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Relationship between Different Brain Regions in Hierarchial Order of Neurofibrillary Tangles and Senile Plaques in Alzheimer Brain

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Background : Neurodegenerative process in AD is characterized by progressive neuronal and synaptic loss with gliosis and formation of senile plaque and neurofibrillary tangles. Relationship between severity of NFTs and SPs has still intriguing aspect. **Methods** : Hierarchial rank order of NFT and SPs was done instead of absolute morphometric quantitation to find out severity of each pathologic changes in regions of frontal, temporal, hippocampus, amygdala, entorrhinal cortex, piriform cortex, basal nucleus of Meynert (BNM), substantia nigra and locus coeruleus. Also age and brain weight were analyzed to find out relationship to each region. **Results** : Weight of brain showed significant correlation with neuronal loss in frontal, temporal and BNM but it did not show any correlation with the neuronal loss in LC, SN and amygdala. In amygdala NFT were high in cortical and cortical transitional nuclei. SPs were heavily accumulated in basomedial, cortical and cortical transitional nuclei. In hippocampus, entorrhinal cortex, H1 and subiculum of the hippocampus were the most consistent and severely affected regions. BNM did not show any correlation with laterobasal nucleus of amygdala which projects to BNM. SN, LC as well as BNM were interrelated in the severity of these changes. Nevertheless, these changes were not correlated with the brain weight. **Conclusion** : Different regional vulnerability even in a single area is demonstrated in a hierarchial order. But it is still a perpetuating question how these regional vulnerability occurs.

Key Words : *Alzheimer's disease, Neurofibrillary tangle, Senile plaque*

INTRODUCTION

The neurodegenerative process in Alzheimer's disease (AD) is characterized by progressive neuronal and synaptic loss with gliosis, and formation of senile plaques (SPs) and neurofibrillary tangles (NFTs)[1]. Many regional vulnerability to these pathologic changes are known, such as hippocampus, hippocampal formation, amygdala, basal nucleus of Meynert (BNM), locus coeruleus and also importantly substantia nigra. Recent studies have shown that neuronal loss initiates in the entorrhinal cortex layer II, progresses to the hippocampus, and then to the temporal, frontal and parietal neocortex[2]. Hippocampal formation is known to be one of the first and, in advanced cases, one of the most severely affected structure[3].

The possibility of histopathological staging of Alzheimer's disease (AD) and related lesions has intrigued neuropathologists since a report in a correlation between the number of SPs and dementia scores has been made[4]. But still the relationship of NFTs and SPs to the severity in AD remains controversial. Accurate understanding of the degree and extent of neuronal loss or other cellular changes such as NFTs and SPs in Alzheimer's disease is largely dependent upon the quantitative method. But absolute morphometric quantitation of NFTs and SPs has its side of unclarification. So instead of usual quantitative analysis we sought to find out whether NFTs, SPs and neuronal loss appears independently in a rank order according to a consistent pattern of hierarchic vulnerability in various brain regions including hippocampus and amygdaloid nucleus. And special attention has been paid to the correlation between age and brain weight with each regional vulnerability .

MATERIALS AND METHODS

Brains were obtained from the 84 cases in the Brain Bank of University of Minnesota, Medical School, U.S.A. Each

case met the National Institute of Neurologic and Communicative Disorders and Stroke and Alzheimer's disease and Related disorders's Association (NINCDS-ADRDA) criteria for the definite diagnosis of Alzheimer's disease[5] based on the presence of NFTs and neuritic plaques observed in the hippocampal formation and neocortical areas, as recommended.

After at least 2 weeks of fixation in 10% buffered formalin, brains were divided at midbrain level to weight cerebrum. Each hemisphere were cut coronally into 1 cm thick slabs and transversely into 3 mm thick slabs of brainstem and cerebellum. Samples were taken from these sections for paraffin embedding from the following regions : frontal cortex; temporal cortex; hippocampus (presubiculum, subiculum, H1, H2, H3, H4, H5 and dentate nucleus); amygdala (lateral, medial, basal, accessory basal, medial, central and cortical subnuclei); entorhinal cortex; piriform cortex; basal nucleus of Meynert (BNM); brainstem (substantia nigra (SN) and locus ceruleus (LC)). Sections from each paraffin-embedded block were cut at 12 μm and stained with either hematoxylin-eosin (H & E) and modified Bielschowsky's silver stain. The clinical diagnosis of AD was confirmed histopathologically according to the guidelines suggested by CERAD[6]. Neuropathologic evaluations were performed by the 2 neuropathologist and 1 neurologist who was trained in that special field. Specimens were rank ordered from No. 1 to No. 84. Entorrhinal cortex and hippocampal subnucleis were rank ordered from No 1 to No 9 in the severity of NFTs and SPs. The specimens which were difficult to identify the rank differences in the severity of neuronal loss, SPs and NFTs, the numberings were done equally.

STATISTICAL ANALYSIS

The brain weight and ages were compared to the neuronal loss and severity of SPs and NFTs in each brain region in each 84 cases. We used Spearman correlation test (p<0.05) in SPSS 9.0 for windows to analyze the correlation between regional neuronal loss and severity of SPs and NFTs in frontal cortex; temporal cortex; hippocampus (each in presubiculum, subiculum, H1, H2, H3, H4, H5); entorrhinal cortex; amygdala (each in lateral, medial, basal, accessory basal, medial, central and cortical subnuclei); piriform cortex; BNM; brainstem (SN and LC). As for the subnucleis of hippocampal area and amygdalar area, the Friedman test were used to compare the degree of pathologic changes of SPs and NFTs.

RESULTS

Brain weight (Table 1)

Of the total 84 cases, male and female was equal in 42. The mean age was 80.17±7.68 (male 81.79±7.68, female 78.55±7.16). The mean brain weight was 987.20±148.49 gm (male 998.45±136.09 gm, female 975.95±160.80 gm), slight heavier in men (Table 1).

Relationship between age, brain weight and neuronal loss of each region (Table 2)

Age revealed significant negative correlation with the brain weight. But it did not show any correlation with the regional neuronal loss, nor with the degree of SPs and NFTs. Nevertheless weight of brain showed significant correlation with neuronal loss in frontal cortex, temporal cortex, BNM, SN but not with the neuronal loss in LC. Although neuronal loss in frontal cortex revealed certain relationship to the regional neuronal loss in temporal cortex and BNM, it did not show correlation with the neuronal loss in SN and LC. Instead

Table 1. Brain Weight

	Male	Female	Total
Case	42 (50 %)	42 (50 %)	84 (100 %)
Mean Age (yr)	81.79±7.68	78.55±7.16	80.17±7.68
Brain Weight (gm)	998.45±136.09	975.95±160.80	987.20±148.49

Table 2. Relationship between age, brain weight and neuronal loss of each region. There were negative correlation between age and brain weight, some positive correlation between frontal and temporal cortex to BNM, BNM to SN and LC, strong positive correlation between SN and LC. (Spearman's correlation)

	Age	Weight	Frontal	Temporal	BNM	SN
Age	1.000	–	–	–	–	–
Weight	-0.277*	1.000	–	–	–	–
Frontal	-0.52	-0.500†	1.000	–	–	–
Temporal	0.095	-0.439†	0.503†	1.000	–	–
BNM	0.007	-0.533†	0.626†	0.692†	1.000	–
SN	0.022	-0.251*	0.152	0.509†	0.624†	1.000
LC	0.043	-0.203	0.096	0.515†	0.564†	0.842†

BNM: Basal Nucleus of Meynert; SN: Substantia Nigra; LC: Locus Ceruleus. *: Correlation is significant at the .05 level (2-tailed). †: Correlation is significant at the .01 level (2-tailed).

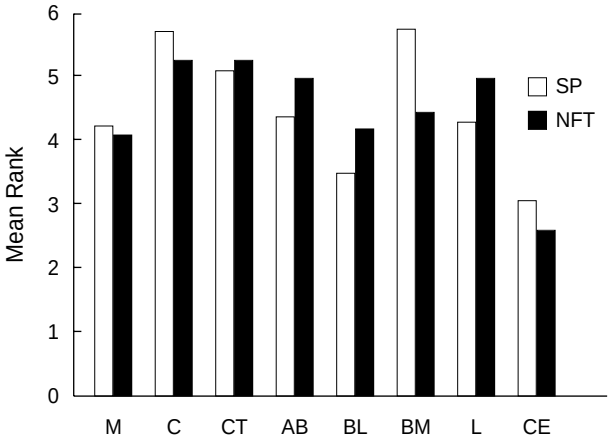

Fig. 1. Rank order of Accumulation of SPs & NFTs in Amygdaloid Nucleus.
SPs were depositied in rank order in decreasing frequency from basomedial, cortical, cortical transitional, anterior basal, lateral, medial, basolateral and central nucleis. NFTs were depositied in rank order in decreasing frequency from cortical, cortical transitional, lateral, anterior basal, basomedial, basolateral, medial and central nuclei.
M: medial, C: cortical, CT: cortical transitional, AB: anterior basal, BL: basolateral, BM: basomedial, L: lateral, CE: central.

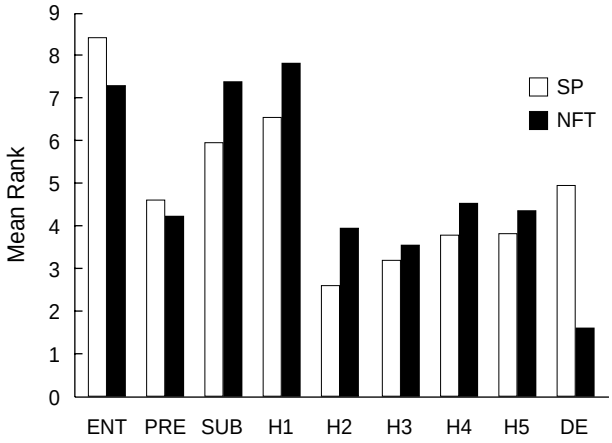

Fig. 2. Rank order of Accumulation of SPs & NFTs in hippocampus.
SPs were depositied in rank order in decreasing frequency from entorrhinal cortex, H1, subiculum, dentate nucleus, presubiculum, H4, H5, H3, H2. NFTs were depositied in rank order in decreasing frequency fromH1, subiculum, entorrhinal cortex, H4, H5, presubiculum, H2, H3, dentate nucleus.
ENT: entorrhinal cortex, PRE: presubiculum, SUB: subiculum, DE: dentate nucleus.

neuronal loss in temporal cortex showed a significant correlation with the BNM, SN and LC.

Alteration of Neurofibrilllary tangle and Senile plaque in amygdaloid nucleus (Fig. 1)

All cases had NFTs in the amygdala. The most pronounced density of NFTs were in the cortical and cortical transitional nuclei. There was consistent and heavy NFTs involvement in accessory basal and lateral nuclei. The medial, basolateal, and central nuclei were largely spared. But there was no significant correlation between the degree of NFTS and age, nor to the brain weight. SPs showed heavy accumulation in the basomedial, cortical and cortical transitional nuclei with relative few in basolateral, medial and central nuclei. In general, the relative densities of NFTS and NP in a given nucleus were almost the same. The only exceptions to the latter observation were the basomedial nucleus, which generally had more SPs than NFTs.

Alteration of Neurofibrilllary tangle and Senile plaque in hippocampus (Fig. 2)

Although a considerable variability in the cortical patterns of NFTs formation was noted previously, a certain regular pattern of vulnerability to NFTs and SP could be established on hippocampus. The entorhinal cortex , H1 and subiculum of the hippocampus were the most consistent and severely affected regions. Relative sparing of SPs in the dentate nucleus were also noted.

Correlation between BNM, Amygdaloid subnuclei and Hippocampal subnuclei

There were no correlation between the neuronal loss in BNM and the severity of NFTs and SPs in laterobasal nucleus of amygdala where the projecting cholinergic area to the BNM. And the laterobasal nucleus and accessory basal nucleus where the projecting cholinergic area to hippocampus, did not show significant correlation with the H1 or subiculum of the hippocampus.

Frontal, temporal, BNM correlation in Substantia Nigra & Locus Coeruleus

Substantia nigra showed significant correlation in view of neuronal loss, severity of NFTs, and SPs with the Locus coeruleus. And both the SN and LC were significantly correlated to the changes of BNM. Only the changes in temporal cortex were significantly interrelated to the changes in SN and LC. But none of these regions were related to brain

weight.

DISCUSSION

We observed some findings that has been prescribed before and some findings that has not been. Our findings are as follows. 1) In hippocampus, entorrhinal cortex, H1 and subiculum were the most consistent and severely affected regions. Dentate gyrus were the least affected area. 2) In amygdala, NFTs were high in cortical and cortical- transitional nuclei. SPs were heavily accumulated in basomedial, cortical and cortical transitional nuclei. 3) BNM did not show any correlation with laterobasal nucleus of amygdala which projects to BNM. 4) Weight of the brain showed significant correlation with neuronal loss in frontal, temporal and BNM but not with the neuronal loss in LC, SN and amygdala. 5) SN, LC as well as BNM were interrelated in the severity of these changes. But neverthless these changes were not correlated with the brain weight.

We now know NFTs and SPs, the two major neuropathological hallmarks of Alzheimer's disease are not evenly distributed throughout the brain. Since the topographic study of Hirano and Zimmerman[7], it is well known that the layer II of the entorhinal cortex, the subiculum, and the CA1 subfield of the hippocampus represent particularly vulnerable sites that display very high NFTs densities in AD, while adjacent areas such as the pre- and parasubiculum or the dentate gyrus are virtually free of tangles[7]. Other studies has also shown NFTs were most pronounced at entorrhinal cortex and CA1, least pronounced at dentate gyrus[8, 9]. We found the differences in the magnitude of NFT formation among anatomically and functionally connected area in hippocampus in decreasing order of frequency from entorhinal cortex, prosubiculum, subiculum, presubiculum and dentate cortex. The most severely affected area was the entorhinal cortex which is affected even in the preclinical stage of AD[5, 22]. As for the dentate gyrus, it was the latest and the least affected area. We also observed that the severity of NFTs were in proportion to neuronal loss.

Other limbic forebrain structure such as the amygdala and BNM, locus ceruleus also constantly contains a high density of tangles. From the review of recent semiquantative study[10] of the pathologic alterations in the amygdala of Alzheimer's disease, NTFs were found to be most pronounced at accessory basal and cortical nuclei, and were not profound at central and laterobasal nucleus. SPs were found to be most pronounced at mediobasal nuclei and relatively less at lateral and laterobasal nuclei. The findings were in concordance with our data.

Amygdaloid involvement in Alzheimer's disease is known, but poorly understood. Brockhaus[11] reported that the amygdala is a consistent site of severe neuropathological alterations in Alzheimer's disease. There was pronounced NFTs formation in the accesory, lateral and cortical nuclei with relative sparing of medial, lateral, laterobasal and central nuclei as L.J. K. Vogt and B.T. Hyman[12] has observed. Recently several hypothesis has been emerged to note that connectional pattern of the amygdaloid nucleus to the entorhinal cortex and to the hippocampus. As noted previously, amygdala has projections to entorrhinal cortex terminating primarily in layer III, with lesser component terminating in layer I and II. The lateral and accessory basal nuclei, cortical nuclei of the amygdala give rise to major entorrhinal afferents forming the terminal entrance to amygdaloid-hippocampal circuit[12]. In Alzheimer's disease NFT pathology of entorrhinal cortex is probably associated with the destruction of this amygdaloid area where is the consistent lesion to be affected.

But the laterobasal nucleus-CA1/subicular zone, accessory basal nucleus-CA1[13] and mediobasal nucleus, cortical transitional area-CA1/subiculum area[14], the connecting subnuclei between amygdala and hippocampus, did not show significant correlation in regard to severity of NFTs and SPs. The NFTs and SPs of laterobasal nucleus of amygdala which receives strong projection from the basal nucleus of Meynert, were not significantly correlated with the neuronal loss of basal nucleus of Meynert.

The degree of AD-related neuronal cell loss in the locus ceruleus has been also been reported [15, 16] and the hypothesis is that as a part of anterograde degeneration, originating in the brainstem, and disrupting metabolic and functional interactions between neurons and glial cell[15-17]. Reduced norarenergic and/or of the cholinergic nucleus of basal forebrain may affect potassium regulating channel of Na^+, K^+-ATPase thus impairing astrocyte reuptake of glutamate [18]. It could be that the increased extracellular glutamate which brings the death of neuronal cells. Alterations in locus ceruleus can lead to Alzheimer's pathology in the brain but it should be expected that these morphological effects may occur somewhat more slowly[19]. These possibilities might explain our findings of relative in-significant correlation of brain

weight reduction to the neuronal loss in locus ceruleus. It should be emphasized that the nucleus basalis of Mynert (BNM) receives an important direct input from locus coeruleus [20] and our data showed significant correlation between the locus ceruleus and BNM. Severity of AD pathology seemed to be more related to the pathologic change of LC and BNM.

Regarding all these results, it is true that increased number of NFTs, SPs with pronounced neuronal loss is in Alzheimer's disease, but it is not likely that these regional vulnerability is the result of disconnection in the amygdala-hippocampal, amygdala-entorhinal cortex and basal nucleus of Meynert-amygdala connecting circuit.

Other assumption is that different regions of the brain have different kinetic roles for the development of NFTs and SPs. There is wide agreement that the entorrhinal cortex and hippocampus would be particularly vulnerable and primary sensory and motor cortices particularly resistant to NFTs. Then what makes these different regional vulnerability for the accumulation of NFTs and SPs in different brain regions?

It might be that as some has proposed that majority of tangle-bearing nuclei in AD belong to the poorly myelinated cortical projection system such as cholinergic basal forebrain [21], norarenergic neurons of the locus coeruleus and serotonergic neurons of the raphe nuclei. And the resistant area such as primary motor cortex and primary sensory cortex belongs to the highly myelinated cortial projection system. This inverse relationship between neurofibrillary pathology and myelinization could possibly indicate some relationship between NFT formation and dendritic plasticity. But still there is perpetuating question why these regional vulnerability exhist.

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