RESEARCH ARTICLE

Inhomogeneous distribution of Alzheimer pathology along the isocortical relief. Are cortical convolutions an Achilles heel of evolution?

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Keywords
amyloid plaques, cortical surface, gyrus, neurofibrillary tangles, sulcus.

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Received 5 July 2016
Accepted 19 August 2016
Published Online Article Accepted 26 August 2016
doi:10.1111/bpa.12442

Abstract
Alzheimer’s disease (AD) is neuropathologically characterized by neuritic plaques and neurofibrillary tangles. Progression of both plaques and tangles throughout the brain follows a hierarchical distribution which is defined by intrinsic cytoarchitectonic features and extrinsic connectivity patterns. What has less well been studied is how cortical convolutions influence the distribution of AD pathology. Here, the distribution of both plaques and tangles within subsulcal gyral components (fundi) to components forming their top regions at the subarachnoidal brain surface (crowns) by stereological methods in seven different cortical areas was systematically compared. Further, principle differences in cytoarchitectonic organization of cortical crowns and fundi that might provide the background for regionally selective vulnerability were attempted to identify. It was shown that both plaques and tangles were more prominent in sulcal fundi than gyri crowns. The differential distribution of pathology along convolutions corresponds to subgyral differences in the vascular network, GFAP-positive astrocytes and intracortical and subcortical connectivity. While the precise mechanisms accounting for these differences remain open, the presence of systematic inhomogeneities in the distribution of AD pathology along cortical convolutions indicates that the phylogenetic shaping of the cortex is associated with features that render the human brain vulnerable to AD pathology.

INTRODUCTION

Alzheimer’s disease (AD) is a neurodegenerative disorder, neuropathologically characterized by fibrillar aggregates of the Aβ-peptide and the microtubule-associated protein tau giving rise to the typical histopathological hallmarks, that is, Aβ plaques and neurofibrillary tangles. Formation of both lesions, most likely, represents a late stage event in a pathological cascade of progressive conversion from oligomeric toward fibrillary aggregates of Aβ and tau, respectively (13, 41, 60, 104). The mechanism of formation and the pathological role of insoluble deposits of Aβ and tau in the brain tissue in form of plaques and tangles remain unclear. Still, their density and distribution provides the basis for postmortem diagnosis (76), as well as more recently, for in vivo diagnosis with PET tracers (61, 78).

The progressive formation of both Aβ plaques and neurofibrillary tangles throughout different brain areas is not random but rather follows a hierarchical distribution which is used to define the neuropathological stage of the disease (18, 75, 97). Apparently, both intrinsic cytoarchitectonic features and extrinsic pattern of connectivity shape the distribution of AD pathology throughout the cortex (8, 19, 33, 80, 89). Moreover, it has not escaped the attention of investigators over the last 100 years that histopathological hallmarks of AD are not evenly distributed within the affected cortical fields but rather follow the cortical surface (4, 5, 10, 17, 18, 46, 47, 49, 71, 86, 90, 103).

Systematic differences in the distribution of specific pathological features might provide valuable insights both into disease mechanism and into principles of pathophysiological organization of the cerebral cortex. To further unravel how neuroanatomy shapes neuropathology, we systematically analyzed the distribution of both Aβ plaques and neurofibrillary tangles along cortical convolutions of affected areas. To this end, we compared subsulcal gyral components (fundi) to components forming their top regions at the subarachnoidal brain surface (crowns) by stereological methods. Further, we attempted to identify principle differences in cytoarchitectonic organization of cortical crowns and fundi that might provide the background for selective neuronal vulnerability.
**METHODS**

Brain tissue of 20 AD patients and 20 healthy controls dying without any history of neurological or psychiatric illness was used (Table 1). The diagnosis of AD was made on the basis of both clinical and neuropathological evidence according to the criteria of the International Working Group (IWG) for New Research Criteria for the diagnosis of AD (34, 35) in the revision of 2014 (IWG-2) (36), the NIA-AA diagnostic criteria in the revision of 2011 (3, 59, 72, 93) and the NIA-AA guidelines for the neuropathological assessment of AD (57, 76). Only cases with typical AD according to IWG-2 criteria were included. All cases underwent neuropsychological assessment within the last 6 months prior to their death. Clinical Dementia Rating (CDR) scale scoring was based on neuropsychological testing (CERAD) (77), MMSE (42) and rating scales (83). CDR scale score was used to identify patients with mild dementia (CDR 1) (56). All cases were neuropathologically assessed for neurofibrillary tangle stage according to Braak and Braak (18) and Braak et al (16), for Aβ/amyloid plaque score according to Thal et al (97) and for neuritic plaque score according to CERAD (75). Neurofibrillary tangles and Aβ/amyloid plaques were detected by immunocytochemical labeling of phospho-tau (anti-human PHF-tau monoclonal antibody AT8; Thermo Scientific) and Aβ (beta amyloid monoclonal antibody, 6E10; BioLegend), respectively. Severity of AD pathology was scored following the consensus guidelines for the neuropathologic evaluation of AD according to Hyman et al (58) and Montine et al (77).

Case recruitment, autopsy and data handling have been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments as well as with the convention of the Council of Europe on Human Rights and Biomedicine and had been approved by the responsible Ethics Committee of Leipzig University.

**TISSUE PROCESSING AND STEREORELOGICAL ANALYSIS**

Brains were immersed in 4% formaldehyde in phosphate buffer (0.1 M, pH 7.4) for one month. Tissue blocks of the agranular cortex (Brodmann area 8, area frontalis intermedia, Brodmann area 6, area frontalis agranularis), primary visual cortex (Brodmann area 17, area striata), primary sensory association cortex (Brodmann area 7, area parietalis superior; Brodmann area 22 area temporalis superior) and proisocortex (Brodmann area 11, area prefrontalis; Brodmann area 23 area cingularis posterior ventralis) were paraffin embedded. Corresponding pairs of 5-μm sections were immunocytochemically processed for phospho-tau (anti-human PHF-tau monoclonal antibody AT8, Thermo Scientific; rabbit-anti-phospho-tau 205, Invitrogen), Aβ (beta amyloid monoclonal antibody, 6E10, BioLegend; mouse-anti-pE3Aβ, Synaptic Systems); NeuN (anti-neuronal nuclei mouse monoclonal antibody; Millipore; clone 60); GFAP (rabbit anti-GFAP antibody; DAKO), synaptophysin (rabbit anti-synaptophysin antibody; DAKO) and rabbit-anti-

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**Table 1. Synopsis of cases.**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>20</td>
<td>20</td>
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<tr>
<td>Gender: male/female</td>
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<td>9/11</td>
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<tr>
<td>Age in years (±SD)</td>
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<td>83.2 ± 8.5</td>
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<tr>
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<tr>
<td></td>
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<td>7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>13</td>
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</tr>
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</tr>
<tr>
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</tr>
<tr>
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<tr>
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<td>10</td>
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<td></td>
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<tr>
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<td>Myocardial infarction</td>
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<tr>
<td></td>
<td>Embolism of lung</td>
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<tr>
<td>Postmortem delay in h (±SD)</td>
<td>20.6 ± 5.9</td>
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<tr>
<td>Fresh brain weight in g (±SD)</td>
<td>1278 ± 73</td>
<td>1216 ± 57</td>
</tr>
</tbody>
</table>

*According to Montine et al (76).
laminin (DAKO). Subsequent sections were Nissl stained with cresyl echt violet. Selected sections were stained by the Klüver–Barrera-method (68).

The numerical density of objects was determined by the unbiased method of physical dissector (20, 52, 95) as described previously (45). In brief, a distance of 3 µm (h value) was chosen between reference section and look-up sections. Six pairs of adjacent sections per block were selected randomly according to the method of West (107) for each region. Images were taken using the digital microscope camera AxioCam HRc running on the AxioVision v.4.3 software (both Carl Zeiss Vision) and analyzed subsequently with Adobe Photoshop v.7.0 software (Adobe Systems, Inc.). Objects were counted within superimposed frames of 200 µm × 100 µm placed to the crowns and fundi. Several dissectors were aligned serially, perpendicular to the pial surface, to cover the entire depth of the cortex. Sampling was carried out at three different sites of the maximum curvature of each crown and fundus. On average, 300–600 profiles were counted in each case and region.

RESULTS

Distribution of Aß plaques and neurofibrillary tangles in Alzheimer’s disease follows the cortical relief

In all cortical regions investigated (Brodmann’s areas 6, 7, 8, 11, 17, 22 and 23) numerical density of Aß plaques in AD was significantly higher in fundi than in crowns (Figures 1 and 2). In some regions (Brodmann’s areas 6, 11 and 22) plaque density in fundi was about twice as high as in crowns. The density of neurofibrillary tangles was also significantly higher in fundi than in crowns of gyri (Figures 1 and 2). Differences were in the same order of magnitude as for plaques, that is, for some areas reached about nearly twice the number in fundi compared with crowns.

Cytoarchitectonic differences between fundi and crowns of gyri in normal human brain

In an attempt to characterize the background for differences in vulnerability between fundi and crowns, we searched for differences in cytoarchitectonic organization associated with cortical convolutions.

First, we analyzed whether a differential thickness of layers which are preferentially affected by Aß plaques and neurofibrillary tangles might contribute to the observed differences in AD pathology between crowns and fundi.

We found a reduced cortical thickness in fundi compared with crowns associated with a shift in the thickness of individual cortical layers, replicating previous findings (15, 23, 38–40, 98). Supragranular layers dominated over infragranular layers in fundi. Vice versa, in crowns, infragranular layers were more prominent than supragranular layers (Figure 3). This clearly shows that layers highly affected by pathology, that is, in particular layers III and V/VI, contribute more to overall cortical thickness in crowns than in fundi. Thus, the shift in thickness of layers can be ruled out as correlate for the higher abundance of pathology in fundi.

We than analyzed potential differences between fundi and crowns in the normal human brain with respect to numerical density of neurons, synapses, GFAP-positive astrocytes and vessels. Corresponding to the higher density of both plaques and neurofibrillary tangles in fundi, we observed a higher density of vessels and of GFAP-positive astrocytes in fundi as compared with crowns of gyri (Figures 2 and 4), providing a potential correlate for the distribution of pathology. On the contrary, no significant differences in the overall density of neurons, of synapses or of the synapse-to-neuron ratio were observed between gyral fundi and crowns (Figure 5).

DISCUSSION

One of the most prominent evolutionary features of the mammalian brain is the surface expansion of the cerebral cortex resulting in cortical convolutions. Here, we show systematic inhomogeneities in the distribution of AD pathology along cortical convolutions indicating that the phylogenetic shaping of the cortex is associated with a mechanism that renders the human brain vulnerable to AD pathology.

Both Aß plaques and neurofibrillary tangles were found concentrated in sulcal fundi compared with gyral crowns. This confirms...
previous observations on the distribution of plaques made by several groups including our own (4, 10, 47, 72) and extends it toward the distribution of neurofibrillary tangles which had not been analyzed systematically before. A preferential involvement of sulcal fundi with both tau pathology and Aβ pathology has similarly been observed in chronic traumatic encephalopathy (CTE) (71, 94) where it may result from stress concentration at the sulcal depth following trauma (32). While traumatic brain injury has long been

Figure 2. Comparative distribution of Aβ plaques, tau pathology and blood vessels in gyral fundi and crowns of the human temporal cortex (Brodmann area 22). Images of Aβ (monoclonal mouse-anti-pE3Aβ antibody) and phospho-tau (rabbit-anti-phospho-tau 205 antibody) pathology were taken from an AD case, while the distribution of blood vessels (rabbit-anti-laminin antibody) is shown for a control brain. Insets in the upper panel are shown below at higher magnification (middle: crowns; bottom: fundi). Scale bars: upper panel 1mm; lower panels 300 μm.
thought to be an epigenetic risk factor for AD, a definitive link has yet to be established (65), and mechanisms that predispose the fundi to lesion formation in AD might be more complex. In any case, the inhomogenic distribution of AD pathology along the isocortical relief clearly indicates a role of those intrinsic cortical features that differ between fundi and crowns. Considering these intragyrif differences of pathology will thus be a challenge to pathogenetic concepts of AD.

The potential relevance of systematic differences in AD pathology between fundi and crowns in an order of magnitude of 100% becomes apparent if translated into terms of diagnostic assessment. In the setting of low Thal phases (1 or 2) and Braak stages III or higher, differences in CERAD plaque scoring between sparse and moderate would give rise to a difference in the neuropathological diagnostic “ABC” score as defined by Montine et al (77) between “Low” and “Intermediate.”

**VULNERABILITY TO AD PATHOLOGY IS LINKED TO GYRIFICATION**

Mammals can be distinguished on the basis of the cortical surface area of their brains and the degree of convolutions (21, 22, 55, 56). Gyrencephalics are characterized by folded or convoluted (gyrified) brains, in contrast to lissencephalics which have smooth-surfaced or non-convoluted brains (7, 63, 64, 67, 102). Larger primate brains are usually more convoluted than smaller ones (84, 107). Moreover, the degree of convolution accelerates with evolution, that is,
cortical folding increases more rapidly with brain size among anthropoids compared with prosimians (109, 110). Accordingly, it has been suggested that the particularly high degree of cortical folding in humans may represent a morphological correlate that supports human-specific cognitive abilities (69).

The primary reason for convolutions is that cortical surface area increases disproportionally with brain size. Convolutions are, thus, formed in a growth process with preference for tangential vs. radial cortical expansion (88, 101). This permits cortical enlargement while keeping the overall increase of brain volume and total fiber length to a minimum (80, 96, 111). These mechanisms, thus follow the requirements of “component placement optimization” (28) as an important design principle of neuronal architecture.

The precise mechanism leading to cortical convolutions is not entirely clear. There still exist two major concepts (24, 44), which attribute cortical folding primarily either to compressive forces induced by differential tangential expansion of the cortex (27, 70, 83, 87, 88, 91) or to tension along axons in the white matter (53, 54, 101). Still, there is consensus that cortical gyration is based on an interplay between genetic determinants and self-organizational principles that involve mechanical factors (6, 11, 24, 27, 30, 33, 44, 46, 49, 51, 54, 82, 92, 98, 105, 108, 109). Mechanical forces associated with folding may have a wide variety of effects on signal transduction, gene expression, differentiation and apoptosis (29, 59). The exposure to different tensional forces of the same classes of neurons in fundi and crowns during the process of gyrogenesis, thus, most likely results in different functional properties of these neurons [see also (1, 14)].

During the formation of folds, also cellular morphology and the thickness of different layers is altered (105). Cortical thickness is higher in crowns of gyri than in fundi, and their laminar diameters show considerable differences which is linked to the different connectivity of these layers (27, 100, 105). In general, infragranular layers are thin along fundi and thick along crowns, while in reverse, supragranular layers are relatively thin along crowns and thick along fundi (15, 23, 39–41, 100; present study Figure 3). Infragranular layers are involved with specific thalamic input and cortical output, while supragranular layers are more restricted to cortico-cortical connections (31, 99).

Gyrification, thus, seems to result in spatial constellations of network components in fundi that compared with crowns modify functional interactions between intrinsic and extrinsic tissue elements. The disproportionally higher intracortical connectivity within fundi may potentially promote intracortical transsynaptic spreading of pathological compounds including fibrillary aggregated Aβ and tau (38, 66) which would agree with a more dense pathology in fundi. On the contrary, the strongly diminished infragranular part of the fundus may facilitate functional deprivation of cortical–subcortical cross-talk. The lower content of the α1-GABA_A receptor subunit in fundi of the human prefrontal cortex (2), for example, indicates strongly diminished intrinsic GABA-mediated inhibitory actions in the fundus. A decrease of synaptic inhibition has also been suggested to play a role in the formation of Aβ-plaques (26).

Mechanical impact during folding not only determines the shape and spatial orientation of neurons and dendrites, it also affects the density and layout of blood vessels (75). Here, we identified higher densities of blood vessels in fundi, that is, those areas that are also characterized by higher densities of plaques and tangles. The nature of this correlate and whether this means a causal relationship warrants further investigation. Still, current concepts of Aβ plaque formation as a consequence of a disturbed balance between Aβ-production and clearance (106) as well as previous descriptions on a link between plaque formation and vascularization (9, 12, 74) concur with this observation.

Only little is known on specific architectonical characteristics of gyral subdivisions, and an in depth characterization of fundus-crown differences was well beyond the aim of the current study.
The differential distribution of AD-related pathology between gyral subdivisions still indicates that features associated with cortical folding might be deleterious providing a basis toward vulnerability against AD pathology.

ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

ACKNOWLEDGEMENT

Support by the Fritz-Thyssen-Foundation (Az.10.13.1.144) is gratefully acknowledged.

CONFLICT OF INTEREST

The authors herewith declare that there are no financial, personal or professional interests that could be construed to have influence on the work.

STATEMENT OF AUTHOR CONTRIBUTIONS

TA, MM, UG, NF, FS, NW, CJ, ChE, H-JG, WM, KB carried out experiments, study design and data analysis; TA, MM, UG, H-JG, WM, KB interpreted the data and were involved in writing the article.

REFERENCES

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