral cortex, caudate nucleus, and SN pars compacta from HD patients was not altered compared to control values (Fig 6A). There was a marked reduction in the GSSG (50%) content in the caudate nucleus from HD patients compared to control

Fig 2. Linear regression analysis for (A) reduced glutathione (GSH) levels ($\mu\text{mol/gm wet weight}$) with postmortem delay (time between death and removal of brain) and (B) oxidized glutathione (GSSG) levels ($\mu\text{mol/gm wet weight}$) with postmortem delay (hours) in the total substantia nigra and putamen from control subjects. There was no correlation between glutathione levels and postmortem delay (regression coefficient < 0.01 ; $n = 13-20$).



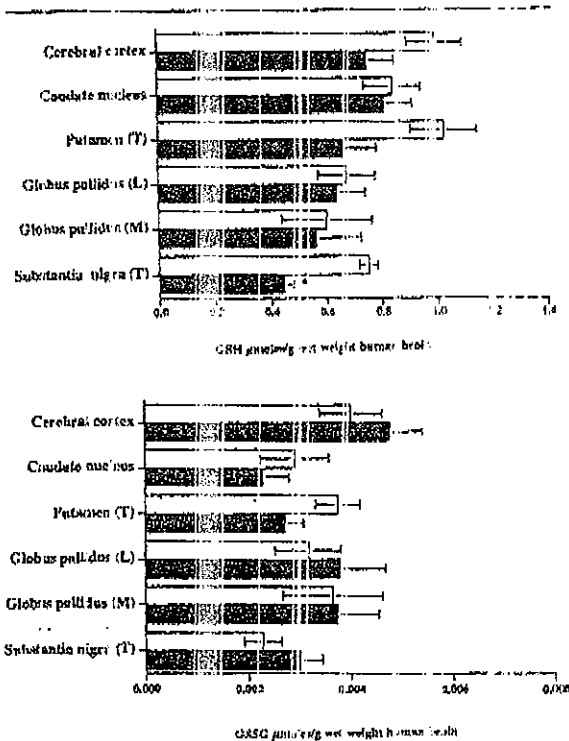


Fig 3. Levels of (top) reduced glutathione (GSH) ($\mu\text{mol/gm}$ wet weight) and (bottom) oxidized glutathione (GSSG) ($\mu\text{mol/gm}$ wet weight) in Parkinson's disease (hatched bars) and age-matched control (open bars) human autopsy brains. Values are represented as mean \pm standard error of mean. Asterisk indicates $p < 0.01$ compared with controls, Student's t test. Number of samples is shown in each bar. T = total; L = lateral; M = medial; C = zona compacta.

subjects (Fig 6B). (There was not sufficient tissue to measure nigral GSSG levels.)

GSH/GSSG Ratio in Substantia Nigra in Parkinson's Disease and Multiple-System Atrophy

The nigral GSH/GSSG ratio tended to be decreased in both PD and MSA patients compared to control subjects (Fig 7). The reduction of the GSH/GSSG ratio in PD patients reached statistical significance ($p < 0.05$); that in MSA patients only just failed to meet statistical significance ($p = 0.051$). No ratios could be calculated for PSP or HD patients since nigral GSSG levels could not be measured due to the limited sample available.

Discussion

Methodological Issues

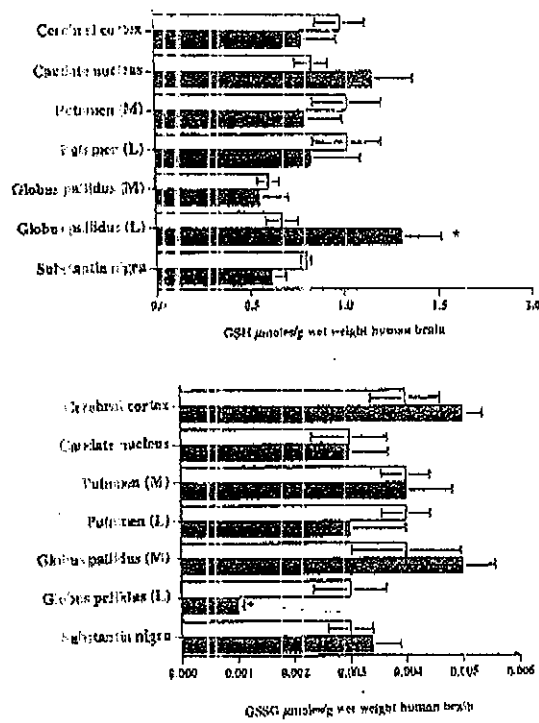
With the glutathione analysis method of Sivka and colleagues [15], a high GSH/GSSG ratio was observed in the brains of all control subjects. This indicates that

little autolytic loss of glutathione had taken place as a result of postmortem delay. Indeed, in this study there was no correlation between glutathione levels and post-mortem delay in control subjects. The greater part (>98%) of total glutathione was in the reduced form (GSH), which is consistent with the normal oxidative state of the brain [12]. The levels of GSH and GSSG found were in accordance with those previously reported [15]. The much higher levels of GSSG previously reported by Perry and coauthors [14] must have been due to postmortem delay or methodological artifacts. In the PSP group studied here, there was a significant difference in age between disease brains and control brains. However, there was no correlation between GSH or GSSG levels and age in any group studied.

Alterations in Glutathione in the Substantia Nigra

The present data indicate a selective reduction of GSH in the SN of PD brains that occurs without a corre-

Fig 4. Levels of (top) reduced glutathione (GSH) ($\mu\text{mol/gm}$ wet weight) and (bottom) oxidized glutathione (GSSG) ($\mu\text{mol/gm}$ wet weight) in multiple-system atrophy (hatched bars) and age-matched control (open bars) human autopsy brains. Values are represented as mean \pm standard error of mean. Asterisk indicates $p < 0.05$ compared with controls, Student's t test. The difference for GSH in the substantia nigra compared to controls was not significant ($p > 0.1$). Number of samples is shown in each bar. L = lateral; M = medial; T = total.



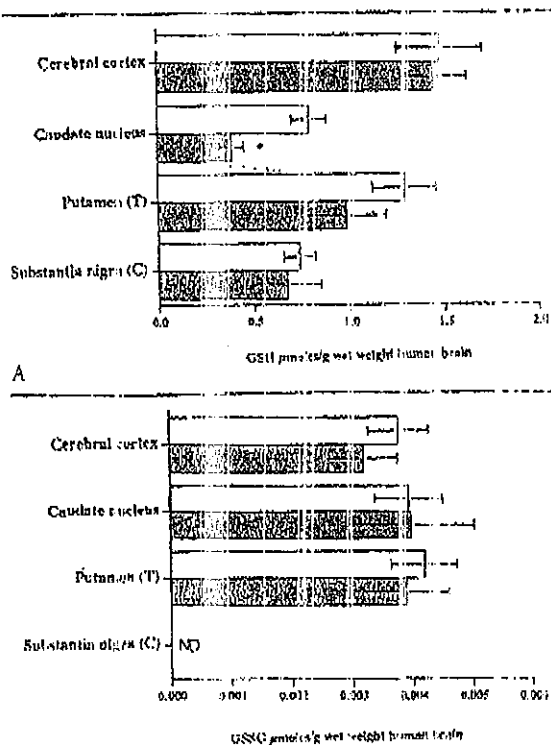


Fig 5. Levels of (A) reduced glutathione (GSH) ($\mu\text{mol/g wet weight}$) and (B) oxidized glutathione (GSSG) ($\mu\text{mol/g wet weight}$) in progressive subcortical palsy (stippled bars) and age-matched control (open bars) human autopsy brains. Values are represented as mean \pm standard error of mean. Asterisk indicates $p < 0.05$ compared with controls, Student's *t* test. The difference for GSH in substantia nigra compared to controls was not significant ($p > 0.05$). Number of samples is shown in each bar. T = total; C = compacta; ND = not determined.

sponding significant increase in GSSG content. There were no other changes in glutathione content in PD brains. However, the ratio GSH/GSSG in the SN of PD brains was altered in favor of the oxidized form and in a manner consistent with the involvement of oxidative stress in nigral cell loss in PD. The present findings are in agreement with a recent report from Riederer and colleagues which also showed a similar decrease of total glutathione [2] and GSH [18] levels in SN in PD.

The marked nigral depletion of GSH appears to be selective to PD, since it was not observed in the SN of MSA, PSP, or HD brains, although there was a trend toward a reduction in MSA and PSP brains. This suggests that the alterations in GSH levels in PD are not a general consequence of neurodegeneration whatever the cause, but are much more evident in the pathological process underlying PD. There are equivalent degrees of cell loss, around 70%, in the lateral

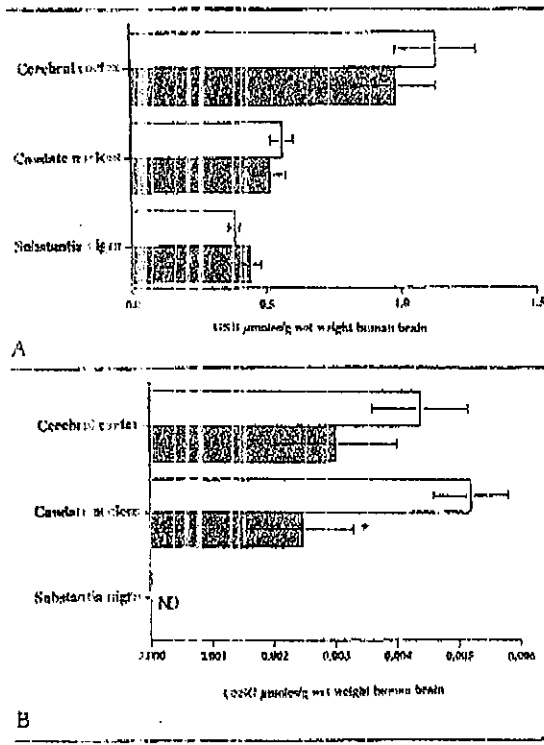


Fig 6. Levels of (A) reduced glutathione (GSH) ($\mu\text{mol/g wet weight}$) and (B) oxidized glutathione (GSSG) ($\mu\text{mol/g wet weight}$) in Huntington's disease (stippled bars) and age-matched control (open bars) human autopsy brains. Asterisk indicates $p < 0.05$ compared with controls, Student's *t* test. Number of samples is shown in each bar. ND = not determined; C = compacta.

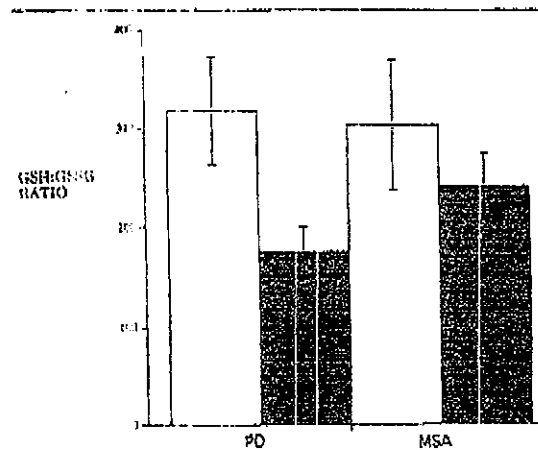


Fig 7. Reduced-oxidized glutathione (GSH/GSSG) ratio in the substantia nigra of Parkinson's disease (PD, $n = 16$) and multiple-system atrophy (MSA, $n = 4$) patients (hatched bars) compared to age-matched control ($n = 10-13$, open bars) human autopsy brains. Values are represented as mean \pm standard error of mean. Asterisk indicates $p < 0.05$ for PD compared with controls, Student's *t* test (for MSA compared to controls, $p = 0.051$).

ventral tier of the zona compacta of the SN in PD, MSA, and PSP [19]. Support for this conclusion comes from HD studies, where not only were nigral levels of GSH unchanged but also there were no alterations in GSH levels in the caudate nucleus despite the marked pathology found in this brain area [20]. The change in GSH in the SN in PD was unlikely to have been due to L-dopa treatment. Patients with MSA also were taking this drug in larger doses on average, yet there was a smaller and insignificant fall in GSH content in MSA brains. However, there was a fall in the ratio of GSH to GSSG both in PD and MSA brains (although that in MSA brains just failed to meet significance); this is compatible with oxidative stress in both conditions, so we cannot rule out a contribution from L-dopa therapy.

Alterations in Glutathione in Other Brain Regions

There were some interesting changes in GSH and GSSG content in other areas in MSA, PSP, and HD brains, but these are difficult to interpret. The most dramatic occurred in the lateral globus pallidus in MSA brains where there was an elevation of GSH levels coupled to a reduction of GSSG content. This area of the globus pallidus is specifically affected in MSA, showing a marked loss of myelinated fibers and gliosis [21]. This finding appears to signify an altered redox state that serves to maintain glutathione in its reduced form [12]. Why this should occur is not clear.

There was a reduction in GSH levels in the caudate nucleus in PSP brains but no equivalent change in GSSG in this area. This change is similar to that observed in the SN in PD brains, and may reflect the presence of oxidative stress. However, the striatum in PSP brains exhibits only mild pathological changes [20]. In HD brains there was a reduction in GSSG in the caudate nucleus but no change in GSH in this area. The reduction of GSSG in HD again is unexplained, but may be secondary to the macroscopic atrophy and loss of the GABAergic medium-sized spiny neurons [20].

Possible Causes of Nigral Glutathione Deficiency in Parkinson's Disease

The decrease of GSH in PD might be the result of defective synthesis, excessive metabolism, or abnormal utilization. Activity of the rate-limiting synthetic enzyme γ -glutamylcysteine synthetase in the SN in PD remains to be determined, as does the enzyme mainly responsible for the translocation and breakdown of both GSH and GSSG, namely γ -glutamyltranspeptidase. The ability to form mercapturate conjugates from glutathione appears unchanged since glutathione transferase activity in the SN in PD reportedly is normal [22].

Given the current concept of oxidative stress as a contributory factor to nigral cell death in PD, changes

in GSH utilization through its oxidative-reductive pathway might be relevant. However, there seems to be no overall change in tissue levels of glutathione peroxidase activity in the SN in PD [9]. Recently, however, a marked elevation was found in glutathione peroxidase-containing glial cells in the SN from parkinsonian patients [23]. Under conditions of intense oxidative stress, utilization of GSH via glutathione peroxidase leads to an irreversible loss of intracellular GSH [24]. Despite the decreased GSH/GSSG ratio in the SN in PD, there was no absolute increase in GSSG levels that might argue against the decrease in GSH arising from oxidative stress. However, GSSG is transported from cells under conditions of oxidative stress as protection against its cytotoxic effects [25].

The precise localization of altered GSH levels in the SN in PD is not known. Since neurons only constitute approximately 1% of the total number of nigral cells, it is likely that the marked nigral GSH depletion found in PD occurs in nonneuronal populations such as glia. Indeed, histochemical studies have shown that GSH is localized mainly in glial cells and the neuropil [26–28]. This raises fundamental questions of the role played by glial cells in the neuronal degeneration in SN that characterizes PD.

Relationship Between Depletion of GSH and Other Indices of Oxidative Stress in the Substantia Nigra

The depletion of GSH in the SN in PD adds to the growing list of biochemical changes (increased lipid peroxidation, raised iron levels, inhibited complex I activity) that suggest oxidative stress as a component of the degenerative process [29]. Recently, we investigated the same biochemical parameters in subjects with incidental Lewy body disease (presymptomatic PD) [30]. In the SN from these subjects, there was no alteration in iron levels and no significant decrease in complex I activity but the levels of GSH were decreased to the same extent as in advanced PD [31]. This suggests that the reduction of GSH is the earliest index of oxidative stress so far uncovered and that only subsequently do alterations in iron metabolism and mitochondrial function become apparent. Interestingly, depletion of GSH in brain using an inhibitor of γ -glutamylcysteine synthetase, namely buthionine sulfoximine, can itself lead to mitochondrial damage [12].

There may, however, be the opposite connection between the depletion of GSH in the SN in PD and inhibition of complex I activity. Thus, hepatocytes exposed to 1-methyl-4-phenylpyridinium ion (MPP⁺) and other mitochondrial toxins show a decrease in GSH content with no corresponding rise in GSSG levels, resulting in a fall in the GSH/GSSG ratio [32]. This effect was attributed to a combination of decreased cellular metabolism and an efflux of GSH from the cells. So even in incidental Lewy body disease

there may be alterations in mitochondrial function that remain to be detected but that lead to GSH depletion.

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