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Featured Article

Locus coeruleus volume and cell population changes during Alzheimer's disease progression: A stereological study in human postmortem brains with potential implication for early-stage biomarker discovery

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Abstract

Introduction: Alzheimer's disease (AD) progression follows a specific spreading pattern, emphasizing the need to characterize those brain areas that degenerate first. The brainstem's locus coeruleus (LC) is the first area to develop neurofibrillary changes (neurofibrillary tangles [NFTs]).
Methods: The methods include unbiased stereological analyses in human brainstems to estimate LC volume and neuronal population in controls and individuals across all AD stages.
Results: As the Braak stage increases by 1 unit, the LC volume decreases by 8.4%. Neuronal loss started only midway through AD progression. Age-related changes spare the LC.
Discussion: The long gap between NFT accumulation and neuronal loss suggests that a second trigger may be necessary to induce neuronal death in AD. Imaging studies should determine whether LC volumetry can replicate the stage-wise atrophy observed here and how these changes are specific to AD. LC volumetry may develop into a screening biomarker for selecting high-yield candidates to undergo expensive and less accessible positron emission tomography scans and to monitor AD progression from presymptomatic stages.

Keywords: Alzheimer's disease; Brainstem; Locus coeruleus; Human; Neurofibrillary tangles; Unbiased stereology; Postmortem; Neuron counts; Volumetry

1. Introduction

The United Nation projects that >200 million people will suffer from neurodegenerative diseases by 2050. In the United States alone, the cost of dementia is >$160 billion a year and is projected to reach $1.1 trillion by 2050 [1]. Effective disease-modifying treatments for Alzheimer's disease (AD) remain elusive. In recent years, all promising therapies for AD that appeared efficacious in animal models fell short when tested in humans [2]. Oversimplification and incomplete modeling of AD pathophysiology are, at least partly, to blame for this failure [3]. AD features double-nature lesions. Besides accumulation of neuritic plaques

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and neurofibrillary tangles (NFTs), neuronal loss plays a critical role [4]. In fact, neuronal loss is considered the best correlate of cognitive deterioration in AD [5]. Furthermore, AD follows a predictable anatomical sequence, with lesions spreading along specific neuronal pathways [6]. Understanding the pathobiology of AD in the brain areas that degenerate first is critical for developing treatments to halt the spread of AD before it causes an irrevocable neuronal loss.

Braak and Braak (BB) designed a universally used 7-point system for staging AD that recapitulates the stereotypical spreading pattern of tau cytoskeletal pathology in the cortex [7]. NFTs limited to transentorhinal/entorhinal cortex and hippocampus represent stages I/II. In 2009, our group demonstrated that tau cytoskeletal pathology in the dorsal raphe nucleus, a serotonin-producing nucleus in the midbrain, precedes the development of NFTs in transentorhinal cortex [8]. Reexamination of the temporal involvement of subcortical structures in AD followed [9,10], including Braak's group revision of their classical staging system in 2011 which now incorporates brainstem structures as precortical staging a–c [11]. They demonstrated that the locus coeruleus (LC), a noradrenergic nucleus located at the pons, shows NFTs as early as the fourth decade of life, corroborating previous findings of early LC involvement in AD [12–14]. The LC belongs to the isodendritic core, a phylogenetically conserved nuclei network [14]. NFT development in components of the isodendritic core consistently precedes NFTs in any cortical areas [8,10,11].

Understanding the changes in LC associated with AD progression may represent a window of opportunity for identifying biomarkers to detect AD in prodromal stages and inform on therapeutic targets to halt AD progression. Meticulous neuropathological investigations that could be criticized as "descriptive" were critical for creating the necessary foundation for further studies utilizing cutting-edge methods such as proteomics, high-resolution imaging, and improved animal models that advance the understanding of neurodegenerative diseases.

Aiming to understand the impact of AD in the LC, we used design-based stereology to investigate LC volume and neuronal population changes in a sample of 68 subjects, enriched for controls and early AD stages.

2. Participants and methods

2.1. Participants

The majority of the cases (65) was sourced from the Brain Bank of the Brazilian Brain Aging Study Group (BBBABSG [15,16]). The Neurodegenerative Disease Brain Bank (NDBB) from the University of California, San Francisco (UCSF) supplied three cases (#62, 63, and 68, Table 1). The institutional review boards of both participating institutions approved this study. The BBBABSG is supplied by the São Paulo City Autopsy Service (SPAS). Autopsies are mandatory for determining the cause of death in Sao Paulo, and the SPAS performs approximately 13,000 autopsies per year. All cases autopsied during morning hours are candidates for the BBBABSG after donation by next of kin [15]. The BBBABSG receives approximately 300 cases per year. A random rotation list among the studies supplied by the BBBABSG was created to accommodate all the studies requiring a modified protocol, including this one. For the present study, we received cases collected from 2010 to 2013. The NDBB receives brain and spinal cord donations from patients enrolled in the UCSF Memory and Aging Center. The great majority of UCSF/NDBB cases developed late-stage dementia, and most of the cases show more than one neurodegenerative condition. We collected the first three consecutive cases meeting the study criteria. The selection criteria for both centers included the absence of non–AD-related neurodegenerative pathology or significant cerebrovascular lesions and availability of an intact brainstem. Subjects were excluded if they had a history of seizures, other neurological diseases, a primary axis 1 psychiatric diagnosis, or gross non-degenerative structural pathology. For all cases, the neuropathological assessment was based on analysis of dementia-related structures embedded in paraffin wax, cut into 8-μm-thick sections, and stained with immunohistochemistry. BBBABSG and NDBB neuropathological protocols are similar. AD pathology was staged according to the new NIA-AA guidelines [17]. Cases were categorized by the BB staging system for neurofibrillary changes [7]. Subjects were considered to be BB stage 0 or free of cortical NFTs when at least four sections across the transentorhinal cortex were negative for phospho-tau immunostaining (CP-13, gift of Peter Davies) [8]. Approximately 500 cases were screened for this study.

2.2. Tissue processing and staining

The tissue processing and staining methods used in this study have been previously described [18]. Briefly, each brainstem block was separated from the brain, fixed in 10% formalin, and embedded in 8% celloidin for subsequent sectioning [19]. Blocks were sectioned horizontally in serial sets, each one containing one 300-μm-thick and five 60-μm-thick sections. For cytoarchitectonic visualization of the LC, all odd-numbered sections were stained with gallocyanin-chromalum. Parallel sections were immunostained for phospho-tau (CP-13) after antigen retrieval [18]. Selected sections were immunostained for tyrosine hydroxylase (TH, 1:00; Millipore, Billerica, MA, USA) for assisting with the LC border segmentation [18](Fig. 1).

2.3. Stereological analyses

Stereological analyses of unilateral LC neuronal population were performed using the optical fractionator probe of the StereoInvestigator software (MBF StereoInvestigator,