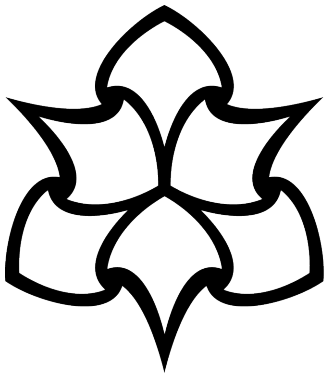

New Developments in Cholinergic Imaging in Alzheimer and Lewy Body Disorders

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Abbreviations
AD: Alzheimer disease; cBF: cholinergic basal forebrain; DTI: diffusion tensor imaging; PD: Parkinson disease; LBD: Lewy body dementias; Ch4: nucleus basalis of Meynert; PPN: pedunculopontine nucleus; VAChT: Vesicular Acetylcholine Transporter

Abstract
Purpose of Review This paper aims to review novel trends in cholinergic neuroimaging in Alzheimer and Lewy body parkinsonian disorders.

Recent Findings The spectrum of cholinergic imaging is expanding with the availability of spatially more precise radioligands that allow assessment of previously less recognized subcortical and cortical structures with more dense cholinergic innervation. In addition, advances in MRI techniques now allow quantitative structural or functional assessment of both the cholinergic forebrain and the pedunculopontine nucleus, which may serve as non-invasive prognostic predictors. Multimodal imaging approaches, such as PET-MRI or multiligand PET offer new insights into the dynamic and interactive roles of the cholinergic system at both local and larger-scale neural network levels.

Summary Our understanding of the heterogeneous roles of the cholinergic system in age-related diseases is evolving. Multimodal imaging approaches that provide complimentary views of the cholinergic system will be necessary to shed light on the impact of cholinergic degeneration on regional and large-scale neural networks that underpin clinical symptom manifestation in neurodegeneration.

Keywords Alzheimer's disease, cholinergic, imaging, Lewy body dementias & Parkinson's disease

Introduction

Degeneration of the cholinergic system has been identified as a major component of the neurodegenerative dementia process in Alzheimer's disease (AD) and Lewy body parkinsonian disorders, including Parkinson's disease (PD) and in particular Parkinson's disease dementia (PDD) and Lewy body dementia (LBD), see [1] for review. The cholinergic hypothesis of dementia is evolving from a primary focus on memory and a traditional view of a more diffuse neuromodulator system, toward more diverse cognitive and behavioural functions modulated by regionally smaller and larger scale neural networks [1].

Prior in vivo cholinergic imaging studies in Lewy body parkinsonian disorders, for example, have shown both cortical and subcortical gray matter cholinergic losses that associate with postural instability and gait difficulties, in particular falls or freezing of gait (FOG;see [2] for review). The subcortical denervation differences between Alzheimer and Lewy body disorders suggest selective vulnerability of subcortical cholinergic cell groups between these two types of neurodegenerations [3–5], which may underpin the distinct cognitive changes seen in these conditions.

In vivo cholinergic molecular imaging studies, such as positron emission tomography (PET) or single photon computed emission tomography (SPECT) imaging, allow specific assessment of the major cholinergic cell groups either directly (requiring higher spatial resolution imaging) or indirectly (by quantifying cholinergic binding in cortical or subcortical cholinergic projection target area (Table 1).

-- Insert Table 1 about here.

Technical advances in magnetic resonance imaging (MRI) allow quantitative assessment of the cholinergic basal forebrain (cBF) nuclei and more recently of the brainstem pedunculopontine nucleus (PPN) (Table 1). Despite relative lack of specificity due to non-cholinergic tissue components in these areas, clinical studies have shown sufficient sensitivity to establish robust cognitive imaging biomarkers in neurodegeneration [6].

The goal of this review is to identify emerging trends in recent molecular and MRI cholinergic imaging studies in AD and Lewy body disorders. Review findings are organized around the major themes of the recent literature (within the last few years).

Cholinergic Imaging Biomarkers

Cholinergic cell groups and their projections

The major cholinergic nuclei are organized by numbered cell groups that have specific cholinergic projections to cortical and subcortical target regions [7] (Figure 1). Magnocellular neurons of the cBF provide the major cholinergic input to the telencephalon [7]. Ch1 (medial septal nucleus) and Ch2 (vertical limb nucleus of the diagonal band) cholinergic neurons provide the major input of the hippocampus; Ch3 (horizontal limb nucleus of the diagonal band) cholinergic neurons project to the olfactory bulb; and Ch4 cholinergic neurons of the nucleus basalis of Meynert project to the remainder of the cerebral cortex, as well as the amygdala [8]. The Ch5 (PPN) and Ch6 (laterodorsal tegmental complex; LDTC) provide cholinergic input to the thalamus, several brainstem nuclei, spinal cord, cerebellum (via medial vestibular nucleus projections) and some striatal projections [9–11]. Intrinsic populations of cholinergic interneurons are also present in the striatum [12,13]. Ch5 and Ch6 striatal projections follow a topographic pattern where rostral PPN (Ch5) neurons preferentially innervate the dorsolateral striatum, and LDTC (Ch6) neurons preferentially innervate the medial striatum and nucleus accumbens core [14,15].

- Insert Figure 1 about here.

Molecular imaging biomarkers (PET and SPECT)

Radioligands have been developed to label specific components of the cholinergic nerve terminal (table 1). Acetylcholinesterase (AChE) PET ligands were the first cholinergic imaging ligand used in

human studies [16]. Although a synaptic enzyme, AChE has been recognized as a reliable marker for the integrity of cholinergic neurons [17]. Example ligands include ^{11}C-donepezil, ^{11}C-MP4A and ^{11}C-PMP. However, the presynaptic vesicular acetylcholine transporter (VAChT) is a more robust marker of presynaptic cholinergic integrity. Example ligands include ^{123}I-IBVM (SPECT) and ^{18}F-FEOBV (PET). Binding differences in AChE may be more difficult to interpret in neurodegeneration as lower levels may reflect loss of cholinergic nerve terminal integrity or possible functional downregulation due to regulation. In contrast, possible compensatory responses in cholinergic functions will be more straightforward when using VAChT ligands due to upregulation. Another advantage of VAChT ligands is that these allow more accurate non-invasive quantification of cholinergic changes in high binding subcortical gray matter structures, such as the basal ganglia, thalamus and cerebellum [18]. There are also radioligands that label muscarinic (e.g., ^{123}I-QNB M1/M4 receptor SPECT) and nicotinic receptors (e.g. 2-[18F]FA, ^{18}F-flubatine PET and ^{123}I-5IA SPECT α4β2 receptor ligands), see [2] for review.

MRI imaging biomarkers

Structural imaging via MRI and diffusion tensor imaging (DTI) can provide an in vivo measure of cholinergic system integrity. They offer complimentary information about the cholinergic system to PET, but are non-invasive, less expensive, and widely available.

The cBF nuclei (Ch1-4) are the most well-studied with structural MRI [1,19,20]. Early in vivo cBF imaging with voxel based morphometry (VBM; a technique that can index brain atrophy), relied on mapping of a relatively gross region, defined as the 'substantia innominata' [21]. However, the availability of stereotactic cytoarchitectonic maps of the cBF in humans now permits morphometric assessment of the cBF at a sub-regional scale [22,23]. Maps of the cBF that allow subregional precision have demonstrated that degeneration is pronounced in the posterior region of the Ch4 (Ch4p) in mild cognitive impairment (MCI) stages of AD, progressing towards more anteriomedial cBF regions in later disease [22]. More recently, studies using these maps have further suggested that atrophy of the cBF subregions coexists with cortical atrophy corresponding to previously documented corticotopic cBF projections [24].

VBM is difficult within the PPN however, as this region cannot be segmented from the surrounding white matter in the brain stem using standard protocols [25]. Instead, researchers have used DTI to index the structural health of the PPN. While DTI has traditionally been used to index the structural integrity of white matter, it is increasingly used to assess grey matter structural changes at a microscopic scale [26].

Emerging themes in the recent literature

Advances in VAChT radioligands demonstrate dense cholinergic innervation in previously under-examined regions

Albin et al.[27] recently presented a detailed anatomic biodistribution assessment of ^{18}F-FEOBV brain PET imaging in neurologically normal adults. In line with post-mortem human, as well as animal studies, binding was highest in the striatum followed by the thalamus, amygdala, hippocampus, vermis, cerebellar nuclei, dorsal rostral brainstem (likely associated with Ch5-Ch6), cerebellar cortex and neocortex. Neocortical binding was lowest in the posterior cortical regions, including the visual cortex. However, within the range of neocortical binding, regionally distinct and more prominent binding was seen in the anterior to mid cingulum, insula, and primary sensorimotor cortex. Thalamic binding was also heterogeneous but with distinct uptake in the lateral geniculate nuclei. Cerebellar binding was highest in the vermis. Focal distinct uptake was also seen the flocculonodular lobes (vestibular nuclei). The patterns of cerebellar and thalamic bindings may be unique to humans [27].

Clinical and behavioural correlates of cholinergic bindings in these particular structures remain largely unexplored in the context of neurodegeneration. Therefore, VAChT PET studies may allow clinical correlation studies of previously less recognised structures, like the lateral geniculate nucleus and vestibular cerebellum.

Spatial covariance studies suggest cholinergic contributions to large scale neural network changes in dementia

Another emerging trend is the use of spatial covariance analysis of whole brain parametric cholinergic PET or SPECT, which suggests an important role of the cholinergic system in cognitive decline in neurodegeneration [28]. For example, Colloby et al.[28] found an α4β2 nicotinic receptor spatial covariance pattern characterized by relative decreases in receptor binding in the cBF, PPN, limbic, thalamic and parietofrontal regions together with relatively preserved or increased binding in occipital, midbrain, cerebellar and sensorimotor cortical regions using 123I-5IA-85380 SPECT. The covariant pattern converged on various subcortical and neocortical regions, implicating a cholinergic network that mapped onto default mode network (DMN) hubs, namely, medial prefrontal, posterior cingulate, precuneus, and inferior parietal [29].

The same authors also reported reduced M1/M4 muscarinic receptor 123I-QNB SPECT binding in basal forebrain, temporal, striatal, insula, and anterior cingulate regions together with concomitant preserved or increased binding in frontal and parieto-occipital areas in patients with PDD [30]. These regions overlapped with key nodes of the DMN and frontoparietal networks.

We recently reported on topographic cholinergic changes in patients with LBD using VAChT PET [31]. Binding reductions were prominent in anterior-to-mid cingulate cortices, bilateral insula, right more than left lateral geniculate nuclei, bilateral anterior and superior thalami, and posterior hippocampal fimbria and fornices. The spatial pattern of the observed cholinergic vulnerability may involve key hubs of large-scale neural networks including the cingulo-opercular, visual attention, saliency and spatial navigation networks. These topographic changes may suggest a cholinergic vulnerability underlying clinical phenotypic features, such as fluctuating cognition, falls and visuospatial/visuoperceptual cognitive in LBD [31].

Bidirectional spatial covariance changes suggest possible cholinergic compensatory processes

Spatial covariance studies of cholinergic receptors have shown evidence of topographic binding changes in patients with AD or LBD [28], that may reflect compensatory and/or decompensatory processes. In early stage disease compensatory processes may limit symptom progression [28]. A recent ^{18}F-FEOBV PET study in patients with idiopathic REM sleep behaviour disorder, a common prodromal stage of Lewy body disorders, found significantly higher VAChT binding in specific brainstem areas corresponding to the bulbar reticular formation, pontine coeruleus/subcoeruleus complex, tegmental periacqueductal grey, and mesopontine cholinergic nuclei [32]. Furthermore, binding in the ventromedial area of the thalamus, deep cerebellar nuclei, and some cortical territories (including the paracentral lobule, anterior cingulate, and orbitofrontal cortex) was higher in the patients compared to controls [32]. Higher than normal range binding in these regions suggests a compensatory cholinergic upregulation associated with the initial phases of Lewy body neurodegenerations. Our voxel-based VAChT PET changes showed evidence of cholinergic vulnerability of key hub regions of different large scale neural networks in LBD [31]. Further research is needed to determine whether cholinergic changes in brain regions that spatially overlap with key hubs in important large-scale brain networks may result in altered compensatory or decompensatory functions of such networks

MRI forebrain measures may expand our understanding of early pathology in AD

AD is characterised by amyloid-β and tau deposition traditionally thought to originate in the entorhinal cortex (EC). However, recent MRI evidence now suggests that AD pathology in the cBF may occur earlier than in the EC. Schmitz and Spreng [33] used longitudinal VBM with cerebrospinal fluid (CSF) markers of amyloid-β. They demonstrated that Ch4 degeneration, but not EC degeneration, could be detected in cognitively healthy individuals with increased amyloid-βCSF markers, indicating prodromal AD. More recent evidence in two independent samples (N>800 combined) endorsed the finding that Ch4 atrophy proceeds and predicts EC degeneration, which in turn predicts neocortical atrophy over two years [34]. In addition, Cantero et al [35] demonstrated that Ch4 atrophy occurs in asymptomatic AD with isolated tauopathy but not isolated amyloidosis, while the extension of atrophy throughout the cBF depends on the presence of both proteinopathies in later AD. Together, this suggests that a new model of AD pathophysiology should consider the initiation of proteinopathies (especially tauopathy) in Ch4, which leads to cholinergic denervation in this region before cognitive symptoms are presented. This degeneration then extends to the EC, which is associated with neocortical atrophy and memory impairment. Combined proteinopathies are then associated with degeneration throughout the cBF in more advanced AD.

A recent ^{18}F-FEOBV VAChT PET study also reported evidence that the cBF degeneration covaries with longitudinal gray matter loss in the basal forebrain, cortex, and amygdala in early AD [36]. This covariation reflects the organization of the cBF projections [36]. The same group of authors also reported that ^{18}F-FEOBV is more sensitive than either glucose metabolic or β-amyloid PET to distinguish AD patients from control subjects. This supports MRI evidence that cholinergic imaging provides novel insight into AD pathology, beyond traditional markers of amyloidosis.

MRI offers novel non-invasive cholinergic prognostic markers in neurodegenerative conditions

Recently, cBF volumetry has been extended to PD, providing the first evidence that Ch4 atrophy may also predict cognitive decline in this neurodegenerative disease. Barrett et al. [37] report that Ch4 integrity is associated with impaired global cognition, attention and visuospatial function in de novo and more advanced PD. In a longitudinal cohort, we recently demonstrated that baseline Ch4 volumes in newly diagnosed PD could predict risk of MCI over 5 years follow-up [6]. This finding was replicated by Schulz et al. [38], who additionally found that mean diffusivity of the Ch4 (measured with DTI) was a stronger predictor of future cognitive impairment in PD.

More recently, Pereira et al. [39] published the first longitudinal study of conversion to PD with dementia (PDD) [39], in which cBF volumes were assessed over 10 years. At baseline, only those with current PDD or those who would later convert to PDD showed reduced Ch4 volumes compared to controls. Both PDD groups showed cBF atrophy beyond the Ch4, extending to Ch1/2 over time compared to controls and those without cognitive progression. In addition, longitudinal changes in Ch4 volumes significantly predicted conversion to PDD over 10 years.

In AD, Jethwa et al. [40,41] found that a simple linear measurement of Ch4 thickness showed excellent diagnostic accuracy for differentiating controls from those with AD and late MCI [40]. In addition, cognitive improvements following treatment with the acetylcholinesterase inhibitor, Donepezil, were found to be associated with significantly reduced annual cBF atrophy in patients with prodromal AD [42], implying that structural MRI measures may provide an objective marker of cholinergic treatment responses. Further research is required to investigate the utility of cBF volumetry as a theragnostic marker in neurodegenerative conditions treated with cholinergic medications.

Recent evidence in non-pathological ageing [43] and vascular cognitive impairment [44] suggests that integrity in cBF white matter projections, but not cBF volumes, predict cognitive outcomes. White matter changes, which are thought to represent demyelination and axonal damage, may be witnessed earlier than grey matter atrophy measured with volumetry [45]. As such, longitudinal multimodal approaches, which include DTI-based metrics, are necessary to improve our understanding of the spatiotemporal patterns of neurodegeneration.

Recent VAChT PET and PPN MRI studies provide novel insights into cholinergic system changes underlying postural instability and gait difficulties in PD

Postural instability and gait difficulties (PIGD), in particular falls and freezing of gait (FOG), are among the most disabling motor features in PD and generally have little response to dopaminergic therapy in advancing disease [46]. Additionally, PIGD symptoms are associated with poorer cognitive outcomes and rapid development of PDD [47]. We previously associated PPN/LTDC-thalamic and cBF corticopetal cholinergic projection system degeneration with falls and slow gait speed in PD, respectively [48,49]. These studies were performed using AChE PET, which limits accurate quantification of high binding subcortical areas.

Recently, we reported on ^{18}F-FEOBV VAChT regional binding changes in PD patients with falls and FOG [50]. We were able to confirm our previous observation of reduced PPN/LTDC-thalamic binding in fallers compared to non-fallers. A novel observation was that there was more prominent reduction of binding in the lateral geniculate nucleus, caudate nucleus (both more abnormal in the right hemisphere) and bilateral prefrontal cortices. In the same study, freezers had lower VAChT binding in the bilateral striatum, limbic archicortices and temporal and mesiofrontal limbic regions compared to non-freezers. These observations suggest different cholinergic systems changes underpin falls and FOG in PD.

In line with these PET findings, previous MRI research demonstrated that reduced connectivity between the PPN and cerebellum [51] and prefrontal regions [52] is associated with freezing of gait in PD. A striking finding from the latter paper was that degeneration of the right PPN in particular was associated with FOG.

Recently, Alho et al [53] developed a stereotactic map of the bilateral PPN to facilitate targeting of this nucleus for deep brain stimulation. Using this map along with DTI in a longitudinal cohort, we found that diffusivity in the PPN, but not Ch4, predicted future PIGD symptoms in PD [54]. This relationship was independent from the relationship between caudate dopamine deficiency and future PIGD, suggesting dopaminergic and cholinergic contributions to PIGD are distinct. Importantly, these data imply that microstructural changes in the PPN could be used to predict future PIGD symptoms.

Taken together, right hemispheric predominance of VAChT system in fallers and PPN changes associated with future PIGD motor features in PD could support a selective right hemispheric vulnerability. This is consistent with a growing body of literature suggesting that right-hemisphere circuitry seems to be particularly implicated in these symptoms [55–57]. The ventral attention network, which responds to unexpected stimuli and is highly integrated with the visual system, lateralizes to the non-dominant hemisphere [58]. This may suggest that deficiency of saliency detection may be a mechanism underlying fall risk in PD. Vulnerability of the right lateral geniculate nucleus may possibly reflect a prior observation of a subtle left hemi-neglect consisting of a directional (right hemi-field) bias of initial visual exploration in PD [59]. Alternatively, there is also evidence of right hemispheric dominance of vestibular cortical functions [60]. This may suggest vestibular dysfunction as a contributing factor to fall risk in PD. In this respect and in view of the predominant right PPN MRI findings, cholinergic losses in Ch5 may result in impaired integrity of the medial

vestibular nucleus which is the major source of cholinergic cerebellar innervation, including the vestibulocerebellum [11].

Multimodal approaches provide further insight into the spatiotemporal patterns of cholinergic degeneration and its role in cognitive decline

Considering the many overlapping themes from molecular and MRI cholinergic imaging studies, it is unsurprising that there is a growing trend for research to employ multimodal imaging techniques, which can provide a more holistic view of subcortical and cortical, as well as structural and functional, cholinergic integrity. For example, research combining MRI and PET [61] found that cBF atrophy in MCI correlated specifically with memory and attentional deficits, and these dissociated deficits were mediated by hypometabolism in specific cortical regions. Similar evidence of dissociated cognitive changes were reported in research combining diffusion-based tractography and resting state functional MRI in non-demented PD [26]. Reduced structural and functional connectivity between Ch3/4(Ch4) and associative frontal, occipital, peri-insular areas and medial/pulvinar thalamus were associated with deficits in global cognition and fronto-executive functions. On the other hand, reduced functional connectivity between Ch1/2 and the medial temporal lobe was associated with memory deficits [26]. This supports the PD literature that suggests that Ch4 degeneration is associated with global cognition and attention deficits [6,37,38], whereas in PDD [39] and AD [22,33,34] degeneration extends throughout the cBF to Ch1/2 and their associated projections when clinical memory deficits present.

Conclusions

The spatial brain regional detail of cholinergic imaging has vastly improved in recent years through the increasing use of VAChT PET and detailed MRI cytoarchitectonic maps of the cBF, both of which enable examination of small structures with dense cholinergic innervation. Recent VAChT PET studies show regionally distinct and more prominent cholinergic binding in cortical and subcortical gray matter structures, such as the lateral geniculate nucleus, vestibular cerebellum and the primary sensorimotor cortex. cBF volumetric MRI has shown utility as a non-invasive prognostic marker in AD and parkinsonian Lewy body disorders. Both techniques show promise to further clinical phenotyping studies to elucidate specific roles in the neurodegenerative process.

In addition to advances in regional imaging, whole brain PET/SPECT imaging or combined PET-MRI imaging suggests cholinergic contributions to large scale neural network changes. Multimodal approaches suggest that cBF atrophy may proceed symptom presentation and cognitive deficits are associated with cortical atrophy corresponding to corticotopic cBF projections. Furthermore, recent advances in PPN MRI and whole brain VAChT PET suggest that a cholinergic vulnerability in regional neural system changes may also underlie dopaminergic-resistant gait and balance impairment in PD. Taken together, these findings endorse the recent revival and evolution of the cholinergic hypothesis of dementia [1,20,62]. AD and PD may lie on a cholinergic spectrum, whereby PD without cognitive complaint show spared cBF nuclei but reduced midpontine cholinergic integrity, whereas Lewy body parkinsonian disorders, including LBD and PDD, show both cBF and midpontine degeneration, associated with their motor and cognitive symptoms [20].

Future multimodal PET-MRI imaging approaches will help elucidate the spatiotemporal gradient of cholinergic degeneration across different neurodegenerative diseases and possible large or regional scale network hub roles of cholinergic structures. Inclusion of DTI metrics may identify whether white matter microstructural changes precede the documented GM atrophy in the cBF, aiding earlier

prognosis. Early prognostic biomarkers would allow potentially disease-modifying interventions to be applied before more debilitating symptoms develop.

Compliance with Ethics Guidelines

Conflict of Interest The authors declare no competing interests. The authors have received research funding support from the National Institutes of Health, Department of Veterans Affairs, the Michael J. Fox Foundation and the Wellcome Trust.

Human and Animal Rights and Informed Consent All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

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• First study to assess the effect of cholinergic medication on cBF integrity in AD. The acetylcholinesterase inhibitor (AChEI), Donepezil, significantly reduced annual cBF GM atrophy in patients with prodromal AD, with the largest treatment effects witnessed in Ch4 (Ch4) and Ch1/2.

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Table and figure legends

Table 1. Major cholinergic imaging biomarkers and functions. Abbreviations: AChE, acetylcholinesterase; DTI, diffusion tensor imaging; PPN: pedunculopontine nucleus; VAChT: Vesicular Acetylcholine Transporter

Figure 1. **The Human Cholinergic System.** Cholinergic nuclei are primarily located in numbered cell groups in the basal forebrain (cBF [in red]; including Ch1-4) and brainstem (Ch5/PPN, Ch6/LDT [in blue]). Ch1/2 mainly project to the hippocampus; Ch3 projects to the olfactory bulb and Ch4 has projections throughout the cerebral cortex and to the amygdala. Ch5 (PPN) and Ch6 (LDT) have cholinergic projections to the thalamus, brainstem, spinal cord, cerebellum and striatum. Recent advances in VAChT radioligands demonstrate dense cholinergic innervation in previously under-examined cortical and subcortical regions, including the lateral geniculate nucleus (LGN), vestibular cerebellum and primary sensorimotor cortex (PSC). The lower pons medial vestibular nucleus (MVN [in yellow]) provides the major cholinergic input to the cerebellar vestibular nuclei. Ch5 likely provides cholinergic input to the MVN. Other intrinsic populations of cholinergic neurons include interneurons in the striatum [in purple].