Primary Motor Cortex Involvement in Alzheimer Disease

DOMIZIO SUVÀ, MD, ISABELLE FAVRE, RUDOLF KRAFTSIK, MONICA ESTEBAN, ALEXANDER LOBRINUŚ, MD, AND JUDITH MIKLÓSSY, MD

Abstract. In Alzheimer disease (AD) the involvement of entorhinal cortex, hippocampus, and associative cortical areas is well established. Regarding the involvement of the primary motor cortex, the reported data are contradictory. In order to determine whether the primary motor cortex is involved in AD, the brains of 29 autopsy cases were studied, including 17 cases with severe cortical AD-type changes with discrete to moderate cortical AD-type changes, and 5 control cases without any AD-type cortical changes. Morphometric analysis of the cortical surface occupied by senile plaques (SPs) on β-amyloid-immunostained sections and quantitative analysis of neurofibrillary tangles (NFTs) on Gallyas-stained sections was performed in 5 different cortical areas including the primary motor cortex. The percentage of cortical surface occupied by SPs was similar in all cortical areas, without significant difference and corresponded to 16.7% in entorhinal cortex, 21.3% in frontal associative, 16% in parietal associative, and 15.8% in primary motor cortex. The number of NFTs in the entorhinal cortex was significantly higher (41 per 0.4 mm²), compared with those in other cortical areas (20.5 in frontal, 17.9 in parietal and 11.5 in the primary motor cortex). Our findings indicate that the primary motor cortex is significantly involved in AD and suggest the appearance of motor dysfunction in late and terminal stages of the disease.

Key Words: Alzheimer disease; Associative cortex; Neurofibrillary tangles; Primary motor cortex; Primary sensory cortex; Pyramidal signs; Senile plaques.

INTRODUCTION

Since its first description at the beginning of this century (1–6), the clinico-pathologic entity of Alzheimer disease (AD) (7–9) continues to raise many questions, well summarized in a report of Khachaturian published in 1985 (8).

In AD, the involvement of the entorhinal cortex, the hippocampus, as well as the frontal and parietal associative cortical areas is well established (10–20). It is generally accepted that the primary motor cortex is less involved or even spared in AD (21–28). Only few case reports have described severe involvement of the primary motor cortex in AD (29, 30). As the reported data are contradictory, the aim of this study was to perform a quantitative analysis of senile plaques (SPs) and neurofibrillary tangles (NFTs) to answer the question whether or not the primary motor cortex is involved in AD. The severity of the cortical changes in the primary motor cortex was compared with the severity of those in the entorhinal cortex and in the frontal and parietal associative areas known to be severely involved in AD.

MATERIALS AND METHODS

The brains of 29 autopsy cases were analyzed. The brains were fixed in 10% formalin for 4 weeks. From all the 29 brains about 3 × 3 × 0.5 cm large samples were taken from the following cortical regions: entorhinal cortex, hippocampus, frontal associative cortex (Brodmann’s areas Br 8, 9), parietal associative cortex (Br 39, 40), primary motor cortex (Br 4), and primary sensory cortex (Br 3, 1, 2). In 9 cases an additional sample from the primary visual cortex (Br 17) was also taken. After embedding in paraffin, from all these blocks 5 μm thick paraffin sections were cut and stained with hematoxylin and eosin, Thioflavin S, Congo red, Gallyas silver technique (30), and immunostained with monoclonal antibody to β-amyloid protein (DAKO, M872, dil.1:100). In some cases, sections were also stained with the cresyl-violet technique and immunostained with a rabbit polyclonal anti-myelin basic protein (MBP) (DAKO, A623, dil. 1:100) for the analysis of cyto- and myeloarchitectonics, respectively.

For immunostaining the avidin-biotin-peroxidase technique was used. The sections were deparaffinized, rehydrated, and incubated in 0.3% methanolic peroxide for 30 minutes in order to eliminate endogenous peroxidase activity. The sections were incubated overnight at 4°C with the primary antibody. The biotinylated secondary antibody and the avidin-biotin complex were used following recommendations of the manufacturer (DAKO, ABC-Complex/HRP-Kit, K0355). For the visualization of the immunoreaction dianaminobenzidine was used as chromogen. After immunostaining, the sections were counterstained with hematoxylin. For the detection of β-amyloid, the sections were pretreated with formic acid for 20 minutes before immunostaining.

For the neuropathological diagnosis of AD, in all cases, a semiquantitative analysis of SPs and NFTs was performed on different cortical regions (entorhinal cortex, hippocampus, and frontal and parietal associative areas), as described in a previous study (32). For the diagnosis of AD, criteria proposed by the Consortium to Establish Registry for Alzheimer Disease (CER-AD) (9) and those proposed by Khachaturian were both used (8). The severity of cortical involvement by NFTs was graded
following Braak (20). Based on these staging procedures 17 cases with severe AD-type cortical changes fulfilled the criteria of the neuropathological diagnosis of AD following Khachaturian (Table, cases 1–17). The severity of the cortical AD-type changes in these AD cases corresponded to Braak stages V–VI. The age of the patients varied between 28 and 94 years. Dementia was clinically documented in all cases, and therefore they also fit the diagnosis of AD according to the guidelines of CERAD (9). In addition to dementia, signs of pyramidal involvement were clinically documented in 3 cases, including Babinski sign and/or spasticity. Seven patients, aged 76 to 89 years, had discrete to moderate AD-type cortical changes, insufficient for a diagnosis of AD (Table, cases 18–24). These patients were not demented, except 1 case (Table, case 24), in which the neuropathological examination revealed discrete hypertensive encephalopathy. In this group the severity of cortical lesions following Braak corresponded to stages I–II in 5 cases (Table, cases 18–22) and to III–IV in 2 cases (Table, cases 23–24). The remaining 5 nondemented cases, aged 37 to 61 years, without any AD-type cortical changes formed the third group and served as controls (Table, cases 25–29). Cases with other pathologies, such as Lewy bodies and multi-infarcts were excluded from this study and there were no concomitant vascular or other demenitizing pathologies, with the exception of 1 case. In this case (Table, case 24), the histological examination showed a hypertensive microangiopathy associated with a discrete perivascular pallor of the myelin in the subependymal regions of the deep white matter without lacunar infarcts. The severity of the AD-type cortical changes was not sufficient to explain dementia.

In all 29 cases, a quantitative analysis of SPs and NFTs was performed on sections derived from the following cortical regions: entorhinal, frontal associative (Br. 8, 9), parietal associative (Br. 39, 40), primary motor (Br. 4), and primary sensory cortex (Br. 3, 1, 2). In order to verify the reproducibility and reliability of the results, the quantitative analysis of plaques and tangles (in all cases and on all sections) was repeated independently by another investigator.

Morphometric analysis of SPs was performed on β-amyloid stained sections. Immunostained deposits of β-amyloid of all types of senile plaque were considered (33). The analyzed cortical areas were always selected from regions without vessels containing β-amyloid deposits. Using a computer assisted Leitz microscope, images (each corresponding to 0.16 mm² of cortical surface) were visualized using 10X power objective. These on-line images were analyzed using Samba Immuno v.4.05 software for Microsoft Windows. The morphometric analysis was made on these online images. In selecting the brown color of β-amyloid deposits we were able to eliminate the blue color of hematoxylin-stained nuclei. After the subtraction of background noise and histology artifacts, the total surface occupied by β-amyloid plaques was measured and expressed as a percentage of the total cortical area studied. The analysis was performed in 5 fields selected from the most severely affected parts of each cortical area and a mean was calculated from these fields.

The quantitative analysis of NFTs was performed on Gallyas-stained sections using a Leitz microscope. The number of tangles was counted in 0.4 mm² microscopic fields obtained using 10X power eyepiece and 25X power objective lens (250X magnification). Similar to SPs, the quantitative analysis of NFTs was performed in 5 different fields of the most severely affected regions of each cortical area. Tangles localized on the border of the upper half field were counted and those situated on the borderline of the lower half field were ignored. The results were expressed as the mean number of tangles per 0.4 mm² microscopic field.

In order to determine the reliability of the quantitative study, the analysis of variance (ANOVA) was used to compare the results obtained by the 2 investigators. The same statistical method was used to compare the mean percentage of cortical surface occupied by SPs and the mean number of tangles between the 5 different cortical areas.

The Pearson correlation was calculated and tested for significance in order to analyze if an association exists between the severity of cortical changes among the different cortical areas. β-amyloid and Gallyas-stained sections were used to analyze the laminar distribution of plaques and tangles with the aid of a Zeiss computer microscope (34). On cresyl-violet stained sections, the neuronal loss in layer Ya and Yb of the primary motor cortex was documented using the same computer microscope and the Neurolucida mapping software from Microbrightfield, Inc.

RESULTS

The results of the quantitative analysis of plaques and tangles obtained by both examiners were without statistically significant difference, indicating their reproducibility and reliability. Therefore we used both datasets, giving us a mean value for 10 cortical fields for both plaques and tangles. These data are presented in the Table.

In the brains of all 17 AD cases the primary motor cortex was severely affected by SPs. The percentage of cortical surface occupied by plaques in the primary motor cortex was similar to the one found in other cortical areas (Table; Figs. 1, 2A). In 2 cases, the percentage of cortical surface occupied by SPs was even higher in the primary motor cortex than in the associative cortical areas (Table, cases 4 and 5), and in 3 other cases it was higher than in the parietal associative cortex (Table, cases 3, 6, 16).

The number of NFTs in the primary motor cortex was lower than in the entorhinal cortex, and slightly lower than in the associative cortical areas (Table; Fig. 2B) with the exception of 3 cases. Case 9 showed a higher number of tangles in the primary motor cortex than in the associative cortical areas. In 2 other cases the number of tangles in the primary motor cortex was higher than in the entorhinal cortex (Table, cases 12 and 13).

The majority of cases with discrete to moderate AD-type cortical changes showed the presence of SPs in all examined areas including the primary motor cortex, with the exception of 1 case in which no plaques were found (Table, case 21) in the primary motor and sensory cortex. Neurofibrillary tangles in these 7 cases were restricted to the entorhinal cortex, except in the 2 cases where few tangles were counted in the associative cortical areas. In
# TABLE
Quantitative Analysis of Senile Plaques and Neurofibrillary Tangles in Several Cortical Areas, Including the Primary Motor Cortex

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Senile plaques: For each cortical region, the mean percentage of cortical surface occupied by SPs of 10 different microscopic fields, each corresponding to 0.16 mm², was calculated in 17 AD cases with severe cortical changes (cases 1-17, AD), in 7 cases with discrete to moderate AD-type cortical changes (cases 18-24, DM-AD), and in 5 control cases without any AD-type cortical changes (cases 25-29, CTR). Note the important involvement of the primary motor cortex, as compared with the other analyzed areas. Neurofibrillary tangles: The mean number of tangles per 10 microscopic fields, each corresponding to 0.4 mm², was calculated. Neurofibrillary tangles also accumulate in the primary motor cortex. The highest number of tangles was found in the entorhinal cortex, followed by a significantly lower number of tangles in the associative cortical areas. Compared with the associative areas, the number of tangles was slightly lower in the primary motor cortex. The involvement of the primary sensory cortex and of the primary motor cortex was comparable.
Fig. 2. Cortical surface occupied by SPs and number of NFTs in the primary motor cortex in AD compared with other cortical areas. Mean percentages and standard errors of cortical surface occupied by SPs (A) and mean numbers of NFTs (B) (with standard errors) in AD cases 1–17) in 5 different cortical areas (Table). There was no significant difference between the 5 cortical areas including the primary motor cortex with respect to the percentage of SPs. The number of tangles was significantly lower in the associative cortical areas than in the entorhinal cortex. Compared with the associative areas, the number of tangles was slightly lower in the primary motor cortex, with a difference of borderline significance. EC = Entorhinal cortex; FA = frontal associative cortex (Brodman’s areas 8, 9); PA = parietal associative cortex (Brodman’s areas 39, 40); PM = primary motor cortex (Brodman’s area 4); PS = primary sensory cortex (Brodman’s areas 3, 1, 2).

1 case (Table, case 24), some NFTs were also found in the primary motor cortex. There were no plaques or tangles in any cortical areas of the brain in the control group.

There were some regional differences in the distribution of plaques and tangles with respect to different cortical areas. In 1 case (Table, case 3) the cortical surface occupied by plaques was more than 5 times higher in the frontal than in parietal cortex.

The distribution of the cases with respect to the increasing percentage of cortical surface (by 2% intervals) occupied by plaques and the increasing number of NFTs (by intervals of 3 tangles) in the primary motor cortex is illustrated in Figure 3A and B. In 11 of 17 AD cases, the percentage of cortical surface occupied by SPs in the primary motor cortex was high (14%–20%). In the 7 cases with discrete to moderate cortical changes the number of tangles was less than 3, and in the 5 control cases it was zero. The primary sensory cortex was also severely affected by AD-type changes by both plaques and tangles in proportions similar to that of the primary motor cortex (Table; Fig. 2).

In the 17 AD cases, the statistical analysis did not reveal significant difference between cortical areas regarding the percentage of cortical surface occupied by SPs in the primary motor cortex and in the other cortical areas. The number of tangles was significantly higher in the entorhinal cortex when compared with the other cortical areas ($p < 0.001$). Their number was somewhat lower in the primary motor and primary sensory cortex than in the associative cortical areas, with a difference of borderline significance ($p = 0.04$). There was no significant difference between the number of tangles in frontal and parietal associative cortical areas, or between the primary motor and sensory cortex.

The severity of the primary motor cortex involvement by SPs strongly correlated with that of primary sensory (R = 0.8; $p < 0.001$), entorhinal (R = 0.7; $p = 0.004$), and frontal associative cortex (R = 0.6; $p = 0.01$) (Fig. 4). Concerning the severity of the cortical involvement by NFTs, a high correlation was found between the primary motor cortex and the sensory (R = 0.9; $p < 0.001$), parietal (R = 0.7; $p = 0.009$), and frontal associative cortex (R = 0.6; $p = 0.007$).

Due to the severe degenerative process, the cyto- and myeloarchitectonic hallmarks that delineate cortical layers are less distinct in the 17 AD cases than in the control cases. We did not observe laminar distribution of SPs in the primary motor cortex (Fig. 5). In a few cases a slight tendency toward a laminar distribution with a higher density of plaques in layers III and V was noticed. A tendency for a laminar distribution of NFTs was observed in the primary motor cortex, where tangles were more numerous in layer II, the superior part of layer III, and in layer V (Fig. 5). In the primary sensory cortex, a laminar distribution with a high density of plaques in the inferior part of layer III and in layer IV was observed. We did not observe laminar distribution of NFTs in the primary sensory cortex. In the 17 AD cases, hematoxylin
DISCUSSION

It is generally accepted that the entorhinal cortex, hippocampus, and the frontal and parietal associative areas are severely involved in AD. The primary motor cortex is known to be less involved or even spared in AD (11, 13–16, 18, 20, 23, 24, 28). Based on these data, several authors have proposed that the distribution of plaques and tangles through the cerebral cortex may follow neuronal connections (12, 17, 19, 22, 26), and that the involvement of the associative cortical areas is correlated with their connections to the limbic areas.

Senile plaques are generally reported as being the most abundant in the associative neocortical areas, and less numerous in entorhinal cortex and hippocampus, with the lowest density in the primary cortical areas (12, 16, 20, 21). Neurofibrillary tangles are consistently reported as being very numerous in the entorhinal cortex, followed by hippocampus, the neocortical associative areas, and finally the primary projection areas.

Severe involvement of the primary motor cortex in AD was only occasionally reported (29, 30, 35). Perretti (36) suggested that, after obtaining abnormal electrophysiologic responses in abductor pollicis brevis and tibialis anterior muscles in AD patients using transcranial magnetic stimulation of the motor cortex, sub-clinical dysfunction of the motor cortex neurons is present in AD before the clinical signs become apparent. Specific signs of pyramidal involvement, including Babinski sign, increased deep tendon reflexes, and spasticity have been reported to occur in rare AD cases (29, 30, 37). Moreover, the occurrence of AD-type cortical changes in the primary motor cortex has been described in progressive supranuclear palsy and in amyotrophic lateral sclerosis (38, 39).

Here, using a quantitative analysis, the primary motor cortex of 17 AD cases was analyzed for the occurrence of cortical AD-type changes. The great variation in size and shape of SPs for a same patient and from a patient to another (20, 33) makes counting of plaques poorly reproducible. We therefore have chosen to measure the percentage of cortical surface occupied by SPs.

The shrinkage of the cerebral cortex secondary to techniques used (formalin fixation, paraffin embedding, staining procedures) was neglected, as all brains were processed in the same conditions. Severe atrophic shrinkage of the cerebral cortex secondary to the degenerative process occurs in AD. This severely reduced cerebral cortex will maintain remaining cortical functions. Therefore, in this study, the cortical fields used for morphometric analysis (0.16 mm² for plaques and 0.4 mm² for tangles) in all 29 cases always corresponded to the functionally available cortical surface (whether normal or atrophic).

As there were no other dementing pathologies in the 29 cases used for this study, except 1 case (Table, case 24),

and eosin and cresyl-violet-stained sections showed neuronal loss in all cortical layers of the primary motor cortex including layers Va and Vb. The loss of neurons in layer V, including the Betz cells in layer Vb in a familial AD case, is illustrated in Figure 1E and F. The severity of neuronal loss, including that of Betz cells, varied in different cases. We did not observe neuronal loss in the 5 control cases.

In the 9 severe AD cases (Table, cases 1, 7–12, 14, 16) where the primary visual cortex was available for analysis, a high number of SPs and a high to moderate number of NFTs were observed in this area, except 1 case (case 10), where tangles were not found.
Fig. 4. Correlation between the severity of the cortical changes in the primary motor cortex (PMC) and the frontal associative cortex (FC). A: Shows the correlation between the percentages of cortical surface occupied by SPs ($R = 0.6; p = 0.001$) and (B) shows the correlation between the numbers of NFTs ($R = 0.65; p = 0.006$).

Fig. 5. Illustration of the distribution of SPs and NFTs with respect to the different layers in the primary motor cortex. A and C: The cyto- and myeloarchitecture of the primary motor cortex in a sporadic AD case. Cresyl-violet and immunostaining to myelin basic protein (MBP), respectively. B: The position and the size of NFTs were entered using a computer microscope system (Gallyas-stained section) from a small part of the primary motor cortex, in a sporadic AD case. Only a slight tendency to a laminar distribution is observed; the number of tangles being more numerous in layers II, in the upper part of layer III and in layer V. D: Photomicrograph of a β-amyloid immunostained section showing a diffuse distribution of SPs in the primary motor cortex. Arrowheads in (A) correspond to the borderline between cortex and white matter. Magnification is ×40.
the atrophic shrinkage of the cerebral cortex, if present, was always due to the degenerative process of AD.

The results of the morphometric analysis showed that in the 17 AD cases the primary motor cortex was severely affected. The percentage of cortical surface occupied by SPs was as high as in the other cortical areas, including the associative frontal and parietal areas. In a few AD cases the number of plaques and/or tangles was even higher in the primary motor cortex than in the entorhinal or associative cortical areas.

In agreement with the generally accepted view our quantitative analysis of NFTs showed that the most involved cortical region is the entorhinal cortex. The number of tangles was significantly lower in the associative cortical areas followed by the primary motor and primary sensory cortex. The difference between the number of tangles in associative areas and primary motor cortex showed only borderline significance.

The distribution of the cases with respect to the severity of the AD-type changes showed that the involvement of the primary motor cortex was more severe in the AD group than in the group with discrete to moderate AD type changes, suggesting that AD is a progressive degenerative process. Variation of the severity of cortical changes in the primary cortex in the group of the 17 AD cases was also observed. These data, together with the high correlation of the severity of cortical involvement between different cortical areas (e.g. frontal associative and primary motor cortex), suggest that cortical involvement may further progress even in patients with the definite diagnosis of AD.

Based on the clinical data available, 2 of the 3 AD cases with pyramidal signs had the highest number of tangles in the primary motor cortex. This would suggest that in AD the impairment of cognitive functions will be followed by impairment of motor functions and, that the motor dysfunction may better correlate with the number of NFTs than with the accumulation of SPs.

In the majority of cases with discrete to moderate AD-type cortical changes, SPs were found in reduced number in all cortical areas including the primary motor cortex, suggesting an early appearance of plaques in the primary motor cortex. In 1 case with Braak stage IV, a few NFTs were also present in the primary motor cortex. In 2 AD cases the accumulation of plaques (but not of tangles) in the frontal cortex was much more severe than in the parietal cortex, indicating that regional variations of senile plaque accumulation occur in AD.

The severity of the primary motor cortex involvement by SPs was strongly correlated with that of primary sensory, entorhinal, and frontal associative cortex. The correlation was also high between the number of NFTs in the primary motor cortex and that of the sensory, parietal, and frontal associative cortex.

The other primary cortical areas, the primary sensory and visual cortex, were also involved in AD. There was a high correlation between the involvement of the primary motor and sensory cortex.

If we consider the histopathological criteria of AD according to Khachaturian (8) or CERAD (9) where the diagnosis of AD depends particularly on the number of SPs, our results indicate that the involvement of the primary motor cortex is as severe as that of the frontal and parietal associative areas. However, if we consider the number of NFTs, which is even more significantly correlated with dementia severity (41), in spite of an important number of tangles in the primary motor cortex in all AD cases, their number is somewhat lower than in the associative areas. The distribution of NFTs suggests a progressive involvement of the cerebral cortex from the entorhinal cortex, hippocampus, through the associative cortical areas, to the primary motor and sensory cortical areas. Further analysis of the distribution of tangles in a high number of cases with discrete, moderate, and severe AD-type cortical changes would be of interest to address this point more accurately.

The association between laminar distribution of NFTs and cortico-cortical circuits was reported by several authors (42). The observation that layer V contains a slightly higher density of tangles than the superficial layers in the associative neocortical areas suggested that feedback and lateral projections may be more affected in AD. The preferential distribution of tangles in layers II, III, and V in the primary motor cortex, found in the present study, indicates that neurons of forward, lateral, and feedback projections may all be involved in AD.

In addition, we have observed neuronal loss in all layers of the primary motor cortex, including Betz cells loss in layer Vb. A quantitative analysis of neuronal loss in the primary motor cortex would add further information concerning the severity of the primary motor cortex involvement in AD.

Our results indicate that the primary motor cortex is affected in AD and may well lead to severe motor dysfunction in the late stages of the disease. An assessment of the severity of the motor dysfunction would be necessary to correlate the quantitative results obtained in this study with clinical findings. For a final conclusion, a prospective study with a well-defined protocol for the detection of the severity of motor dysfunction would be essential. The detailed neurological examination, followed by the neuropathological analysis of the primary motor cortex in AD patients would permit a more accurate definition of the clinical significance of the involvement of the primary motor cortex observed in this study.

Our findings suggest that not only phylogenetically new and vulnerable associative brain regions and their
connected areas are affected in AD (24). With the progression of the disease the primary motor cortex also becomes severely involved, suggesting that motor dysfunction will appear in late and terminal stages of the disease.

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