Biol Trace Elem Res (2013) 151:18–29
DOI 10.1007/s12011-012-9535-1

Mineral Composition of and the Relationships Between Them of Human Basal Ganglia in Very Old Age

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Received: 10 July 2012 / Accepted: 18 October 2012 / Published online: 1 November 2012

Abstract Trace elements and the relationships among them were investigated by direct chemical analysis in three basal ganglia regions in very old age individuals and age- and gender-related differences were assessed. After ordinary dissections at Nara Medical University were finished, the caudate nucleus, putamen, and globus pallidus belonging to the basal ganglia were removed from the identical cerebra of the subjects who consisted of 22 men and 23 women, ranging in age from 70 to 101 years (average age=83.3± 7.5 years). After incineration with nitric acid and perchloric acid, the element contents were determined by inductively coupled plasma-atomic emission spectrometry. It was found that the Ca, P, and Mg contents increased significantly in the putamen with aging and the Mg content increased significantly in the globus pallidus with aging, but no elements increased significantly in the caudate nucleus with aging. Regarding the relationships among elements in the basal ganglia, extremely significant direct correlations were found among the Ca, P, and Mg contents in the putamen. These results suggested that slight calcification occurred in the putamen in very old age. With regard to seven elements of Ca, P, S, Mg, Zn, Fe, and Na, it was examined whether there were significant correlations among the caudate nucleus, putamen, and globus pallidus. It was found that there were extremely significant direct correlations among all of the three basal ganglia in the P content. Likewise, with regard to the Fe content, there were extremely or very significant direct correlations among all of the three basal ganglia. Regarding the gender difference in elements, it was found that the Ca content of the caudate nucleus was significantly higher in women than in men.

Keywords Basal ganglia · Caudate nucleus · Putamen · Globus pallidus · Calcium · Phosphorus · Magnesium · Iron · Aging

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Introduction

To elucidate compositional changes of the brain with aging, the authors previously investigated age-related changes of elements in the corpus callosum [1], anterior commissure [2], and fornix [3] of the white matter and the hippocampus [3], dentate gyrus [3], pineal body [4], olfactory bulb and tract [5], mammillary body [6], lateral geniculate body [7], and superior colliculus [7] of the gray matter. It was found that elements changing significantly with aging were different among the various brain regions. In addition, it was found that there were significant gender differences in the elements of the various brain regions [8, 9].

There are several studies [10–14] on age-related changes of the Fe content in the basal ganglia. Postmortem [10] and in vivo [11–14] studies have demonstrated that although Fe

is not present at birth, progressive Fe deposition occurs in different structures of the brain during aging process and the extent of Fe deposition with aging is markedly higher in the basal ganglia than in most other brain structures. However, little work had been done to study age-related changes of other elements except for Fe in the basal ganglia. Therefore, the authors investigated age-related differences of the seven elements, such as Ca, P, S, Mg, Zn, Fe, and Na, in the basal ganglia from the identical cerebra used to investigate other brain regions [2, 3, 6]. It was found that the Ca, P, and Mg contents increased significantly in the putamen with aging and the Mg content increased significantly in the globus pallidus with aging, but no elements increased significantly in the caudate nucleus with aging.

To explore the connection among the basal ganglia, it was examined whether there were significant correlations among the three basal ganglia from a viewpoint of elements and it was found that there were extremely or very significant direct correlations among all of the three basal ganglia with regard to both the P and Fe contents. In addition, a significant gender difference was found in the Ca content of the caudate nucleus.

Materials and Methods

Sampling

Japanese cadavers were treated by injection of a mixture of 36 % ethanol, 13 % glycerin, 6 % phenol, and 6 % formalin through the femoral artery [15]. After ordinary dissections by medical students at Nara Medical University were finished, the caudate nucleus, putamen, and globus pallidus were removed from the identical cerebra of the subjects who consisted of 22 men and 23 women, ranging in age from 70 to 101 years (average age=83.3±7.5 years).

Determination of Elements

After the brain samples were treated with 99.5 % ethanol three times to remove lipids, they were washed thoroughly with distilled water and were dried at 80 °C for 16 h. One-milliliter concentrated nitric acid was added to the samples to incinerate, and the mixtures were heated at 100 °C for 2 h. After the addition of 0.5-ml concentrated perchloric acid, they were heated at 100 °C for an additional 2 h. The samples were adjusted to a volume of 10 ml by adding ultrapure water and were filtered through filter paper (No. 7; Toyo Roshi, Osaka, Japan). The resulting filtrates were analyzed by inductively coupled plasma-atomic emission spectrometry (ICPS-7500; Shimadzu, Kyoto, Japan) [16]. The conditions were 1.2 kW of power from a radiofrequency generator, a plasma argon flow rate of 1.2 l/min, a cooling gas flow of 14 l/min, a carrier gas flow of 1.0 l/min, an entrance slit of 20 μm, an exit slit of 30 μm, a height of observation of 15 mm, and an integration time lapse of 5 s. Especially prepared standard solutions of Ca, Mg, Zn, Fe, and Na for atomic absorption spectrometry and phosphate and sulfate ions for ion chromatography were purchased from Wako Pure Chem. (Osaka, Japan) and were used as standard solutions. The detection limits of elements were determined to be 100 ng/ml for Ca, 50 ng/ml for P, S, Mg, and Na, and 25 ng/ml for Zn and Fe, respectively, from the standards. The element amount was expressed on a dry-weight basis.

Statistical Analysis

Statistical analyses were performed using the GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA). Pearson's correlation was used to investigate the association between parameters. A paired or unpaired Student's t test was used to analyze differences between groups. A p value of less than 0.05 was considered to be significant. Data were expressed as the mean±standard deviation.

Results

Table 1 indicates ages, sexes, and causes of deaths of the subjects used in the present study. The average age (83.3± 7.5 years) of the subjects at their deaths was similar to the mean lifespan (82.6 years old) of Japanese at present.

Element Contents

Table 2 indicates the average contents of seven elements in the caudate nucleus, putamen, and globus pallidus. In all of the caudate nucleus, putamen, and globus pallidus, major elements were Ca, P, and S, whereas minor elements were Mg, Zn, Fe, and Na.

With a paired Student's t test, it was analyzed whether there were significant differences in the average contents of seven elements among the caudate nucleus, putamen, and globus pallidus. The following significant differences were found in the average contents of elements among the basal ganglia: The average content of P was the highest in the globus pallidus, followed by the putamen and caudate nucleus. The average content of S was less in the globus pallidus than in the caudate nucleus. The average content of Zn was significantly higher in the caudate nucleus compared with the globus pallidus. The average content of Fe was higher in both the globus pallidus and putamen compared with the caudate nucleus.

Age-Related Differences of Elements

Figure 1 shows age-related differences of Ca in the caudate nucleus, putamen, and globus pallidus. The correlation coefficients were estimated to be 0.046 (p=0.766) in the caudate

Table 1 The subjects used in the present study

Age (Years)	Sex	Cause of death
70	M	Intestinal obstruction
71	M	Malignant lymphoma
71	W	Acute renal failure
73	M	Lung cancer
74	M	Acute respiratory failure
74	M	Cardiac failure
75	W	Hepatic failure
76	W	Chronic respiratory failure
76	M	Sigmoid cancer
77	M	Septicemia
78	W	Respiratory failure
78	W	Myocardial infarction
79	M	Pneumonia
79	W	Cerebellar hemorrhage
79	M	Thoracic abscess
80	W	Bronchial asthma
80	M	Septicemia
80	M	Prostate cancer
81	W	Pneumonia
81	W	Lung cancer
81	W	Lung cancer
81	W	Pneumonia
83	W	Gall bladder cancer
84	W	Acute drug poisoning
84	M	Pancreatic cancer
85	M	Respiratory failure
85	W	Acute cardiac failure
85	M	Pneumonia
86	W	Acute cardiac failure
86	M	Pneumonia
86	M	Aspiration pneumonia
88	M	Pneumonia
88	M	Respiratory failure
88	M	Stomach cancer
89	W	Acute renal failure
90	W	Pneumonia
90	W	Pancreatic cancer
91	W	Chronic cardiac failure
92	M	Septicemia
92	W	Pneumonia
93	W	Myocardial infarction
94	M	Pneumonia
97	W	Acute respiratory failure
97	W	Senile
101	M	Coronary heart disease

M men, *W* women

nucleus, 0.332 (p=0.026) in the putamen, and 0.140 (p=0.361) in the globus pallidus. A significant direct correlation was found between age and Ca content in the putamen, but no significant correlations were found between them in the caudate nucleus and globus pallidus.

Table 2 Average content of elements in the caudate nucleus, putamen, and globus pallidus

Element	Average content (mg/g)		
	Caudate nucleus	Putamen	Globus pallidus
Ca	4.112±0.719	3.990±0.946	3.972±0.735
P	2.309±1.001	2.843±1.242	3.718±1.287
S	7.553±1.437	7.114±0.820	6.967±0.689
Mg	0.428±0.090	0.441±0.104	0.428±0.087
Zn	0.112±0.069	0.108±0.113	0.091±0.038
Fe	0.525±0.154	0.683±0.176	0.729±0.243
Na	0.056±0.043	0.052±0.089	0.037±0.026

Figure 2 shows age-related differences of P in the caudate nucleus, putamen, and globus pallidus. The correlation coefficients between age and P content were estimated to be 0.230 (p=0.128) in the caudate nucleus, 0.317 (p=0.034) in the putamen, and 0.268 (p=0.075) in the globus pallidus. A significant direct correlation was found between age and P content in the putamen, but no significant correlations were found between them in the caudate nucleus and globus pallidus.

Age-related differences of Mg in the caudate nucleus, putamen, and globus pallidus are shown in Fig. 3. The correlation coefficients between age and Mg content were estimated to be 0.023 (p=0.881) in the caudate nucleus, 0.346 (p=0.020) in the putamen, and 0.356 (p=0.016) in the globus pallidus. Although no significant correlation was found in the caudate nucleus, significant direct correlations were found between age and Mg content in both the putamen and globus pallidus.

Figure 4 shows age-related differences of Fe in the caudate nucleus, putamen, and globus pallidus. The correlation coefficients between age and Fe content were estimated to be −0.205 (p=0.176) in the caudate nucleus, 0.101 (p=0.510) in the putamen, and 0.111 (p=0.468) in the globus pallidus. No significant correlations were found between age and Fe content in the caudate nucleus, putamen, and globus pallidus.

Likewise, no significant correlations were found between age and element contents, such as S, Zn, and Na, in the caudate nucleus, putamen, and globus pallidus.

The age-related differences of elements were also examined in the three basal ganglia of the subject group deleted the eight subjects who died of cardiovascular diseases. In the caudate nucleus, a significant direct correlation was found between age and P content (p=0.025). In the putamen, significant direct correlations were found between age and all of Ca (p=0.016), P (p=0.010), and Mg contents (p=0.019). Very significant direct correlations were found

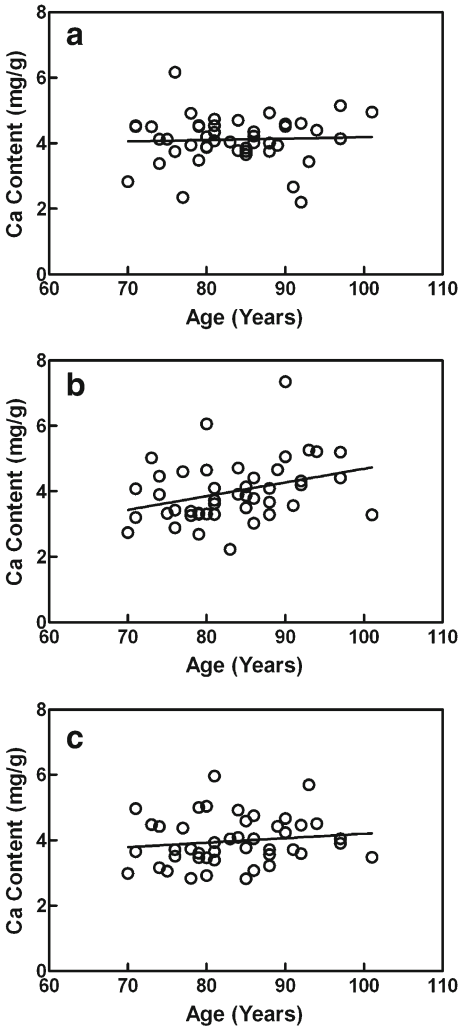

Fig. 1 Age-related differences of the Ca content in the caudate nucleus (**a**), putamen (**b**), and globus pallidus (**c**)

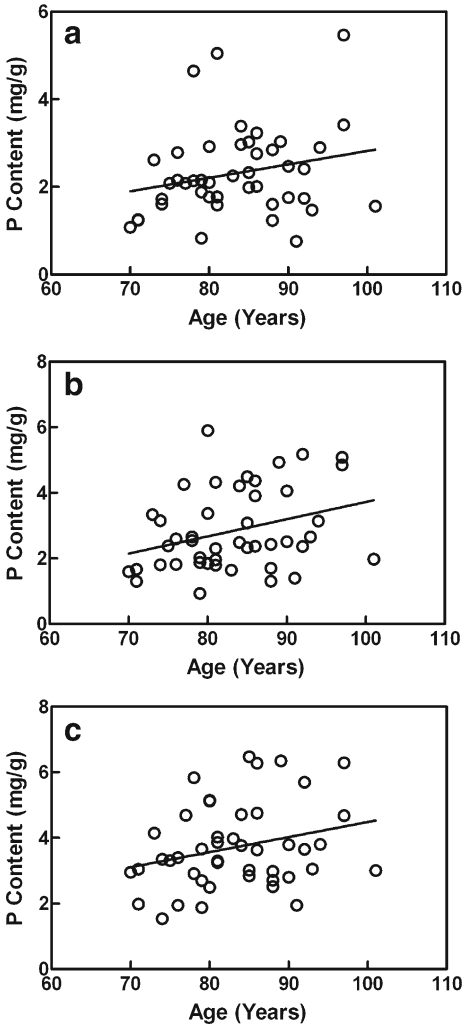

Fig. 2 Age-related differences of the P content in the caudate nucleus (**a**), putamen (**b**), and globus pallidus (**c**)

between age and either P (p=0.005) or Mg content (p= 0.009) in the globus pallidus.

Compared with the results in all the subjects, additionally significant and very significant direct correlations were found between age and P content in the caudate nucleus and globus pallidus of the subject group deleted the eight subjects, respectively.

Mass Ratios of Ca/P and Mg/Ca in the Basal Ganglia

To examine whether calcification occurred in the basal ganglia in very old age, the mass ratios of Ca/P and Mg/Ca were estimated. As shown in Table 3, the average mass ratios (in milligrams per milligram) of Ca/P were 2.07±0.92 in the caudate nucleus, 1.61±0.64 in the putamen, and 1.19 ±0.43 in the globus pallidus. The average mass ratios (in percent) of Mg/Ca were 10.48±1.59 % in the caudate nucleus, 11.17±1.95 % in the putamen, and 10.92±1.94 % in the globus pallidus. The average mass ratios of Mg/Ca were higher than 10 % in all of the caudate nucleus, putamen, and globus pallidus.

Relationships Among Elements in the Basal Ganglia

Tables 4, 5, and 6 indicate the relationships among element contents in the caudate nucleus, putamen, and globus pallidus. In the caudate nucleus, extremely significant direct correlations were found between Ca and S, Mg, or Fe contents, between P and Mg contents, between S and either Mg or Fe contents, and between Zn and Na contents (Table 4). A very significant direct correlation was found between Mg and Fe contents. In addition, significant direct correlations were found between Ca and either P or Zn contents and between P and S contents. Therefore, significant direct correlations were found among the Ca, P, S, and Mg contents in the caudate nucleus.

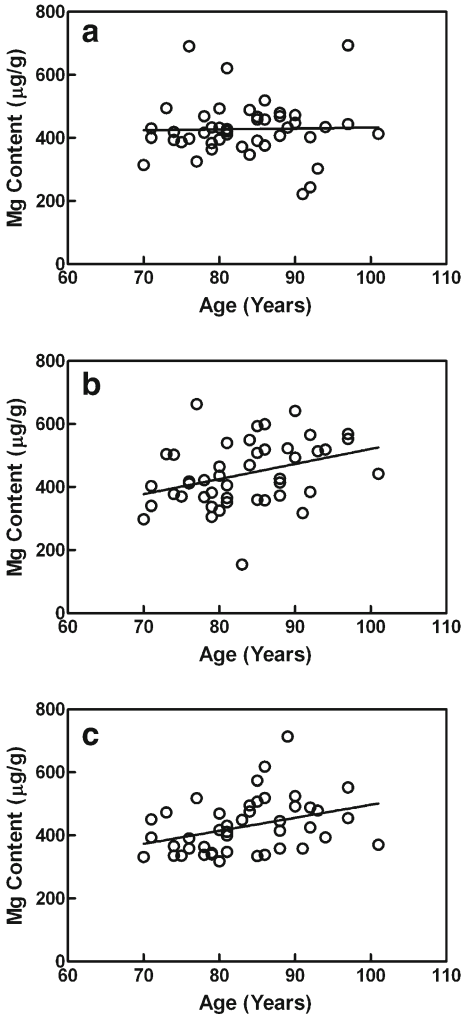

Fig. 3 Age-related differences of the Mg content in the caudate nucleus (**a**), putamen (**b**), and globus pallidus (**c**)

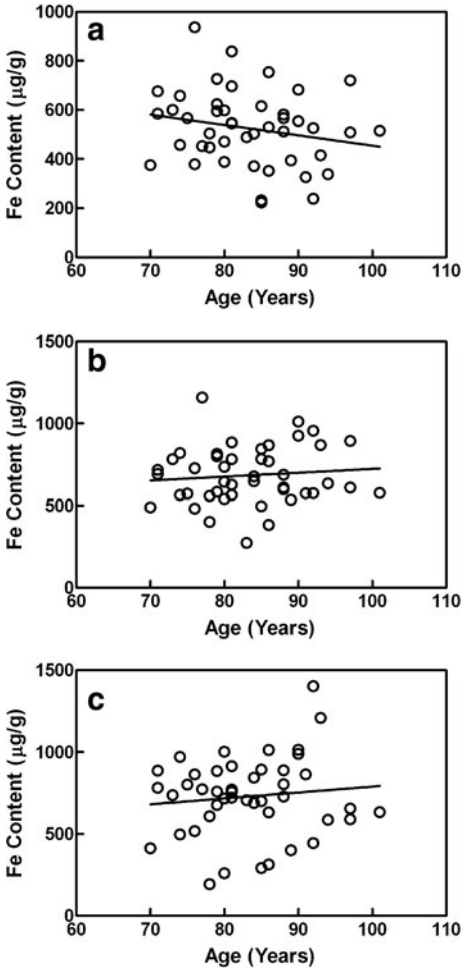

Fig. 4 Age-related differences of the Fe content in the caudate nucleus (**a**), putamen (**b**), and globus pallidus (**c**)

In the putamen, extremely significant direct correlations were found between Ca and either P or Mg contents, between P and Mg contents, and between Zn and Na contents (Table 5). Very significant direct correlations were found between Ca and either Zn or Fe contents and between S and Fe contents. In addition, significant direct correlations were found between Mg and either S or Fe contents, whereas a significant inverse correlation was found between S and Na contents. Therefore, significant direct correlations were found among the Ca, P, and Mg contents in the putamen.

Extremely significant direct correlations were found between Ca and Zn contents, between P and Mg contents, between S and Zn, Fe, or Na contents, and between Zn and either Fe or Na contents in the globus pallidus (Table 6). Extremely significant inverse correlations were found between P and S, Fe, or Na contents and between Mg and Na contents. A very significant direct correlation was found between Ca and Mg contents, whereas a very significant inverse correlation was found between P and Zn contents. In addition, a significant direct correlation was found between Ca and Fe contents, whereas a significant inverse correlation was found between S and Mg contents.

Elements with Significant Correlation in the Basal Ganglia

Table 7 summarizes elements with significant correlation in the caudate nucleus, putamen, and globus pallidus. The findings that there were significant direct correlations between Ca and Mg, Zn, or Fe contents, between P and Mg contents, between S and Fe contents, and between Zn and Na contents were commonly obtained in all of the caudate

Table 3 Mass ratios of Ca/P and Mg/Ca in the basal ganglia

Basal ganglia	Average mass ratio	
	Ca/P (mg/mg)	Mg/Ca (%)
Caudate nucleus	2.07±0.92	10.48±1.59
Putamen	1.61±0.64	11.17±1.95
Globus pallidus	1.19±0.43	10.92±1.94

Table 4 Relationships among elements in the caudate nucleus

Element	Correlation coefficient and p value					
	P	S	Mg	Zn	Fe	Na
Ca	0.314 (0.036)	0.689 (<0.0001)	0.687 (<0.0001)	0.379 (0.010)	0.576 (<0.0001)	0.292 (0.052)
P		0.302 (0.044)	0.616 (<0.0001)	−0.060 (0.696)	0.041 (0.791)	0.036 (0.813)
S			0.787 (<0.0001)	−0.067 (0.661)	0.648 (<0.0001)	−0.201 (0.186)
Mg				−0.066 (0.665)	0.433 (0.003)	−0.033 (0.828)
Zn					0.022 (0.885)	0.845 (<0.0001)
Fe						−0.063 (0.680)

p values are indicated in parentheses

nucleus, putamen, and globus pallidus. In addition, the finding that there was a significant correlation between S and Mg contents was commonly obtained in all of the caudate nucleus, putamen, and globus pallidus. However, significant direct correlations were found between S and Mg contents in both the caudate nucleus and putamen, whereas a significant inverse correlation was found in the globus pallidus.

Relationships Among the Basal Ganglia with Regard to Seven Element Contents

To elucidate whether there were significant relationships among the basal ganglia with regard to elements, the relationships among the basal ganglia were analyzed with Pearson's correlation with regard to seven elements of Ca, P, S, Mg, Zn, Fe, and Na.

1. Ca

   The relationships were examined among the Ca contents of the basal ganglia. The correlation coefficients in the Ca content were estimated to be 0.012 (p=0.938) between the caudate nucleus and putamen, 0.059 (p=0.700) between the caudate nucleus and globus pallidus, and 0.407 (p=0.006) between the putamen and globus pallidus. A very significant direct correlation was found between the Ca contents of the putamen and globus pallidus, but not between those of the caudate nucleus and either putamen or globus pallidus.
2. P

   Figure 5 shows the relationships among the P contents of the basal ganglia. The correlation coefficients in the P content were estimated to be 0.641 (p<0.0001) between the caudate nucleus and putamen, 0.505 (p=0.0004) between the caudate nucleus and globus pallidus, and 0.768 (p<0.0001) between the putamen and globus pallidus. It should be noted that extremely significant direct correlations were found among the P contents of the three basal ganglia.
3. S

   The relationships were examined among the S contents of the basal ganglia. The correlation coefficients in the S content were estimated to be 0.065 (p=0.672) between the caudate nucleus and putamen, 0.204 (p=0.179) between the caudate nucleus and globus pallidus, and 0.261 (p=0.084) between the putamen and globus pallidus. No significant correlations were found among the S contents of the three basal ganglia.
4. Mg

   The relationships were examined among the Mg contents of the basal ganglia. The correlation coefficients in

Table 5 Relationships among elements in the putamen

Element	Correlation coefficient and p value					
	P	S	Mg	Zn	Fe	Na
Ca	0.520 (0.0002)	0.171 (0.261)	0.632 (<0.0001)	0.402 (0.006)	0.402 (0.006)	0.249 (0.098)
P		−0.154 (0.312)	0.746 (<0.0001)	0.286 (0.057)	0.036 (0.816)	0.272 (0.071)
S			0.331 (0.027)	−0.280 (0.063)	0.414 (0.005)	−0.321 (0.032)
Mg				−0.038 (0.802)	0.329 (0.027)	−0.111 (0.468)
Zn					−0.013 (0.935)	0.953 (<0.0001)
Fe						−0.088 (0.564)

p values are indicated in parentheses

Table 6 Relationships among elements in the globus pallidus

Element	Correlation coefficient and p value					
	P	S	Mg	Zn	Fe	Na
Ca	0.156 (0.307)	0.167 (0.272)	0.457 (0.002)	0.529 (0.0002)	0.373 (0.012)	−0.027 (0.859)
P		−0.572 (<0.0001)	0.667 (<0.0001)	−0.414 (0.005)	−0.501 (0.0005)	−0.492 (0.0006)
S			−0.356 (0.016)	0.489 (0.0007)	0.524 (0.0002)	0.497 (0.0005)
Mg				−0.268 (0.075)	−0.090 (0.556)	−0.611 (<0.0001)
Zn					0.524 (0.0002)	0.637 (<0.0001)
Fe						0.202 (0.183)

p values are indicated in parentheses

the Mg content were estimated to be 0.306 (p=0.041) between the caudate nucleus and putamen, 0.249 (p=0.099) between the caudate nucleus and globus pallidus, and 0.687 (p<0.0001) between the putamen and globus pallidus. A significant direct correlation was found between the Mg contents of the caudate nucleus and putamen and an extremely significant direct correlation was found between those of the putamen and globus pallidus. However, no significant correlation was found between the Mg contents of the caudate nucleus and globus pallidus.

5. Zn

The relationships were examined among the Zn contents of the basal ganglia. The correlation coefficients in the Zn content were estimated to be −0.151 (p=0.322) between the caudate nucleus and putamen, 0.474 (p=0.001) between the caudate nucleus and globus pallidus, and 0.049 (p=0.747) between the putamen and globus pallidus. A very significant direct correlation was found between the Zn contents of the caudate nucleus and globus pallidus, but not between those of the caudate nucleus and putamen and between those of the putamen and globus pallidus.

6. Fe

Figure 6 shows the relationships among the Fe contents of the basal ganglia. The correlation coefficients in the Fe content were estimated to be 0.434 (p=0.003) between the caudate nucleus and putamen, 0.427 (p=0.003) between the caudate nucleus and globus pallidus, and 0.612 (p<0.0001) between the putamen and globus pallidus. Very significant direct correlations were found between the Fe contents of the caudate nucleus and either the putamen or globus pallidus, and an extremely significant direct correlation was found between those of the putamen and globus pallidus.

7. Na

The relationships were examined among the Na contents of the basal ganglia. The correlation coefficients in the Na content were estimated to be −0.141 (p=0.357) between the caudate nucleus and putamen, 0.193 (p=0.203) between the caudate nucleus and globus pallidus, and 0.168 (p=0.270) between the putamen and globus pallidus. No significant correlations were found among the Na contents of the three basal ganglia.

Table 8 indicates elements with significant correlation among the three basal ganglia.

Table 7 Elements with significant correlation in the basal ganglia

Element	P	S	Mg	Zn	Fe	Na
Ca	C^a, P^c	C^c	C^c, P^c and G^b	C^a, P^b, and G^c	C^c, P^b, and G^a	
P		C^a and g^c	C^c, P^c, and G^c	g^b	g^c	g^c
S			C^c, P^a, and g^a	G^c	C^c, P^b, and G^c	p^a and G^c
Mg					C^b and P^a	g^c
Zn					G^c	C^c, P^c, and G^c
Fe						

The capital and small letters indicate significant direct and inverse correlations, respectively

C caudate nucleus, *P* putamen, *G* globus pallidus

[a] Significant

[b] Very significant

[c] Extremely significant

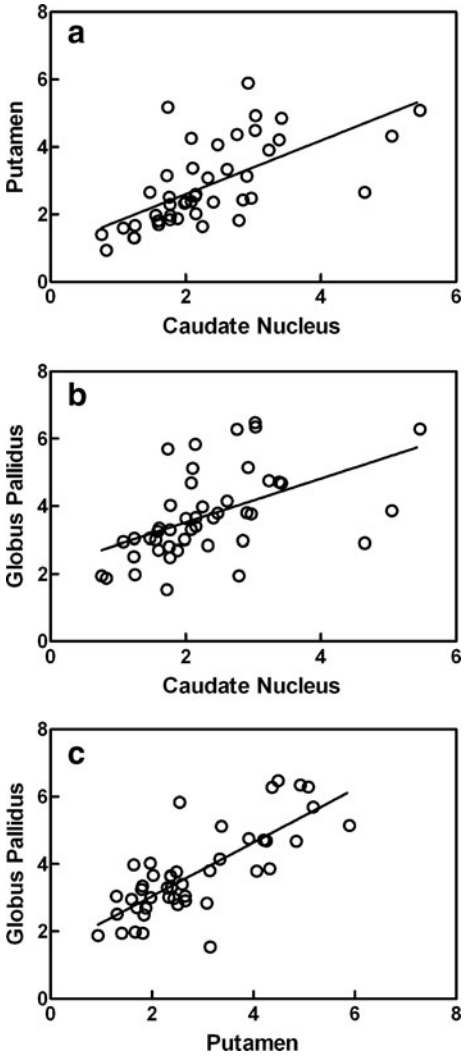

Fig. 5 Relationships between the P contents (in milligrams per gram) of the caudate nucleus and putamen (**a**), between the P contents (in milligrams per gram) of the caudate nucleus and globus pallidus (**b**), and between the P contents (in milligrams per gram) of the putamen and globus pallidus (**c**). The relationships between the P contents (in milligrams per gram) of two brain regions were analyzed with Pearson's correlation

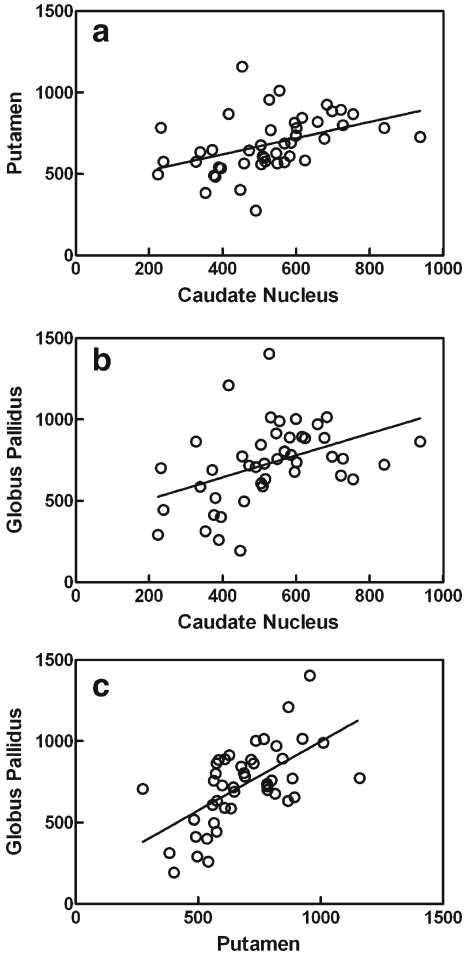

Fig. 6 Relationships between the Fe contents (in micrograms per gram) of the caudate nucleus and putamen (**a**), between the Fe contents (in micrograms per gram) of the caudate nucleus and globus pallidus (**b**), and between the Fe contents (in micrograms per gram) of the putamen and globus pallidus (**c**). The relationships between the Fe contents (in micrograms per gram) of two brain regions were analyzed with Pearson's correlation

Gender Differences in the Elements

It was examined whether there were gender differences in the seven elements of the caudate nucleus, putamen, and globus pallidus. The average ages of 22 men and 23 women's subjects were 82.3±7.9 and 84.3±7.1 years old, respectively. The average age was 2 years higher in women than in men.

Table 9 indicates the average contents of seven elements in the caudate nuclei of both men and women. The differences were observed in the average contents of Ca, P, Zn, and Na. With two tailed unpaired Student's *t* test, a significant difference was found only in the Ca content (p=0.028) between the caudate nuclei of men and women, but no statistically significant differences were found in the P (p=0.161), Zn (p=0.167), and Na contents (p=0.383). Therefore, a significant gender difference was found only in the Ca content of the caudate nucleus. The Ca content of the caudate nucleus was higher in women than in men.

Figure 7 shows age-related differences of the Ca content in the caudate nuclei of both men and women. The correlation

Table 8 Elements with significant correlation among the basal ganglia

Basal ganglia	Putamen	Globus pallidus
Caudate nucleus	P[c], Mg[a], and Fe[b]	P[c], Zn[b], and Fe[b]
Putamen		Ca[b], P[c], Mg[c], and Fe[c]

[a] Significant

[b] Very significant

[c] Extremely significant

Table 9 Comparison in the average contents of elements between the caudate nuclei of men and women

Element	Average content (mg/g)		Ratio
	Men	Women	Men/Women
Ca	3.874±0.719	4.340±0.655	0.89*
P	2.094±0.651	2.514±1.228	0.83
S	7.364±1.030	7.734±1.746	0.95
Mg	0.412±0.066	0.444±0.106	0.93
Zn	0.097±0.033	0.126±0.089	0.77
Fe	0.513±0.136	0.537±0.171	0.95
Na	0.050±0.028	0.061±0.053	0.82

*p value<0.05

coefficients between age and Ca content were estimated to be 0.233 (p=0.297) in the caudate nuclei of men and −0.271 (p=0.211) in those of women. No significant correlations were found between age and Ca content in the caudate nuclei of men and women. Furthermore, the difference between the two slopes in Fig. 7 was not significant because a p value was 0.104.

Regarding both the putamen and globus pallidus, no significant gender differences were found in the seven elements.

Discussion

To elucidate the manner of element accumulation in the arteries with aging, the authors [17–19] investigated age-related changes of elements in the arteries and found that when calcification occurred in the arteries, a high accumulation of Ca, P, and Mg occurred simultaneously in the arteries and both the Ca and P contents were well correlated with the Mg content. In addition, the mass ratio of Ca/P was constant and the mass ratio (in percent) of Mg/Ca was low [20].

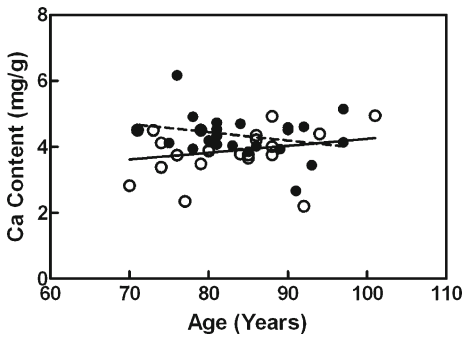

Fig. 7 Age-related differences of the Ca content in the caudate nuclei of men (*open circles*) and women (*solid circles*). The *straight and dotted lines* of trend with age indicate men and women, respectively

In the putamen, the following results were obtained in the present study: all of the Ca, P, and Mg contents increased significantly with aging. There were extremely significant direct correlations among the Ca, P, and Mg contents. The mass ratio of Ca/P was almost constant, being independent of the Ca content, and the average mass ratio of Ca/P was 1.61. However, the average mass ratio (in percent) of Mg/Ca was very high, being 11.2 %. Therefore, it was suggested that slight, but not moderate, calcification occurred in the putamen in very old age.

In both the caudate nucleus and globus pallidus, the Ca content did not increase significantly with aging. Therefore, it was suggested that calcification scarcely occurred in the caudate nucleus and globus pallidus in very old age.

There are several clinical studies [21–24] on calcification in the basal ganglia by CT scan. The prevalence of calcification in the basal ganglia ranged from 0.24 to 2.0 % [21–24]. In a histopathological series using the Von Kossa method for calcium detection, Wegiel et al. [25] reported calcification in the globus pallidus and putamen in 60 and 36 %, respectively, of the subjects who ranged in age from 33 to 83 years. Our results are not consistent with the finding by Wegiel et al. [25] that the incidence of calcification was 1.6 times higher in the globus pallidus than in the putamen. The present study indicated that the average content of Ca was less in the globus pallidus than in the putamen and no significant correlation was found between Ca and P contents in the globus pallidus.

Histological analysis of the basal ganglia concretions demonstrated that mucopolysaccharide deposition preceded that of minerals in the basal ganglia, and this matrix was followed by a deposition of Fe and then of Ca [26–28].

The present study revealed that there were extremely or very significant direct correlations among all of the caudate nucleus, putamen, and globus pallidus in both the P and Fe contents. The authors [29] previously investigated whether there were significant correlations among the hippocampus, dentate gyrus, mammillary body, and fornix belonging to the limbic system using the anterior commissure as a control with regard to the seven elements and found that there were extremely or very significant direct correlations among all of the five brain regions of the hippocampus, dentate gyrus, mammillary body, fornix, and anterior commissure in the P content. Likewise, with regard to the Fe content, there were significant direct correlations among all of the four brain regions belonging to the limbic system, except for the anterior commissure. It is necessary for significance of P and Fe to examine whether there are significant correlations between the brain regions belonging to both the basal ganglia and limbic system with regard to the P and Fe contents.

It is well known that phosphate containing P has five major functions: (1) as a part of large molecules or molecular assemblies, e.g., DNA, RNA, and membranes, (2) as a

carrier of substrates, e.g., in glucose phosphate and coenzyme, and as a carrier of chemical energy, e.g., ATP, (3) as a signaling device in the cytoplasm, e.g., in cAMP and IP_3, (4) as a reversible chemical modification of proteins, and (5) as a constituent of biominerals [30].

The P content of tissue is mostly determined by the nucleic acid content (DNA and RNA) and the phospholipid content of tissue. Nucleic acids in the cell nucleus and the cytosol and phospholipids in the cell membrane including myelin are indicators of metabolically active cells [31, 32]. Taking these into consideration, it is reasonable to presume that the P content in the basal ganglia indicates the active cell density, namely, the number of active cells per volume.

Because there were extremely or very significant direct correlations among all of the caudate nucleus, putamen, and globus pallidus in the P content, it is likely that the active cell density of the caudate nucleus correlates well with those of the putamen and globus pallidus. Based on the average contents of P shown in Table 2, the respective active cell densities of the putamen and globus pallidus were 23 and 61 % higher compared with that of the caudate nucleus.

There are several reports [33–37] on age-related changes of the volume in the basal ganglia by MRI. Raz et al. [33] revealed that age-related shrinkages occurred significantly in both the caudate nucleus and putamen and slightly in the globus pallidus [33]. It is thought that the active cell densities of the basal ganglia change with aging.

Postmortem [10] and in vivo [11–14] studies have demonstrated that although Fe is not present at birth, progressive Fe deposition occurs in different structures of the brain during aging process and the extent of Fe deposition with aging is markedly higher in the basal ganglia than in most other brain structures [10]. Our results that no significant correlations were found between age and Fe content in the three basal ganglia over 70 years old are compatible with the finding by Hallgren and Sourander [10].

Fe is critical for normal neuroembryogenesis and physiology and participates in a wide spectrum of cellular functions including cytokinesis, myelination, electron transport, antioxidant enzyme activity, and biogenic amine metabolism [38–42]. It is well known that ferritin of the Fe storage protein, transferrin of the Fe transport protein, and free Fe are present throughout all brain regions [38]. Oligodendrocytes have the highest Fe content within all brain cell types and as much as 70 % of brain Fe is associated with myelin [39].

Cass et al. [43] investigated the relationships among the motor performance, striatal dopamine release, and striatal iron level using monkeys, and proposed that striatal Fe levels might be a biomarker of motor dysfunction in aging. It is thought that as Fe participates in a wide spectrum of cellular functions, the Fe content is one of indicators of metabolic activity of cells, namely, cells with a high Fe content have a metabolically high activity. Therefore, in the case that the caudate nucleus has a high metabolic activity, both the putamen and globus pallidus also have a high metabolic activity, because significant direct correlations are found among all of the three basal ganglia in the Fe content.

Rajan et al. [44] determined the concentrations of 15 elements, Na, K, P, Ca, Mg, Si, Cr, Cu, Ni, Zn, Fe, Al, Cd, Pb, and As, in 12 regions of eight normal human brains by inductively coupled plasma-atomic emission spectrometry and reported that there were significant direct correlations in all of 15 trace element concentrations between one region of brain and other region, that is, thalamus vs. hippocampus, hypothalamus vs. thalamus, mid-brain vs. pons, somatosensory cortex vs. occipital cerebrum, parietal cerebrum vs. somatosensory cortex, temporal vs. parietal cerebrum, and frontal vs. temporal cerebrum. The six elements, such as Ca, P, Mg, Zn, Fe, and Na, were commonly studied by us and by Rajan et al. [44]. It is noteworthy that although the brain regions examined were different, significant direct correlations regarding the P content were found among all of the brain regions examined by us [29] and by Rajan et al. [44].

The present study revealed that there was the significant gender difference in the Ca content of the caudate nucleus. The Ca content of the caudate nucleus was higher in women than in men. The authors [8, 9] previously investigated whether there were gender differences in the corpus callosum, anterior commissure, and fornix of the white matter and the hippocampus, dentate gyrus, pineal body, olfactory bulb and tract, mammillary body, lateral geniculate body, and superior colliculus of the gray matter and found that there were significant gender differences in the elements of the anterior commissure (Zn), olfactory bulb and tract (Ca, P, and Zn), dentate gyrus (S), and lateral geniculate body (Ca and Zn). It should be noted that there are mainly significant gender differences in the Ca and/or Zn contents of the brain regions examined.

There are some clinical reports [12, 13, 45] on the gender difference in the elements of the human brain. Bartzokis et al. [12] measured the Fe amount in ferritin molecules (ferritin Fe) in vivo in 165 healthy adults aged 19–82 years by MRI utilizing the field dependent relaxation rate increase method and reported that women had significantly lower ferritin Fe amount than men in the five brain regions, such as the caudate nucleus, thalamus, white matter region of the frontal lobe, and genu and splenium of the corpus callosum. Xu et al. [13] studied the brain Fe levels in vivo in 78 healthy adults aged 22–78 years by using magnetic susceptibility-weighted phase imaging and reported that although the left hemisphere had higher Fe levels than the right in the putamen, globus pallidus, substantia nigra, thalamus, and frontal white matter, no gender-related differences in brain Fe levels were detected. The present study revealed that there were no significant gender differences in

the Fe content of the three basal ganglia over 70 years of age. However, the age range of the subjects used in the present study is different from those of Bartzokis et al. [12] and Xu et al. [13].

Riehemann et al. [45] investigated the gender influence on the concentrations of different P compounds in the frontal lobes of healthy subjects by using an image-selected in vivo spectroscopy on a whole body scanner and reported that healthy women exhibited increased values of inorganic phosphate and decreased values of phosphocreatine in the frontal lobe in comparison with their men counterparts.

There are the following two types [8, 9] on the gender difference in the elements of the brain regions: the first type is that the gender difference in the element has already been present in the adulthood and persists up to old age. The second type is that although the gender difference in the element is absent from the adulthood, it occurs in old age. Namely, age-related changes of the element after adulthood are different between men and women. Although the correlations between age and Ca content in the caudate nuclei of both men and women (Fig. 7) were not statistically significant, the caudate nucleus appeared to belong to the first type.

It is necessary to further investigate whether there are gender differences in elements of the various brain regions and to elucidate the physiological significance.

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