

Age-Related Changes of Elements and Relationships Among Elements in Human Hippocampus, Dentate Gyrus, and Fornix

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Abstract To elucidate compositional changes of the limbic system with aging, the authors investigated age-related changes of elements in the hippocampus, dentate gyrus, and fornix and the relationships among elements by direct chemical analysis. After ordinary dissections at Nara Medical University were finished, the hippocampi, dentate gyri, and fornices were resected from identical cerebra of the subjects which consisted of 23 men and 23 women, ranging in age from 70 to 101 years. After ashing with nitric acid and perchloric acid, element contents were determined by inductively coupled plasma-atomic emission spectrometry. The average contents of P, Zn, and Na were significantly less in both the hippocampi and dentate gyri compared with the fornices. It was found that the Ca and Mg contents increased significantly in the hippocampus with aging; the P content increased significantly in the dentate gyrus with aging, whereas the Na content decreased in the dentate gyrus with aging; and the Mg content increased significantly in the fornix with aging. Regarding the relationships among elements, a significant direct correlation between Ca and Fe contents and an extremely significant inverse correlation between P and Zn contents were found in both the hippocampi and dentate gyri. In addition, a significant direct correlation between P and Mg contents was found in both the hippocampi and fornices. Pearson's correlation was used to examine whether there were elements with significant correlation among the hippocampus, dentate gyrus, fornix, and mammillary body. Significant correlations were found in five elements of Ca, P, Mg, Zn, and Fe except for S and Na among the hippocampus, dentate gyrus, and mammillary body with one

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exception. Regarding the fornix, significant correlations were found in two elements of P and Fe between the fornix and hippocampus, dentate gyrus, or mammillary body.

Keywords Hippocampus · Dentate gyrus · Fornix · Limbic system · Calcium · Element · Aging

Introduction

To elucidate compositional changes of the brain with aging, the authors previously investigated age-related changes of elements in the corpus callosum [1] and anterior commissure [2] of the white matter and the pineal body [3], olfactory bulb and tract [4], and mammillary body [5] of the gray matter.

There are several reports [6–10] on age-related changes of the hippocampus, dentate gyrus, and fornix which belong to the limbic system. However, little work had been done to study age-related changes of the human hippocampus, dentate gyrus, and fornix by direct chemical analysis. Therefore, the authors investigated age-related changes of elements in the hippocampus, dentate gyrus, and fornix and relationships among elements.

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 [11]. After ordinary dissections by medical students at Nara Medical University were finished, the hippocampi, dentate gyri, and fornices were resected from the cerebra. The pes hippocampi was used as the hippocampus in the present study.

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 dry samples, 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-7510; Shimadzu, Kyoto, Japan) [12]. 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. Specially 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. Ind. (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, USA). Pearson's correlation was used to investigate the association between parameters. A paired 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 \pm standard deviation.

Results

Element Contents

The hippocampus, dentate gyrus, and fornix derived from the same cerebrum. The subjects consisted of 23 men and 23 women for the hippocampi, ranging in age from 70 to 101 years (average age = 83.5 ± 7.5 years). The subjects consisted of 21 men and 23 women for the dentate gyri, because two subjects were not analyzed. The subjects consisted of 14 men and 16 women for the fornices, ranging in age from 70 to 101 years (average age = 83.6 ± 7.5 years), because 16 subjects were not analyzed.

Table 1 indicates the average contents of seven elements in the hippocampi, dentate gyri, and fornices. Paired Student's *t* test was used to examine whether there were significant differences in the average contents of seven elements among the hippocampi, dentate gyri, and fornices. The average content of Ca was significantly higher in both the dentate gyri and fornices compared with the hippocampi. The average content of P was the highest in the fornices and decreased in the order of the hippocampi and dentate gyri. The average content of Mg was significantly higher in both the hippocampi and fornices than in the dentate gyri. The average content of Zn was significantly higher in the order of the fornices, hippocampi, and dentate gyri. The average content of Na was significantly lower in both the hippocampi and dentate gyri compared with the fornices. With regard to the average contents of S and Fe, no significant differences were found among the hippocampi, dentate gyri, and fornices.

Age-Related Changes of Elements

Figure 1 shows age-related changes of the Ca and Mg contents in the hippocampi. The correlation coefficients were estimated to be 0.343 ($p=0.020$) between age and Ca content and 0.335 ($p=0.023$) between age and Mg content. Significant direct correlations were found between age and either Ca or Mg content. However, no significant correlations were

Table 1 The Average Contents of Seven Elements in the Hippocampi, Dentate Gyri, and Fornices

Brain region	Average content (mg/g)			Average content (μ g/g)			
	Ca	P	S	Mg	Zn	Fe	Na
Hippocampus	4.479 \pm 0.791	3.333 \pm 1.085	8.314 \pm 0.848	426.3 \pm 93.98	79.67 \pm 25.39	324.8 \pm 80.99	27.93 \pm 44.56
Dentate gyrus	5.568 \pm 0.920	2.672 \pm 0.868	8.189 \pm 0.483	293.0 \pm 104.9	60.89 \pm 44.30	325.6 \pm 126.1	33.37 \pm 73.50
Fornix	5.481 \pm 1.473	5.135 \pm 0.966	7.944 \pm 0.520	402.8 \pm 34.51	198.8 \pm 142.7	332.8 \pm 84.92	473.9 \pm 391.7

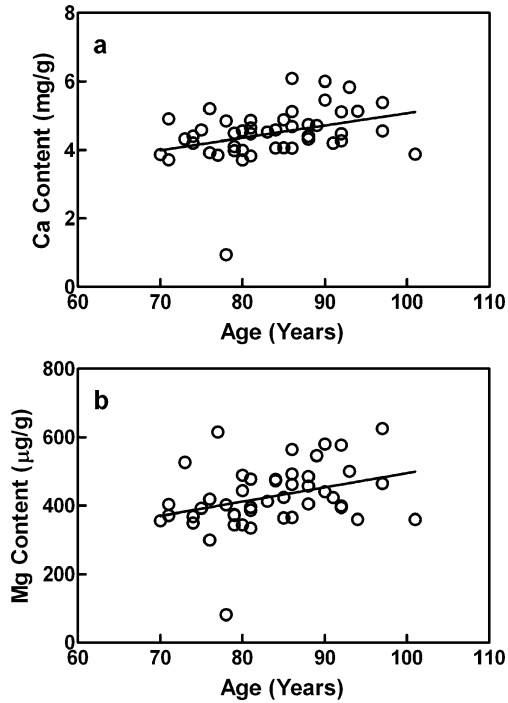


Fig. 1 Age-related changes of the Ca (a) and Mg (b) contents in the hippocampi

found between age and the other element contents, such as P ($p=0.096$), S ($p=0.756$), Zn ($p=0.742$), Fe ($p=0.583$), and Na ($p=0.264$).

In comparison with the average contents of elements by age group, the average content of Ca in the hippocampi was 10% and 21% higher in the 80's and 90's of the subjects, respectively, compared with that in the 70's. Likewise, the average content of Mg in the hippocampi was 16% and 23% higher in the 80's and 90's, respectively, compared with that in the 70's.

Figure 2 shows age-related changes of the P and Na contents in the dentate gyri. The correlation coefficients were estimated to be 0.361 ($p=0.016$) between age and P content and -0.376 ($p=0.012$) between age and Na content. A significant direct correlation was found between age and P content in the dentate gyri, whereas a significant inverse correlation was found between age and Na content. However, no significant correlations were found between age and the other element contents, such as Ca ($p=0.071$), S ($p=0.470$), Mg ($p=0.195$), Zn ($p=0.086$), and Fe ($p=0.297$).

The average content of P in the dentate gyri was 26% and 32% higher in the 80's and 90's of the subjects, respectively, compared with that in the 70's. The average content of Na in the dentate gyri decreased to 16% and 12% in the 80's and 90's, respectively, compared with that in the 70's.

Age-related changes of the Mg content in the fornices are shown in Fig. 3. The correlation coefficient was estimated to be 0.437 ($p=0.016$) between age and Mg content, indicating that there was a significant direct correlation between them. However, no significant correlations were found between age and the other element contents, such as Ca ($p=0.122$), P ($p=0.423$), S ($p=0.988$), Zn ($p=0.174$), Fe ($p=0.637$), and Na ($p=0.719$).

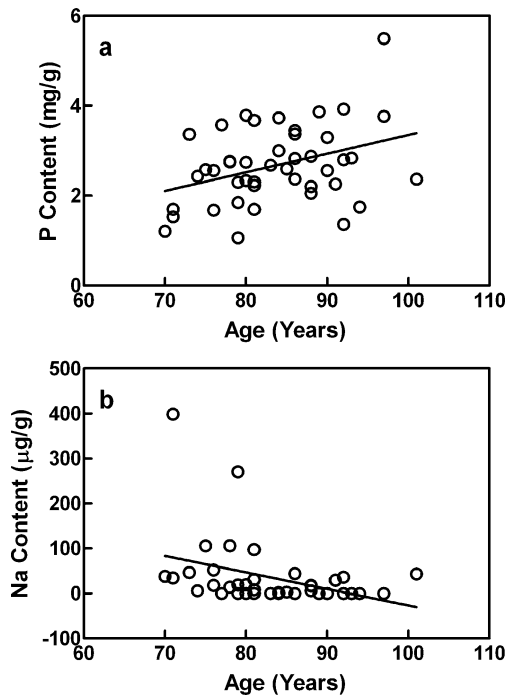


Fig. 2 Age-related changes of the P (a) and Na (b) contents in the dentate gyri

The average content of Mg in the fornices was 5% and 9% higher in the 80's and 90's of the subjects, respectively, compared with that in the 70's.

Relationships Among Element Contents

Tables 2, 3, and 4 indicate the relationships among element contents in the hippocampi, dentate gyri, and fornices. In the hippocampi, extremely significant direct correlations were found between Ca and either Mg or Fe contents, and between P and Mg contents, and extremely significant inverse correlations were found between P and Zn contents and between Mg and Na contents. A very significant inverse correlation was found between Ca and Na contents. In

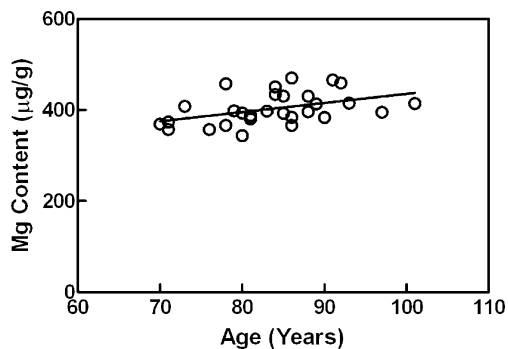


Fig. 3 Age-related changes of the Mg content in the fornices

Table 2 Relationships Among Elements in the Hippocampi

Element	Correlation coefficient and <i>p</i> value					
	P	S	Mg	Zn	Fe	Na
Ca	0.134 (0.376)	0.226 (0.131)	0.541 (0.0001)	0.276 (0.064)	0.486 (0.0006)	-0.421 (0.004)
P		-0.261 (0.080)	0.742 (<0.0001)	-0.582 (<0.0001)	-0.261 (0.079)	-0.342 (0.020)
S			0.106 (0.483)	0.363 (0.013)	0.034 (0.822)	-0.105 (0.488)
Mg				-0.269 (0.071)	0.046 (0.762)	-0.475 (0.0008)
Zn					0.376 (0.010)	0.204 (0.175)
Fe						-0.375 (0.010)

p Values are indicated in parentheses

addition, significant direct correlations were found between Zn and either S or Fe contents, whereas significant inverse correlations were found between Na and either P or Fe contents.

In the dentate gyri, an extremely significant inverse correlation was found between P and Zn contents, whereas an extremely significant direct correlation was found between Zn and Na contents. A very significant direct correlation was found between Ca and P contents, whereas a very significant inverse correlation was found between Ca and Zn contents. In addition, significant direct correlations were found between Ca and Fe contents and between Mg and Zn contents.

Extremely significant direct correlations were found between Ca and Zn contents and between Fe and Na contents in the fornices. A very significant direct correlation was found between S and Na contents. In addition, significant direct correlations were found between P and Mg contents and between S and Fe contents, whereas a significant inverse correlation was found between P and S contents.

Accordingly, a significant direct correlation between Ca and Fe contents and an extremely significant inverse correlation between P and Zn contents were found in both the hippocampi and dentate gyri. In addition, a significant direct correlation between P and Mg contents was found in both the hippocampi and fornices.

Comparison in Elements with Significant Correlation Among Eight Brain Regions

Table 5 indicates elements with significant correlation in the corpus callosum [1], anterior commissure [2], and fornix of the white matter and the pineal body [3], olfactory bulb and

Table 3 Relationships Among Elements in the Dentate Gyri

Element	Correlation coefficient and <i>p</i> value					
	P	S	Mg	Zn	Fe	Na
Ca	0.433 (0.003)	0.217 (0.157)	-0.181 (0.239)	-0.404 (0.007)	0.326 (0.031)	-0.236(0.124)
P		0.215 (0.160)	-0.215 (0.162)	-0.624 (<0.0001)	-0.160 (0.300)	-0.267 (0.080)
S			-0.156 (0.313)	-0.084 (0.588)	0.091 (0.558)	-0.094 (0.545)
Mg				0.317 (0.036)	0.195 (0.204)	0.064 (0.681)
Zn					-0.043 (0.783)	0.660 (<0.0001)
Fe						-0.057 (0.712)

p Values are indicated in parentheses

Table 4 Relationships Among Elements in the Fornices

Element	Correlation coefficient and <i>p</i> value					
	P	S	Mg	Zn	Fe	Na
Ca	-0.080 (0.676)	0.065 (0.735)	0.302 (0.105)	0.964 (<0.0001)	0.163 (0.390)	0.317 (0.087)
P		-0.372 (0.043)	0.395 (0.031)	-0.150 (0.430)	-0.327 (0.077)	0.152 (0.423)
S			-0.100 (0.601)	0.060 (0.752)	0.436 (0.016)	0.541 (0.002)
Mg				0.165 (0.384)	-0.090 (0.636)	-0.106 (0.577)
Zn					0.208 (0.271)	0.341 (0.065)
Fe						0.586 (0.0007)

p Values are indicated in parentheses

tract [4], mammillary body [5], hippocampus, and dentate gyrus of the gray matter. Significant correlations were found between Ca and P contents, between Ca and Mg contents, between P and Mg contents, and between Ca and Na contents in more than five out of the eight brain regions. Furthermore, all of significant correlations found between Ca and P contents and between P and Mg contents were direct.

Relationships in Seven Elements Among the Hippocampi, Dentate Gyri, Fornices, and Mammillary Bodies

Pearson's correlation was used to analyze whether there were significant correlations in seven elements among the hippocampus, dentate gyrus, fornix, and mammillary body [5] which belonged to the limbic system. Table 6 indicates elements with significant correlation among the hippocampi, dentate gyri, fornices, and mammillary bodies. Significant correlations were found in five elements of Ca, P, Mg, Zn, and Fe except for S and Na among the hippocampi, dentate gyri, and mammillary bodies, with one exception that there was no significant correlation in the Mg content between the hippocampi and dentate gyri. Regarding the fornix, significant correlations were found in two elements of P and Fe between the fornices and hippocampi, dentate gyri, or mammillary bodies.

Table 5 Elements with Significant Correlation in the Eight Brain Regions

Element	P	S	Mg	Zn	Fe	Na
Ca	C,D,M,O,P	A,c,m	a,C,H,m,O,P	A,d,F,O	A,c,D,H	A,h,M,O,P
P		c,f,m,O	A,C,F,H,O,P	d,h,O	c	h,O,P
S			c,M,O	H,P	A,C,F,P	A,F
Mg				a,D,M,O	a,c	a,h,m,P
Zn					H	A,D,O
Fe						c,F,h,M

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

A anterior commissure, *C* corpus callosum, *D* dentate gyrus, *F* fornix, *H* hippocampus, *M* mammillary body, *O* olfactory bulb and tract, *P* pineal body

Table 6 Elements with Significant Correlation Among the Hippocampi, Dentate Gyri, Mammillary Bodies, and Fornices

Brain region	Dentate gyrus	Mammillary body	Fornix
Hippocampus	Ca ^b , P ^c , Zn ^c Fe ^c	Ca ^b , P ^c , mg ^b Zn ^b , Fe ^c	P ^c , s ^a , Fe ^b
Dentate Gyrus		Ca ^c , P ^c , Mg ^a Zn ^b , Fe ^b	P ^b , Fe ^a
Mammillary Body			P ^c , Fe ^b

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

^a Significant correlation

^b Very significant correlation

^c Extremely significant correlation

Discussion

The present study revealed that the Ca and Mg contents increased progressively in the hippocampus with aging; in the dentate gyrus, the P content increased significantly with aging, whereas the Na content decreased with aging; and the Mg content increased significantly in the fornix with aging.

There are some reports [6–8] on age-related changes of elements in the hippocampus, dentate gyrus, and fornix. To determine intracellular Ca²⁺ levels in neurons, Raza et al. [6] isolated acutely CA1 hippocampal neurons from young and mid-age rats and demonstrated that mid-age neurons in comparison with young neurons manifested significant elevations in basal intracellular Ca²⁺ levels. Although the elevations in basal intracellular Ca²⁺ levels did not always indicate an increase of Ca accumulation in the hippocampus, our finding is compatible with the finding by Raza et al. [6].

Yoo et al. [7] studied age-dependent changes of Fe deposition in the gerbil hippocampus and found that the increase of Fe deposition might be associated with normal aging and that the Fe deposition in the aged hippocampus was different according to the hippocampal subfields.

Bartzokis et al. [8] measured the Fe amount in ferritin molecules in vivo by magnetic resonance imaging (MRI) utilizing the field-dependent relaxation rate increase method in 165 healthy adults aged 19–82 years and reported that there were the following significant age-related changes of ferritin Fe: The ferritin Fe increased in the hippocampus, caudate, putamen, and globus pallidus with aging, whereas it decreased in the white matter region of the frontal lobe with aging. The present study revealed that the Ca and Mg contents increased significantly in the hippocampus with aging, but the Fe content did not change significantly in the hippocampus with aging. Our finding seems to be different from the finding by Bartzokis et al. [8].

The authors previously investigated age-related changes of elements in the corpora callosa [1] and anterior commissures [2] of the white matter and the pineal bodies [3], olfactory bulbs and tracts [4], and mammillary bodies [5] of the gray matter. It was found that a very high accumulation of Ca and P occurred in the pineal body at old age. In contrast, an accumulation of Ca and P hardly occurred in the corpus callosum, anterior commissure, and olfactory bulb and tract with aging. In the mammillary body, the Ca content increased slightly and significantly with aging, and the P content tended to increase

with aging. In the present study, the Ca and Mg contents increased progressively in the hippocampus with aging. As there was an extremely significant direct correlation between Ca and Mg contents in the hippocampi, it was reasonable that both the Ca and Mg contents increased in the hippocampus with aging. The finding that the P and Na contents increased significantly in the dentate gyrus with aging was first found in the brain regions examined by us.

Ca dysregulation has been extensively investigated in brain aging, but the role of Mg has received less attention though aging constitutes a risk factor for Mg deficit [13]. One of general properties of Mg at presynaptic fiber terminals is to reduce transmitter release. At the postsynaptic level, it closely controls the activation of the N-methyl-D-aspartate, a subtype of glutamate receptor, which is critical for the expression of long-term changes in synaptic transmission. In addition, Mg is a cofactor of many enzymes localized either in neurons or in glial cells. Billard [13] has suggested that Mg is involved in age-related deficits in transmitter release, neuronal excitability, and in some forms of synaptic plasticity such as long-term depression of synaptic transmission [13]. In the present study on the hippocampus and fornix, the Mg content increased significantly with aging, but it did not decrease. It is ambiguous whether the Mg accumulation may affect the function of the hippocampus and fornix.

Regarding element contents, there were significant differences in the average contents of P, Zn, and Na between the hippocampus and dentate gyrus of the gray matter and the fornix of the white matter in the present study. Therefore, the authors examined in detail the differences in the average contents of P, Zn, and Na between the gray and white matters, including the anterior commissure, corpus callosum, mammillary body, olfactory bulb and tract, and pineal body analyzed previously by us. The average contents of P were 5.14 ± 0.97 mg/g in the fornix, 7.67 ± 1.33 mg/g in the corpus callosum (trunk) [1], and 5.51 ± 1.04 mg/g in the anterior commissure [2] of the white matter and 3.33 ± 1.09 mg/g in the hippocampus, 2.67 ± 0.87 mg/g in the dentate gyrus, 3.20 ± 0.86 mg/g in the mammillary body [5], 2.11 ± 0.79 mg/g in the olfactory bulb and tract [4], and 28.79 ± 32.88 mg/g in the pineal body [3]. Namely, the average content of P was higher than 5.1 mg/g in all of three white matters, whereas the average content of P was less than 3.3 mg/g in all of four gray matters except for the pineal body. Therefore, the average content of P was higher in the white matter than in the gray matter except for the pineal body. Our finding is compatible with the finding by LoPachin et al. [14, 15] that the peripheral nerves with the myelin sheath contained a high content of P and a low content of Ca. Furthermore, the P content increased remarkably in the pineal body at old age.

Regarding Zn and Na, no clear differences in the average contents were found between the gray and white matters, taken those of the anterior commissure, corpus callosum, pineal body, olfactory bulb and tract, and mammillary body into consideration.

There are many reports [16–23] on volume changes of the brain regions with aging. Tisserand et al. [10] investigated global and limbic brain volumes by MRI in 61 healthy persons aged 21–81 years and reported that the volumes of the hippocampus and parahippocampal gyrus declined significantly with aging. Several authors have reported similar age-related volume decreases in these regions [16–21]. Studies measuring directly brain volume have found age-related reductions in total and gray (but not white) matter volumes [16, 21–23].

In the adult brain, the dentate gyrus within the hippocampus generates new neurons throughout the lifetime of the animal, including the human [24]. In the dentate gyrus, neuronal stem/progenitor cells (NSCs) proliferate within the subgranular zone, migrate into the granular cell layer, and undergo neuronal or glial differentiation. The newly generated

neurons are capable of integrating into neural networks as fully functional neurons [25]. It is well known that neurogenesis in the dentate gyrus decreases greatly by middle age [26, 27]. Therefore, the aforementioned hippocampal atrophy may result in part from decreased neurogenesis in the dentate gyrus.

A recent study in rats suggests that an age-related decrease in dentate neurogenesis is primarily attributable to decreased production of new cells, as the extent of neuronal differentiation from newly born cells, and the migration and long-term survival of newly born neurons are analogous among young, middle-aged, and aged groups [28]. Nevertheless, the precise reasons for striking decreases in the production of new cells from NSCs at middle age are unknown. The present study revealed that the Ca content increased significantly in the hippocampus with aging and tended to increase in the dentate gyrus with aging ($r=0.275$, $p=0.071$). Therefore, it is likely that NSCs may be affected by a high Ca content in the hippocampus and dentate gyrus, and the production of new cells from NCSs diminishes remarkably by middle age.

Stadlbauer et al. [9] investigated age-related changes of quantitative diffusivity parameters and fiber characteristics within the fornix and cingulum of the limbic system in 38 healthy subjects aged 18–88 years and found that the fornix revealed moderate correlations with age for all diffusivity parameters of fractional anisotropy (FA), mean diffusivity (MD), and eigenvalues (λ_1 , λ_2 , and λ_3), whereas the cingulum revealed no association for FA and a weak positive correlation for MD and the three eigenvalues. They suggested that degradation of limbic fiber bundles occurred within the fornix and the cingulum due to normal aging. The fornix is a massive fiber bundle that follows the hippocampal formation to the hypothalamus, where it terminates in the mammillary bodies and in septal nuclei. Regions connected by the fornix such as the hippocampus are more affected by normal aging than regions connected by the cingulum [21, 29, 30]. As a result, the number of fibers is more strongly reduced in the fornix. The present study on the fornix revealed that although the Mg content increased significantly in the fornix with aging, the P content containing predominantly in the nerve fibers did not decrease in the fornix with aging. Therefore, it was thought that although the number of fibers decreased in the fornix with aging, qualitative changes hardly occurred in the fornix with aging.

The present study revealed that the hippocampus, dentate gyrus, and mammillary body of the gray matter correlated significantly with one another with respect to five elements of Ca, P, Mg, Zn, and Fe except for S and Na, whereas they correlated significantly with the fornix of the white matter with respect to two elements of P and Fe. Namely, as Ca increased in the hippocampus, Ca also increased in the dentate gyrus and mammillary body, but not in the fornix. It is noteworthy that regarding the correlations in the Ca, P, Mg, Zn, and Fe contents, there were clear differences between the hippocampus, dentate gyrus, and mammillary body of the gray matter and the fornix of the white matter.

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