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SEXUAL DIMORPHISM IN VOLUME OF INSULAR CORTEX IN NORMAL AND NEURODEGENERATVIE HUMAN BRAINS: A STEREOLOGIC AND MACROSCOPIC STUDY

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Abstract-Little is known about the sexual differences in volume of human insular cortex in normal subjects and those suffering from neurodegenerative diseases. The objective of this study is to investigate the sex difference in volume of the left insular cortex in normal right-handed subjects versus subjects suffering from Alzheimer and Parkinson diseases. This study was performed on 72 normal human brains (38 males, 34 females), 11 brains suffered from Alzheimer (4 males, 7 females), and 13 brains suffered from Parkinson (9 males, 4 females). The right hemispheres were used for neuropathologic studies. The volumes of the left insular cortex in the male and female normal subjects were 6.65 ± 1.55 cm^3 and 5.83 ± 1.12 cm^3, respectively ($P = 0.01$). The volumes of the left insular cortex in the male and female subjects suffering from Alzheimer were 5.68 ± 1.49 cm^3 and 4.49 ± 0.86 cm^3, respectively ($P = 0.21$). The volumes of the left insular cortex in the male and female subjects suffering from Parkinson were 5.99 ± 1.05 cm^3 and 5.37 ± 0.51 cm^3, respectively ($P = 0.18$). The present study shows a significant larger left insular cortex volume in normal right-handed males than in females. No significant sexual difference in volume of the left insular cortex in subjects suffering from Alzheimer and Parkinson diseases was observed. Disappearance of the normal sexual dimorphism in the volume of the insular cortex may be due to a more severe degeneration of this cortical area in males during the neurodegenerative disorders.

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INTRODUCTION

It is now an established fact that there are sex differences in the cognitive functions, the brain physiology and its structure. A few studies have shown that men might have better intellectual skills in spatial cognition and numerical information (1, 2), whereas women have better speech abilities (3, 4). These cognitive differences between the two sexes are also reflected in the physiological (5-7) and structural (8-15) differences of their brains.

Over a century after the discovery of sex differences in the structure of the human brain, and the fact that men have a bigger brain than women (16) even after the adjustment of the body size factor (17-18), there have only been a few systematic morphometric studies on a sufficient number of autopsy samples. Therefore, some of the fundamental questions about the sex differences in the brain structure are still unanswered. For example, whether the sex differences in volume of the cerebral cortex are regional or generalized (19). Furthermore, it is important the scientists to be aware of and to appropriately control for gender differences when conducting morphologic studies of disease pathology in the brain (19).

On the other hand, epidemiologic studies have revealed a higher prevalence and a faster progression of Parkinson disease (PD) in males as compared to

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females (20, 21). The research for a definitive gender difference in Alzheimer disease (AD) has resulted in a multitude of epidemiological findings that point to a higher prevalence and incidence of AD in females than in males (22). Although it is known that there are gender differences in prevalence (and probably in progression) of AD and PD, the effects of these two neurodegenerative disorders on normal pattern of sexual dimorphism in volume of different areas of cerebral cortex have not been studied as yet.

The insular cortex is one of the most important areas of cerebral cortex. According to Brodmann's classification, the insular lobe is the fourth principle region of the brain including the areas 13 through 16, and is known as the fifth lobe of the brain (23). The insula is buried deep in the lateral sulcus. In primates, such as humans, the insula has extensive connections with the other cerebral cortices, the basal nuclei, the amygdaloid body, limbic areas and the dorsal thalamus (24). These extensive connections along with various clinical and laboratory evidences have led to the contribution of different functions to the insular cortex. It has been considered a visceral sensory area, a visceral motor (autonomic) area, a motor association area, a vestibular area, a language area, and recently, a somatosensory area (25). Lately, it has also been mentioned as an integration of the limbic cortex (25). On the other hand, the insular cortex is also known as one of the regions which is involved in the early stages of the AD (26).

There are only two studies that investigate sexual dimorphism in insular volume of normal human brains (27, 28). These two studies investigate sex differences in volume of insular cortex using magnetic resonance imaging (MRI). MR images, even under best resolution, never have as good quality as photographs obtained directly from macroscopic sections in autopsied brains. Since there was no postmortem study on sexual dimorphism in volume of insular cortex in normal and neurodegenerative subjects, a study on sufficient number of autopsied brains could be useful to determine whether a sex difference in volume of insular cortex is present in normal subjects and if neurodegenerative disorders can alter the normal sex difference or not.

This study was designed to identify the normal pattern of sexual dimorphism in volume of left insular cortex in human autopsied brains obtained from normal right-handed subjects and to detect the effects of AD and PD on this normal pattern of gender difference using an unbiased design-based stereologic method.

MATERIALS AND METHODS

This is a descriptive cross-sectional study on three groups of human brains obtained postmortem at autopsy. The first group consisted of 72 normal human brains (38 males, 34 females). The ages of men in this group were from 58 to 84 (mean ± S.D. was 69.3 ± 7.5). The women in normal group were between the ages of 61-83 (mean ± S.D. was 67.2 ± 5.5). The second group consisted of 11 human brains suffering from AD (4 males, 7 females). The ages of men in this group were from 61 to 81 (mean ± S.D. was 69 ± 7.7). The women in AD group were between the ages of 60-79 (mean ± S.D. was 68 ± 4.6). The third group consisted of 13 human brains suffering from PD (9 males, 4 females). The ages of men in this group were from 63 to 78 (mean ± S.D. was 69 ±71.8 ± 5.1). The women in PD group were between the ages of 65-81 (mean ± S.D. was 72.5 ± 6.8). All samples were collected among right-handed subjects from the Department of Neuropathology, Munich University, Germany.

The brains were extracted from the cranium in a maximum of 24 hours after death, were weighed and then suspended with a thread passing beneath the basilar artery in a container of 4-5 liters of 4% formalin solution (29). The minimum fixation time was four weeks. The brains were kept in the formalin solution long enough to ensure the resumption of the brains to normal state from the initial swelling caused by fixation (30). Upon completion of the fixation, the brains were prepared for neuropathological and morphometrical studies. The two hemispheres were separated with a midsagittal section. The right and left hemispheres were used for neuropathological and morphometrical studies, respectively. The right hemispheres were examined with routine postmortem tests to verify the diagnosis of AD and PD.

Right-handed subjects who had no evidence of neurologic or psychiatric disorders and head trauma in their past medical histories with non-neurologic causes of death were included as normal group in this study. The autopsied brains were excluded from normal group if they had any definitive or suspicious abnormality in routine postmortem tests.

Right-handed subjects with clinical diagnosis of AD without any other neurologic or psychiatric disorders in their past medical histories were included as AD group in this study. The autopsied brains were excluded from AD group if the clinical diagnosis was not confirmed or any other disorder was found in routine postmortem tests. Right-handed subjects with clinical diagnosis of PD without any other neurologic or psychiatric disorders in their past medical histories were included as PD group in this study. The autopsied brains were excluded from PD group if the clinical diagnosis was not confirmed or any other disorder was found in routine postmortem tests.

The left hemisphere of each brain was then sectioned in a series of parallel 5 mm slices in coronal plane using a macrotome. The first coronal section was randomly passed through the frontal pole. Each slice was individually numbered and photographed against a ruler (Fig. 1).

The insular cortex over the coronal sections of the left hemisphere can be discriminated from the neighboring areas with the insular circular sulcus. The border between the gray matter and the white matter was also distinctly visible in these sections. After determining the outline of insular cortex on photographs (Fig. 2), the Cavalieri's Principle was employed to measure the volume of the insular cortex. According to this principle, we can measure the volume of a structure from the total sum of a set of equally and sufficiently spaced parallel sections of that structure.

The volume (V) can be calculated by multiplying the sum of the surface areas of all the sections (ΣAi) by the thickness of the sections (t). The Cavalieri's Principle yields this formula: $V = t. \Sigma A_i$. To measure the surface area of each of the sections of the structure, the point counting method is usually used (Fig. 3).

The formula for the calculation of the volume based on the point counting method will be: $V = t. \Sigma P_i.\alpha_{(p)}$; where the volume of the structure (V) is calculated by multiplying the thickness of the sections (t) by the sum of all the points in the point grid that meet the surface area of the structure in all sections (ΣP_i) by the surface area of one point in the point grid ($\alpha_{(p)}$) (31).

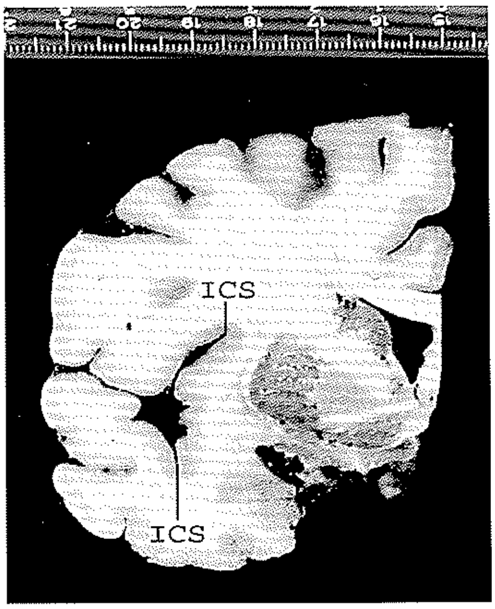

Fig. 1. Coronal macroscopic section of left hemisphere in an autopsied brain against a ruler: the insular cortex is well-defined in this photograph; ICS = Insular circular sulcus.

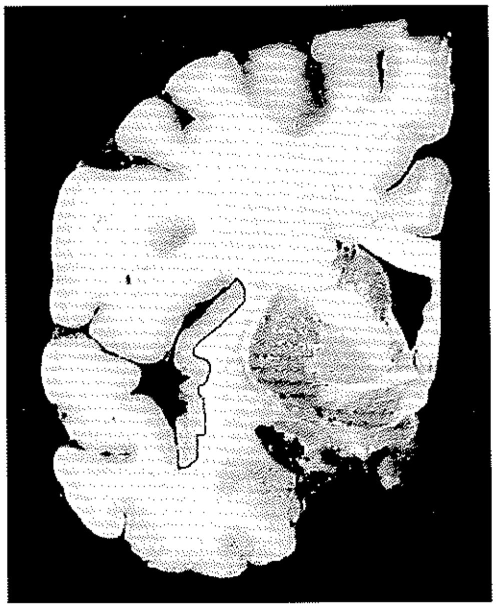

Fig. 2. Determining the outline of insular cortex on macroscopic photographs. the Cavalieri's principle was employed to measure the volume of the insular cortex.

Fig. 3. Calculation of the surface area of insular cortex using point counting of a point grid on macroscopic photograph.

The appropriateness of the thickness of sections and the point distances of the point grid were checked by calculating the CE (coefficient of error) according to Gundersen-Jensen formula (32-34). The appropriateness and efficiency of the 5 mm sections had also already been proved in the studies of Weis *et al.* (33, 34). The data provided by this study were finally analyzed employing the statistical software SPSS in a PC (pentium 4). To identify the significance of the difference between the two sexes, *t* Student and non-parametrical Mann-Whitney tests were used (35). *P* value of 0.05 was accepted as the level of significance.

RESULTS

Table 1 shows the volume of left insular cortex in all three groups of brains. The left insular cortex in normal right-handed subjects was 12.33% smaller in females than in males ($P = 0.01$). The volume of left insular cortex in right-handed subjects suffering from AD showed no significant difference between two genders ($P = 0.21$). The volume of left insular cortex in right-handed subjects suffering from PD was also similar between two sexes ($P = 0.18$).

DISCUSSION

The results of the present study show that there is a sex difference in volume of the left insular cortex in right-handed normal subjects.

Although the brains of men are larger than that of women, this sexual dimorphism is not generalized and differs from one area to another. For example, despite the significant sexual difference in the volume of the frontal lobe cortex, the volume of the precentral gyrus, one of the main substructures of the frontal lobe, is similar between the two sexes (36, 37). Therefore, it is essential to study the sexual differences in the volume of each cortical area or gyrus separately.

The results of current study, which are obtained from postmortem brains, confirm the results of the two recent MRI studies on normal right-handed subjects by Allen *et al.* (27,28), which have also shown that the insular cortex volume of healthy men is significantly higher than that of healthy women. Nevertheless, the present study had some advantages compared with Allen *et al.* studies, *i.e.* it was performed on a larger sample size (72 subjects versus 46 subjects) and with better resolution of images (direct photographs from macroscopic brain slices versus MR images).

Despite all the effort, no other paper was found to have studied the sex difference in the insular cortex volume in healthy subjects in order to compare with the results of the current study.

Table 1. The volume of left insular cortex in right-handed subjects in normal, Alzheimer, and Parkinson groups in two genders (cm^3)

Group	Males		Females		Percentile decrease in females compared with males	*P* value
	Mean	S.D.	Mean	S.D.		
Normal	6.65	1.55	5.83	1.12	12.33	0.01*
Alzheimer	5.68	1.49	4.49	0.86	20.95	0.21
Parkinson	5.99	1.05	5.37	0.51	0.18	0.18

* $P < 0.05$, significant difference between two sexes

The insula is considered as a limbic integration cortex in view of its extensive connections with different parts of the limbic system (25). Because of their role in excitements, areas connected to the limbic system have always been a center of attention for sex differences. Some authors have mentioned the insula as a language area (24). The insular cortex also has a role in memory tasks related to language and auditory processing underlying speech (25). Furthermore, the insula participates in the verbal component of working memory and selective visual attention (25). Although the language areas of cerebral cortex, such as the dorsolateral prefrontal cortex and the superior temporal gyrus, are usually larger in women than in men (38), based on the present results in autopsied brains and the results of Allen *et al.* (27, 28) using in vivo MR images, it can be argued that the insula is the only language area with a larger volume in men than in women.

The insula is one of the first areas of the cortex to be inflicted in the AD (28). Foundas *et al.* using volumetric MRI study have shown that the patients with AD compared with the age and sex matched controls undergo an atrophy of the insular cortex in early stages of disease, a fact that can be used as a diagnostic marker (26). On the other hand, it is proved that AD and PD have a sex difference in their prevalence (20-22). Furthermore, it is suggested that the progression of PD might be more rapid in males as compared with females (20). Nevertheless, the changes in normal sexual dimorphism in volume of specific cortical areas had not been studied in subjects suffering from these two neurodegenerative disorders.

The results of present study show that AD and PD diminish the normal pattern of sexual dimorphism in volume of insular cortex. Disappearance of normal sexual dimorphism in volume of insular cortex exhibits a more severe atrophy of insular cortex in men as compared with women. This is the first study which investigates the sexual dimorphism in volume of insular cortex in AD and PD.

In conclusion, this study showed that the volume of left insular cortex was larger in normal right-handed males than females, a difference whose functional significance remains to be discovered. This normal sexual dimorphism is diminished in subjects suffering from AD and PD. This may be due to a more severe degeneration of insular cortex in men than in women. So it is postulated that these two neurodegenerative disorders have a gender difference not only in prevalence but also in severity. The results of this study concerning about gender difference in severity of insular atrophy during AD and PD must be considered preliminary and further research in this area is needed.

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