**A case-control study of the locus coeruleus degeneration in** **Alzheimer’s disease**

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**Abstract**

The locus coeruleus (LC) is the major source of noradrenaline, which plays a key role in cognition. We aimed to detect the extent of the LC signal attenuation in Alzheimer's disease (AD) patients using a neuromelanin (NM)-sensitive MRI and how it may correlate with inflammatory and autonomic measures. An individually matched case-control study design was employed. 24 patients with AD and 24 age and gender matched controls with no cognitive impairment were recruited. The primary outcome measure was the LC signal intensity indicated by the LC contrast ratio (CR) and measured by the NM-sensitive MRI. Secondary outcome measures included neuropsychometric tests of cognitive state, peripheral inflammatory and autonomic measures. Conditional logistic regression analysis revealed a significant 22% LC-CR reduction in the AD group compared with the control group. However, there was no statistical significance from inflammatory or autonomic measures. This is the largest individually-matched case-control study to visualise the LC degeneration in AD patients. The study revealed significant LC degeneration which holds promise to stratify patients who may benefit from treatment targeting noradrenergic dysfunction.

**Key words:**

Alzheimer’s disease; locus coeruleus; neuromelanin; magnetic resonance imaging; noradrenaline

**Main text**

**1. Introduction**

The locus coeruleus (LC) is a bilateral nucleus located in the dorsal pontine tegmentum (PT) and is the major source of noradrenaline (NA), a neuromodulator that plays a key role in cognition. While cognitive decline in Alzheimer disease (AD) has primarily been related to dysfunction within the cholinergic system in the nucleus basalis, there is considerable research evidence indicating extensive LC degeneration in AD (Zarow et al. 2003; Lyness et al. 2003), with some suggesting that it is among the earliest pathologies(Braak and Del Tredici 2011; 2012). Therefore, the early vulnerability of the LC to AD is of considerable clinical significance(Betts et al. 2019a), as this raises the possibility that changes of the LC activity may provide early detection markers for diagnosis as well as early intervention targets to delay AD progression. However, the contribution of LC degeneration to cognitive decline in the development of AD has been underappreciated due to methodological difficulties, with most evidence coming from animal and post-mortem studies. The absence of reliable non-invasive direct measures of the LC remains the biggest challenge.

Recent research indicates that LC visibility is driven by neuromelanin content of noradrenergic neurons and the intrinsic neuromelanin (NM)-sensitive MRI technique enables direct visualisation of the LC (Sasaki et al. 2006; Shibata et al. 2006; Keren et al. 2009; Betts et al. 2017; Priovoulos et al. 2018; Trujillo et al. 2019). In addition, experimental lesions of the LC in animal models of AD lead to increased inflammation and Aβ plaque burden (Heneka et al. 2010), but this association has not been examined in AD patients. It is also well recognised that the LC plays an important role in controlling autonomic function and sleep/arousal (Hou et al. 2006; Samuels et al. 2008). Therefore, the primary aim of the study was to detect the extent of the LC signal attenuation in AD using the NM-sensitive MRI technique. The secondary aim was to examine how the LC signal attenuation may be linked to cognitive, peripheral inflammatory and autonomic measures.

**2. Methods**

*2.1 Participants*

24 patients who met the NINCDS-ADRDA criteria for probable AD were recruited from the Memory Assessment and Research Centre at Southampton Moorgreen Hospital. 24 age (within 1-year difference) and gender matched controls with no cognitive impairment were recruited from the Older Adult Volunteer database and individually matched to AD patients. Participants with any medical condition or taking any medication which might potentially affect the LC-NA pathway were excluded.

*2.2 Study design and procedure*

A cross-sectional and individually-matched case-control study design was employed. All potential participants were screened, and AD diagnoses were validated. All eligible participants gave their written, informed consent before taking part in assessments in the Memory Assessment and Research Centre at Southampton Moorgreen Hospital and the Radiology Department at University Hospital Southampton, including 1) clinical assessment of cognitive state by standard neuropsychometric tests; 2) the NM-sensitive MRI brain scan; 3) peripheral inflammatory markers (serum cytokines) measured using the Meso Scale Discovery panel; and 4) physiological measures of autonomic function including resting heart rate, blood pressure, and the cold pressor test. Full ethical approval was obtained from the UK Health Research Authority and National Research Ethics Service (16/NW/0675).

*2.3 Neuropsychometric measures*

Standardised Mini-Mental State Examination(SMMSE), Montreal Cognitive Assessment(MOCA), and Alzheimer’s Disease Assessment Scale cognitive section(ADAS-cog), and Pittsburgh Sleep Quality Index(PSQI) were used.

*2.4* *MRI measures of the LC*

An optimised, high resolution T1-weighted turbo spin echo (TSE) sequence was employed to provide NM-sensitive contrast in order to evaluate the LC signal intensity (SI). All participants underwent an hour-long scan on a Siemens 3T Skyra MRI scanner, using a 32 channel head coil. The LC images were acquired in the oblique axial plane as shown in Figure 1a, which allows the LC to be visualised as two small hyperintense areas, shown in Figure 1b. The SI of the LC (SILC) and the SI of the pontine tegmentum (SIPT) as the reference region, were taken on the axial slice, approximately 7mm below the inferior colliculi. Threshold-based region of interest measures were developed where ImageJ was used to find the two maxima values located anatomically where the LC would be seen based on previous post-mortem studies. A circular ROI of 1.67mm² was centred on these brightest pixels on both the left and right side to calculate LC signal intensity. The signal intensity of the PT was calculated using a circular ROI of 99.701mm² which was placed at the same co-ordinates on each cropped pons image, approximately equidistant from the LC ROIs. This is a similar method to those previously reported to calculate LC-CR on NM-MRI (Takahashi et al. 2015; Shibata et al. 2006; Sasaki et al. 2006). The average of the left and right LC-contrast ratio (LC-CR) was then calculated using the following formula (SILC-SIPT)/ SIPT. The imaging processing procedure was illustrated in Figure 1c.

*2.5 Biological and autonomic measures*

Blood was collected to measure serum levels of cytokines including TNF-α, IL-6, and IL-10 by Meso Scale Discovery and ApoEe4 by the RayBio ELISA Kit in the laboratory at University Hospital Southampton. Blood pressure and heart rate were taken. The cold pressor test (CPT) was conducted which consisted of immersing the left hand up to the wrist crease in a bucket containing crushed ice and water (4°C) for a period of 90 s and recording sympathetically mediated responses (rise in blood pressure and heart rate) (Tavernor et al. 2000).

*2.6 Data analysis*

To allow for the matched design, conditional logistic regression analysis was used. Effect size was measured as the odds ratio for being a case rather than a control for combinations of predictor variables including sociodemographic variables, cognitive measures, LC-CR, biological and autonomic measures. Analyses were also stratified according to BMI and years of memory problems. Variables were log-transformed or converted into *z*-score when necessary. Analyses were performed using IBM SPSS version 26.

**3. Results**

*3.1 Demographic, cognitive, and biological characteristics of study participants*

24 AD participants (aged between 58-82) were recruited, but two were excluded due to poor quality MRI scans. Therefore, data from 11 mild AD (scoring 21-25 on the SMMSE) and 11 moderate AD (scoring 10-20) and 22 age and gender matched controls were entered into final analysis. Among 22 AD patients, 11 patients reported shorter memory problem and 11 patients reported longer memory problem using 3 years as a cut-off point. More descriptive data are shown in Table 1.

*3.2 Comparison of the LC contrast ratio (LC-CR) between groups*

MRI brain imaging scans were acquired from 22 AD and 22 controls without significant motion artefact (see Figure 2a). The signal intensity of the pons was not significantly different in the control group (mean = 240.87, SD = 16.51) to the AD group (mean = 239.73, SD = 27.96), t(42) = 6.887, p = 0.869 indicating differences observed were due to differences in the LC signal rather than being driven by differences in pons signal. LC-CRs across all four consecutive slices were calculated to compare the signal throughout the rostrocaudal extent of the LC to check for any differences between groups at the rostral end. Although the trend was for the LC signal to be diminished across all 4 slices in the AD group compared to the control group, it was at the single slice approx. 7mm below the inferior colliculi as previously reported that conditional logistic regression analysis revealed a significant 22% LC-CR reduction in the AD group (see Table2 and Figure 2b). The effect was similar: in men and in women, in those with longer and shorter duration of memory problems; and in mild and moderate AD subgroups.

*3.3 Comparison of biological and autonomic measures between groups*

Cox regression analysis did not reveal any group differences in terms of APOEe4, IL-6, IL-10, TNFα, heart rate, blood pressure, or the CPT effects (*p*>0.05 in all cases) (see Table 2).

*3.4 Correlation analysis between cognitive, biological and autonomic measures and LC-CR*

Correlation analysis was conducted to examine how the LC-CR was associated with cognitive, biological, and autonomic measures across all participants. There was a significant positive correlation between LC-CR and SMMSE total score (*r*=0.384, *p*=0.009) and a negative correlation between LC-CR and ADAS total score (*r*=-0.298, *p*=0.049) at the non-corrected level. However, there were no statistically significant correlations of LC-CR with other inflammatory or autonomic measures.

**4. Discussion**

The current study successfully adopted a NM-sensitive MRI imaging technique to directly visualise the LC degeneration in AD patients which provides strong support for the use of this LC imaging technique in future AD research. The study reveals a significant 22% LC signal attenuation in AD patients compared with matched controls, along with significant associations between LC-CR and cognitive measures. These findings have important implications for future work into the role of the LC as potential diagnostic markers, predictors for treatment response, and novel intervention targets.

So far, only 4 studies have detected the LC signal attenuation using NM-sensitive MRI imaging technique in AD patients. However, variations in imaging processing and calculations were used when examining LC signal attenuation. After reviewing the sensitivity and specificity, we adopted and optimised the imaging protocol developed by Takahashi and colleagues (2015).Our study is the first imaging study in AD patients using an individually matched control group.The significant LC signal attenuation in AD patients is strongly in line with previous LC imaging work (Takahashi et al. 2015; Betts et al. 2019b; Dordevic et al. 2017; Olivieri et al. 2019), plus recent evidence showing the significant correlation between LC attenuation and CSF Aβ level (Betts et al. 2019b), further supports the use of this imaging technique to visualise the LC as a biomarker for AD neuropathology. The imaging protocol utilised in this study can be used to inform how future scanning parameters could be optimised. It should be noted that one recent study suggests that LC hyperintensity is due to high water proton density in cell bodies rather than neuromelanin (Watanabe et al. 2019). As the study used a mouse AD model, the finding has to be cautiously translated into human due to differences in terms of the amount and effect of NM. The findings from our study also suggests that the degree of the LC attenuation was not affected by gender, BMI or duration of memory problems. The significant associations between the LC-CR and cognitive measures (including both SMMSE and ADAS) in this study indicate the potential of the LC as a biomarker for cognitive decline in AD patients. Future studies correcting for multiple comparisons is needed to confirm this finding. If the association between the LC degeneration and AD neuropathology can be confirmed in clinical longitudinal studies, treatment interventions targeting the LC-NA pathway may have the potential to delay or prevent AD progression in which promising results have been shown in experimental studies (Rorabaugh et al. 2017; Franco et al. 2014). The current study is the first to examine the association between LC signal attenuation and peripheral inflammatory markers in AD patients. There were no significant associations identified which are not what we have predicted. Likewise, no significant associations were found with sleep and autonomic measures. This could be due to the relatively small sample examined and/or the lack of sensitivity of the chosen measures. These preliminary findings should be further tested in future larger sample studies.

**5. Conclusions**

This study is the first individually-matched case-control study to visualise the LC in AD patients adopting a neuromelanin-sensitive imaging protocol. The study revealed a significant LC signal attenuation in AD measured by the NM-sensitive MRI. Significant efforts were taken to achieve an optimally-matched study population, pairing highly compatible datasets between case and controls. This, alongside the specialised statistical analytical approach, allowed valid comparisons to be made. The study strongly supports the use of the imaging technique to visualise the LC degeneration as a biomarker for AD neuropathology and holds promise to stratify patients who benefit from treatment targeting noradrenergic dysfunction. Future longitudinal studies are warranted to characterize how LC signals evolve at different stages of AD, which cloud lead to a new pathway for neuropharmacological interventions.

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**Figure legends:**

**Figure 1a**. Placement of imaging block (yellow box) and saturation bands (crossed area) for the locus coeruleus imaging protocol. After optimisation, the neuromelanin-sensitive MRI protocol was a T1 weighted turbo spin echo sequence with TR=600ms, TE = 16ms, flip angle = 90°, 5 signal averages, 220mm FOV, pixel size = 0.4mm x 0.4mm, slice thickness 2.5mm, 12 slices, acquisition time 10 minutes 21 seconds. The locus coeruleus images were acquired in the oblique axial plane, through the brain stem, perpendicular to the fourth ventricle, with coverage from the inferior colliculi to the base of the fourth ventricle. **Figure 1b**. The locus coeruleus seen as two hyperintense areas on the MRI scan with red arrows pointing to the left and the right locus coeruleus indicated in blue circles; the reference region of the pontine tegmentum indicated in white circle. **Figure 1c**. Imaging processing of the locus coeruleus using NM-MRI. Including 1) draw around pons; 2) smooth section; 3) Find brightest points; 4) draw circular ROIs centred on brightest points; 5) Reference region (99.7mm2) imported to same co-ordinates centred between LC ROIs.

**Figure 2a.** Images of the locus coeruleus from all participants (n=44). The top 2 rows are images from the control group (n=22), the 3rd row is mild AD (n=11) and the 4th row is moderate AD (n=11). **Figure 2b.** Comparison of the locus coeruleus-contrast ratio between the AD group and the control group. A significant locus coeruleus signal attenuation was shown in the AD group compared with age and gender matched control group (*p*=0.008).