Archival Report

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Resilience of Precuneus Neurotrophic Signaling Pathways Despite Amyloid Pathology in Prodromal Alzheimer's Disease

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ABSTRACT

BACKGROUND: Reduction of precuneus choline acetyltransferase activity co-occurs with greater beta-amyloid (Aβ) in Alzheimer's disease (AD). Whether this cholinergic deficit is associated with alteration in nerve growth factor (NGF) signaling and its relation to Aβ plaque and neurofibrillary tangle (NFT) pathology during disease onset is unknown.

METHODS: Precuneus NGF upstream and downstream signaling levels relative to Aβ and NFT pathology were evaluated using biochemistry and histochemistry in 62 subjects with a premortem diagnosis of non–cognitively impaired (NCI; $n = 23$), mild cognitive impairment (MCI; $n = 21$), and mild to moderate AD ($n = 18$).

RESULTS: Immunoblots revealed increased levels of proNGF in AD subjects but not MCI subjects, whereas cognate receptors were unchanged. There were no significant differences in protein level for the downstream survival kinase-signaling proteins Erk and phospho-Erk among groups. Apoptotic phospho-JNK, phospho-JNK/JNK ratio, and Bcl-2 were significantly elevated in AD subjects. Soluble $A\beta_{1-42}$ and fibrillar Aβ measured by [^{3}H] Pittsburgh compound-B ([^{3}H]PiB) binding were significantly higher in AD subjects compared with MCI and NCI subjects. The density of plaques showed a trend to increase, but only 6-CN-PiB–positive plaques reached significance in AD subjects. AT8-positive, TOC-1–positive, and Tau C3–positive NFT densities were unchanged, whereas only AT8-positive neuropil thread density was statistically higher in AD subjects. A negative correlation was found between proNGF, phospho-JNK, and Bcl-2 levels and phospho-JNK/JNK ratio and cognition, whereas proNGF correlated positively with 6-CN-PiB–positive plaques during disease progression.

CONCLUSIONS: Data indicate that precuneus neurotrophin pathways are resilient to amyloid toxicity during the onset of AD.

Keywords: Alzheimer's disease, Amyloid, Mild cognitive impairment, Neuropathology, Neurotrophic factors, Tau

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The precuneus, a component of the default mode network (DMN), is implicated in episodic memory retrieval (1) and displays high metabolic activity during conscious rest and selectively deactivates during non–self-directed cognitive tasks in the healthy brain (2–4). However, the precuneus is dysregulated in aging (5), and its ability to inactivate during cognitive tasks is compromised at the earliest stages of Alzheimer's disease (AD) even before cognitive impairment (6–8). [^{3}H] Pittsburgh compound-B ([^{3}H]PiB) amyloid imaging reveals that the default mode network is vulnerable to beta-amyloid (Aβ) deposition in the earliest, preclinical stages of the disease (9–11). There is also an overlap between synaptic failure, functional disconnection, and amyloid in the DMN in mild cognitive impairment (MCI) (12), linking Aβ and connectivity disruptions within the default mode network before clinical onset of dementia (13). The precuneus also displays a greater degree of atrophy in early-onset compared with late-onset AD (14–16) and a reduction in synapse number in patients with AD but not MCI individuals (17).

Precuneus cholinergic activity is reduced in AD but not in MCI subjects and co-occurs with increased amyloid burden (18). Aβ plays a role in cholinergic dysfunction by alterating interactions between nerve growth factor (NGF)/proNGF and its cognate high-affinity TrkA and low-affinity $p75^{NTR}$ receptors (19–21), which underlie cholinergic basal forebrain neuron survival. We have shown a shift in upstream and downstream proNGF signaling from cell survival to cell death within the lateral parietal cortex (22,23) and hippocampus (24) during progression of AD. Whether precuneus neurotrophic NGF signaling pathways are altered in relation to amyloid and neurofibrillary tangle (NFT) deposition during AD progression is unknown. Expression levels of proNGF, TrkA, and $p75^{NTR}$ as well as cell survival and proapoptotic downstream NGF activated pathways relative to Aβ and tau pathology were examined in precuneus tissue from people who died with a clinical diagnosis of no cognitive impairment (NCI), MCI, or AD. Neurochemical changes were correlated with cognitive and neuropathologic variables.

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METHODS AND MATERIALS

Subjects

The study included 62 cases diagnosed as NCI (18 women /5 men), MCI (15 women/6 men), and AD (10 women/8 men) from the Rush Religious Order Study (RROS) (25–27) and University of Kentucky Alzheimer's Disease Center (UKADC) (Table 1) (28,29). Participants agreed to an annual clinical evaluation and brain donation at death. Human Investigations Committees of Rush University and University of Kentucky approved the study.

Clinical and Neuropathologic Evaluations

Clinical criteria for diagnosis of AD, MCI, and NCI have been reported (25,27–30). Five RROS and six UKADC cases were diagnosed as amnestic MCI (31). Mini-Mental State Examination (MMSE) was performed within 2 years of death. A global cognitive *z* score comprising 19 tests and an episodic memory z score was available for RROS cases (Supplement 1).

Tissue

Frozen precuneus was homogenized (150 mg/mL) on ice in phosphate-buffered saline and processed for [^{3}H]PiB, Aβ enzyme-linked immunosorbent assay (ELISA) and Western blotting (Supplement 1). Precuneus from the other hemisphere was dissected and fixed in 4% paraformaldehyde (pH 7.4) for 5 days, cryoprotected and cut frozen into 40-μm sections, and stored until processing (24,30).

Table 1. Clinical, Demographic and Neuropathologic Characteristics by Clinical Diagnosis Category

	NCI (n = 23)	MCI (n = 21)[a]	AD (n = 18)	Total (n = 62)	p Value	Pair-wise Comparison
Age (Years) at Death, Mean ± SD (Range)	86.3 ± 3.9 (78–92)	86.8 ± 5.1 (75–96)	88.9 ± 7.1 (73–100)	87.2 ± 5.4 (73–100)	.2[b]	—
Number (%) of Males	5 (21.7%)	6 (28.5%)	8 (44.4%)	18 (29%)	.4[c]	—
Years of Education, Mean ± SD (Range)	16.0 ± 3.1 (10–21)	17.2 ± 3.1 (10–25)	17.1 ± 3.4 (12–26)	16.7 ± 3.2 (10–26)	.5[b]	—
Number (%) with ApoE ε4 allele	1 (4.3%)	10 (47.6%)	5 (27.7%)	16 (25.8%)	.001[c]	NCI < MCI
MMSE[d], Mean ± SD (Range)	28.3 ± 1.5 (26–30)	27.0 ± 2.5 (22–30)	18.0 ± 6.0 (10–28)	24.9 ± 5.6 (10–30)	<.001[b]	(NCI, MCI) > AD
Global Cognitive *z* Score[e], Mean ± SD (Range)	.37 ± .17 (.06–.59)	.17 ± .35 (−.53–.91)	−.62 ± .44 (−1.18–.06)	−.03 ±.54 (−1.18–.91)	<.001[b]	(NCI, MCI) > AD
Episodic Memory *z* Score[e], Mean ± SD (Range)	.73 ± .37 (.08–1.37)	.36 ± .47 (−.25–1.68)	−.64 ± .76 (−1.99–.65)	.12 ± .80 (−1.99–1.68)	<.001[b]	NCI > MCI > AD
Postmortem Interval (Hours), Mean ± SD (Range)	4.3 ± 2.1 (1.0–9.0)	4.8 ± 2.4 (2.0–10.6)	4.5 ± 2.1 (1.5–8.6)	4.5 ± 2.2 (1.0–10.6)	.9[b]	—
Brain Weight (g), Mean ± SD (Range)	1174.1 ± 115.3 (940–1473)	1163.2 ± 140.0 (890–1350)	1132.7 ± 83.6 (975–1320)	1158.4 ± 116.0 (890–1473)	.4[b]	—
Distribution of Braak Scores						
No AD	3	0	0	3		
I/II	6	8	1	15	.008[b]	NCI < AD
III/IV	12	9	11	32		
V/VI	2	4	6	12		
NIA-Reagan Criteria Diagnosis (Likelihood of AD)[f]						
No AD	6	0	0	6		
Low	11	10	2	23	<.001[b]	NCI < MCI < AD
Intermediate	5	7	10	22		
High	1	3	6	10		
CERAD Diagnosis[f]						
No AD	10	6	0	16		
Possible	5	1	1	7	<.001[b]	NCI < AD
Probable	7	8	9	24		
Definite	1	5	8	14		

AD, Alzheimer's disease; ApoE, apolipoprotein E; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; MCI, mildly cognitively impaired; MMSE, Mini-Mental State Examination; NCA, non–cognitively impaired; NIA, National Institute on Aging; RROS, Rush Religious Order Study.

[a]n = 11 MCI cases were amnestic.

[b]Kruskal-Wallis test, with Dunn's correction for multiple comparisons.

[c]Fisher's exact test.

[d]MMSE of 2 NCI cases were unavailable.

[e]Cognitive *z* scores were available only for the 12 NCI, 14 MCI, and 14 AD RROS cases with additional cognitive testing within 2 years before death. Among them, 1 AD case completed less than half of the testing battery, and no global cognitive *z* score was computed for this case.

[f]Reagan and CERAD diagnoses of 1 MCI case were unavailable.