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Age-related Changes in Locus Ceruleus on Neuromelanin Magnetic Resonance Imaging at 3 Tesla

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Purpose: To investigate age-related changes in the locus ceruleus (LC) in healthy subjects using neuromelanin magnetic resonance (MR) imaging at 3 Tesla.

Methods: We examined 64 healthy volunteers (aged 23 to 80 years) using neuromelanin-sensitive T₁-weighted images and measured the contrast of areas of high signal intensity corresponding to the LC.

Results: A pair of punctate areas of high signal intensity that represented neuromelanin within the noradrenergic neurons of the LC was easily recognized in all subjects. The contrast ratio of the LC to the adjacent pontine tegmentum increased to the age of 40 to 59 years and gradually and significantly decreased in elderly subjects. This correlates well with pathologically proven age-related changes in neuromelanin content within the LC.

Conclusion: Age-related variance should be considered when determining the existence of abnormalities in the LC.

Keywords: *locus ceruleus, neuromelanin, noradrenaline, high field MRI*

Introduction

The locus ceruleus (LC) is a small blue-black nucleus on the floor of the fourth ventricle that comprises noradrenergic neurons containing neuromelanin, a byproduct of monoamine synthesis.^{1,2} Although neuromelanin is said to have paramagnetic T₁ shortening effects when combined with metals,^{3,4} the LC has been an invisible nucleus on conventional magnetic resonance (MR) imaging. Recently, we reported that the LC can be identified using neuromelanin MR imaging at 3 Tesla (3T) and that it is obscured in Parkinson's disease (PD), in which the LC is selectively affected.⁵ However, the age-dependent changes in LC signal on neuromelanin MR imaging have not been determined. In this study, we quantitatively examined the signal intensity of the LC in young to elderly adults to elucidate whether the neuromelanin-generated contrast depends on age-related changes in neuromelanin concentration, which has been pathologically proven.^{6–8}

Materials and Methods

We examined 64 healthy volunteers (28 men, 36 women) 23 to 80 years old (mean age 50.3) using a superconductive 3T MR scanner (Signa VH/i, GE, Milwaukee, Wisconsin, USA). Pulse sequence was T₁-weighted fast spin-echo (FSE), 600/14 repetition time/effective echo time (TR/TE), 2-echo train length, 2.5-mm section thickness with 1-mm intersection gaps, matrix size 512 × 320, 220-mm field of view (FOV; pixel size 0.43 × 0.69 mm), and acquisition time 12 min, as previously described.⁵ The sections were in the oblique axial direction perpendicular to the fourth ventricle floor and covered from the posterior commissure to the pontine inferior border, with one slice at the inferior edge of the inferior colliculus. We also obtained proton-density and T₂-weighted FSE images (3000/16, 96; 8-echo train length) with the same parameters to exclude pathological lesions in the brain stem. All examinations were performed after approval of the institutional review board. Written informed consent was obtained from all subjects.

One of the authors, who was blinded, measured the signal intensity of the LC at 7 mm below the

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Fig. 1. Age-related changes in the locus ceruleus (LC) on neuromelanin images
A: 27-year-old man, contrast ratio (CR) of 7.87% of the LC; **B:** 33-year-old man, CR of 9.34%; **C:** 46-year-old woman, CR of 16.86%; **D:** 56-year-old woman, CR of 16.39; **E:** 66-year-old woman, CR of 11.20%; **F:** 70-year-old man, CR of 10.10. In all age ranges, we recognized symmetric high signal spots in the upper pontine tegmentum, suggesting the existence of neuromelanin at the LC (arrows). The signal intensity appears to be relatively low in both young and elderly subjects relatively high in middle-aged subjects.

section through the inferior edge of the inferior colliculus, where the signal was most evident. Signal intensity of the LC and mid portion of adjacent pontine tegmentum were measured 3 times on a liquid crystal display using round cursors of 1 mm² and oval cursors of 10 mm², respectively; and the values were averaged.

The contrast ratio (CR) of the LC was calculated as $(S_{LC} - S_{PT})/S_{PT}$, where S_{LC} is the signal intensity of the LC and S_{PT} is the signal intensity of the pontine tegmentum. Polynomial regression analysis with quadratic curve fitting and alpha level of 0.05 was performed to determine whether the contrast ratio showed age-dependent changes.

Results

On axial neuromelanin-sensitive images of healthy volunteers of all age groups, we recognized symmetric spots of high signal in the upper pontine tegmentum just anterolateral to the fourth ventricle floor (Fig. 1). This corresponds well with the location of the LC, as previously reported.⁵

Our quantitative analysis revealed that the average contrast ratio of the LC in the 64 healthy subjects was $10.86\% \pm 3.68\%$. By decade of life, contrast ratios were: 8.53 ± 3.41 for those in their

20 s ($n = 9$ [3 men, 6 women], 23 to 28 years [mean 26.0 years]); 9.49 ± 3.67 for those in their 30 s ($n = 11$ [5 men, 6 women], 30 to 39 years [mean 35.0]); 12.88 ± 3.01 for those in their 40 s ($n = 11$ [4 men, 7 women], 41 to 49 years [mean 44.7]); 12.06 ± 4.28 for those in their 50 s ($n = 11$ [5 men, 6 women], 50 to 58 years [mean 54.4]); 11.21 ± 4.03 for those in their 60 s ($n = 11$ [5 men, 6 women], 60 to 68 years [mean 64.8]); and 10.55 ± 2.38 in those 70 and older ($n = 11$ [6 men, 5 women], 70 to 80 years (mean 73.2)).

Polynomial regression analysis revealed increased contrast ratio of the LC up to the 40 s and 50 s that gradually decreased significantly in the 60 s and 70 s ($P = 0.01$); the data can be fitted to a quadratic curve: $y = 0.58x - 0.0055x^2 - 3.3$ with a multiple correlation coefficient of 0.38 (Fig. 2).

The contrast ratios of men were 10.53 ± 3.45 ($n = 28$, 26 to 80 years, mean 52.3) and of women were 11.10 ± 3.78 ($n = 36$, 23 to 75 years, mean 48.7), with no significant difference between them.

Discussion

Recently, we visualized neuromelanin-containing nuclei, the LC, and the substantia nigra pars compacta (SNc) using T₁-weighted FSE at 3T, presuma-

Fig. 2. Contrast ratio (CR) of the locus ceruleus (LC) in normal healthy subjects. CR of the LC gradually increases up to 59 years of age and gradually decreases significantly in the elderly. An estimated quadratic curve is shown with its 95% confidence intervals.

bly permitted by synergic effects from high spatial resolution, signal attenuation of the brain tissue from T_1 prolongation, and FSE-related magnetization transfer effect.⁵ We demonstrated reduced signal intensity of these structures in patients with Parkinson's disease patients compared with age-matched healthy controls.⁵ In this study, we also elucidated an age-related difference in signal intensity of the LC among healthy subjects. To the best of our knowledge, this is the first report concerning *in vivo* imaging of age-dependent alteration in the LC.

Histological studies have revealed that the number of adrenergic neurons and amount of intraneuronal neuromelanin in the LC both increase until age 60 and then tend to decrease,^{6,7} although in some study reports disagree.⁸ In this study, the contrast ratios of the LC tended to be relatively low in young and elderly subjects and relatively high in middle-aged subjects. This age-related tendency was statistically significant and can be fitted to an upwardly convex quadratic curve. This result corresponded well with age-related changes reported in previous histological studies,^{6,7} suggesting that LC signals on neuromelanin MR imaging reflect changes in neuronal number and intracellular neuromelanin concentration that occur during aging. It also indicates that we should consider patient age when evaluating for signal abnormalities in the LC.

Pathologically, the LC has been known to be affected in degenerative diseases, such as Parkinson's and Alzheimer's,⁹⁻¹¹ as well as in psychiatric disorders, such as depression and schizophrenia.¹²⁻¹⁵ In Parkinson's and Alzheimer's diseases, neurons are lost and intraneuronal neuromelanin is diminished.⁹⁻¹¹ On the other hand, in depression and schizophrenia, subtle changes were observed in neuronal number or size and in enzyme activities that suggested dysfunction of the noradrenergic system.¹²⁻¹⁵ We believe that neuromelanin MR imaging has the potential to detect organic or functional changes in the LC in the above-cited disorders as a reduction in the neuromelanin-produced contrast from decreased cell number and/or intracellular neuromelanin concentration.⁵ However, signal changes that occur during pathological conditions should be carefully evaluated by considering age-dependent signal fluctuations.

One limitation of this study is the absence of data in children, teenagers, and elderly over 80 years, resulting mainly from difficulties recruiting healthy volunteers of these ages. We speculate that the signal intensity of the LC in these age ranges is relatively low^{6,7} and may be roughly estimated by extrapolation of the quadratic curve, which we obtained. Another limitation is the pulse sequence we used. The T_1 -weighted FSE sequence used for neuromelanin imaging appears to have insufficient spatial resolution in both the in-plane and slice directions to avoid errors from partial volume effects when measuring the signal intensity of the LC. Uneven signal from the inhomogeneity of a local magnetic field (B_1) is remarkable,¹⁶ which may also affect the quantitative values of the LC. To overcome this limitation, we need a new sophisticated sequence with 3-dimensional acquisition capability and less sensitivity to B_1 inhomogeneity.

In this study, we did not measure the SNc because of the possible influence of intraneuronal iron that can accelerate neuromelanin-related T_1 relaxation.^{17,18} Dopaminergic neurons in the SNc are well known to contain abundant iron (approximately 20 mg/100 g tissue) that increases with aging.¹⁸ Thus, because the iron concentration within the SNc can strongly affect the neuromelanin-generated T_1 contrast, it may mask age-related changes, particularly in elderly subjects. Thus, iron-compensation/normalization techniques are needed to quantitatively evaluate neuromelanin within the SNc, which we are currently investigating.

The biological implications of the age-related alteration of neuromelanin remain unknown. Although little has been elucidated regarding the

physiological roles of neuromelanin, it is speculated that neuromelanin can attenuate neuronal damage by binding with metals or mediating intracellular oxidation.¹⁹ Thus, the age-related changes may reflect not only changes in noradrenergic activity but also reinforcement and degradation of the neuronal protection mechanism during aging.

Conclusion

Using neuromelanin imaging at 3T, we visualized the LC in normal subjects aged 23 to 80 years, and its signal intensity showed fluctuations that correlated with age-dependent changes in the neuromelanin concentration. It appears that neuromelanin MR imaging is capable of assessing degenerative or functional changes in the LC; however, age-related signal alterations should be considered when evaluating abnormalities in disorders affecting the LC.

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References

1. German DC, Walker BS, Manaye K, Smith WK, Woodward DJ, North AJ. The human locus coeruleus: Computer reconstruction of cellular distribution. *J Neurosci* 1988; 8:1776–1788.
2. Graham DG. On the origin and significance of neuromelanin. *Arch Pathol Lab Med* 1979; 103:359–362.
3. Enochs WS, Petherick P, Bogdanova A, Mohr U, Weissleder R. Paramagnetic metal scavenging by melanin: MR imaging. *Radiology* 1997; 204:417–423.
4. Tosk JM, Holshouser BA, Aloia RC, et al. Effects of the interaction between ferric iron and L-dopa melanin on T₁ and T₂ relaxation times determined by magnetic resonance imaging. *Magn Reson Med* 1992; 26:40–45.
5. Sasaki M, Shibata E, Tohyama K, et al. Neuromelanin magnetic resonance imaging of locus coeruleus and substantia nigra in Parkinson's disease. *Neuroreport* 2006; 17:1215–1218.
6. Manaye KF, McIntire DD, Mann DM, German DC. Locus coeruleus cell loss in the aging human brain: a non-random process. *J Comp Neurol* 1995; 358:79–87.
7. Mann DM. The locus coeruleus and its possible role in ageing and degenerative disease of the human central nervous system. *Mech Ageing Dev* 1983; 23:73–94.
8. Zucca FA, Bellei C, Giannelli S, et al. Neuromelanin and iron in human locus coeruleus and substantia nigra during aging: consequences for neuronal vulnerability. *J Neural Transm* 2006; 113:757–767.
9. Mann DM, Yates PO. Possible role of neuromelanin in the pathogenesis of Parkinson's disease. *Mech Ageing Dev* 1983; 21:193–203.
10. Zarow C, Lyness SA, Mortimer JA, Chui HC. Neuronal loss is greater in the locus coeruleus than nucleus basalis and substantia nigra in Alzheimer and Parkinson diseases. *Arch Neurol* 2003; 60:337–341.
11. Busch C, Bohl J, Ohm TG. Spatial, temporal and numeric analysis of Alzheimer changes in the nucleus coeruleus. *Neurobiol Aging* 1997; 18:401–406.
12. Baumann B, Danos P, Krell D, et al. Unipolar-bipolar dichotomy of mood disorders is supported by noradrenergic brainstem system morphology. *J Affect Disord* 1999; 54:217–224.
13. Craven RM, Priddle TH, Crow TJ, Esiri MM. The locus coeruleus in schizophrenia: a postmortem study of noradrenergic neurones. *Neuropathol Appl Neurobiol* 2005; 31:115–126.
14. Marner L, Soborg C, Pakkenberg B. Increased volume of the pigmented neurons in the locus coeruleus of schizophrenic subjects: a stereological study. *J Psychiatr Res* 2005; 39:337–345.
15. Zhu MY, Klimek V, Dilley GE, et al. Elevated levels of tyrosine hydroxylase in the locus coeruleus in major depression. *Biol Psychiatry* 1999; 46:1275–1286.
16. Alecci M, Collins CM, Smith MB, Jezzard P. Radio frequency magnetic field mapping of a 3 Tesla birdcage coil: experimental and theoretical dependence on sample properties. *Magn Reson Med* 2001; 46:379–385.
17. Hallgren B, Sourander P. The effect of age of the non-haemin iron in the human brain. *J Neurochem* 1958; 3:41–51.
18. Vymazal J, Righini A, Brooks RA, et al. T₁ and T₂ in the brain of healthy subjects, patients with Parkinson disease, and patients with multiple system atrophy: relation to iron content. *Radiology* 1999; 211:489–495.
19. Fedorow H, Tribl F, Halliday G, Gerlach M, Riederer P, Double KL. Neuromelanin in human dopamine neurons: comparison with peripheral melanins and relevance to Parkinson's disease. *Prog Neurobiol* 2005; 75:109–124.