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MACROSCOPIC MORPHOMETRY OF THE HUMAN BRAIN IN NEURODEGENERATION

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ABSTRACT

Human brains were analysed, by means of stereology, at the macroscopic level, for the following reasons: (1) to compare Alzheimer's disease (AD) and Parkinson's disease (PD) and to determine the presence of AD changes in PD, and vice-versa, (2) to detect signs of atrophy in the brains of HIV-1 infected patients, and (3) to provide baseline quantitative data for further morphometric analyses using MR-imaging. The volumes of 20 cortical and 17 subcortical brain structures were estimated using Cavalieri's principle. Furthermore, the surface area and the mean cortical thickness of all cortical structures were measured.
In Alzheimer's disease, the volume of the hippocampus, the parahippocampal gyrus, the basal and medial aspects of the frontal lobe, the insula, the cingulate gyrus as well as the frontal and parieto-occipital white matter were significantly reduced. No changes were seen in subcortical brain structures. In Parkinson's disease, no significant reductions in volume were found. No significant changes were found in the cerebral cortex of HIV-1 infected patients as compared to age- and sex-matched controls. Only a significant reduction in volume was found in the internal capsule. The lack of significant changes in HIV-1 infected brains might be attributed to the selection of the sample which was composed of brains with the neuropathological diagnosis of HIV-1 encephalitis but showing no remarkable gross-anatomical changes.

Key words: Alzheimer's disease, Cavalieri's principle, HIV-1 Infection, Parkinson's disease, Volume estimation

INTRODUCTION

Neurodegenerative disorders of the human brain encompass a variety of clinical and neuropathological entities. They include, among others, Alzheimer's disease (AD) and Parkinson's disease (PD). Qualitative description of atrophic changes at the gross-anatomical level as well as neuronal loss, occurring in various brain regions at varying degrees, at the light-microscopic level, was done.
The infection of the nervous system with the human immunodeficiency virus 1 (HIV-1) leads to the degeneration of different neuronal systems with neuronal loss (Ketzler et al., 1990;

Weis et al. 1993a; Weis, 1992), reactive astrocytosis (Weis et al., 1993b), activation of microglia (Weis, 1992), and vascular changes (Weis, 1992).

With the advent of nuclear magnetic resonance imaging (MRI), it became possible to visualise the brains of living persons and patients without using any radiation at all. The MRI-scans can be generated multiplanarly, and a good resolution between gray and white matter is possible. Since no radiation is applied, the section thickness can be chosen to be appropriate for morphometric measurements. Stereologic investigations on the brains of normal controls and persons with Down's syndrome have already been performed and gave good quantitative descriptions of changes occurring in the brains of persons with Down's syndrome (Weis et al., 1991).

In the present study, quantification of brain structures was done on autopsy brains using the Cavalieri principle. This method was applied to detect differences in the volume of various brain regions in patients with AD, PD, and HIV-1 infection as compared to normal controls.

MATERIALS AND METHODS

The autopsy brains were derived from normal controls (n=78), patients with Alzheimer's disease (n=20), patients with Parkinson's disease (n=12), and from HIV-1 infected patients (n=15). One hemisphere was randomly chosen and used for morphometry, whereas the other hemisphere was processed for routine neuropathological diagnosis.

Volume measurements were based on Cavalieri's principle (Cavalieri, 1635; Gundersen and Jensen, 1987; Cruz-Orive, 1987, Weis et al., 1991). The first cut through the brain was done randomly followed by systematic sections with a constant section thickness. All sections were photographed with a linear ruler. The measurements were done on photographs by point-counting using a grid with a 4mm space between the points. The surface area was estimated using a linear grid following the outlines of Elias et al. (1969). The combination of the volume and the mean surface area of the same brain region resulted in the mean cortical thickness of that region.

The volumes of the following brain structures were determined (abbreviations in parentheses): the medial (fo med), the lateral (fo lat), and the basal (fo bas) aspects of the frontal lobe; the frontal lobe (frontal) composed of fomed+folat+fobas, the precentral gyrus (precent); the postcentral gyrus (postcen); the lateral aspect (te lat), the basal aspect (te bas) of the temporal lobe, and the parahippocampal gyrus (te para); the temporal lobe (temporal) composed of telat+tebas+ tepara; the hippocampus (hippo); the medial aspect (pa med), and the lateral aspect (pa lat) of the parietal lobe; the parietal lobe (parietal) composed of pamed+palat; the medial aspect (oc med), the basal aspect (oc bas), and the lateral aspect (oc lat) of the occipital lobe; the occipital lobe (occipital) composed of ocmed+ ocbas+oclat; the insular gyrus (insula), the cingulate gyrus (cingulate), and the amygdaloid body (amygdala). Among the subcortical structures, the caudate nucleus, the putamen, the globus pallidus, and the thalamus were investigated. The white matter was subdivided into a frontal, a temporal, and a parieto-occipital part. The ventricles were subdivided following the same pattern.

The coefficient of error (CE), as an indicator of the precision in the measurement of individual brain structures, was calculated for each brain structure. Details regarding CE are given in Gundersen and Jensen (1987) and in Weis (1991).

For the statistical analysis of the data, the non-parametric Mann-Whitney-U-test was applied using the SPSS/PC package (Statistical Package for the Social Sciences).

RESULTS

Differences between the right and the left hemisphere were statistically assesed. In this sample, the side of the hemisphere had no influence on the noted differences between control brains and the neuropathological groups. This is in accordance with previous results of our group (Weis et al., 1989).

The results of the volume estimation of cortical regions in Alzheimer's disease as compared to age-matched controls are given in Tbl. 1. In AD, the volume of the frontal lobe was significantly reduced; this was attributed to a significant reduction in volume of its lateral and basal aspects. The hippocampus, the parahippocampal gyrus, and the medio-basal aspect of the temporal lobe showed a significantly reduced volume. The volume of the lateral aspect of the occipital lobe was significantly reduced in AD. Likewise, the volume of the insular lobe and the cingulate gyrus were significantly reduced in AD. In the parieto-occipital white matter, a significant volume reduction was also noted (data not shown). No significant changes were found in the parietal lobe. No changes were seen in subcortical brain structures (data not shown).

Table 1. Volume estimation of the cortical and subcortical brain regions in Alzheimer's disease. Volumes are given in cm^3. Measurement was done on one hemisphere.

	Controls		Alzheimer		
	mean	(sd)	mean	(sd)	p
frontal	64.64	(17.0)	51.98	(10.3)	0.03
fo med	17.23	(4.8)	13.75	(2.8)	0.01
fo lat	36.13	(11.9)	28.94	(7.4)	0.09
fo bas	11.24	(2.1)	9.30	(1.1)	0.00
precent	13.90	(5.7)	10.89	(2.2)	0.13
postcent	13.82	(3.0)	11.61	(2.8)	0.06
temporal	50.69	(12.4)	41.09	(6.7)	0.02
te lat	25.10	(6.7)	20.82	(4.3)	0.07
te bas	21.97	(5.4)	18.07	(3.9)	0.03
te para	3.81	(1.0)	2.20	(0.7)	0.00
hippo	2.74	(0.9)	1.71	(0.6)	0.00
parietal	33.72	(9.1)	31.19	(9.8)	0.57
pa med	8.58	(3.6)	7.46	(2.8)	0.48
pa lat	25.14	(6.6)	23.74	(7.5)	0.50
occipital	44.37	(10.5)	36.01	(8.6)	0.03
oc med	12.74	(4.0)	11.47	(3.9)	0.43
oc bas	7.57	(3.5)	5.70	(2.5)	0.12
oc lat	24.06	(6.3)	18.84	(4.8)	0.02
insula	5.54	(1.6)	4.64	(1.9)	0.04
cingulate	8.56	(1.9)	6.77	(1.4)	0.00
amygdala	0.98	(0.4)	0.72	(0.4)	0.07

In Parkinson's disease, no significant reductions in volume were found (data not shown). Upon analysing the volume, surface area, and the mean cortical thickness of various brain

regions, no significant changes were found in the cerebral cortex of HIV-1 infected patients as compared to age- and sex-matched controls (Tbl. 2). A significant reduction in volume was found only in the internal capsule.

Table 2. Volume estimation of the cortical and subcortical brain regions in HIV-1 infected patients. Volumes are given in cm^3. Measurement was done on one hemisphere.

	Controls		HIV-1		
	mean	(sd)	mean	(sd)	p
frontal	62.47	(9.7)	58.29	(8.9)	0.24
fo med	16.42	(3.5)	15.38	(2.6)	0.44
fo lat	35.09	(5.4)	33.20	(6.2)	0.48
fo bas	10.97	(2.5)	9.72	(1.6)	0.23
precent	11.86	(2.4)	12.01	(3.0)	0.98
postcen	12.08	(3.9)	11.18	(2.0)	0.59
temporal	46.35	(7.4)	47.42	(5.7)	0.44
te lat	24.02	(4.6)	25.89	(5.2)	0.26
te bas	19.17	(3.1)	18.09	(2.8)	0.29
te para	3.16	(0.7)	3.47	(0.8)	0.36
hippo	3.34	(0.6)	2.74	(0.9)	0.08
parietal	28.85	(7.5)	26.38	(6.3)	0.47
pa med	8.63	(2.6)	8.39	(3.0)	0.47
pa lat	20.22	(5.5)	17.99	(4.1)	0.31
occipital	51.85	(10.1)	48.15	(8.3)	0.38
oc med	18.96	(4.4)	16.12	(3.4)	0.12
oc bas	4.65	(1.9)	5.42	(1.7)	0.17
oc lat	28.24	(5.8)	26.62	(6.2)	0.47
insula	5.30	(1.1)	5.29	(0.7)	0.96
cingulate	8.06	(1.8)	8.30	(1.7)	0.70
amygdala	0.94	(0.4)	0.95	(0.4)	0.90

DISCUSSION

When analysing the brain at the gross-anatomical level, the volume, the surface area, and the mean cortical thickness have to be determined for cortical regions, whereas, for subcortical structures, the volume is the only meaningful parameter.

The brain must be subdivided into various regions. Measuring the volume of the whole hemisphere or the four major lobes, i.e. the frontal, the parietal, the temporal, and the occipital lobe does not give enough information. Although the assessment of the neuropathological changes of degenerative processes necessitates precise localisation of the changes, recent investigations lack a thorough subdivision of the control and diseased brain into well-defined regions (Henery & Mayhew, 1989; Regeur & Pakkenberg, 1989; Mayhew et al, 1990; Pakkenberg, 1991; Oster et al., 1993).

In some studies, quantification of changes occurring in AD at the gross-anatomical level was done (De la Monte, 1989; Gelman & Guinto, 1992; Mann, 1991). However, the basic requirements of stochastic geometry were not fulfilled. In none of these studies the three-dimensional parameter, i.e. the volume, of a brain structure was measured. The authors

claimed to cut the brain at "standardized points" or "standardized landmarks" and, thus, to make the measurements done on a brain section comparable to the same section of other brains. However, a standardized landmark does not really exist in the human brain. In all of these studies, the profile area of brain regions on the section was measured. Thus, De la Monte (1989) described global cerebral atrophy of both cortex and white matter as well as a selective atrophy of the amygdala and hippocampus, all being findings also described in the present study. Mann (1991) reported that atrophy of the cerebral cortex was globally distributed, although the temporal lobe was most severely affected. Grey and white matter was in general affected equally. Furthermore, Mann (1991) described that atrophy was also present within the basal ganglia, particularly the caudate nucleus and putamen. The latter changes were not observed in our study. It is obvious that the changes reported in both papers lack the subdivisions necessary for a more precise localisation of atrophic changes. The significant reduction in volume of the hippocampus and the parahippocampal region in Alzheimer's disease was recently shown on MRI-scans (Kesslak et al., 1991). Although, from a clinical standpoint, it is known that AD patients develop PD signs and vice-versa, we could not show at the gross-anatomical level that specific AD changes are found in PD or vice-versa. This is supported by the finding that the histopathologic changes differ between AD and PD, and that only in a small number of cases with PD AD-specific changes are found, but might not contribute to atrophic changes in PD.

No significant changes were found in the cerebral cortex of HIV-1 infected patients as compared to age- and sex-matched controls. Only a significant reduction in volume was found in the internal capsule. Our findings differ from those reported by Oster et al. (1993). The lack of significant differences might be attributed to the sample which, in our investigated group, was composed of brains with the neuropathological diagnosis of HIV-1 encephalitis but showing no remarkable gross-anatomical changes. Howerver, exact comparison of the data between both investigation groups is not possible, since Oster et al. (1993) did not use any elaborate subdivision of the brain. In a recent study, Gelman & Guinto (1992) quantitated the size of cerebrospinal fluid (CSF) spaces on autopsy brains by measuring the profile area on several brain sections, and correlated the autopsy findings with data obtained by analysing CT-scans. They reported that in 58% of the AIDS cases, the CSF space index was two standard deviations above the mean of the age-matched control subjects. The CSF spaces were expanded the most in the frontal and temporal lobes. However, it is very difficult to follow this approach since delineation of CSF spaces on autopsy brain slices is not possible as the natural boundaries, i.e. the bony skull cannot be considered.

Cavalieri's principle for volume estimation of structures is a very powerful stereological tool and, when applied to well delineated brain structures obtained from autopsy slices or MRI scans, provides objective data. The application of stereological techniques on autopsy material provides baseline data for further investigations using neuroradiologic imaging modalities. Then, the use of stereologic methods to investigate MR scans is the most suitable method for studying quantitative morphologic changes in the central nervous system in a living population for cross-sectional as well as longitudinal studies.

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