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Turning on the light within: subcortical nuclei of the isodentritic core and their role in Alzheimer's disease pathogenesis

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Abstract

Pharmacological interventions in Alzheimer's disease (AD) are likely to be more efficacious if administered early in the course of the disease, foregoing the spread of irreversible changes in the brain. Research findings underline an early vulnerability of the isodendritic core (IC) network to AD neurofibrillary lesions. The IC constitutes a phylogenetically conserved subcortical system including the locus coeruleus in pons, dorsal raphe nucleus and substantia nigra in the midbrain, and nucleus basalis of Meynert in basal forebrain. Through their ascending projections to the cortex, the IC neurons regulate homeostasis and behavior by synthesizing aminergic and cholinergic neurotransmitters. Here we reviewed the evidence demonstrating that neurons of the IC system show neurofibrillary tangles in the earliest stages of AD, prior to cortical pathology, and how this involvement may explain pre-amnestic symptoms, including depression, agitation and sleep disturbances in AD patients. In fact, clinical and animal studies show a significant reduction of AD cognitive and behavioral symptoms following replenishment of neurotransmitters associated with the IC network. Therefore, the IC network represents a unique candidate for viable therapeutic intervention and should become a high priority for research in AD.

Keywords

Aging; Alzheimer's disease; neurodegeneration; brainstem nuclei; human; neurofibrillary tangles; early diagnosis; monoamines; neuromodulation; pathology

Introduction

The neurodegenerative dementia epidemic that is accompanying the global exponential increase of the aging population has an extremely high economic, political and social burden [1,2]. Alzheimer's disease (AD) is the primary cause of dementia and disability in older adults with costs reaching $160 billion/year in the USA alone [3]. Thus far, treatment to

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cure, delay or prevent the progression of AD is elusive and only symptomatic therapies are available.

AD features two specific neuropathological hallmarks which are opposite in nature: a) positive lesions (caused by extracellular accumulation of β-amyloid (Aβ) peptides in neuritic plaques (NPs) and intracellular accumulation of phosphorylated tau protein in neurofibrillary tangles (NFTs) and dystrophic neurites and b) negative lesions (neuronal and synaptic loss; [4]. Positive and negative AD lesions do not necessarily progress in parallel [5]. Nevertheless, the vast majority of studies have only focused on the positive lesions as a proxy of the AD progression. In the same line, the neuropathologic diagnosis for AD is based on a combination of Aβ plaques and NFTs [6]. According to these criteria, a case can only be called AD if deposition of plaques are present. In fact, for decades, the amyloid cascade hypothesis dominated the AD field [7]. However, in the light of recent failures in clinical trials based on Aβ modifying drugs, research is shifting its attention to understanding the characteristics of tau pathology in AD. In effect, the spread pattern of phospho-tau neurofibrillary changes is highly predictable and strongly correlates with cognitive decline [8–10], whereas, the spread pattern of Aβ plaques is predictable to a lesser extend [11,12]. This shift of focus to tau protein enhanced the interest on large group of older adults that show phospho-tau deposition in the brain without any trace of Aβ plaques. Recently, in an attempt to acknowledge these cases, a neuropathological consensus coined the term primary age related tauopathy (PART) [13,14]. Further studies will determine whether PART represents early AD stages or whether a proportion of them actually shows benign changes resembling AD tau deposition.

AD presents a long and gradually progressing a/pre- symptomatic phase [15]. Treatment is likely to be more effective if started early, before the neurons die irrevocably. Therefore, the growing effort to elucidate the role and spread of tau pathology during AD pre-symptomatic phases is very timely. Until recently, based on studies of the Braak couple, NFT pathology was believed to develop first at the transentorhinal/ entorhinal cortex in the temporal lobe (Braak stage I–II). From there, it would spread to the hippocampus and the adjacent limbic areas (Braak stage III–IV), and finally to primary neocortical areas (Braak stage V–VI; [16]). This cortical-based view of the disease origins resulted in a focused effort to these areas, in detriment of subcortical structures [17,18]. In the last years, clinical and animal studies underscore that NFT develop in subcortical nuclei related to the isodendritic core (IC) even before they are seen in the transentorhinal cortex (IC; [12,19–25]). Interestingly, all these IC structures are interconnected to the entorhinal cortex.

The aim of this review is to discuss the role and significance of the interconnected and evolutionary conserved IC network to AD pathogenesis. In this context, we will explore the selective vulnerability of each nucleus individually with respect to neuronal loss and its effects on neurotransmission in AD. Examination of the brainstem circuitries is significant as it may clarify the mechanisms of disease progression prior to clinical symptoms in AD and expedite the development of novel biomarkers and diagnostic strategies.

Brainstem and the isodentritic core network

Derived from the ectoderm in the early embryonic stages, the brainstem is continuous rostrally with the diencephalon and caudally with the spinal cord. It comprises the mesencephalon or midbrain, pons and medulla (Figure 1A, B). Despite its relatively small size, the brainstem provides the main relay point of information arriving and leaving the central nervous system (CNS) and vice versa. It also serves as an integrative hub for major critical processes for the individual's survival and homeostasis [26,27].

The gray matter of the brainstem contains the cranial nerve nuclei, the reticular formation and the pontine nuclei, while the white matter comprises fiber tracts connecting the cortex with the peripheral nerves and spinal cord. The reticular formation consists of a long column of diffusely organized neurons of different types and sizes aligned in parallel to its long axis and **occupies** the central and most conserved part of the brainstem, the tegmentum. The dendritic processes branching from specific neuronal groups located mostly within the reticular system intersect into a characteristic net-like pattern that justifies the name `isodendritic', while their extensive axonal outflow allows the release of neurotransmitters to very large projection areas [27,28]. Research findings underscore the significance of the reticular formation in regulating primary homeostatic functions, such as respiration and circulation, as well as the refinement of afferent and efferent signals ranging from maximum alertness to relaxed wakefulness, sleep and coma [29].

With the exception of the basal nucleus of Meynert in basal forebrain, the IC broadly corresponds to regions of the brainstem's reticular formation. The IC represents a group of interconnected nuclei with characteristic cytoarchitectonic and morphological features [30,31]. IC neurons show minimal specialization and retain an undifferentiated morphology especially regarding their dendritic features. In the coronal plane, Golgi impregnation of the IC group reveals a shared pattern of overlapping and symmetrically extending dendritic fields that form extensive interconnections between cells and expand diffusely to subcortical nuclei and the cortex. Cell bodies are mostly polygonal and their poorly myelinated axons project to the cortex. These characteristics resemble reptilian neurons. Actually, morphological similarities among IC neurons across species, ranging from humans to lower vertebrates, suggest a phylogenetically conserved network with a highly symmetric dendritic arrangement [30,31]. In contrast, neurons located at the “allodendritic” and “idiodentritic” centers display “different” or “peculiar” dendritic morphologies, respectively, heavily myelinated axons and round or ovoid cell bodies [30]. In his seminal 1960's publication, Mannen was the first to highlight this difference between generalized and specialized neuronal groups of the brainstem using the terms “noyaux ouverts” (broad or open core) and “noyaux fermés” (firm core), respectively [32].

The principal nuclei within the IC network include the noradrenergic locus coeruleus (LC) in pons, the serotoninergic dorsal raphe nucleus (DRN) and dopaminergic substantia nigra (SN) in the midbrain; and the cholinergic basal nucleus of Meynert (NbM) in the basal forebrain [30,31,33] (Figure 1 C–F). Early studies by Dahlström and Fuxe using formaldehyde-induced fluorescent histochemistry in the rat brain classified the monoaminergic nuclei based on their chemoarchitectural properties into A6 (LC), A9 (SN)

and B7 (DRN) groups; A and B designate catecholaminergic and serotonergic neurons respectively, and numbers represent the order of nuclei groups beginning caudally from medulla oblongata [34]. Likewise, Mesulam and colleagues categorized NbM into the Ch4 group of the basal forebrain based on studies in monkeys, with Ch representing cholinergic neurons (Figure 1F) [35].

IC neurons, endowed with long axons and disproportional bigger cell bodies globally innervate the entire CNS via volume transmission, thus having a widespread effect in the brain following neurotransmitter release to diverse neuronal populations [36,37]. In contrast to classic synaptic transmission where neurons locally project to neighboring cells, non-synaptic/volume intercellular communication allows modulation of large areas via a long distance systemic flow of neurotransmitters released in the extrasynaptic space or cerebrospinal fluid (CSF). This characteristic organizational pattern allows a relatively small group of neurons to receive input from multiple sources and promote rapid signaling modulation in dispersed cortical regions.

Vulnerability of the isodentritic core network in early AD stages

The vulnerability of the IC network to NFT lesions in AD was recognized as early as 1960s in cases with senile dementia, showing accumulation of NFTs in the DRN, LC and SN as well as in the hypothalamus [38]. The vulnerability of the IC components to NFTs, even prior to Aβ accumulation or onset of cognitive decline, can directly impact the brain's neurochemical balance because these structures are a major source of neuromodulatory neurotransmitters [18,19,39–42](Figure 2).

The relationship between NFTs and neuronal loss in AD is still not completely understood. On one hand, studies have shown a positive correlation with increase of positive lesions paralleling a reduction in neuronal numbers as AD advances [43,44]. Others have shown that phospho-tau may accumulate in the absence of cell loss [5]. This could further explain why subjects with histopathological features of AD can be initially asymptomatic. These discrepancies may be related to methodological problems such as selection biases, the lack of an integrative approach for studying neuronal loss and protein accumulation in the same brain, as well as the use of unbiased stereology [18,20,45,46].

It is not well understood why the IC neurons are consistently susceptible to AD lesions while other neuronal types remain resistant to NFTs. Anatomically, the IC nuclei project to the cortex directly, without intermediate relay points, in contrast to the majority of ascending and descending tracts that follow a transthalamic route [47]. For instance, primary sensory cortices, except the primary olfactory cortex are very resistant to AD changes. Out of them, the olfactory cortex is the only one without relay [27]. In addition, the IC tracts have disproportionally long and thin axons that traverse the medial forebrain bundle to innervate virtually the entire cortex. Their poor or incomplete myelination, as well as being key sources of diffuse (volume) autonomic neurotransmission could expose these nuclei to triggers of AD pathology [27,48,49]. As a possible counterproof, neurons with heavy axonal myelination offer greater stability to the parent neuron by allowing efficient conduction of

action potentials with reduced energy costs and possibly less exposure to oxidative stress [50]. These neurons are notably spared until later AD stages [27,49].

Although the IC is involved in AD from its earliest stage, probably years before the onset of disease-defining clinical symptoms, such lesions are unlikely benign and may explain behavioral, mood and sleep disturbances seen in AD prodromal stages and along the disease progression, although this need to be confirmed by structured studies [12,18,21,45,51]. With **this** in mind, the IC network represents a unique candidate for viable therapeutic intervention and should become a high priority for research in AD [12,19–21,52]. In the following sections, we will discuss how AD pathology affects each of these four IC nuclei and their corresponding neurotransmitters, aiming to lay foundations for future studies targeting early AD stages.

1. Locus coeruleus and the norepinephrine system

1.1 Anatomy and function

In the adult brains, the LC (“blue spot” in Latin) comprises a relatively homogeneous group of conspicuously pigmented noradrenergic neurons. Together with the SN, the LC makes the largest brainstem nuclei containing neuromelanin [53,54]. As neuromelanin-bearing neurons can be recognized even at gross examination, the LC and SN vulnerability to AD and Parkinson's disease (PD) have been noted since the first descriptions of these diseases [47,55].

The LC is situated laterally in the pontine central gray of the upper pons and its neurons infiltrate the nearby tegmentum (Figures 1B, C). Despite its small size in cross sections, the LC extends rostocaudally into a continuous 11–15 mm column from the upper pons to caudal midbrain. The LC harbors two primary neuronal types: medium (35–45 μm in diameter) multipolar neurons with round or oval somata and long dendrites that often extend to adjoining structures, and small, spindle-shaped neurons (15–25 μm). Both types display highly ubiquitous synaptic connections throughout the CNS [26,28]. Neurons of the LC and adjacent subcoeruleus (SubC) are the major sources of norepinephrine (NE); a catecholamine synthesized from dopamine by dopamine β-hydroxylase (DBH) with multiple biological roles including **those of** a hormone and neurotransmitter. Upon binding to adrenergic receptors, NE regulates critical behavioral and physiologic processes including wakefulness and attention, in both humans and animals [29,54,56]. For example, studies in rats and monkeys confirm that the LC activity increases during waking state, and it is absent during REM sleep. Furthermore, increased external stimulation induces activity in the LC, suggesting a role in vigilance and alertness [57].

1.2 Pathophysiology in Alzheimer's disease

The LC degenerates in several neurodegenerative diseases, particularly in AD and Parkinson's disease [12,18,42,58–62]. Early in the course of AD, the neurons of LC display characteristic morphological and chemical changes, including swollen cell bodies, contracted dendrites and depleted aminergic transmission [58,62–64]. The rostral portion of the nucleus that projects to the cortex and the hippocampus shows the greatest vulnerability in AD, whereas caudal cells and surrounding nuclei are relatively spared ([62,65]; Table 1).

In this context, due to the LC's contribution to arousal state and mental wellbeing via noradrenergic projections, it is no surprise that non-cognitive symptoms in patients with prodromal AD include disruption of the sleep/wake cycle, lack of alertness and vigilance, and mood disturbances including depression [63,66–68]. Neuropathologically, the LC consistently shows NFTs, even in the absence of Aβ deposition in the brain and as early as 10 years prior to the expected onset of cognitive changes [12,69,70]. Braak and colleagues recently updated their staging system for neurofibrillary changes to reflect LC early involvement in AD [12]. Furthermore, atrophy in the LC is positively correlated to increased frequency of cortical NFTs, as well as severity and duration of AD [71,72]. The selective NFT propagation from LC to cortical and subcortical regions in AD may include a trans-cellular spreading of tau protein isoforms from axonal terminals to neighboring cells in a 'prion-like' manner [73,74].

Regarding the effects of AD in the LC neurotransmission, NE availability and its synthesis are reduced in the LC cortical projection areas in postmortem brains of AD patients [75–77]. Moreover, NE transport inhibitors and therapies aiming at NE augmentation have shown promising results in animal models of AD and are candidates for therapeutic trials in early AD stages [78]. Particularly interesting are studies in postmortem AD brains which identified compensatory mechanisms in surviving neurons of the LC network by means of upregulated synthesis of NE and/or a reduced reuptake of the neurotransmitter at the synaptic terminals [79]. This upregulation could explain the unexpected increase of NE levels in cerebrospinal fluid in AD patients with marked LC pathology [80,81].

In agreement to clinical findings, studies on animal models of AD demonstrate that LC pathology and related decline of NE levels predispose neurotoxicity, suppress anti-inflammatory responses and negatively impact cognition - all central hallmarks of early AD stage [82,83]. Furthermore, brain-derived neurotrophic factor (BDNF), the most abundant neurotrophic factor in the brain, is closely related to the LC neuronal growth and survival upon binding to TrkB tyrosine kinase receptors [84,85]. In this context, decreased levels of BDNF, and reduced expression of its TrkB tyrosine kinase receptor in patients with AD as well as in mouse models of the disease may accelerate AD pathogenesis. This may be due to lack of trophic support to specific neuronal populations, including the LC and other IC components such as the DRN, leading to monoamine enervation to the cortex [85–87]. Consequently, the delivery of BDNF to the brain represents an active area of translational research in the early AD stages [88].

2. Dorsal raphe nucleus and the serotonergic system

2.1 Anatomy and function

The human DRN is a heterogeneous nucleus located dorsally to the medial longitudinal fasciculus at the level of the midbrain and rostral pons ([26,54]; Figures 1B, D). The DRN can be divided into four sub-groups: the supratrochlear, interfascicular, caudal compact and lamellar subnuclei [89,90]. Its two morphological neuronal types show eccentrically located nuclei and are arranged in an irregular fashion: medium-sized neurons (22–31 μm in diameter) are round or ovoid, and smaller neurons (11–24 μm in diameter) are fusiform or triangular [91].

The DRN neurons are not confined to the midline as their Greek name raphe or “seam” implies or as is the case in the other components of the raphe system, but rather it extends laterally into the ventral periaqueductal gray and around the medial longitudinal fasciculus [91]. The DRN contains the largest population of serotonergic (5-Hydroxytryptamine; 5-HT) neurons, a monoamine neurotransmitter biochemically derived from tryptophan with essential homeostatic and behavioral functions. Upon its release, serotonin has both a direct and indirect effect in the brain via activation of serotonergic receptors and/or modulation of complementary systems related to cholinergic, glutamatergic, dopaminergic and GABAergic neurotransmission [92]. The rostral (oral) raphe complex, including the DRN, the median raphe nucleus (MnRN) and caudal linear nucleus (RL) form the main serotonergic projections to the forebrain, specifically to areas critical for cognitive functions, including the hippocampus, striatum, hypothalamus and amygdala [91,93–95].

2.2 Pathophysiology in Alzheimer's disease

DRN vulnerability to AD and increased NFT burden as disease progresses have been demonstrated by independent investigations since decades ([19,45,69,89,96]; Table 1). However, only recently a study was able to show that the DRN accumulates NFTs before the transentorhinal region does [21]. This finding restored the interest on DRN vulnerability in AD. From the rostral raphe complex, DRN consistently shows the earliest and most severe NFT lesions in AD and for this reason it is one component of the IC network most intensely studied for the development of therapeutic innervations in the initial stages of AD pathogenesis [21,89]. In this context, DRN degeneration may be partially responsible for mood disorders in prodromal AD. In particular, there is a significant correlation between DRN pathology, serotonergic denervation and behavioral changes in AD patients [89,92]. Furthermore, AD-related deficits in serotonin neurotransmission are associated with accelerated cognitive decline as determined by the Mini-Mental State Examination (MMSE) scores [97]. In advanced AD, different serotonin receptor agonists show promising effects in enhancing memory and learning capacity according to clinical and animal studies [92,98]. In the same line, administration of serotonin re-uptake inhibitors improved memory function in AD patients [99]. Additionally, deficits in serotonergic innervation, serotonin transporters and tryptophan hydroxylase may contribute to anxiety and changes in waking/sleep cycle seen in AD [100,101]. Studies using immunohistochemistry for tryptophan hydroxylase as a marker for serotonin synthesizing neurons, points to a significant decrease of this neuronal population in AD brains [101,102]. On the other hand, in response to AD lesions, the DRN shows a high degree of plasticity via terminal sprouting as a possible adaptive strategy to take over lost cells [103].

Loss of DRN neurons and their projections to both cortical and subcortical brain regions overlap with defects in the serotonergic transmitter system and the accumulation of NFT in early AD. Dysfunction of the serotonergic transmitter system features decrease in serotonin levels and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in serotonergic nerve terminals and in cerebrospinal fluid of AD patients. This effect has been shown in vivo by positron emission tomography (PET) [104]. Decreased axonal transport causes enzymes and their metabolites to accumulate in the cell bodies. In fact, AD patients show a two-fold

increase of toxic serotonin oxidative metabolites in the DRN cells that may explain the selective vulnerability of these neurons [105].

On the other hand, PET studies also identified upregulated serotonin metabolism and an increase of 5-HT1A serotonin receptor densities in the hippocampus of patients with mild cognitive decline. This may reflect a possible compensatory mechanism for reduced serotonergic transmission from compromised neurons in DRN. In contrast, a dramatic decrease in receptor numbers was observed at advanced stages of AD [106,107], also in the cortex [108,109]. Further experiments using spectrophotometric analysis of cortical tissue from patients at late AD stages suggested that a reduced level of serotonin in cerebrospinal fluid and platelets correlated with AD pathology and cognitive decline severity[110,111]. Therefore, 5-HIAA should also be explored as a possible early biomarker of AD [105,112].

3. Substantia nigra and the dopaminergic system

3.1 Anatomy and function

The SN lies in the mesencephalon, dorsal to cerebral peduncles and extends ventrally along the entire midbrain tegmentum (Figure 1B, E). It is divided into two sub-regions comprising triangular/ fusiform shaped cells: the cell-sparse ventral (pars reticularis; SNR) and cell-dense dorsal regions (pars compacta; SNC) [26,113]. Similar to the LC in pons, the SN is a large nucleus, easily recognizable macroscopically, with high levels of neuromelanin in the dopaminergic neurons that give its Latin name “black substance”. Both LC and SN neurons stain positive for tyrosine hydroxylase, a key-synthesizing enzyme of dopamine and norepinephrine synthesis from L-tyrosine [54,114]. Neurons of the SNC mediate reward, addiction behavior, and movement by releasing dopamine via ascending projections to the basal ganglia and cortex [115–118]. In contrast, dopaminergic neurons from the neighboring ventral tegmental area (VTA) modulate cognitive processes via connections to nucleus accumbens and medial temporal lobe [119]. The SN comprises the largest network of dopaminergic cells in the brain and neuronal loss in this nucleus is hallmark of major neurodegenerative disorders including PD, other movement disorders and AD. Often AD and PD pathological lesions coexist, as in dementia with Lewy bodies, especially in advanced AD stages [120].

Five metabotropic receptors divided in D1 and D2 groups bind to dopamine. Dopamine synaptic levels depend on dopamine transporter (DAT), responsible for dopamine reuptake and termination of its activity at the synapses [121]. DAT became a common imaging biomarker of dopaminergic neuron density.

3.2 Pathophysiology in Alzheimer's disease

SN lesions in AD have been well documented in clinical and neuropathological studies ([122–127]; Table 1). Clinically, SN involvement in AD may include extrapyramidal motor symptoms (EPS) such as bradykinesia, muscular hypertonia, and gait difficulties. However, in contrast to PD, tremor is rare [128]. EPS signs increase in prevalence as AD advances and correlated with tau accumulation and dopaminergic neuronal loss [125,126]. EPS signs often parallel neuropsychiatric symptoms including depression, hallucination and cognitive decline, possibly reflecting deficiency of dopamine neurotransmission. Apathy, a common

behavioral symptom of AD, is attributed to defects in ascending nigrostriatal projections that mediate feelings of motivation and reward-seeking behavior. Therapeutic strategies using psychostimulants can improve apathy in AD by restoring dopamine availability [129].

Neuropathological studies confirmed SN susceptibility to NFTs and neurodegeneration in AD, but severe cell loss is rarely present in this nucleus in contrast to the rest of the IC [16,124,125,127,130]. On the other hand, in pre-immunohistochemistry era studies, AD cases with SN pathology are often misclassified as having PD and are excluded from the analysis, leading to an underestimation of SN involvement in AD pathology [125]. Another confounder in SN analysis in AD is that neuromelanin may obscure visualization of intraneuronal NFTs and initial lesions could be easily overlooked [131]. Concerning chronological involvement of the SN in AD, a recent study corroborates previous findings showing NFT in the SN in a subset of early AD. They also identified a linear correlation between AD stage and NFT burden in the SN [51].

SN pathology may affect striatal function due to loss of projections on the nigrostriatal pathway in AD. For example, accumulation of NFTs in the SN parallels dopamine deficiency in the caudate nucleus and putamen [124,125]. Several studies show movement abnormalities including EPS in the majority of AD patients with neurofibrillary tau in the SN. The latter correlated best with loss of pigmented neurons in SN, suggesting tau pathology to be the morphological substrate for ESP in AD patients, in absence of PD pathology [124,127,132].

Despite evidences of SN involvement in AD, in vivo experiments show contradictory results. A study using single-photon emission tomography (SPET) with a selective D2 receptor found a significant reduction in striatal receptor density in the absence of clinical EPS signs in AD, possibly due to reduced dopaminergic projections from the SN [133]. On the other hand, another study using a ligand that binds to DAT reuptake sites found no correlation between tau pathology, neuronal death in SN, and dopamine depletion in the striatum of AD cases [134].

4. Basal nucleus of Meynert and the cholinergic system

4.1 Anatomy and function

The NbM is located within the substantia innominata of the basal forebrain, ventrally to the anterior part of the thalamus and putamen ([27,135]; Figure 1F). There is an evolutionary trend towards size increase and differentiation in this nucleus, with primates and cetaceans showing the highest development [136]. NbM neurons are heteromorphic in shape and display a characteristic pattern of overlapping and sparsely ramified dendrites, features also present in the IC neurons of the brainstem, suggesting that NbM is a telencephalic extension of brainstem's reticular core [30].

Cholinergic neurons of the NbM form wide projections to the cortex and are the activity sites of the cholinergic synthetic enzyme choline acetyltransferase (ChAT) and the hydrolytic enzyme acetylcholinesterase (AChE;[137]). Constituting a major source of cortical acetylcholine, the NbM plays a significant role in learning and memory, as well as

regulation of motor control, arousal and sleep cycle, and motivation [136]. For example, stimulation of NbM in rodents induces acetylcholine release in the cortex and promotes learning [138]. In contrast, lesions of NbM or blockers of central ACh muscarinic receptors, such as scopolamine, result in cognitive deficits and transient amnesia [139]. Since deterioration of memory is a critical aspect of AD, abnormalities in central cholinergic neurotransmission originating from NbM may partly underlie the amnestic manifestations of the disease.

4.2 Pathophysiology in Alzheimer's disease

The impact of early NbM vulnerability and depletion of cholinergic transmission in AD has been well noted over the years particularly in relation to NFT burden, cell loss and cortical atrophy ([70,139–145]; Table 1). Importantly, AD lesions in the NbM were identified in clinical stages as early as MCI [52,140,144,146,147]. These cases had low ChAT, cytoplasmic loss of Nissl substance and fiber abnormalities including thickened axons and ballooned terminals, all pointing to defective NbM activity in early AD [52,146]. Moreover, Sassin and colleagues identified NFT lesions in the NbM in the tissue of preclinical stages of AD and these gradually increased in severity as AD pathology spread to the cortex [144]. Finally, cases with MCI-early AD showed reduction of low affinity p75 neurotrophin receptors in neurons of the basal forebrain including NbM, which coincides with cognitive and attention abnormalities [148]. These findings are the rationale for cholinergic therapy in AD. In fact, acetylcholinesterase inhibitors (tacrine, rivastigmine, galantamine and donepezil;) are widely used in moderate AD and promote temporary relief of symptoms [149]. Furthermore, cholinergic receptor antagonists have been shown to produce significant cognitive impairments similar to those in AD, affecting attention and memory encoding. This observation, combined with evidence of loss of cholinergic neurons, supports the cholinergic hypothesis of AD [150].

Postmortem studies indicate that large cholinergic neurons of NbM are particularly vulnerable to AD [141,142]. These findings suggest that reduced responsiveness to the nerve growth factor and loss of its neuroprotective effects may mediate to neurodegeneration in NbM and promote AD pathogenesis.

Magnetic resonance imaging (MRI) studies identified a significant volume reduction in substantia innominata, suggesting neuronal loss in NbM and impaired cholinergic transmission in AD [151,152]. Reduced thickness was significantly correlated with scores from cognitive tests in patients with AD. In addition, cognitively intact subjects with basal forebrain thinning showed significant risk for developing dementia in follow-up tests, indicating the potential use of NbM as a marker for risk of cognitive decline. By integrating *in vivo* morphometric analysis and diffusion tensor imaging (DTI) in the basal forebrain of MCI and advanced AD patients, Teipel and colleagues identified significant atrophy in substantia innominata, including the NbM. Although similar regions were involved, atrophic changes in MCI were less pronounced than in patients with AD and were both significantly different from age-matched controls [152]. Finally, a recent study on a large sample of MCI and advanced AD subjects revealed atrophy in the NbM and adjacent basal forebrain regions and its severity was positively linked to Aβ accumulation in the cortex and clinical diagnosis

of AD. The authors concluded that the volume reduction in NbM was a superior predictor of early AD pathology than hippocampal volume [145]. Overall, these findings suggest that volumetric changes in the NbM and adjacent areas precede cognitive decline in patients in the initial stages of AD.

Conclusions

The full picture of AD etiopathogenesis is yet to be revealed and treatment of this disease is still elusive. AD-type neurofibrillary lesions are found in a network of subcortical structures belonging to the IC system, in brains with no cortical neurofibrillary changes, suggesting that IC pathology represents the earliest AD stage. Being a signaling hub of neuromodulatory neurotransmission ascending to the cortex, neuronal loss and synaptic dysfunction in the IC may mediate behavioral and psychological disturbances common in patients in the earliest stages of AD by depriving the cortical neurons from their signaling input. Therefore, better understanding of how and when the subcortical nuclei of the IC become vulnerable to AD pathology will provide the necessary foundation for the development of therapies targeting these systems that may be effective for treating AD.

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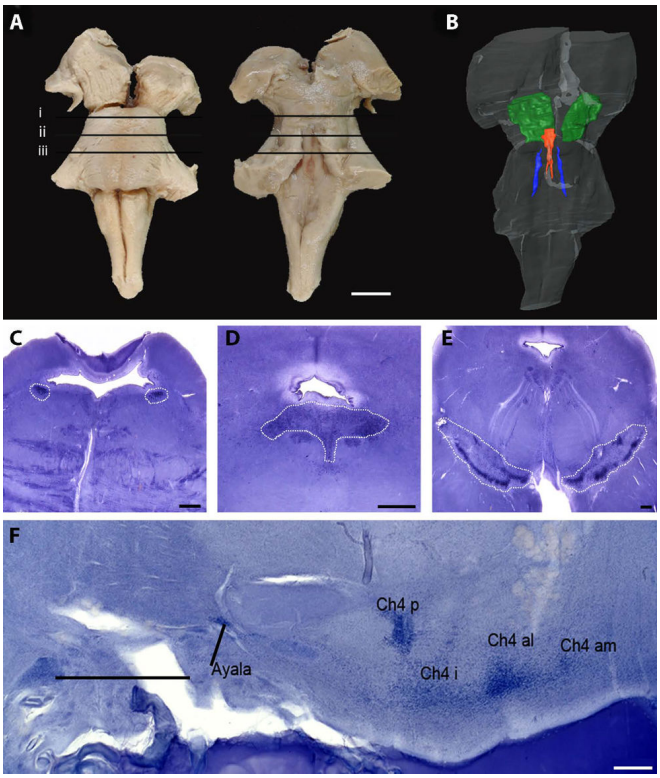

Figure 1.

The human isodentritic core network extends from the brainstem to the basal forebrain. (A) Ventral (left) and dorsal (right) views of the human brainstem, depicting the caudal midbrain (i) and the rostral (ii) and mid-pons (iii) at the rostrocaudal axis. (B) 3D reconstruction of the isodentritic core nuclei in the brainstem. Despite their relative small size on the horizontal plane, each nucleus occupies large areas within the brainstem's core. Note the overlapping between the structures as well as the idiosyncrasy of their shape and location. The transparent surface represents the brainstem boundaries; green, orange and blue represent the substantia nigra, the dorsal raphe nucleus and the locus coeruleus, respectively. (C–F) Delineation of the isodentritic core nuclei in thick histological sections (300 μm) of the human brainstem and the basal forebrain stained with gallocyanin (Nissl). (C) Locus coeruleus situated laterally to the midline and ventrally to the fourth ventricle in the pons. (D) The dorsal raphe nucleus is located medially in the midbrain ventrally to the aqueduct. (E) The substantia nigra extends bilaterally in the rostral and caudal midbrain, dorsally to the cerebral peduncles. (F) The spatial arrangement of the subnuclei of the nucleus basalis of Maynert in a horizontal section through the left hemisphere. Ayala: Ayala's nucleus; Ch4 al, nucleus basalis of Meynert anterolateral part; Ch4 am: nucleus basalis of Meynert, anteromedial part; Ch4 i: nucleus basalis of Meynert, intermediate part; Ch4 p: nucleus basalis of Meynert, posterior part. Scale bars: (A) 1 cm, (C–F) 1 mm.

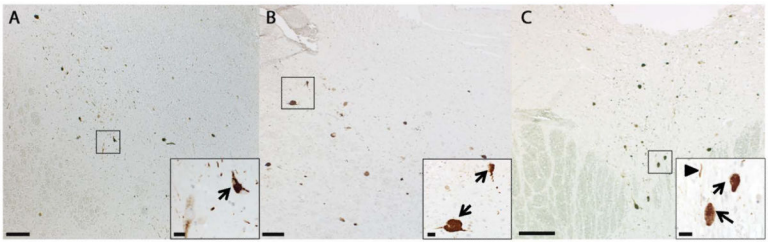

Figure 2.

Immunostaining against hyperphosphorylated tau in the isodentritic core nuclei of the brainstem. Thin paraffin sections (5 μm) from the substantia nigra (A), locus coeruleus (B) and dorsal raphe nucleus (C) show prominent neurofibrillary lesions in Alzheimer's disease brains. Insets correspond to the framed areas and show neurofibrillary tangles (arrow) and threads (arrowhead). Scale bars: 200 μm, Insets 20 μm.

Table 1

Selected articles reporting susceptibility of the brainstems' isodentritic core nuclei in AD.

Studies on the LC	Objectives	Subjects	Materials	Results
Tomlinson, 1981 [59]	Compare the neuronal loss in the LC in cognitively healthy controls in middle-late life versus individuals with AD.	10 middle-aged (32–59 years), 15 old control cases(62–93 years); 15 AD cases(76–88 years)	Postmortem tissue, paraffin embedded; cresyl violet staining used for identification of the LC neurons.	The mean neuronal count of control cases above 60 years was significantly higher compared to the AD group; the two groups were not matched for age or sex.
Chan-Palay & Asan, 1989 [63]	Examine the degree and topographical distribution of neuronal loss between the LC in normal and AD brains.	12 controls (43–89 years), 8 AD cases (71–85 years).	Postmortem tissue, vibratome sectioned; NE neurons stained against TH; NFTs and NPs identified with Bodian stain.	3.5% to 87.5% reductions of neuron numbers in the LC of the AD cases. Neuronal loss was greatest in the rostral, less in middle and least in the caudal part of the LC. Morphological changes of LC neurons included swollen somata with thick dendrites.
Zweig *et al*., 1988 [69]	Examine neuronal loss and NFT accumulation in the neuromelanin containing neurons of the LC in AD vs control cases.	22 AD cases (mean age 72.5 years); 12 age-matched control subjects (mean age 68.9 years)	Postmortem tissue, paraffin embedded; cresyl violet staining for neuronal counts in the LC; Congo red for NFT staining.	Significant neuronal loss and NFT burden in the LC of AD vs control cases. Loss was more severe at mid-level than at caudal or rostral levels. No positive correlation was found between neuronal and NFT counts in AD.
German *et al*., 1992 [65]	Compare the topographic patterns of cell loss in AD patients versus aged controls. Utilize computer graphics to map and quantify the LC neurons.	8 AD brains (61–82 years), 7 controls (62–76 years). 1 AD case had also PD.	Postmortem tissue, microtome sectioning; cresyl violet staining for neuronal counting; Schmorl's ferricyanide for neuromelanin staining and an antibody against the norepinephrine-synthesizing enzyme, TH.	Approximately 60% loss in the LC compared to aged normal subjects; positive correlation between the magnitude of LC cell loss and AD duration. Cell loss was prominent at the rostral portion of the nucleus compared to the relatively preserved caudal cells.
Grudzie *et al*., 2007 [62]	Determine whether the LC displays NFTs early in the course of AD.	7 cognitively normal controls (age 83–95 years) and 5 MCI or early AD cases (age 89–99 years).	Postmortem tissue, microtome sectioning; TH and cresyl violet staining of the LC neurons; AT8 staining for NFTs and their precursors. Thioflavine-S labelled mature tangles.	AT8-positive labeling and thioflavine-S positive tangles were present in both groups of specimens and became significantly more prominent at the stages of MCI and early AD, compared to controls. Moreover, MMSE scores displayed a negative correlation with both markers of cytopathology.
Braak & Del Tredici, 2011 [42]	Examine the AD pathogenesis based on intraneuronal tau lesions in 42 individuals under the age of 30	42 cases (ages of 4–29): 14 non-demented females and 28 non-demented males (age range 4–29 years).	Postmortem tissue, microtome sectioning. AT8, 4G8 and silver stains against NFT and NP aggregates associated with AD.	19 of the 22 cases that lacked abnormal tau in the transentorhinal region but showed subcortical lesions confined to non-thalamic nuclei had pretangle material confined to the noradrenergic LC-subcoeruleus complex.

Studies on the DRN	Objectives	Subjects	Materials	Results
Curcio & Kemper, 1984 [96]	Quantitatively study the neurofibrillary changes and neuronal packing density in the DRN of AD patients.	7 AD brains and 6 controls, matched for age (mean age 87.7 vs 86.5 years) and brain weight (1,076 vs 1,145g).	Postmortem tissue; paraffin sectioning. Staining with Luxol Fast Blue, cresyl violet and HE; NFTs stained with the Bodian silver method.	A 6-fold increase of NFTs in the AD brains compared to controls; no significant difference in neuronal packing density between the two groups.
Zweig *et al*., 1988 [69]	Examine neuronal loss and the accumulation of NFTs in the DRN of AD patients.	22 patients with AD (mean age 72.5 years) and 12 age-matched controls (mean age 68.9 years)	Postmortem tissue, paraffin sectioning; cresyl violet staining for neuronal counts; Congo red for NFT staining	Neuronal loss was most severe in the caudal DRN. AD duration correlated inversely with NFT counts although neuronal and NFT counts did not correlate at individual levels.

Studies on the DRN	Objectives	Subjects	Materials	Results
Aletrino *et al*., 1992 [100]	Determine the total neuronal population in the DRN in AD using a 3D sampling scheme throughout the entire rostrocaudal length of the nucleus.	3 cases of early onset AD and 5 late AD (age range 65–93 years), 10 control brains (age range 60–91 years).	Postmortem tissue, paraffin sectioning; cresyl violet staining used for neuronal counts and the KliJver-Barrera staining used to identify DRN neurons.	An overall 39.4% reduction in the DRN neurons of AD brains was demonstrated and was accompanied by cell shrinkage. No rostrocaudal gradient in neuronal loss was identified.
Rub *et al*., 2000 [89]	Determine in which BB stage the raphe nuclei display AD-related cytoskeletal lesions and if these are correlated with the AD-related cytoskeletal pathology in the cortex.	27 cases (average age 79.6 ± 7.5 years) with BB stages I ± VI of cortical cytoskeletal lesions were examined.	Postmortem tissue embedded in polyethylene glycol. Aldehydfuchsin-Darrow red used for delimiting the raphe nuclei. Silver iodide and Gallyas technique used to visualize NFTs. 23 cases were treated with AT8 to visualize phosphorylated tau.	The DRN showed cytoskeletal lesions early in BB I±II. In stages V and VI, the DRN displayed the most severe cytoskeletal pathology within the raphe system.
Parvizi *et al*., 2001 [19]	Find a correlation between the severity or absence of AD-related changes in the brainstem nuclei of AD patients vs age and gender-matched control cases.	32 AD patients (ages of 58–89) and 26 controls (ages of 56–98).	Postmortem tissue, microtome sectioning. Nissl staining for nuclei identification; thioflavin S for NFTs and NPs. Gallyas silver impregnation or staining with 10D5 for Aβ and ALZ50/AT8 for phosphorylation -independent and -dependent tau inclusions, respectively.	The DRN, along with the central subnucleus of the inferior colliculus, was positive for NFTs and NPs in 100% of the AD patients. No NFTs or NPs were found in the caudal raphe nuclei. The authors found severe pathological alterations in the rostral nuclei (DRN, paramedian, median and linear raphe nuclei) in AD cases.
Grinberg *et al*., 2009 [21]	Determine during which BB stage and how frequently the DRN is affected by NFTs	118 cases: 38 staged as BB = 0 and 80 as BB ≥1; (mean age 75.98 ± 11.63 years).	Postmortem tissue, paraffin sectioning; HE used for the delineation of the ST-DR. PHF-1/ AT8 counterstained with haematoxylin was used to identify NFTs in the DRN.	Neurofibrillary changes were detected in the ST-DR in all of the BB ≥ 1 cases and in more than 1/5 of the BB = 0.

Studies on the SN	Objectives	Subjects	Materials	Results
Tabaton *et al*., 1985 [122]	Study the neuronal loss and NFT burden in the SN and NCS in AD patients.	4 AD cases (mean age 63.2 ± 3), 3 controls (62.6 ±2.8)	Postmortem tissue, paraffin sectioning; Sections stained with cresyl violet for neuronal counts and Bodian stain for NFTs identification.	Significant neuronal loss in both nuclei was present in AD cases, while the NFT burden was remarkably higher in NCS. No significant correlation between neuronal loss and NFT numbers was detected.
Gibb *et al*., 1989 [124]	Establish the frequency and severity of AD pathology in the SN and VTA.	104 AD cases (38–99 years), 31 controls (61–88 years)	Postmortem tissue, paraffin sectioning; Stains included HE for neuronal counts, Bielschowsky and Palmgren's stains for quantitation of NFTs and NPs.	54% frequency of NFTs was present in AD cases versus controls. 50% of these cases had excess SN cell loss but no PD-related syndromes.
Kazee *et al*., 1995 [125]	Determine the association between AD and SN pathology.	48 AD cases (mean age 81.2 years) and 18 normal elderly controls (mean age 75.3 years).	Postmortem tissue, paraffin sectioning; Stains for neuronal and NFT counts included HE, Bodian, and modified Bielschowsky. For amyloid staining, periodic acid-Schiff, Congo red, or Thioflavin S.	The prevalence of SN lesions was much greater in AD cases than in controls. 31% of the AD cases showed severe loss in the SN neurons, while 29% had moderate neuropathology in the SN. Most control brains (89%) had normal or mild pathology in the SN. EPS signs were positively correlated to increasing SN pathology.
Pizzolato *et al*., 1996 [133]	Determine the relative striatal uptake of the dopamine receptor D_2 receptor ligand [1231]-IBZM Using SPECT in AD patients vs controls.	15 AD patients (mean age was 67.1 ± 6.9 years) without overt EPS; 9 controls (mean age, 64 ± 6 years).	*In vivo* imaging. Central dopamine D_2 receptors were evaluated using SPECT with [1231J-IBZM as a tracer.	Striatal regions of the AD patients showed a significant reduction of mean specific activity of the D_2 receptors compared to controls. The authors suggest that alterations of striatal D_2 receptors may be part of the pathologic abnormalities of AD, in particular alterations of the nigrostriatal dopaminergic system.
Zarow *et al*., 2003 [130]	Compare the severity of neuronal loss in AD and PD	86 AD (mean age 76.8 years), 19 idiopathic PD	Postmortem tissue, paraffin sectioning; Cresyl violet staining used to visualize neurons.	The AD cases showed the greatest neuronal loss in the LC, followed by the NbM but variable loss in the SN. PD cases

Studies on the SN	Objectives	Subjects	Materials	Results
	relative to healthy elderly control subjects across 3 nuclei (NbM, LC, and SN).	(mean age 70 years), and 13 healthy controls (mean age 71.1 years).		also showed the greatest neuronal loss in the LC, followed by the SN and the NbM. The duration of illness correlated with greater neuronal loss in the LC and NbM in AD, and greater neuronal loss in the SN in PD.
Attems *et al*., 2007 [127]	Identify the differences in tau and α-synuclein in the SN and LC of AD patients with and without EPS.	160 AD cases (61–102 years), 94.4% being demented, and 21.9% with clinically reported EPS.	Postmortem tissue, paraffin sectioning; Stains included HE, cresyl violet, Kluver-Barrera, Bielschowsky's silver stain. Immunohistochemistry for 4G8 (NPs) and AT8 (NFTs).	Neuronal loss in the SN and the frequency and intensity of tau aggregates in both the SN and LC increased with advancing BB stages, α-synuclein pathology in various brain regions was seen in 25.6% of the total cohort of AD patients. Neuronal loss in the SN was more severe in EPS+ than in EPS- cases.

Studies on the NbM	Objectives	Subjects	Materials	Results
Arendt *et al*., 1983 [141]	Investigate the involvement of the NbM in AD by focusing on neuronal population estimates in the substantia innominata, the nucleus of the diagonal band of Broca and in the nucleus septi medialis.	14 AD (age 60.8±1.3 years) and 14 control cases (age 59.3±2.6 years), matched for gender.	Postmortem tissue from the basal forebrain, embedded in celloidin and stained with cresyl violet for neuronal identification.	Neuronal numbers of the NbM were reduced by 70% compared to controls.
Vogels *et al*., 1990 [146]	Estimation of the total neuronal number of the entire rostrocaudal extent of the NbM and of its subdivisions in cases with AD.	5 cases of early onset AD, 5 cases of late onset AD (age 65–93 years), 1 case of PD without cognitive impairment (age 74 years), and 8 healthy controls (age 60–91 years).	Postmortem tissue, paraffin sectioning; Nissl stain was used for neuronal counting. Kltiver-Barrera-stained sections were used for identification of the NbM and its subdivisions. A 3D sampling scheme was used throughout the whole nucleus.	Total neuron numbers in controls and AD (early and late onset together) indicated neuron loss for the NbM complex as a whole and for all its subdivisions in AD except for the Ch1 + Ch2 regions. In AD, the length of the NbM was significantly reduced bilaterally. In the single PD case no significant neuronal loss in NBMC was observed.
Geula *et al*., 1998 [143]	Determine the relationship between loss of cholinergic axons and density of NFTs/ NPsinthe cortex of AD brains.	13 aged controls (mean age 76.23 ± 9.8) 15 cases AD (mean age 76.6 ± 8.8)	Postmortem tissue; Microtome sectioning Sections stained with HE, Bielschowsky silver stain and thiofiavin-S for neuropathological analysis. Cresyl violet used for delineation of cytoarchitectonic boundaries. PHF-tau and the 1282 polyclonal antibody were used against tau and Aβ respectively.	The Ch1–Ch4 cholinergic cells were stained positive for both plaques and tangles within the basal forebrain. In contrast to plaque density, tangle density displayed a strong correlation with cholinergic loss in the AD cases versus controls.
Mufson *et al*., 2002 [148]	Examine if the reduction of the neurotrophin receptor $p75^{NTR}$, a marker of cholinergic basal forebrain neurons, in the NbM of cases with late AD, extends to individuals with MCI and mild AD. Moreover, how this reduction relates to cognitive performance in these groups.	11 cases with no cognitive impairment (mean age 82 years), 12 with MCI (mean age 84.6 years) and 6 with AD (mean age 88.3 years). Groups were comparable for age, education, and ApoE allele status but not for gender.	Postmortem tissue, Paraffin sectioning. Sections throughout the NbM were processed for $p75^{NTR}$ staining with a monoclonal antibody raised against human $p75^{NTR}$. Cresyl violet stain was used to visualize cytoarchitectonics. Quantitative estimates of the total number of neurons containing p75NTR. An optical dissector cell counting procedure was used.	The MCI (38%) and mild AD(43%) groups showed a significant reduction in the number of NbM p75NTR-immunoreactive neurons, compared to the NCI cases. The number of p75NTR-immunoreactive neurons in the NbM was significantly correlated with MMSE scores
Mesulam *et al*., 2004 [70]	Determine if cholinergic denervation occurs in early versus late stages in AD	5 early AD/MCI cases (age 89–99 years); 7 age-matched cognitively healthy controls (age 88–100 years).	Postmortem tissue, microtome sectioning. The NbM was analyzed unilaterally in 1 hemisphere with the following stains: thioflavin-S fluorescence against NFTs and two tau	NFT and Alz-50/AT8 positive neurons were found in the NbM in both AD and control groups. However, the NbM tau cytopathology was significantly more pronounced in the cognitively

Studies on the NbM	Objectives	Subjects	Materials	Results
			antibodies (AT8, Alz-50) for labeling pre-tangles.	impaired than in the cognitively intact subjects.
Teipel *et al*., 2011 [152]	Investigate the in vivo changes of the cholinergic basal forebrain in AD and MCI patients using MRI.	21 patients with a clinical diagnosis of probable AD (age 58–87 years), 16 subjects with MCI (age 60–88 years) compared to 20 healthy elderly subjects (age 56–83 years).	*In vivo* imaging using a 3.0-Tesla MRI.	AD and MCI subjects showed reduced volumes in basal forebrain areas corresponding to anterior medial and lateral, intermediate and posterior nuclei of the NbM as well as in the diagonal band of Broca nuclei. AD patients showed significant neuronal loss in the cholinergic nuclei Ch2–Ch4. The MCI group showed less pronounced effects than the AD group in similar localizations.

Abbreviations: AChE, Acetylcholinesterase; AD, Alzheimer's disease; ApoE, Apolipoprotein E; Aβ, Amyloid beta; BB, Braak and Braak stage; Ch1–Ch4, Cholinergic groups 1–4 of the nucleus basalis complex; DRN, Dorsal raphe nucleus; EPS, Extrapyramidal symptoms; HE Hematoxylin and eosin stain; LC, Locus coeruleus; MCI, Mild cognitive impairment; MMSE, Mini-mental state examination; MRI, Magnetic resonance imaging; MRN, Median raphe nuclei; NbM, Nucleus basalis of Meynert; NCS, Nucleus centralis superior; NE, Norepinephrine; NFT, Neurofibrillary tangle; NP, neuritic plaques; $p75^{NTR}$ low affinity p75 neurotrophin receptor; PD, Parkinson's disease; PH8, antibody that cross-reacts with the serotonin synthesis enzyme, tryptophan hydroxylase; PHF, paired helical filament; SN, Substantia nigra; SPECT, Singlephoton emission computed tomography; ST-DR Supratrochlear subnucleus of the dorsal raphe nucleus; TH, Antibody against the norepinephrine-synthesizing enzyme, tyrosine hydroxylase; VTA Ventral tegmental area.