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https://escholarship.org/uc/item/8jt540v0

Brain : a journal of neurology, 142(9)

0006-8950

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2019-09-01

10.1093/brain/awz193

Peer reviewed
Locus coeruleus imaging as a biomarker for noradrenergic dysfunction in neurodegenerative diseases

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Pathological alterations to the locus coeruleus, the major source of noradrenaline in the brain, are histologically evident in early stages of neurodegenerative diseases. Novel MRI approaches now provide an opportunity to quantify structural features of the locus coeruleus in vivo during disease progression. In combination with neuropathological biomarkers, in vivo locus coeruleus imaging could help to understand the contribution of locus coeruleus neurodegeneration to clinical and pathological manifestations in Alzheimer’s disease, atypical neurodegenerative dementias and Parkinson’s disease. Moreover, as the functional sensitivity of the noradrenergic system is likely to change with disease progression, in vivo measures of locus coeruleus integrity could provide new pathophysiological insights into cognitive and behavioural symptoms. Locus coeruleus imaging also holds the promise to stratify patients into clinical trials according to noradrenergic dysfunction. In this article, we present a consensus on how non-invasive in vivo assessment of locus coeruleus integrity can be used for clinical research in neurodegenerative diseases. We outline the next steps for in vivo, post-mortem and clinical studies that can lay the groundwork to evaluate the potential of locus coeruleus imaging as a biomarker for neurodegenerative diseases.
Introduction

The number of individuals over the age of 60 years is projected to rise from 841 million in 2013 to over 2 billion by 2050 (World Population Ageing report, United Nations). As the population has continued to age, age-related neurodegenerative diseases have already reached epidemic proportions. Whilst numerous therapeutic strategies are being investigated, current treatments provide only modest symptomatic relief and do not slow or halt ensuing neurodegeneration. A major priority is to develop early disease stage biomarkers that can improve the understanding of the pathophysiology of neurodegenerative diseases, enable earlier detection of pathology and facilitate the application of timely symptomatic interventions.

The locus coeruleus (LC) is the major source of noradrenaline modulation in the brain and has been shown to be involved in regulating a wide range of higher cognitive functions, such as working memory, learning and attention (Robbins, 1984; Aston-Jones and Cohen, 2005; Benarroch, 2009; Mather et al., 2016), memory consolidation and retrieval (Sterpenich et al., 2006; Sara, 2009), vigilance, arousal/wakefulness, rapid eye movement (REM) sleep behaviour, pain modulation, local blood flow and immunological mechanisms in the brain (Aston-Jones and Bloom, 1981; Benarroch, 2009; Espar et al., 2014; Heneka et al., 2015; O’Donnell et al., 2015). Conversely, age-related decline within the LC–noradrenergic system is associated with reduced cognitive abilities relating to episodic memory (Hämmerer et al., 2018,
 Jacobs et al., 2018b; Dahl et al., 2019) and reduced cognitive reserve (Robertson, 2013; Wilson et al., 2013; Clewett et al., 2016; Mather and Harley, 2016). In Alzheimer’s disease, tau aggregates are observed first in the LC, prior to their presence in transcortical/entorhinal cortex and neocortex (Braak et al., 2011; Strattmann et al., 2016; Andrés-Benito et al., 2017; Ehrenberg et al., 2017). In Parkinson’s disease, intraneuronal α-synuclein burden in the LC precedes and may be of even greater magnitude than that in the dopaminergic substantia nigra pars compacta (SNpc) (Braak et al., 2003; Dickson et al., 2008). As a result, the degree of Parkinson’s disease-related neuronal loss is likely just as severe in the LC as in the SNpc (Zarow et al., 2003; Giguère et al., 2018). Pathological changes in the LC have also been reported in other neurodegenerative and neuropsychiatric conditions (Box 1), leading to the notion that the LC may be a vulnerable target for pathology (Sharma et al., 2010). Whether this is related to LC neurons’ high metabolic need in order to maintain essential physiological functions or the close proximity of the LC to the fourth ventricle, exposing it to toxins from the CSF (Mather and Harley, 2016; Weinschenker, 2018), remains to be determined.

With the notable exception of most of the basal ganglia, the LC projects to large portions of subcortical and cortical areas (Berridge and Waterhouse, 2003). Moreover, reminiscent of

Box 1 | Role of the LC in neurological disorders

Structural and functional changes in the human LC leading to deficits in noradrenaline may contribute to the pathophysiology and symptomatology of several neurological disorders:

**Alzheimer’s disease**

Deposition of tau in and degeneration of the LC occurs early in the asymptomatic phase of Alzheimer’s disease (Braak and Del Tredici, 2012; Arendt et al., 2015) suggesting that the LC is the initial site of pathology. Accumulation of tau (doubling from Braak stage 0 to 1; Ehrenberg et al., 2017) and significant volume loss precede neuronal loss in the LC during Alzheimer’s disease progression (Theofilas et al., 2017). Depletion of up to 30% of LC neurons has been reported in prodromal (mild cognitive impairment stage) Alzheimer’s disease, increasing to 55% with diagnosed dementia (Kelly et al., 2017). Neuronal loss is particularly prevalent in the rostral/middle portion of the LC (German et al., 1992; Theofilas et al., 2017), correlating with cognitive dysfunction including memory, perceptual speed, and visuo-spatial ability (Kelly et al., 2017), and with reduced noradrenaline levels in hippocampus and cortex (Lyness, 2003). Compensatory activity, such as increased metabolism in surviving neurons or reorganization of functional networks, can occur in early disease stages (Hoogendijk et al., 1999; Jacobs et al., 2015).

**Synucleinopathies**

In Parkinson’s disease and dementia with Lewy bodies, α-synuclein containing Lewy bodies and neuronal cell loss are seen along the entire length of the LC (German et al., 1992; Theofilas et al., 2017). Intraneuronal Lewy bodies also affect tyrosine hydroxylase (TH) activity, potentially interfering with normal catecholamine biosynthesis (Tabrez et al., 2012), which may distinguish the effects of Parkinson’s disease/dementia with Lewy bodies from Alzheimer’s disease (McMillan et al., 2011). It has also been postulated that LC burden precedes substantia nigra involvement in Parkinson’s disease (Zarow et al., 2003; Braak et al., 2004; Seidel et al., 2015), rendering the LC a good candidate for preclinical diagnosis (Liu et al., 2017). However, the field lacks a comprehensive analysis detailing the stages of neuronal loss in Parkinson’s disease.

Multiple system atrophy (MSA) is another heterogeneous α-synucleinopathy, in which autonomic dysfunction, parkinsonism, and ataxia (Stankovic et al., 2014; Walsh et al., 2018) are associated with severe neuronal loss in the LC and noradrenergic cardiorespiratory brainstem nuclei (A5, A1) (Benarroch et al., 2008). An in vivo neurumelanin-sensitive MRI study has shown that LC contrast is reduced in MSA compared to healthy controls and that the ratio of neurumelanin-sensitive MRI contrast in substantia nigra versus LC could help distinguish MSA from Parkinson’s disease (Matsura et al., 2013). Patients with idiopathic rapid eye movement sleep disorder (iRBD) have a strongly increased risk of developing Parkinson’s disease, dementia with Lewy bodies, MSA, and mild cognitive impairment; 75.7% and 90.9% develop a neurodegenerative syndrome 10 and 14 years from the time of iRBD diagnosis, respectively. Interestingly, iRBD is associated with dysfunction of the LC-noradrenergic system (Knudsen et al., 2018) suggesting that LC imaging might serve as a valuable biomarker in patients at risk of developing neurodegenerative disease.

**Chronic traumatic encephalopathy**

In addition to early pathological affection of cortical sulci, substantial LC damage and tau accumulation has also been reported in early disease stages (Stein et al., 2014). LC pathology may also exacerbate clinical presentation of cognitive complaints and mood disturbances in chronic traumatic encephalopathy (Stern and Daneshvar, 2013).

**Frontotemporal lobar degeneration**

Modest LC neuronal loss occurs in both tau- and non-tau- forms of frontotemporal lobar degeneration (Brunnström et al., 2011; Eser et al., 2018), both in early and late disease stages (Irwin et al., 2016). Clinically, the loss and dysregulation of noradrenaline from LC degeneration may contribute to cognitive decline, including impulsivity and apathy (Passamonti et al., 2018).

**Essential tremor**

A neuropathological study (n = 33) revealed Lewy body pathology in the LC in 25% of the cases with essential tremor (Louis et al., 2007). Noradrenergic LC-cerebellar connections are important for the normal function of Purkinje cells and their inhibitory output (Moises et al., 1981). Degeneration and ensuing LC dysfunction may modulate Purkinje cell activity and contribute to decreased cerebellar inhibition in essential tremor (Louis et al., 2007).

**Non-degenerative, neuropsychiatric illnesses**

LC dysfunction, also without neuronal loss, has also been implicated in post-traumatic stress disorder (Bernard et al., 2011; Pietrzak et al., 2013), addiction (Bernard et al., 2011), depression (Berridge and Waterhouse, 2003), suicidal behaviour (Roy et al., 2017) and chronic pain (Llorca-Torrallba et al., 2016; Taylor and Westlund, 2017).
locally specific connectivity in dopaminergic nuclei (Haber and Knutson, 2010), tracing studies in the rat suggest that there might be distinct projections from rostral lateral LC to hippocampus, amygdala and septum (Van Bockstaele et al., 2006) and from caudal aspects of the LC to the spinal cord (Ennis et al., 1991). This may indicate a stronger involvement of rostralateral LC in modulating memory encoding and caudal LC in modulating pain perception.

While the LC is among the first brain structures to demonstrate pathology in neurodegenerative diseases, it currently remains unclear how alterations in LC structure and function influence pathogenesis and symptom progression. In human studies, neuropsychiatric symptoms associated with LC activity, such as sleep dysfunction, agitation, anxiety, appetite dysfunction and depression, appear as elements of the early clinical phenotypes of neurodegenerative diseases (Assal and Cummings, 2002; Lanctôt et al., 2017; Ehrenberg et al., 2018). In animal models, lesions of the LC have been shown to exacerbate tau and amyloid-β plaque deposition, leading to depletion of noradrenaline in LC target regions and impaired cognitive function (Heneka, 2006; Kalnin et al., 2006, 2012; Jardanhazi-Kurutz et al., 2010; Chalermpanalupap et al., 2017; Rorabaugh et al., 2017). Whether the influence of LC degeneration on pathology is directly related to a dysregulation of noradrenaline, or indirectly related via neuroinflammatory mechanisms or alterations in additional LC neuromodulators, such as brain-derived neurotrophic factor or galanin, requires further investigation (Chalermpanalupap et al., 2017; Betts et al., 2018). Interestingly, there is also evidence of noradrenergic hyperactivity in early disease (as reviewed by Weinschenker et al., 2018), consistent with a number of reports of elevated levels of CSF noradrenaline and/or noradrenaline turnover in Alzheimer’s disease (Palmer et al., 1987; Hoogendijk et al., 1999). Thus, hyperactivity in a structurally impaired LC might further accelerate the propagation of neuropathology in neurodegenerative disease via noradrenergic projections (Weinschenker, 2018). As such, early detection of LC decline using in vivo imaging techniques might contribute to earlier diagnosis, and support personalized pharmacological interventions to alleviate compensatory hyperactivity of the noradrenergic system and potentially slow down disease progression.

Recent advances in non-invasive neuroimaging techniques now permit the in vivo assessment of the LC using MRI (Sasaki et al., 2006; Keren et al., 2009; Clewett et al., 2016; Betts et al., 2017; Priovoulos et al., 2018) opening the possibility to track LC changes as a biomarker for noradrenergic dysfunction. The utility of this approach for clinical research will depend on whether LC imaging in combination with established biomarkers, can help to better stage neurodegenerative diseases and characterize biomarker-positive individuals before the onset of dementia. LC imaging could also have important implications in clinical trials as a stratification tool for predicting treatment success of pharmacological intervention studies targeting the noradrenergic system. This article reports on the consensus reached at the first European Locus Coeruleus Imaging meeting held in Magdeburg in 2018. It describes the challenges for establishing LC MRI measures as a biomarker for diagnosis and targeting therapeutic interventions in neurodegeneration and outlines a strategy for obtaining reliable, biologically validated and clinically suitable imaging approaches.

As discovered over a decade ago (Sasaki et al., 2006), the LC is visualized using MRI scanning protocols that are sensitive to a paramagnetic compound called neuromelanin, which accumulates in noradrenergic neurons, and may be taken as an indicator of LC integrity (i.e. cell density). While neuromelanin-sensitive MRI is a relatively new technique, a number of studies have shown that MRI can detect signal differences in the LC between healthy controls and diseases with known LC involvement (e.g. in major depression, Parkinson’s disease and Alzheimer’s disease as recently reviewed by Liu et al., 2017). Given, however, the substantial interindividual differences in LC measures across healthy older adults (Liu et al., 2017, 2019), further studies are required to establish whether LC degeneration can be reliably detected in vivo (Liu et al., 2017; Sulzer et al., 2018). Furthermore, to clarify whether interindividual variability in LC integrity is related to varying levels of neuropathologies (e.g. tau, amyloid or α-synuclein), studies using LC MRI in combination with in vivo measurements of pathology (e.g. PET and CSF biomarkers) in cognitively normal older adults and patients are required. While these studies are currently underway, first results in ageing and early-stage Alzheimer’s disease indicate that LC MRI contrast is indeed modulated by both tau and amyloid pathology. Recent evidence demonstrating that LC MRI contrast is associated with CSF amyloid (amlod-β42/40) in early-stage Alzheimer’s disease (Betts et al., 2019) and tau pathology at higher levels of amyloid burden using tau and amyloid PET, respectively (Jacobs et al., 2018a), may partially explain the interindividual variability in LC integrity observed in old age. Interestingly, post-mortem studies using 18F-AV-1451 tau PET have also shown off-target binding properties to neuromelanin in the substantia nigra (Marquie et al., 2015; Hansen et al., 2016). Thus 18F-AV-1451 may also be capable of imaging neurodegeneration. However, such changes may be difficult to interpret because of the mixed PET signal generated by on-target binding to tau tangles and off-target binding to neuromelanin, which would likely also be age-dependent.

Practical considerations in in vivo locus coeruleus imaging

The intrinsic neuromelanin-driven contrast that allows us to visualize the LC using MRI was first identified in vivo as hyperintensity on 2D T1-weighted turbo spin echo (TSE) images (Sasaki et al., 2006). These hyperintensities correspond closely with areas of higher concentration of neuromelanin (Keren et al., 2015), suggesting that the high signal intensities in the LC are driven by neuromelanin. In support
of this, a recent study has shown that the neuromelanin concentration in the substantia nigra is linearly related to neuromelanin-MRI contrast and resting blood flow in the substantia nigra (Cassidy et al., 2019). The observation that LC MRI acquisitions are subject to incidental magnetization transfer (MT) effects (Dixon et al., 1990) and that dedicated MT preparation increases neuromelanin-related contrast, have led several authors to credit MT as the primary source of LC contrast in MRI (Nakane et al., 2008; Priovoulos et al., 2018). Recent findings suggest that an interaction of (higher) intracellular water content with paramagnetic ions (such as neuromelanin) may set the LC apart from its surroundings in MT-weighted imaging (Watanabe et al., 2019). Moreover, paramagnetic ions in addition to neuromelanin may contribute to this effect as structures low in neuromelanin (e.g. periaqueductal grey matter) demonstrate similar MRI contrast to the LC using MT-weighted imaging (Cassidy et al., 2019; Watanabe et al., 2019). Despite the different approaches that have successfully visualized the LC (Fig. 1), a precise understanding of the underlying contrast mechanism is thus still lacking. As outlined in more detail below, a combination of histological and post-mortem imaging studies would provide important insights in this regard. Mechanisms involving both neuromelanin concentration and macromolecules in the LC and surrounding tissues may be active simultaneously, and a combination of mechanisms may be required to explain the contrast patterns seen in normal ageing and in pathology. A relevant aspect for imaging neuromelanin is that it is known to scavenge metals both across the lifespan and in disease states (Zecca et al., 2004; Biesemeier et al., 2016). In vitro studies, albeit with synthetic melanin, suggest that T1 shortening in the LC may be driven by compounds of neuromelanin and chelated metals, such as iron and copper (Enochs et al., 1989, 1997; Trujillo et al., 2017) impacting the macromolecule-bound pool, rather than neuromelanin or iron alone (Langley et al., 2015; Trujillo et al., 2017). It has therefore been proposed that complexes of neuromelanin-bound paramagnetic ions are the primary drivers of LC contrast (Enochs et al., 1989; Nakane et al., 2008; Trujillo et al., 2017); however, neuromelanin itself, even free of metals, is paramagnetic (Shima et al., 1997). Indeed, at least in vivo, LC contrast does not seem to benefit from typical iron-sensitive contrasts, such as quantitative susceptibility mapping (QSM) or apparent R2* (Acosta-Cabronero et al., 2016; Betts et al., 2016, respectively) (Fig. 1N and R).

More recently, MT preparation and/or T1-weighting have been combined, both with short repetition time spoiled gradient echo and turbo-flash readouts, to increase spatial resolution and/or shorten the acquisition time compared to TSE acquisitions (Chen et al., 2014; Betts et al., 2017; Hämmrer et al., 2018; Priovoulos et al., 2018). The small size of the LC has motivated the use of high spatial resolution, at least in-plane, while high isotropic resolution minimizes partial volume effects (i.e. inclusion of non-LC tissue within a putative LC voxel). However, this comes at the cost of relatively low signal- and contrast-to-noise ratio (SNR and CNR, respectively) due to smaller voxel sizes and higher vulnerability to subject motion, which has prompted averaging over multiple repetitions. Anisotropic voxels, which mimic the shape and orientation of the LC, have capitalized on the elongated shape of the LC to increase SNR and shorten scan times. Given the tilted and not perfectly cylindrical nature of the LC, this strategy is prone to errors at the most rostral and caudal ends of the structure leading to biases in segmentation and volumetric measurements (Liu et al., 2017). Alternatively, isotropic acquisitions that capture the entire rostrocaudal length of the LC have also been proposed (Betts et al., 2017; Priovoulos et al., 2018) and may be more consistent with in vivo measurements compared to anisotropic scans (Liu et al., 2017). While the increased SNR with ultra-high field MRI (i.e. ≥ 7.0 T) offers higher attainable spatial resolution and/or shorter acquisition times, increased specific absorption rates means that higher power acquisitions, e.g. those using MT preparation pulses or high flip angles, might not be readily available thereby necessitating further optimization (Priovoulos et al., 2018) (see also Box 2 for recommendations on how to use currently available sequences).

Validating in vivo locus coeruleus imaging using post-mortem MRI and histology

A key aim of conducting LC imaging in clinical groups is to capture in vivo disease-related physiological and structural changes such as reductions in neuronal density. Several validation strategies, including a combination of post-mortem magnetic resonance microscopy and histological labelling (Forstmann et al., 2017), have thus been proposed to shed new light into the underlying biological processes driving in vivo imaging results. Such approaches, however, are also technically challenging. Tissue fixation and embedding media can affect the properties of post-mortem tissue and strongly influence the MRI signal (Dusek et al., 2019). Thus, the same tissue properties might be expressed differently in post-mortem and in vivo MRI. For instance, tissue fixation causes strong T1 and T2 shortening that is additionally dependent on the choice of fixative agent and fixation time (Birkel et al., 2016, 2018). Contrast changes also arise if the temperature used for post-mortem scanning differs from 37°C (Birkel et al., 2014). Differences between in vivo and post-mortem tissue could also be driven by changes in metal oxidation states during fixation or by redistribution of iron and other metals across macromolecules (e.g. proteins, neuromelanin, etc.) (Shima et al., 1997; Krebs et al., 2014). It should be considered that iron is bound in brain tissue in different molecular forms such as ferritin, neuromelanin, hemosiderin and others,
Figure 1  Overview of LC visibility using post-mortem and in vivo MRI. The LC can be imaged in post-mortem tissue using numerous MRI protocols [arrows indicate the LC, evident as dark spots in T2* (A and G) and bright spots in T1-weighted (E) and MT-weighted (F) scans] in addition to using histological techniques. (D) The LC is visible as dark spots in a post-mortem slice without any staining, due to neuromelanin deposits, which are thought to contribute to magnetic resonance visibility of the LC. (C) Myelination in the LC area. The LC and central pontine grey show very low myelination, but are surrounded by areas with very high and intermediate myelination (green areas), possibly also contributing to MR visibility of the LC. To image the LC in vivo, T1-weighted (H, O and S) and MT-weighted (I–K) MRI protocols can be used (arrows indicate the LC, evident as bright spots in T1-weighted and MT-weighted scans). Using these protocols, a decline in LC integrity in Alzheimer’s disease dementia (S) compared to healthy elderly adults (O) can be identified (Betts et al., 2019). To fine-tune these scan protocols further, it is necessary to understand the magnetic resonance contrast mechanisms that underlie LC visibility. (L–N and P–R) Quantitative maps which isolate different magnetic resonance contrast effects (R1, MT and R2* effects), show that LC visibility in T1-weighted as well as MT-weighted scans in vivo is mostly due to R1 effects (mean LC visibility across 22 healthy older adults extracted from line regions of interest; see inset on right for position of line regions of interest. Black dots indicate position of maximal signal intensity based on T1-weighted maps. Peaks in signal intensity are apparent in R1.

(continued)
T2*/R2* measurements of the LC return markedly different characteristic T1- and MT-weighted hyperintensity of the showed that areas of LC hyperintensity in T1-weighted sensitive MRI contrast could serve as a proxy measure of containing proteins). Iron, neuromelanin, copper, and iron- and copper-quantitative histological measures (e.g. myelin, lipids, neuromelanin pigments (Engelen et al., 2012; Zucca et al., 2018) that accumulate in specific organelles in the LC as aggregated tau, which are well known markers of ageing and neurodegenerative disease as well as non-pathological proteins and lipids, are additional important constituents of neuromelanin pigments (Engelen et al., 2012; Zucca et al., 2018) that accumulate in specific organelles in the LC as occurs in substantia nigra (Zecca et al., 2004; Braak et al., 2011; Zucca et al., 2017). However, the effect of these constituents on LC MRI contrast has not yet been systematically explored.

Using a combination of histology and post-mortem MRI in the LC, a recent study explored whether neuromelanin-sensitive MRI contrast could serve as a proxy measure of LC ‘integrity’ (i.e. neuronal density in the LC). The authors showed that areas of LC hyperintensity in T1-weighted MRI were co-localized with neuromelanin-rich neurons in the LC region (Keren et al., 2015), suggesting that LC visibility in MRI may indeed be driven by the neuromelanin content of noradrenergic neurons. Similarly, studies using neuromelanin-sensitive MRI could show that age-related cross-sectional patterns in in vivo signal intensity across the lifespan (Shibata et al., 2006; Jacobs et al., 2018a; Liu et al., 2019) replicate the inverted U-shaped cross-sectional development of neuromelanin deposits in the LC observed in post-mortem tissue (Mann and Yates, 1974, but see Ohm et al., 1997; Zecca et al., 2004 for studies reporting a stable level of neuromelanin-containing neurons or linear increase of neuromelanin content in LC tissue across the lifespan, respectively). This inverted U-shaped pattern has been suggested to reflect increasing neuromelanin content due to continuous noradrenaline production during adulthood prior to ensuing cell death and clearance of released intracellular neuromelanin with the advent of age-related neurodegeneration and onset of disease (Mann and Yates, 1974). Indeed, post-mortem data suggest that neuromelanin may be a more suitable indicator of neuronal density in older adults (at least > 55 years) as younger adults’ LC neurons may not yet be sufficiently pigmented with neuromelanin to allow inference on cell numbers in neuromelanin-sensitive MRI (Manaye et al., 1995; Liu et al., 2019). Nonetheless, further studies are warranted to investigate whether higher signal intensities in older adults invariably represent more intact LC neurons in the LC, and whether additional mediating factors for interpreting LC-related MRI signals must be considered in different age groups or clinical populations. For instance, pathologically altered proteins, such as hyperphosphorylated or aggregated tau, which are well known markers of ageing and neurodegenerative disease as well as non-pathological proteins and lipids, are additional important constituents of neuromelanin pigments (Engelen et al., 2012; Zucca et al., 2018) that accumulate in specific organelles in the LC as occurs in substantia nigra (Zecca et al., 2004; Braak et al., 2011; Zucca et al., 2017). However, the effect of these constituents on LC MRI contrast has not yet been systematically explored.

**Outlook and conclusion**

A major goal of in vivo LC imaging in clinical research is to aid differential diagnosis, disease monitoring and stratification to novel treatments. As such, LC imaging has the potential to become a component of a precision medicine approach to neurodegenerative diseases. As brain function adapts to ensuing pathology, such as receptor increases in target areas of noradrenergic LC projections or increased connectivity following a decline in LC function (Herrmann et al., 2004; Ye et al., 2015), LC imaging could provide a stratification tool for predicting treatment success of pharmacological intervention studies targeting the noradrenergic system (Ye et al., 2015, see also Fig. 2). Once its relationship to pathogenic changes (e.g. α-synuclein or tau aggregation) has been determined, it could also support differential diagnosis and be added to the portfolio of longitudinal monitoring staging tools for neurodegenerative diseases. Over the next years, we expect substantial progress towards these goals through a multidisciplinary

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**Figure 1 Continued**

and to some extent in R2* maps (Hämmerer et al., 2018a]). Sequence details/stains: (A) 7 T T2*-weighted (50 μm resolution) FLASH MRI image TE = 19 ms; (B) TH staining for LC neurons (dark); (C) Luxol fast blue staining for myelinated fibres in same slice (green); (D) Block face image after celloidin embedding (LC neurons dark); (E) 7 T T1-weighted (0.2 x 0.2 x 2 mm) TSE image (TE/TR/TI = 11/2000/825 ms); (F) 7 T MT-weighted FLASH MRI image (TE/TR/TL = 5.1/26 ms); (G) 7 T T2*-weighted FLASH image (TE/TR = 21/30 ms) (Otaduy et al., unpublished results); (H) 3 T T1-weighted (0.4 x 0.4 x 3 mm) FLASH image (TE/TL = 3.35–16.95/27) averaged across six repetitions; (I) 3 T MT-weighted (0.4 x 0.4 x 3 mm) FLASH image (TE/TL = 3.35–16.95/30.74); (j) 7 T MT-TFL image (0.4 x 0.4 x 0.5 mm); (K) 3 T MT-weighted (1.5 mm3) SPGR image (TE/TR/TL = 5/30/300; (O and S) 3 T T2*-weighted (2.7 mm3) FLASH image (TE/TL = 5.56 ms/20 ms). Image in J is reproduced with permission from Priovoulos et al. (2018); O is reproduced with permission from Betts et al. (2017). TE/TL/TR = echo time/repetition time/inversion time.
Box 2 Practical suggestions for in vivo LC imaging

Sequences

The LC is most visible using $T_1$- or MT-weighted structural sequences and can be well characterized using 3 T and 7 T MRI (see Betts et al., 2017; Priovoulos et al., 2018 and Liu et al., 2017 for a recent review). To date, there is no single ‘best’ sequence for LC imaging; there is a trade-off between non-quantitative protocols offering easier LC localization, and quantitative magnetic resonance acquisitions that provide more objective biophysical measures of brain tissue but with lower localization ability compared to dedicated LC sequences.

Voxel size

Owing to the small size of the LC (~15 mm in length and 1–3 mm in diameter), larger voxel sizes should be avoided as they result in weaker signals owing to partial volume effects with tissue outside the LC within the voxel. On the other hand, voxel volumes lower than 0.4–0.5 mm$^3$ run the risk of lacking sufficient signal-to-noise ratio, given the current sensitivity of neuromelanin-sensitive magnetic resonance sequences. If more accurate LC volume assessments (with less partial volume contamination) are desired, isotropic voxels sizes are preferable [e.g. 0.75 mm $\times$ 0.75 mm $\times$ 0.75 mm are possible at 3 T (Betts et al., 2017) and 0.4 mm $\times$ 0.4 mm $\times$ 0.5 mm at 7 T (Priovoulos et al., 2018)]. These, however, can make identification of individual LCs harder because of lower signal-to-noise ratio, requiring a group template approach (see below). If reliable identification of individual LCs is desired but accurate volume assessments are less critical, anisotropic voxel sizes (e.g. 0.5 mm $\times$ 0.5 mm in plane resolution $\times$ 2 mm slice thickness) can be used to exploit the quasi-cylindrical shape of the LC and maximize signal-to-noise ratio at the cost of greater partial volume errors in peripheral LC voxels (Liu et al., 2017).

Acquisition

It is recommended that the field-of-view is aligned perpendicular to the dorsal edge of the pons such that cylindrical voxels align with the LC.

Motion artefacts

Great care should be taken to reduce head motion during the MRI acquisition. A thin pillow placed on the base of the coil to ensure tight fixation and patient comfort can help minimize motion and achieve high measurement precision e.g. improving coregistration across/within individuals e.g. between structural and functional data. Shorter acquisition times which are less prone to motion artefacts can be achieved by using a reduced field-of-view comprising the brainstem only and by relying on offline-averaging of scans (Chen et al., 2014). Typical scan durations for smaller field-of-view acquisitions take approximately half the time of whole-brain acquisitions and range between 5 and 9 min at 3 T and 7 T MRI, respectively (Liu et al., 2017; Priovoulos et al., 2018). However, template-based segmentation approaches (see below) are more challenging with reduced field-of-view acquisitions than with whole brain scans (but see Dahl et al., 2019).

Post-processing

Semi-automated LC segmentation can be performed through highly iterative co-registration resulting in a high definition group-average template where the LC can be reliably identified. The resultant template LC region of interest can then be spatially transformed to each individual’s native space (Betts et al., 2017; Liu et al., 2019). When using template-based approaches however, care must be taken to minimize the impact of coregistration inaccuracies and additional user intervention might be required. There are at present differing views on whether LC segmentation should be performed manually or automatically, and on whether LC measurements should encompass the whole structure or favour partial extraction to avoid potential contamination. Chen et al. (2014) argued that automated segmentation approaches might be less error-prone and may thus lead to higher consistency across scans and studies, and that visually locating the LC as the highest-intensity voxel at either side of the fourth ventricle (on the axial plane) might be susceptible to noise-related errors. In contrast, Keren et al. (2009) argued that assessing single voxels might be more reliable in that it does not rely on boundary definitions or signal intensity thresholding, thereby leading to improved sensitivity to overall LC differences. To date, however, no study has systematically compared manual and automated methods.

Reference normalization

An important limitation of MRI is that it produces arbitrarily scaled greyscale images that are suboptimal for intersubject comparisons because normalization to a proximal control region (often the pontine tegmentum) becomes necessary (Betts et al., 2017; Liu et al., 2017; Hammerer et al., 2018). However, the reference tissue may also change throughout the lifespan, or be abnormal in neurodegenerative diseases (Keren et al., 2009; Clewett et al., 2016). Furthermore, asymmetric signal-intensity biases in the LC and surrounding tissue have been reported both in healthy subjects and Parkinson’s disease patients (Keren et al., 2009; Garcia-Lorenzo et al., 2013; Betts et al., 2017; Tona et al., 2017), in apparent contradiction with post-mortem studies that show largely symmetric distributions of neuromelanin-pigmented cells in the LC (German et al., 1988; Baker et al., 1989; Chan-Palay and Asan, 1989; Ohm et al., 1997). It is therefore imperative to avoid instrumental biases such as transmit and receive field inhomogeneities, and ensure asymmetries are not introduced by the methodology. To this end, quantitative (parametric) MRI (Weiskopf et al., 2013) has shown promise by quantifying specific tissue properties in terms of physical units and may now be used for quantitative LC imaging (Fig. IL–N and P–R) (Hammerer et al., 2018a). Moving forward, one may speculate that multi-parametric mapping might be preferred to single-contrast assessments for a more complete view of LC status and greater insight into underlying contrast mechanisms, especially if performed within the population-specific time constraints.

Age comparisons and control groups

Owing to an age-related increase in neuromelanin content in LC cells as well as LC signal intensity using neuromelanin-sensitive MRI, LC imaging may be suboptimal in younger adults, likely due to reduced neuromelanin levels compared to older adults (Zecca et al., 2004, 2006; Liu et al., 2019). For the same reason, interpreting age differences in LC MRI contrast between young and older adults as age differences in LC integrity may be problematic. In a large cross-sectional dataset, a decline in LC integrity was only observed at ~60 years of age and over (Liu et al., 2019). At present it is unclear if neuromelanin-sensitive MRI can detect a reduction in LC integrity below this age range. Moreover, within an ageing sample, control groups must be tightly matched for age to the clinical population under investigation.
collaboration of experts in neurodegenerative diseases, imaging, neuropathology and cognition.

As neuromelanin accumulates in the cell bodies of LC neurons, neuromelanin-sensitive MRI can only inform on changes in cell density but not changes in synaptic density or cell activity. It is known from post-mortem studies that cell numbers in the LC decline with advancing Alzheimer’s disease pathology (Theofilas et al., 2017). However, prior to cell loss, changes in synaptic density in the LC and/or neuronal activity should be considered. Post-mortem and lesion studies suggest that noradrenaline production in remaining LC neurons may increase following degeneration of LC neurons (for a review see Weinshenker et al., 2018). At present, it is not known whether synaptic density is altered prior to or in tandem with cell loss in the LC. It would be interesting to use novel PET tracers of synaptic density (Finnema et al., 2016) in combination with LC imaging to assess how differences in LC synapse density relate to LC MRI contrast in neurodegenerative diseases. Direct and indirect functional measures of LC activity or noradrenaline release can be assessed using high resolution functional MRI, pupillometry, PET measures of noradrenergic transporter levels (Sommerauer et al., 2018), or by assessing noradrenaline levels in CSF. These measures, however, are not short of caveats. PET and functional MRI measures provide noisy assessments given the small size of the LC. Moreover, the LC is also known to release dopamine and GABA (Kempadoo et al., 2016; Takeuchi et al., 2016; Breton-Provencher and Sur, 2019), thus dopamine and GABA levels may also need to be assessed in conjunction with noradrenaline levels when characterizing LC function. Finally, it can also be difficult interpreting functional activation in the LC as an indicator of neuronal capacity since activation will not only depend on the
number of neurons or synaptic density, but also on the
degree in which the LC is engaged during the processing
of the task at hand [i.e. the strength and the nature of the
experimental manipulation, e.g. as suggested previously by
Hämmerer et al. (2018)]. Nonetheless, synaptic density and
functional activation may be superior indicators of cogni-
tive impairment than cell pathology (Terry et al., 1991). As
the specificity of functional and experimental manipulations
for the LC increases, these measures may contribute valu-
able additional data on the role of the LC in neurodegen-
erative diseases.

One of the main tasks ahead for LC research lies in
setting up carefully designed longitudinal studies in healthy
ageing and disease cohorts with deep cognitive and physi-
ological phenotyping (e.g. the DELCODE study; Jessen
et al., 2018) to assess how LC integrity is related to cog-
nitive symptoms and functional brain changes at the ear-
est stages of neurodegeneration. Previous work assessing
the reproducibility of LC imaging by quantifying the sta-
bility of LC contrast across two or more independent scan
sessions has revealed moderate to high reproducibility
(Langley et al., 2017; Tona et al., 2017; Betts et al.,
2017; Dahl et al., 2019). Therefore to achieve reliable lon-
gitudinal testing in the future will require an improvement
in the reproducibility and reliability of LC imaging tech-
niques. To assess how the LC modulates the function of
distributed brain networks at rest and during cognitive
tasks, will require further optimization of existing func-
tional MRI sequences to overcome the challenges of brain-
stem imaging (Düzel et al., 2015) and inclusion of
additional physiological information (see Liu et al., 2017
for recommendations). In addition, experimental tasks that
elicit robust LC signals require development (Mather et al.,
2016; Liu et al., 2017; Clewett et al., 2018).

Second, future studies should test whether LC imaging is
related to disease progression in neurodegenerative diseases.
This will require characterizing the relationship between LC
MRI measures and neuropathology (e.g. with respect to
amyloid, tau or alpha-synuclein using CSF biomarkers or
PET) but also noradrenergic function using noradrenergic
PET-tracers including those to assess noradrenergic trans-
porter density (Sommerauer et al., 2018). However, ad-
vances in analysis (to maximize spatial resolution) and
exclusion of potential off-target binding sites (Lee et al.,
2018) must accompany ligand development. For optimizing
and validating LC MRI further, greater synergy between
in vivo, post-mortem, in vitro and clinical studies is
required for a deeper understanding into how LC MRI
contrast relates to the pathophysiology of different neuro-
degenerative diseases (Fig. 2). Such studies will also benefit
from using clinically informed test beds, i.e. populations,
that show a clear difference in relevant tissue properties,
e.g. between young and older adults but also in neurode-
genative diseases, such as Alzheimer’s disease and beyond
(Box 1).

Finally, a growing body of knowledge on similarities and
differences between neuromelanin deposits in noradrenergic
cells in the LC and dopaminergic cells in the substantia
nigra (Zecca et al., 2004; Zucca et al., 2006; Wakamatsu
et al., 2015) open further avenues for using in vivo neuro-
 melanin-sensitive MRI to also investigate the involvement
of the substantia nigra in tandem with the LC in a wide
range of clinical conditions, i.e. in diseases also affecting
the dopaminergic system, such as Parkinson’s disease
(Sulzer et al., 2018).

In conclusion, there is rapid progress towards achieving
more sensitive and high-resolution in vivo imaging of the
LC. The methods are non-invasive and fast enough to be
well tolerated using high (3 T) or ultra-high (≥7 T) MRI,
and can have great potential to inform the effectiveness
of psychopharmacological probes, therapeutic trials, and
physiological monitoring in clinical populations. We antici-
pate rapid growth in the evidence base for developing LC
imaging as a biomarker in neurodegenerative diseases.

Funding

M.B. and D.H. were supported by the Human Brain
Project (SP3 WP 3.3.1) and CRC 779 (Project A7). E.D.
and D.H. are also supported by the MRC MR/P012698/1.
L.Z. and F.A.Z. were supported by the Italian Ministry of
Education, University, and Research (MIUR) - National
Research Programme (PNR) - National Research Council
of Italy (CNR) Flagship “InterOmics” Project (PB.P05), by
MIUR - PNR - CNR Aging program 2012-2014. L.Z. was
also supported by the Grigioni Foundation for Parkinson’s
Disease (Milan, Italy). C.L. is supported by the MRC (MR/
R006504/1) and L.P. is supported by the MRC (MR/
P01271X/1). J.R. is supported by the MRC, NIHR,
Wellcome Trust (103838), McDonnell Foundation, AZ-
MedImmune and Janssen. M.M. is supported by the
German Research Foundation (DFG) priority program PP
2041 (MO 2249/3-1) and the Alzheimer-Forschungs-
Initiative e.V., (AFI # 18072). H.J. is supported by a
NWO VENI grant [451-14-035], a standard grant of
Alzheimer Nederland [#15007] and by European Union’s
Horizon 2020 Research and Innovation Programme under
the Marie Sklodowska-Curie Grant agreement [IF-2015-
GF, 706714]. N.W. received funding from the European
Research Council under the European Union’s Seventh
Framework Programme (FP7/2007-2013) / ERC grant
agreement n° 616905, and from the European Union’s
Horizon 2020 research and innovation programme under
the grant agreement No 681094, and from the BMBF
Horizon 2020 research and innovation programme under
the Marie Sklodowska-Curie Grant agreement [IF-2015-
GF, 706714]. N.W. received funding from the European
Research Council under the European Union’s Seventh
Framework Programme (FP7/2007-2013) / ERC grant
agreement n° 616905, and from the European Union’s
Horizon 2020 research and innovation programme under
the grant agreement No 681094, and from the BMBF
(01EW1711A & B) in the framework of ERA-NET
NEURON, H.R.S. holds a 5-year professorship in precision
medicine at the Faculty of Health Sciences and Medicine,
University of Copenhagen, which is sponsored by the
Lundbeck Foundation (Grant Nr. R186-2015-2138).
L.T.G. is funded by NIH R01AG056573, K24AG053435
and the BrightFocus Foundation. R.H. is supported
by the UCLH NIHR Biomedical Research Centre. K.F. is
supported by the German Research Foundation (DFG)
Competing interests

H.R.S. has received honoraria as speaker from Sanofi Genzyme, Denmark and Novartis, Denmark, as a consultant from Sanofi Genzyme, Denmark and as senior editor (NeuroImage) from Elsevier Publishers, Amsterdam, The Netherlands. H.R.S. has also received royalties as book editor from Springer Publishers, Stuttgart, Germany. The Max Planck Institute for Human Cognitive and Brain Sciences has an institutional research agreement with Siemens Healthcare.

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