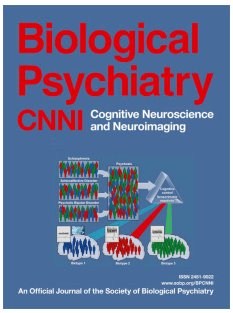

Locus Coeruleus Microstructure and Connectivity as Novel Markers of Depression and Cognitive Dysfunction in Older Adults

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Abstract

Background: Late-life depression (LLD) is a risk factor for age-related cognitive decline. Postmortem studies highlight pathological changes in the locus coeruleus (LC) and its projections as potential early cognitive vulnerability markers. Here, we use a novel individualized multimodal magnetic resonance imaging (MRI) approach to characterize the cognitive correlates of LC microstructure and connectivity in participants with LLD and age-matched never-depressed (ND) controls.

Methods: Diffusion-weighted and LC-sensitive MRI were acquired in 52 participants (LLD: n=26, 19 females, age 67.8+/-5.48; ND: n=26, 12 females, age 69.8+/-7.62). Using LC-sensitive MRI to localize the LC in each participant's native space, we computed diffusion metrics (fractional anisotropy, FA; mean diffusivity, MD) for the LC and its projections to the hippocampus (Hp), reconstructed with constrained spherical deconvolution tractography. Associations of FA and MD with diagnosis and cognitive performance were evaluated with analyses of covariance and Pearson correlations, respectively, adjusted for demographic/disease covariates and multiple testing (p-Bonferroni<0.05).

Results: Higher MD (F=10.07, p=0.003) was observed in the LC of individuals with LLD relative to ND. Conversely, no group differences emerged in the LC-Hp pathway. Across the combined LLD-ND sample, accounting for LLD diagnosis, lower FA in the LC and its hippocampal projections were associated with worse processing speed (LC: $r_{Word\text{-}Reading}$=-0.47; LC-MTL: $r_{Word\text{-}Reading}$=-0.46, $r_{Color\text{-}Naming}$=-0.49; all p≤0.0007) and executive functions (LC-MTL: $r_{Inhibition}$=-0.50, $r_{Inhibition/Switching}$=-0.45, $r_{\mathbf{Number/Letter\text{-}Sequencing}}$=-0.40; all p≤0.0033).

Conclusions: Neuronal injury of the LC may be a marker of LLD. Alternatively, the microstructural status of LC-Hp projections might be a biomarker more specific to age-related cognitive deterioration, irrespective of depression diagnosis.

Introduction

Late-life depression (LLD) is a common psychiatric disorder in older adults, and a major risk factor for the development of mild cognitive impairment (MCI) and subsequent progression to dementia [1]. Older adults with LLD are more likely to experience accelerated cognitive decline than age-matched individuals who have never been depressed. However, the temporal relationships between cognitive and depressive symptoms vary extensively with features such as the onset of the first lifetime depressive episode and an individual's baseline cognition levels [2]. LLD-MCI comorbidity could result from shared pathophysiological mechanisms in the brain such as neuroinflammation, oxidative stress, and age-related neurodegeneration [3–5], which may lead to structural damage, particularly to frontal-striatal and limbic-temporal networks that are crucial for executive functions [6,7]. However, the precise mechanisms linking LLD to risk for cognitive deterioration remain incompletely characterized.

Brainstem neuromodulatory nuclei, especially the locus coeruleus (LC), have been linked to the pathogenesis of neurodegenerative disorders, including preclinical stages of Alzheimer's disease (AD) and neuropsychiatric disorders such as depression and pos-traumatic stress [8,9]. The LC is the largest source of norepinephrine in the human brain with widespread noradrenergic outputs reaching numerous regions and spanning across the entire cerebral cortex [10]. Localized damage to this nucleus might dysregulate norepinephrine signaling, decreasing its levels in response to factors such as stress, which can in turn exacerbate depressive symptoms [11]. Norepinephrine levels in older individuals may also be upregulated in response to tau pathology or stress [12,13]. This upregulation may reflect a compensatory activity increase of a declining noradrenergic neuron population or amplification of LC reactivity and reduced auto-inhibition after chronic stress

[12,13]. These mechanisms are particularly relevant to late-life and stress-induced depression, although they depend on factors such as the type of adrenergic receptors activated, the level of existing pathology, and the use of antidepressant and anxiolytic medications. Post-mortem studies have also demonstrated that the LC is an early site of typical age-related pathology, and the presence of Lewy bodies in this nucleus and neuronal loss in the hippocampus and prefrontal cortex, two of its major output regions, have been associated with depression [14–16]. Moreover, early tau accumulation in the LC triggers norepinephrine dysregulation in the hippocampus, which may later be associated with reduced axonal density, hippocampal degeneration, and cognitive impairment [17,18].

LC structural integrity has been evaluated *in-vivo* with quantitative magnetic resonance imaging (MRI) techniques, including methods typically acquired with either turbo/fast spin or gradient-recalled echo sequences with a magnetization transfer contrast preparation pulse to sensitize the MRI contrast to neuromelanin-iron complexes found inside the LC noradrenergic neurons [19]. LC-sensitive MRI studies have evaluated its integrity by comparing contrast-to-noise-ratio values (CNR; contrast in signal intensity relative to a reference region with minimal neuromelanin content) between healthy controls and patients with several neurological disorders. Loss of LC signal CNR has been reported in individuals with AD and Parkinson's disease (PD), relative to age-matched controls in several studies, and CNR has been proposed as a proxy of LC integrity in these neurodegenerative conditions and of norepinephrine system function in neuropsychiatric disorders, including schizophrenia and LLD [20–24]. Nevertheless, LC-sensitive MRI contrast mechanisms and interpretations are still exploratory and controversial [25]. Although we previously showed a complex relationship between regional LC CNR and cognition in a sample of older adults enriched for LLD, there were no differences in these regional metrics between

participants with LLD and age-matched never-depressed (ND) participants [26]. Therefore, additional microstructural metrics may be needed to confirm, or rule out, the LC's potential involvement in depression and cognition in late life.

Diffusion MRI-derived metrics are sensitive to changes in neural microstructure (e.g., axon/cell loss, demyelination) and may offer an indirect measure of LC integrity complementary to CNR [27]. These metrics have been utilized to evaluate LC microstructure and the status of its projecting white matter pathways in healthy older adults [28], patients with AD [29], epilepsy [30], multiple sclerosis [31], progressive supranuclear palsy [32], and in younger versus older heathy individuals [33–35]. However, previous work is still limited; some results appear contradictory, especially those pertaining to older individuals, and they suggest that the microscopic architecture of the LC and its cortical projections evaluated with diffusion metrics are individually affected by age-related degeneration [36]. For example, one study found lower fractional anisotropy (FA) in the LC nucleus of younger compared to older adults, although the opposite trend (lower FA in older versus younger adults) was observed in the LC's ascending noradrenergic bundle [34]. One study reported higher FA and lower mean (MD) and radial diffusivities (RD) in the LC-thalamus projections in older versus younger healthy subjects, and higher diffusivities positively correlated to better verbal delayed recall only in older adults [33]. Another study from the same group reported higher diffusion tensor imaging (DTI)- and neurite orientation and dispersion density imaging (NODDI)-derived metrics (MD, free diffusion) in older versus younger adults and higher restricted diffusion associated with better recall variability irrespective of age [35].

The mixed nature of these findings could be at least partially explained by the LC's characteristics, namely its adjacency to isotropic, rapidly diffusing cerebrospinal fluid (CSF) of the brain stem and

the small size of the LC/subcoeruleus complex (~2-2.5 mm diameter) [37]. The LC nucleus is challenging to image with the typically poor spatial resolution of diffusion MRI protocols (voxel volumes > 8 mm^3 for most standard acquisitions). Thus, diffusion metrics can be easily contaminated by partial volume effects with CSF and misregistration to atlas standard space, which may contribute to inconsistencies across prior studies. Furthermore, the anatomy of the LC's ascending projections in the human brain is not well-characterized in post-mortem studies, and their trajectories, specifically of those pathways connecting to the hippocampus (Hp) and adjacent medial-temporal structures, are partly informed by functional imaging studies selectively targeting terminal regions receiving noradrenergic innervations [28,29,38,39]. Therefore, given the heightened dementia risk of older individuals with LLD, and histological evidence of decreased neuronal density in LC-Hp projection areas associated with depression and tau pathologies, we aimed to characterize the cognitive correlates of LC microstructure and hippocampal connectivity in older adults with and without LLD. To address the challenges of imaging the LC and its projections, we used an individualized multimodal approach utilizing LC-sensitive MRI to localize this nucleus in each participant's native space. We then applied high angular resolution diffusion MRI to probe microstructural damage in the LC and its connections to the hippocampus and evaluated its associations with cognitive performance in LLD and ND individuals.

Methods

Participants

This study was approved by the Centre for Addiction and Mental Health (CAMH) Research Ethics Board. Fifty-two participants, aged 60-83 years, provided written informed consent, including 26 diagnosed with LLD and 26 never clinically depressed (ND) controls. Subjects were enrolled as part of the Senescence and Depression (SenDep) study at CAMH (Toronto, Canada) to examine the impact of depression on cognitive trajectories in late life. ND subjects were recruited through public advertisements and research registries, while participants with LLD were enrolled at the Geriatric Psychiatry clinic. ND participants had no current or past history of any major psychiatric, neurological or neurodegenerative disorders, head trauma, current unstable medical condition, or alcohol/substance dependence for the past six months before the MRI scan, excluding nicotine. Participants with LLD fulfilled the Diagnostic and Statistical Manual of Mental Disorders 5th Edition diagnostic criteria for current major depressive episode (MDE) without psychotic features [40]. Thirteen participants with LLD (50%) reported symptom onset of the current MDE that spanned at least two years from the date of the MRI scan and were classified as persistently depressed [41]. Fourteen participants with LLD were on antidepressant medications at the time of the MRI scan, from which the majority were taking selective serotonin-reuptake inhibitors (9/14, 64%) and the rest were taking medications with noradrenergic effect (serotonin-norepinephrine reuptake inhibitors + norepinephrine-dopamine reuptake inhibitors + noradrenergic and specific serotonergic antidepressants + tricyclics); ND controls were not taking any antidepressants or mood stabilizers for any medical condition. Antidepressant medication types, names, and daily dosages are reported in Table S1. Comorbidities were evaluated in participants with LLD and ND

across 14 organ systems with the Cumulative Illness Rating Scale for Geriatrics and are reported in Table S2 of the Supplementary Material [42].

Cognitive and Clinical Assessments

Global cognitive performance was evaluated with the Montreal Cognitive Assessment (MoCA) scores adjusted for education level [43]. The following Delis-Kaplan Executive Function System (DKEFS) subtests: Color-Word Interference (CWI) task – word-reading, color-naming, inhibition, and inhibition/switching; Trail Making Test (TMT) – number/letter-sequencing and motor-speed were utilized as proxies of processing and motor speed, and executive function (inhibition, set-shifting) [44,45]. CWI and TMT subscores were measured as time-to-completion in seconds. The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) was utilized to evaluate five cognitive domains: attention, immediate- and delayed-memory, visuospatial/constructional, and language [46]. Depression severity was assessed with the Montgomery-Asberg Depression Rating Scale (MADRS) and the Patient Health Questionnaire (PHQ-9) [47,48].

Magnetic Resonance Imaging Acquisition

Brain MRI was performed on a 3 T Siemens Prisma scanner with a 64-channel head coil at the University of Toronto Neuroimaging Facility (ToNI). Whole-brain diffusion MRI was acquired with a high angular and spatial resolution diffusion imaging multi-shell protocol, LC-sensitive MRI was acquired using a 2D gradient-recalled echo sequence with a magnetization transfer contrast preparation pulse, and 3D magnetization prepared rapid acquisition gradient echo

sequence yielded whole brain T1-weighted anatomical scans. Technical parameters for the three sequences are detailed in the Supplementary Material.

LC-sensitive and Diffusion MRI Processing and Analyses

All LC-sensitive MRI preprocessing and CNR calculations were performed with the SPM12 toolbox and custom MatLab scripts to segment the LC nucleus as in our previous work with a method comparable to a validated version (Figure 1A, B) [24,26,49]. Preprocessing of diffusion MRI was executed with the integrative software pipeline *QSIprep*, while LC nucleus ROIs and LC-Hp tractography analysis workflows were performed with the open-source software *MRtrix3* [50,51]. Two diffusion MRI workflows were followed to calculate metrics from the over-inclusive LC-sensitive MRI ROI masks (Workflow #1 – FA and MD DTI-derived metrics; Figure 1B) and from the ascending fibers projecting from the LC nucleus to the hippocampus (Workflow #2 – tract-weighted FA, MD and fiber bundle capacity (FBC) computed with constrained spherical deconvolution (CSD) probabilistic tractography; Figure 1C). The lower portion of the brainstem (medulla oblongata) served as a control tract to rule out generalized brainstem effects (four participants' tracts are shown in Supplementary Figure S1). LC-sensitive and diffusion MRI processing, and DTI and CSD workflows are described in detail in the Supplementary Material [17,24,26,28,29,34,50–59].

Statistical Analysis

Statistical analyses were performed in R Studio v4.4.1. Group differences (ND, LLD) in the diffusion and CNR metrics in the LC nucleus, LC-Hp and lower brainstem pathways were examined with analysis of covariance (ANCOVA), with age, biological sex, duration of current

MDE since symptoms onset, and the neighboring diffusion correlation (NDC, only for the diffusion MRI metrics to account for any potential motion degradation) as covariates. Subgroup differences considering 'LLD under antidepressant' and 'LLD no treatment' versus ND participants; 'LLD under selective serotonergic antidepressant', 'LLD under noradrenergic antidepressant' and 'LLD no treatment' versus ND participants; and 'Not persistently depressed' (current MDE symptoms duration < 2 years) and 'Persistently depressed' (current MDE symptoms duration ≥ 2 years) versus ND participants were also tested post-hoc with Tukey's tests. Age, biological sex, level of education, and LLD diagnosis were regressed out from each diffusion metric and cognitive score (RBANS, DKEFS) in all participants, then linear associations between them were evaluated with partial Pearson correlations. Follow-up associations accounting for the joint effects of LLD diagnosis and antidepressant use, and of LLD diagnosis and the persistency of depression on diffusion metrics and cognitive scores were also explored with partial Pearson correlations. Then, if significant correlations were found, post-hoc linear regressions evaluated interaction effects between group/subgroups and diffusion metrics on cognitive scores. All statistical tests were family-wise adjusted with Bonferroni correction (ANCOVA tests - $p \leq 0.0063$; partial Pearson correlations –$p \leq 0.0036$). The Bonferroni correction for the partial correlation analyses was performed using the effective number of tests determined by the Galwey eigenvalue-based method, implemented in the 'poolr' package for R [60,61].

Results

Groups Characteristics

Participants did not display any between-group age differences; however, the proportion of biological sex differed by group with 7 more females (+27%) in the LLD group compared to the ND group. The education level was on average 1.6 years lower in LLD compared to ND subjects. Participants with LLD had higher depression scores in both the clinician-administered (MADRS) and the patient-reported (PHQ-9) questionnaires, as expected. There were no group differences in global cognitive status, assessed with MoCA scores adjusted by education level. Five LLD and four ND participants had a MoCA score ≤ 24, which could be indicative of MCI [62]. For specific cognitive domains, there were no group differences for the majority of the DKEFS and RBANS subscores, except for higher delayed-memory subscores of the RBANS ($t=2.07$, $p=0.045$), in the LLD group. Participant demographic and clinical characteristics, and depression and cognitive assessments are reported in Table 1.

Diffusion Qualitative Analysis of the LC-Hp Pathway

White matter projections from the LC nucleus to the hippocampus followed trajectories in agreement with previous tractography studies and described anatomy [28,29,58,63]. Streamlines projected superiorly and rostrally from the LC and then curved laterally toward the medial temporal lobe (Figure 2). They connected to the medial-anterior portion of the hippocampus in all participants, and some streamlines also appeared to reach through the amygdala given the adjacency of these two regions and the use of over-inclusive hippocampus ROIs that may overlapped with the amygdala. The highest FA values along the streamlines were observed on the

LC nucleus and reaching the hippocampus, and FA values and streamline density varied across participants irrespective of age and LLD diagnosis.

Group Differences in Diffusion Metrics in the LC and the LC-Hp Pathway

Overall, there were significant differences in the diffusion metrics calculated from the LC nucleus between the LLD and ND groups. FA [$F(1, 45)=6.34$, $p=0.015$, $\eta^2_p=0.12$] was 8% lower and MD [$F(1, 45)=10.07$, $p=0.003$, $\eta^2_p=0.18$] was 14.5% higher in participants with LLD when compared to ND participants, although FA differences did not survive the conservative Bonferroni threshold for multiple comparisons ($p\leq0.0063$; Figure 3A,B). LC CNR values calculated from LC-sensitive MRI ROIs did not differ by group (Figure 3C). Additionally, there were no group differences for any diffusion metric along the LC-Hp pathway, and no interactions between groups and any of the covariates were observed for the LC or the LC-Hp pathway. There were positive associations between MD and age in the LC nucleus [$F(1, 45)=7.64$, $p=0.008$, $\eta^2_p=0.15$] and in the LC-Hp pathway [$F(1, 45)=13.32$, $p=0.0068$, $\eta^2_p=0.23$] for the entire cohort. MD was also negatively associated with the quality of the diffusion data (NDC) [$F(1, 45)=6.8$, $p=0.012$, $\eta^2_p=0.13$] in the LC nucleus, and positively associated with the duration of current MDE since symptoms onset [$F(1, 45)=4.16$, $p=0.047$, $\eta^2_p=0.09$] in the LC-Hp pathway. None of the diffusion metrics extracted from the lower brainstem fibers differed between groups, highlighting the specificity of our findings to the LC within the brainstem region (diffusion metrics from the lower brainstem pathway reported in Supplementary Figure S2).

Regarding the LLD subgroups classified according to antidepressant use, FA [$F(2, 44)=4.99$, $p=0.011$, $\eta^2_p=0.19$; $t=-3.15$, $p_{Tukey}=0.008$] and MD [$F(2, 44)=5.86$, $p=0.006$, $\eta^2_p=0.21$; $t=3.24$, $p_{Tukey}=0.006$] differences in the LC nucleus were only significant for those patients not taking

antidepressant medication when compared to ND participants (Figure 4A,B). These group differences were only found when patients under treatment were pooled together disregarding antidepressant type. Regarding the LLD subgroups classified according to the persistency of depression, FA [$F(2, 46)=5.74$, $p=0.006$, $\eta^2_p=0.21$; $t=-3.39$, $p_{Tukey}=0.004$] and MD [$F(2, 46)=8.27$, $p=0.0009$, $\eta^2_p=0.27$; $t=4.04$, $p_{Tukey}=0.0006$] differences in the LC nucleus were only significant for those patients with persistent depression when compared to ND participants (Figure 4C,D).

Associations Between Diffusion Metrics and Cognitive Performance

Across the combined LLD-ND sample, and accounting for the effects of LLD diagnosis, slower reading speed (DKEFS-CWI $_{Word\text{-}Reading}$, $r=-0.471$, $p=0.0005$) correlated with lower FA in the LC (Figure 5A). For the LC-Hp pathway, slower naming/reading speed (DKEFS-CWI $_{Color\text{-}Naming}$, $r=-0.493$, $p=0.0002$; DKEFS-CWI $_{Word\text{-}Reading}$, $r=-0.459$, $p=0.0007$; Figure 5B,C)) and worse performance on inhibition and set-shifting (DKEFS-CWI $_{Inhibition}$, $r=-0.495$, $p=0.0002$; DKEFS-CWI $_{Inhibition/Switching}$, $r=-0.451$, $p=0.0009$; DKEFS-TMT $_{Number/Letter\text{-}Sequencing}$, $r=-0.404$, $p=0.0033$; Figure 5D-F) correlated with lower FA. All these associations survived Bonferroni correction ($p\leq0.0036$). There were no significant interactions between LLD/ND groups and diffusion metrics for any of these cognitive scores. All other nominally significant correlations ($p<0.05$) that did not survive Bonferroni correction are reported in Supplementary Table S3. There were no significant correlations between diffusion metrics in the lower brain stem pathway and cognitive scores (data not shown).

Two follow-up exploratory analyses, individually accounting for the effects of antidepressant use and the persistency of depression in LLD, showed equivalent correlations to those found when only accounting for LLD diagnosis between slower reading speed and lower FA, and between

slower naming/reading speed, worse performance on inhibition and set-shifting and lower FA in LC-Hp pathway. All these associations also survived Bonferroni correction ($p \leq 0.0038$). There were no significant interactions between LLD using antidepressants/LLD not using antidepressants/ND group and diffusion metrics for any of these cognitive scores, with the exception of FA in the LC-Hp pathway and set-shifting performance in DKEFS-CWI $_{\text{Inhibition/Switching}}$ task for participants with LLD using antidepressants ($t=-3.25$, $p=0.002$). The negative coefficient for the interaction term between this patient subgroup and FA in the LC-Hp pathway suggests that the effect of higher FA values on better task performance (lower timed scores) is stronger in those participants with LLD using antidepressants. Additionally, there were no significant interactions between LLD persistently depressed/LLD not persistently depressed/ND group and diffusion metrics for any of these cognitive scores.

Discussion

This study is the first to use high angular resolution diffusion MRI and CSD tractography to investigate diffusion metrics of the LC and its ascending hippocampal projections in a cohort of older individuals with and without LLD. Our use of a semi-automated segmentation method allowed the localization of this small nucleus directly in each participant's native LC-sensitive MRI space, and this aided in minimizing errors in the diffusion metrics that may result from mis-registration to standard LC atlas templates to diffusion images compared to native space segmentations [26], providing more accurate and individualized metrics of LC microstructure. These results suggest a link between abnormal LC microstructure and LLD. On the other hand, despite the LC-Hp projections being relatively preserved in participants with LLD when compared to similarly aged ND controls, the structural connectivity of this pathway may be a marker more specific to age-related cognitive deterioration, irrespective of depression diagnosis.

Our results confirm selective microstructural damage to the LC associated with aging and depression. We reported lower FA and higher MD in the LC nucleus of patients with LLD, especially in those not taking antidepressant medications or suffering long-term chronic depression, relative to ND controls, and no differences on LC CNR values. To our knowledge, no prior studies have evaluated diffusion MRI metrics in the LC and its connections to the hippocampus in individuals over 60 years diagnosed with LLD. Furthermore, no differences on bilateral rostral, middle, or caudal LC integrity, indexed by LC-sensitive MRI CNR alone, were identified in previous work in a cohort similar to ours [26]. LC diffusion metrics in our study did not correlate with CNR values and this could indicate that diffusion and CNR metrics might be sensitive to different microstructural properties of neuronal populations in the LC and thus to

complementary pathology (e.g., axonal loss versus neuromelanin-containing cell/dendritic loss) [64]. Our results agree with lower FA reported in the solitary tract of a cohort with major depressive disorders compared to controls, and this brainstem structure connects to the LC [65,66]. Lower FA and higher MD could indicate overall axonal degeneration, demyelination, and inflammation in the LC, but also be a consequence of a decreased population of smaller neurons across this nucleus. These smaller neurons are particularly diminished in older adult brains when chronically depressed, as reported in post-mortem histology [67]. They provide more biological barriers to water motion than larger neurons, thus restricting diffusion at the cellular level [68]. In our study, LC-Hp projections showed no between-group differences in diffusion metrics, and MD values in both the LC nucleus and LC-Hp projections were positively associated with age, irrespective of group diagnosis. Prior work has shown lower FA in the LC ascending noradrenergic bundle of older versus younger healthy adults [34]. These results agree with typical white matter age-related trajectories and indicate a positive relationship between the microstructural degeneration of the LC-Hp pathway and aging, although no direct link with depression.

In addition, our correlation analysis identified robust associations between diffusion metrics of the LC and its hippocampal projections, and several cognitive domains in LLD and ND groups, in agreement with prior work linking LC degeneration, LC-noradrenaline system dysfunction and cognitive decline [69,70]. We found that, lower FA in the LC and particularly in the LC-Hp pathway correlated with slower naming/reading speed, and worse executive functions, specifically inhibition and cognitive flexibility (set-shifting) across all participants. These findings agree with a [^{18}F] Fluoro-m-tyrosine positron emission tomography study in young and old healthy adults, which found positive associations between greater LC integrity, catecholamine synthesis capacity, processing speed, and executive function, including inhibition and set-shifting [71]. Moreover, in

one diffusion MRI study lower FA along these projections was associated with elevated CSF tau biomarkers in healthy older adults, while in another study higher RD was linked to dementia severity in subjects with AD and MCI [28,29]. Our results also complement a study in aging and AD vulnerability that proposes greater LC neuronal density and higher catecholamine synthesis capacity as mechanisms of cognitive resilience in older individuals [72]. Therefore, diffusion metrics in the LC nucleus and LC-Hp projections might be explored as imaging markers of MCI progression to AD in healthy elders and in those with comorbid depressive disorders, given their strong associations with cognitive performance.

There were several limitations of this study. First, our sample size is small and the limited number of participants in the LLD and ND cohorts may have contributed to the inability to find more group differences or cognitive associations with diffusion metrics; accordingly, studies with larger samples are recommended. Second, despite utilizing higher than standard spatial resolution when acquiring diffusion MRI and choosing optimal preprocessing and analysis strategies, diffusion metrics may be still at risk of contamination due to partial volume effects with non-LC tissue such as CSF. When possible, future studies should acquire higher resolution sequences and calculate additional multi-compartment metrics in addition to DTI maps that may be less sensitive to CSF contamination in ROI analyses. Third, diffusion metrics on the LC nucleus were calculated from a single diffusion shell (b=1000 s/mm^2) with only 15 directions so there may be bias in the MD and FA measurements [73]. Fourth, the anatomy of the LC-Hp pathway is not well histologically described and there is no gold standard of the *in-vivo* anatomy of these connections. Future work should acquire ultra-high-resolution diffusion MRI sequences of the LC post-mortem to evaluate the accuracy of the reconstruction of this pathway and adjacent connections as proposed by Sun et al, and to aid disentangle the heterogeneity of its terminative regions [29]. Fifth, we did not provide

any mechanistic evidence into how microstructural degeneration of the LC and its hippocampal pathway contribute to cognitive decline in LLD and ND participants. Therefore, combining amyloid-beta and tau blood-biomarkers with diffusion MRI data might improve these interpretations in future studies. In conclusion, our study found group differences between older adults with LLD and ND that were specific to the LC nucleus. The microstructural status of LC and its hippocampal projections was strongly associated with worse cognitive performance across the combined sample. Taken together, these findings suggest that the noradrenergic system, specifically the connections between the LC and the hippocampus could be relatively preserved in LLD and that abnormal diffusion metrics in these projections might be indicators of cognitive decline in late life.

Author contributions

Diana Valdés Cabrera: Conceptualization, Methodology, Validation, Data curation, Statistical analysis, Writing – original draft. **Navona Calarco:** Methodology, Validation, Writing – original draft. **Clifford M. Cassidy:** Methodology, Software, Validation. **Aristotle N. Voineskos:** Funding acquisition, Investigation, Resources. **Breno S. Diniz:** Funding acquisition, Investigation. **Yuliya S. Nikolova:** Conceptualization, Funding acquisition, Investigation, Resources, Supervision, Writing – original draft. All authors contributed to and have approved the final manuscript draft.

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Competing Interests

BSD serves as consultant to Bough Bioscience Inc. for activities unrelated to this work. CMC reports patents related to the neuromelanin-sensitive MRI analytic methods, licensed to Terran Biosciences, but no royalties received. All other authors report no biomedical financial interests or potential conflicts of interest.

Supplement Description:

Supplement Methods, Figures S1-S2, Tables S1-S3

Tables and Figures

Table 1: Demographics and Clinical Characteristics for LLD and ND groups

Clinical Variables (Mean ± SD; Range)	LLD (n=26)	ND (n=26)
Age (Years)	67.8 ± 5.48 (60-82)	69.8 ± 7.62 (60-83)
Biological Sex (% Females) *	73.1% (19)	46.2% (12)
Education Level (Years) *	14.1 ± 2.50 (8-18)	15.7 ± 1.67 (12-19)
PHQ-9 *	12.7 ± 4.63 (6-22)	1.04 ± 1.43 (0-4)
MADRS § *	16.7 ± 5.58 (6-26)	1.08 ± 1.50 (0-5)
Current MDE duration (years) §§	5.60 ± 10.3 (0-48)	-
MoCA (Education-adjusted)	25.7 ± 3.16 (16-30)	26.3 ± 1.94 (22-29)
DKEFS		
CWI Color-Naming (secs) ‡	34.2 ± 10.1 (23-71)	34.0 ± 6.89 (25-57)
CWI Word-Reading (secs) ‡	24.3 ± 6.23 (18-44)	25.4 ± 5.43 (19-43)
CWI Inhibition (secs) ‡	69.1 ± 23.5 (45-141)	61.7 ± 16.8 (37-108)
CWI Inhibition/Switching (secs) ‡	72.5 ± 28.9 (36-167)	64.4 ± 11.7 (36-90)
TMT Number/Letter-Sequencing (secs) §	127.2 ± 62.2 (60-240)	104.4 ± 46.1 (24-240)
TMT Motor-Speed (secs) §	42.8 ± 16.1 (21-82)	40.2 ± 27.6 (17-140)
RBANS		
Attention (index) †	101.2 ± 16.4 (72-135)	106.5 ± 16.4 (82-138)
Immediate-Memory (index) §	97.5 ± 13.9 (73-129)	97.0 ± 12.2 (73-129)
Delayed-Memory (index) † *	101.0 ± 11.1 (71-121)	96.5 ± 14.5 (60-123)
Visuospatial/Constructional (index) §	92.3 ± 17.0 (58-131)	96.6 ± 15.4 (69-131)
Language (index) §	98.7 ± 10.3 (75-113)	98.7 ± 12.0 (71-129)

* $p<0.05$, independent sample t-tests, ANCOVA with education level as a covariate, or Chi-Square tests.
§ One ND participant did not have subscore.
§§ One LLD participant did not have information regarding duration of depression in years since symptom's onset of the current MDE, but did not have a recurrent MDE. Therefore, it was coded with the mode of the LLD group only for subgroup comparisons.
‡ Two ND participants did not have subscore.
† Two participants (one LLD, one ND) did not have subscore.
DKEFS-CWI/TMT– Higher subscores mean worse performance.
RBANS – Higher subscores mean better performance.

Figure Captions

Figure 1: Locus coeruleus (LC)-sensitive and diffusion MRI analyses. (A) A study-specific template was created by averaging spatially normalized LC-sensitive MRIs from all subjects in MNI space and an over-inclusive LC region of interest (ROI) was delineated in the axial plane of this template. (B) This over-inclusive LC ROI was transformed back to native space where contrast-to-noise-ratio (CNR) was calculated relative to a reference region with minimal neuromelanin content (central pons enclosed in a green circle) and the smaller LC was segmented with an intensity-threshold-free cluster method. LC-sensitive MRIs and over-inclusive ROIs were registered and resliced to 1 mm isotropic diffusion space and mean diffusivity (MD) and fractional anisotropy (FA) were calculated. (C) LC (red), thalamus (cyan) and hippocampus (navy blue) dilated ROIs were utilized to track streamlines ascending from the LC and curving toward the hippocampus. A representative tractography example superimposed with 3D rendered LC and hippocampus ROIs (before the dilation step) is shown.

Figure 2: Diffusion tractography (axial and coronal views) of the pathway projecting from the LC nucleus to the hippocampus (rendered in semi-transparent grey color before ROI dilation step) with a FA color encoding scale in five ND and five LLD representative participants with comparable ages. Streamlines ascended from the LC nucleus and then curved toward the medial anterior portion of hippocampus, often reaching through the amygdala, in all participants.

Figure 3: Bilateral fractional anisotropy (FA, A), mean diffusivity (MD, B), and contrast-to-noise (CNR) ratio of the LC nucleus, and tract-weighted FA (D), MD (E) and fiber bundle capacity (FBC, F) of the LC-Hippocampus (Hp) pathway are shown for ND and LLD groups (mean group values displayed). The central boxes show the median and interquartile range while the whiskers above and below the boxes show the minimum and maximum values, excluding outliers. All participants datapoints are shown with circles. The LC nucleus showed significant differences between the ND and LLD groups for FA (*$p<0.05$)and MD, although FA did not survive corrections for multiple testing (**Bonferroni corrected $p\leq0.0063$), while there were no group differences for any diffusion metrics along the LC-Hp pathway.

Figure 4: Bilateral fractional anisotropy (FA; A, C), and mean diffusivity (MD; B, D) of the LC nucleus are shown for ND and LLD subgroups classified according to antidepressant use (A, B) and the persistency of depression (C, D) (mean group values displayed). The central boxes show the median and interquartile range while the whiskers above and below the boxes show the minimum and maximum values. All participants datapoints are shown with circles. Significant FA and MD differences were found between those patients not taking antidepressant medication (A, B) and those patients with depressive symptoms that were persistent for at least two years (C, D) when compared to ND participants. However, FA differences between those patients not taking antidepressant medication and ND participants did not survive corrections for multiple testing (**Bonferroni corrected $p\leq0.0063$).

Figure 5: Significant linear correlations (Bonferroni corrected at $p\leq0.0036$) between diffusion MRI metrics from the LC nucleus and the LC-Hp pathway in the full LLD+ND cohort, adjusted for the effects of LLD diagnosis, versus cognitive scores. Slower reading/naming speed (higher scores) was negatively associated with lower FA in the LC nucleus (A) and the LC-Hp pathway (B, C), while worse performance on inhibition (higher scores; D) and set-shifting (higher scores;

E, F) were negatively associated with lower FA in the LC-Hp pathway. All these correlations were also significant in the LLD and the ND groups when analyzed separately (data not shown)

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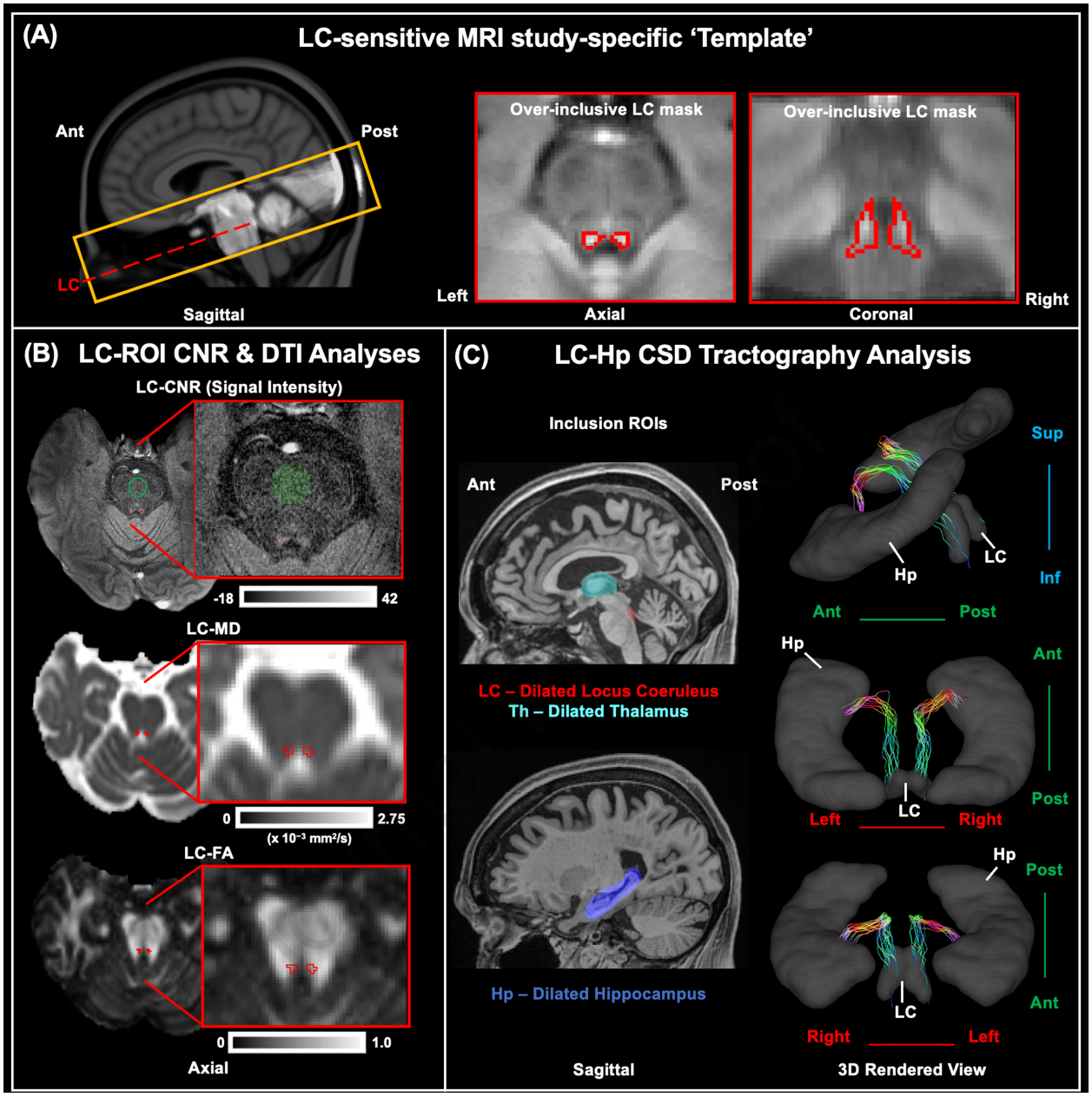
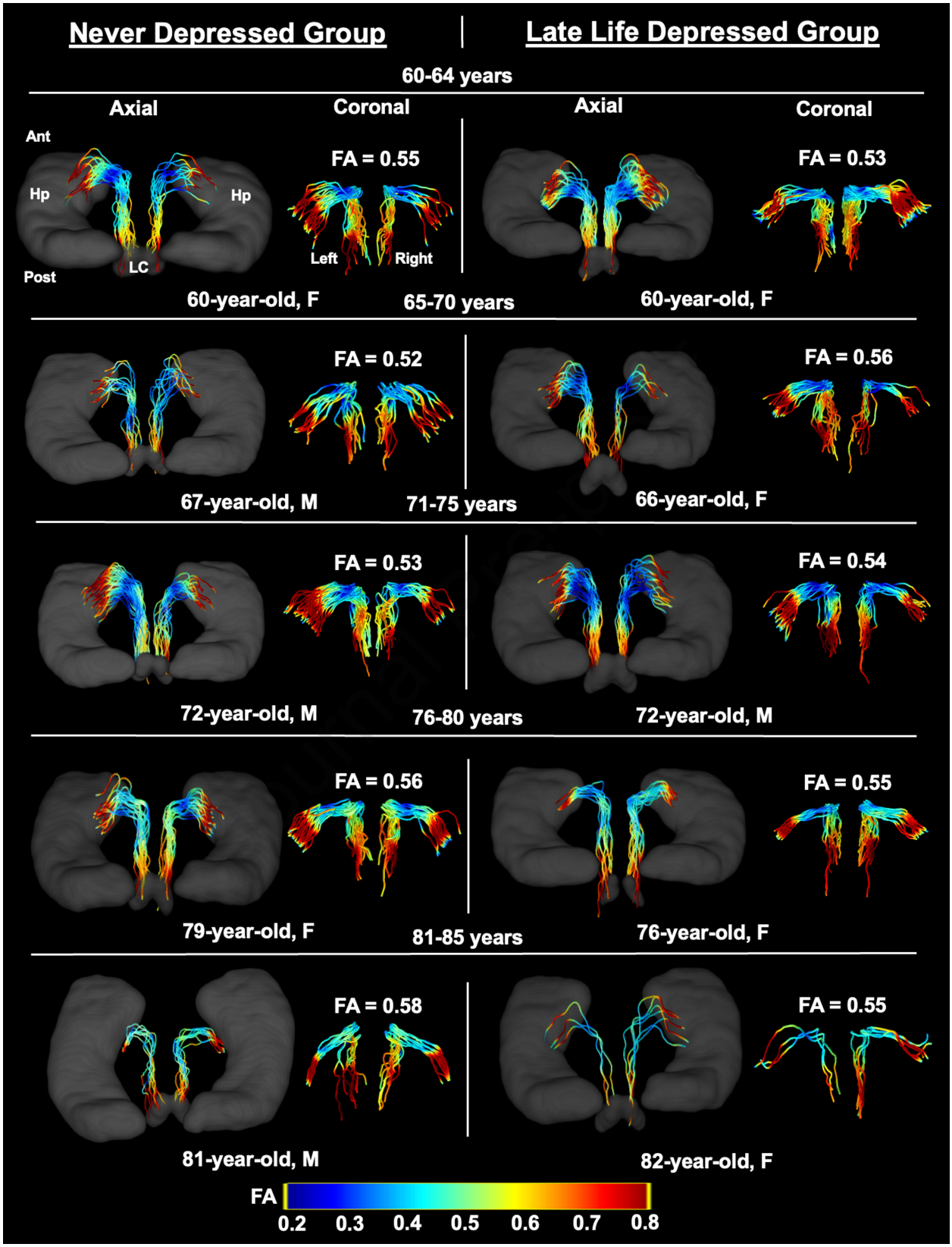

(A)
LC-sensitive MRI study-specific 'Template'
Ant
Post
LC
Sagittal
Over-inclusive LC mask
Left
Axial
Over-inclusive LC mask
Coronal
Right
(B) LC-ROI CNR & DTI Analyses
LC-CNR (Signal Intensity)
-18
42
LC-MD
0
2.75
(x 10⁻³ mm²/s)
LC-FA
0
1.0
Axial
(C) LC-Hp CSD Tractography Analysis
Inclusion ROIs
Ant
Post
LC – Dilated Locus Coeruleus
Th – Dilated Thalamus
Hp – Dilated Hippocampus
Sagittal
Sup
Hp
LC
Inf
Ant
Post
Hp
Ant
Left
LC
Right
Post
Hp
Post
LC
Ant
Right
Left
3D Rendered View


Never Depressed Group
Late Life Depressed Group
60-64 years
Axial
Coronal
Axial
Coronal
Ant
Hp
Hp
Post
LC
FA = 0.55
Left
Right
FA = 0.53
60-year-old, F
60-year-old, F
65-70 years
FA = 0.52
FA = 0.56
67-year-old, M
66-year-old, F
71-75 years
FA = 0.53
FA = 0.54
72-year-old, M
72-year-old, M
76-80 years
FA = 0.56
FA = 0.55
79-year-old, F
76-year-old, F
81-85 years
FA = 0.58
FA = 0.55
81-year-old, M
82-year-old, F
FA
0.2
0.3
0.4
0.5
0.6
0.7
0.8