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An investigation of olfactory bulb and entorhinal cortex volumes in both patients with Alzheimer's disease and healthy individuals, and a comparative analysis of neuropeptides

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Abstract

Alzheimer's Disease (AD) is the most common neurodegenerative disease and is hard to diagnose at the early stages. The pathogenesis of AD is associated with the loss of a sense of smell. Reduction in the volumes of the Olfactory Bulb (OB) and Entorhinal Cortex (EC) is positively correlated with the decline of the smelling function where OB projects to EC. This study aims to detect the early changes in OB and EC volumes in AD patients by comparing them to healthy subjects. This study also aims to make a comparative analysis of plasma levels and the relationship between arginine-vasopressin (AVP) and Oxytocin (OT), which are neuropeptides associated with cognitive functions. The participants comprised 9 AD patients and 12 healthy individuals. We used volumetric methods such as MRICloud and IBASPM to measure the OB and EC volumes with the help of 3D MRI (Magnetic Resonance Imaging) images. We compared the left and right differentiation. Moreover, we investigated the neuropeptide levels in blood samples from the participants. We conducted a correlation analysis for all parameters. Bilateral OB atrophy was discovered in the AD patients in comparison to the control group ($p=0.002$ for right; $p=0.015$ for left). The right OB volume was measured to be larger than the left OB volume in the control group,but this asymmetry was not observed in the AD patients. The right and left EC's of the AD patients were atrophic in comparison to the control ($p<0.001$). The atrophy of the left EC was measured to be higher than that of the right EC ($p=0.0008$). There was no significant difference between the OT and AVP plasma levels of the AD patients and the control group. The study revealed that the OB and EC volumes of the AD patients were bilaterally reduced in comparison to patients of similar ages. This outcome may indicate that an MRI scan examination of OB and EC volumes may help early AD diagnosis.

Keywords: Alzheimer's Disease, olfactory bulb, entorhinal cortex, oxytocin, arginin vasopressin

Introduction

Alzheimer's Disease (AD) is the most common neurodegenerative disease in the elderly, characterized by a clinical progressive impairment in cognitive functions and dementia [1]. It is hard to diagnose AD at early stages because it is an insidious illness with slow progress [2,3]. Sensory impairment has the potential to indicate the early signs of the disease [4]. Various pieces of evidence since the showed that the pathogenesis of Alzheimer-type dementia is associated with a change in smelling function [2, 3, 5]. 85% of early-stage AD patients encounter disorders in smell acuity and odor recognition before a decline in cognitive functions [1, 6]. However, more than 90% of AD patients are unaware of their smelling problems before having a test [1, 6]. The sense of smell has a significant role in the physical and mental well-being of people [7]. The olfactory bulb (OB) is the first step in the smelling function [8]. OB directly projects to the piriform and entorhinal cortex (EC). Secondary projections reach the amygdala and insula. Other secondary and tertiary projections reach structures such as the hippocampus, anterior cingulate cortex, and orbitofrontal cortex [9]. The loss of odor recognition in AD is associated with the olfactory projection region in OB and especially the hippocampus CA1 region [1]. MRI (Magnetic Resonance Imagery) examinations showed a high correlation between smelling function and OB volumes. As age

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increases, OB volume decreases, which causes a decline of smell acuity and odor recognition in the elderly [6, 10]. The sensory system is the first region of the brain that is affected by AD [1]. Post-mortems tudies show that pathological changes, especially neurofibrillary tangles, are observed in the entorhinal and trans-entorhinal regions, anterior olfactory nucleus, and OB at early stages [1, 2, 4]. The long-term memory of cognitive function depends on the hippocampus and EC [6]. The olfactory system is the only primary sensory structure with a direct projection to EC [6]. Afferent inputs to the hippocampus have a significant role in spatial and episodic memory [11]. Moreover, EC activity is modulated with the input received from the hippocampus and EC returns to OB [11]. EC seems to contribute to smelling function as a top-down and bottom-up modulator. EC damage due to olfactory function disorder and deafferentation of the hippocampus impairs the episodic memory consolidation in AD patients [6].

Arginine-vasopressin (AVP) and Oxytocin (OT) are produced in the supraoptic nucleus (SON) and paraventricular nucleus (PVN) of the human hypothalamus [12-14]. These peptides have both central effects and peripheral effects like endocrine effects[12, 13]. AVP has a role in blood pressure and peripheral osmoregulation, whereas OT is associated with birth and lactation [13]. Furthermore, AVP and OT fibers take their role in recognition function by innerving many brain regions [12, 15]. These extra-hypothalamic projections are considered to be anatomic-centered for the effects of AVP and OT on cognitive functions [12].

In light of this information, we may conclude that investigating OB and EC volumes that may imply the early changes in AD patients and making a comparative analysis of AVP and OT which are related to cognitive function gain significant importance. Correlation of volume changes and neuropeptides in patients and control groups may provide a new perspective to reconsider the early-onset indicators of AD. This study aims to identify the relationship of neuropeptides with EC volume with the help of a radiographical analysis of OB in mild AD patients and a healthy control group.

Material and Methods

We conducted this study after ethical approval by the Hatay Mustafa Kemal University Clinical Research Medical Ethics Committee (Ethical permission no:2018/19). We included 9 AD patients at mild stage determined according to the NINCDS-ADRDA Alzheimer's criteria (National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association) [16]. The control group consisted of random individuals with no brain pathology or disease equivalent to the patients' ages and education levels. The exclusion criteria were brain trauma, brain tumor, attacks, or clinical history with other accompanying psychological symptoms. We started with 15 volunteering patients and 15 healthy persons but had to exclude some of them due to the tumor, exitus, and MRI scan limitations yielding 9 patients in the AD group and 12 healthy people in the control group. We received the individual consent of the participants in the healthy group and the legal custodian consent of the participants in the patient group. We examined the 3D axial brain MRI images of both groups to measure EC and OB volumes with the help of and IBASPM (Individual Brain Atlas Using Statistical Parametric Mapping) methods. We used T1 weighted images scanned with a Philips Ingenia 1.5T MRI system (1.5 T, Philips Healthcare, Best, the Netherlands).

MRICloud and IBASPM Analysis

We used MRICloud for Entorhinal Cortex volume measurement in the lobar analysis and IBASPM for olfactory bulb measurement. T1 weighted MRI images are used for both methods. One should convert the images to analysis format (hdr/img) for MRICloud and IBASPM compatibility. MRI_convert or MRIcron is used for the conversion [17]. We selected the T1-MultiAtlas option for segmentation on the website:https://braingps.mricloud.org/ to create the hdr/img images and uploaded them to measure the volume of the EC. We downloaded the result.zip/output file including a statistical analysis of the tissue volume measurements of the uploaded images from "my job/status/action" folder in 24 hours.

IBASPM is a free toolbox in MATLAB for segmenting structures in MRI images (http://www.thomaskoenig.ch/Lester/ibaspm.htm). MRI images are segmented in three different brain tissues as white matter, grey matter, and cerebrospinal fluid [18]. An MRI image converted to the .img format is used for the measurement of the olfactory bulb volume. We uploaded the file to MATLAB for segmentation and opened IBASPM. IBASPM completed the consecutive processes such as segmentation, labeling, and atlasing (Fig. 1). The "volume statistic" of IBASPM computes an individual atlas for each brain structure. After the computation is completed, the function generates a file including the statistical analysis of the volumes. We obtained the results of the participants in both patient and healthy groups from this file.

Neuropeptide Analysis

We collected blood specimens from the participants to evaluate the effects of OB and EC volumes and neuropeptide hormones (OT and AVP). We applied 10 minutes of the bear-hug test to initiate physical contact between the researcher and the participants. We collected blood specimens after 5 minutes considering the half-life of the hormones. We collected the blood specimen in anticoagulant ethylenediaminetetraacetic acid (EDTA) tubes (tubes containing the polypeptide aprotinin) (EDTA Aprotinin Tubes, Greiner Bio-One GmbH, Germany). The specimens were centrifuged at 1500 x g for 15 minutes at 4$^{\circ C}$ to obtain their plasma. We collected supernatants and preserved them in -80°C freezers. We removed the blood specimens from the -80°C freezers and brought them to room temperature to set up an ELISA test. We followed the manufacturer's protocol of the ELISA immunoassay kits (Elabscience ELISA kit) to analyze OT Plasma (Oxytocin EIA (Assay Designs, Ann Arbor, MI)) and AVP. We identified the peripheral OT and AVP hormone levels by the ELISA test of the blood plasma specimens and compared the OB and EC volumes and the hormone levels of both patient and control groups.

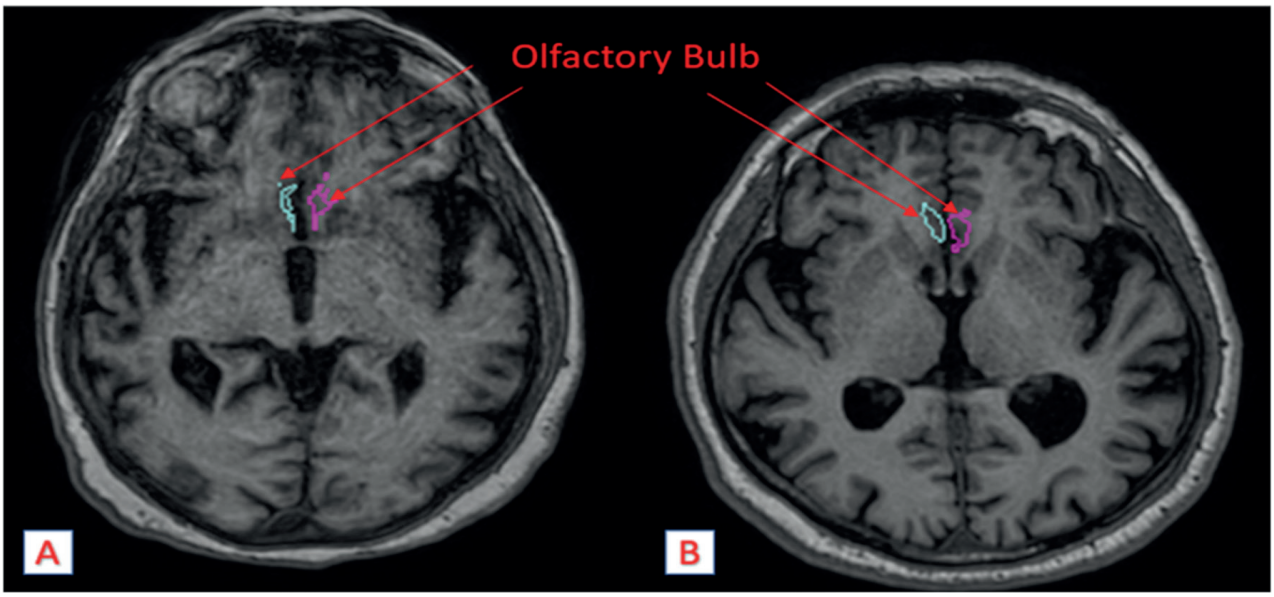

Figure 1. Olfactory bulb images A: Alzheimer's Disease, B: Healthy

Statistical Analysis

The suitability of the data to normal distribution was tested with the Shapiro Wilk test. We used Student's t-test for the normally distributed variables and for comparing the right and left parameters. The relationship between two quantitative variables was analyzed by the Pearson correlation coefficient. The SPSS Windows version 24.0 package program was used for the statistical analyses, and P<0.05 was considered statistically significant.

Results

A total of 21 volunteers, including 9 individuals with AD and 12 healthy people, participated in the study. We used the MRI images of 8 men and 12 women. All participants were right-handed. The mean age of the AD group was 73.13± 4.73, and the mean age of the control group was 72.47± 3.35. We obtained the MRI results of OB and EC for both groups and measured the volumes. After that, we compared the right and left differentiation.

In the general comparison, the OB and EC measures were higher on the right side in comparison to the left with statistical significance (respectively p=0.007; p<0.001) (Table 1).

When we compared the participants in the AD and control groups, we observed differences in the OB_R, OB_L, ENT_R and ENT_L measurements with statistical significance (respectively p=0.002; p=0.015; p<0.001; p<0.001) (Table 1). The other parameters of the AD and control groups showed no statistically significant difference (Table 1).

When the left and right values were compared within the AD and control groups separately, the ENT_R was significantly higher than the ENT_L value in the AD group (p=0.0008) (Table 1). Yet in the control group, the OB_R was higher than OB_L, and ENT_R was higher than ENT_L with statistical significance (respectively p=0.0039; p=0.009) (Table 1).

Table 1. The comparison of the right-left OB and EC and neuropeptide measurements.

Variable	General (n=21)	AD (n=9)	Control (n=12)	AD vs Control
OB_R (cm^3)	1.06±0.28	0.85±0.32	1.21±0.10	*p=0.002*
OB_L (cm^3)	0.95±0.19	0.84±0.18	1.04±0.14	*p=0.015*
OB_R (cm^3) vs OB_L (cm^3)	*p=0.007*	*p=0.1250*	*p=0.0039*	
ENT_R (mm^3)	1332.95±502.32	969.89±197.51	1605.25±491.60	*p<0.001*
ENT_L (mm^3)	946.38±307.66	661.11±105.45	1160.33±217.94	*p<0.001*
ENT_R (mm^3) vs ENT_L (mm^3)	*p<0.001*	*p=0.0008*	*p=0.009*	
OT_Signal	0.56±0.25	0.67±0.33	0.49±0.12	*p=0.310*
OT_Conc. [nmol/ml]	298.63±47.50	303.77±47.40	294.78±49.30	*p=0.602*
AVP_Signal	0.54±0.19	0.62±0.24	0.48±0.13	*p=0.219*
AVP_Conc. [pg/ml]	294.34±31.27	292.87±28.17	295.45±36.69	*p=0.464*

The p-value for comparing parameters between patient and control groups was obtained from the Student t-test. The p-value for the right and left comparisons were obtained from the Wilcoxon test. Where appropriate, values are presented as mean ± standard deviation.

When a correlation analysis was conducted with general parameters, we determined a strong positive correlation between OB_R and OB_L (r=0.859, p=0.001) and a significant positive correlation between ENT_R and ENT_L (r=0.677, p=0.001) (Table 2). We also determined a positive correlation between OB_R and ENT_R (r=0.542, p=0.011), a positive correlation between OB_ R and ENT_L (r=0.519, p=0.016) (Table 2).

Finally, we conducted a correlation analysis of the general parameters related to neuropeptides. We determined a negative correlation between OT_Signal and OT Conc. (r=-0.600, p=0.004) and a strong positive correlation between OT_Signal and AVP Signal (r=0.961, p=0.001) (Table 2). We also determined a moderate negative correlation between OT_Conc. and AVP Signal (r=-0.676, p=0.001), a positive correlation between OT_Conc. and AVP Conc. (r=-0.445, p=0.043), and a negative correlation between AVP Conc. and AVP Signal (r=-0.438, p=0.047) (Table 2).

Table 2. The correlation analysis of OB_R, OB_L, ENT_R, ENT_L measurements, and Neuropeptides.

Variable 1	Variable 2	r	p
OB_R (cm^3)	OB_L (cm^3)	0.859	**0.001**
OB_R (cm^3)	ENT_R (mm^3)	0.542	**0.011**
OB_R (cm^3)	ENT_L (mm^3)	0.519	**0.016**
ENT_R (mm^3)	ENT_L (mm^3)	0.677	**0.001**
OT_Signal	OT_Conc. [nmol/ml]	-0.600	**0.004**
OT_Signal	AVP_Signal	0.961	**0.001**
OT_Conc. [nmol/ml]	AVP_Signal	-0.676	**0.001**
OT_Conc. [nmol/ml]	AVP_Conc. [pg/ml]	0.445	**0.043**
AVP_Signal	AVP_Conc. [pg/ml]	-0.438	**0.047**

r: Spearman'scorrelation coefficient.

Discussion

In this study, we investigated the OB and EC volumes of patients with Alzheimer's disease and healthy people comparatively by using the MRICloud and IBASPM methods. Meanwhile, we collected peripheral blood plasma specimens and investigated the relationship between OT and AVP levels with the related brain regions. The sense of smell and OB integrity is significant for both navigation and episodic memory in the environmental interactions of human beings [8]. OB is the first transportation station in the olfactory pathway [8]. OB, an oval structure located just above the cribriform lamina of the ethmoid bone [19-21], collects the sensory afferents of olfactory receptor cells located in the olfactory epithelium [22]. We investigated the OB volume of people with pathology and healthy people using MRI in different studies [23]. Suzuki et al. are the first researchers to examine the Olfactory System by using MRI scans [24]. After them, Yousem et al. developed a standard process to measure the OB volume [25]. Thus, investigation of OB is considered to be useful for early diagnosis of AD, which has an insidious onset. Since patients with AD suffer navigation and episodic memory disorders, OB and EC may be considered as the primary focus of the pathology of AD [20]. In this study, we measured the OB volume of the AD patients with MRI at he early stages and compared them to the healthy control group who were close in terms of age. Most clinical findings assert evidence showing a relationship between OB volume reduction and loss of sense of smell [6, 9, 10, 19, 22, 26, 27]. The olfactory function declines as the age grows and seems to be a prodromal indication of cognitive impairment in progressive neurodegenerative diseases [3].

OB volume variations among individuals are relatively high. The right OB volume ranges from 41 mm^3 to 97 mm^3, whereas the left OB volume ranges from 37 mm^3 to 98 mm^3 [19]. In this study, the mean OB volume was generally larger on the right side than it was on the left. Yet, some studies show that the right and left OB volumes are symmetrical [27]. In this study, we determined atrophy in both the OB_R and OB_L volumes of the AD patients. The outcomes of this study were corroborated by different previous studies[20, 28]. While the OB_R volume was higher than the OB_L volume in the control group, this asymmetry was not observed in the patients with AD. We could explain this result in the patients with AD with greater atrophy on the right side compared to the left. Bilateral OB volume which is more apparent on the left and observed on the migraine patients of former studies was reduced compared to the control group [27]. This implies that the asymmetry in atrophy varies in different diseases. Thus, OB volume may even have greater values in some patients [21]. OB projects to EC [6, 9] which led us to explore the possible correlation between the OB and EC volumes. According to the results of the study, a positive correlation was determined between the right and left OB volumes and the right and left EC volumes as expected. These results supported establishing a relationship between OB volume change and EC volume [1].

EC is one of the important central recognition regions in processing signals via the OT and AVP receptors, building episodic memory, and especially in direction finding with the help of the sense of smell [29-31]. EC has topographically repetitive and organized connections between the hippocampus and parahippocampal gyrus for spatial memory and spatial representation [30]. Besides,

it is shown that EC damages may cause memory disorders [30]. In this study, both the left and right EC of the AD patients with early-stage cognitive disorders were atrophic in comparison to the control group. This result complied with the olfactory impairment and EC volume reduction claimed for AD patients before [30, 32]. Additionally, we observed that the ENT_L was more atrophic than the ENT_R in patients with AD. The right EC volume was greater than the left also in healthy people at similar ages. Our findings showed that the EC volume bilaterally decreases in patients with AD. Wang et al. associated greater EC volume with better memory [33]. Likewise, Insausti, R et al. stated that the right EC volume was greater than left after both histopathological and MRI scan volumetric comparisons [30]. AD studies have shown that the earliest neuropathologic changes appear in EC and then proceed to the hippocampus [2, 33-35]. Likewise, the outcomes of this study suggested that identifying EC atrophy by volumetric MRI examination may reveal a significant indicator for the early diagnosis of AD.

Although the effect of OT in odor processing has not been thoroughly explained yet, it was observed that it increases the stimulation via OB interneurons in the cortex including the anterior olfactory nucleus [32]. After coding the odor information with mitral and tufted cells in OB, these data are modulated by granule cells with odor interneurons and transported to the olfactory cortex [32]. The bottom-up sensory processing of OT is found to alter the olfactory coding, so, it is necessary to have OT functions in a top-down early sensory cortex system for social recognition [32]. Mitre et al. stated that OT was expressed in 29 different brain regions predominantly in the region for the sense of smell [36]. 10 minutes after the intracerebroventricular OT application, the highest activity increase was observed in EC, olfactory tubercle, dorsal and ventral subiculum, accumbens nucleus, ventral-medial striatum, lateral septum and bed nucleus of the stria terminalis [36]. It is thought that the inhibitor granule cells in OB are innerved by the anterior olfactory nucleus, and the input for the sense of smell in OB is provided by OT regulation [36]. Reduced OT plasma/serum levels are considered to cause some cognitive disorders [37]. Despite this, the OT levels in the AD patient and control groups were not different.

It is asserted that AVP has effects on learning and memory processes. Buijs et al. showed fibers containing AVP in a rat EC [38]. Fujiyoshi et al. stated that AVP secretion was reduced in the human brain cortex during senile dementia [39]. Sorensen et al. reported that AVP decreases in the plasma and cerebrospinal fluid in patients with dementia [40]. In this study, the AVP plasma levels did not show a statistically significant difference between the patient and the control group, but a minimal change in the patient group was remarkable.

This study showed that the OB and EC volumes bilaterally decreased in the AD patients at early stages, and it may have a clinical significance to measure the volumes of these brain regions with MRI for early AD diagnosis. However, we could not find any evidence to use OT and AVP plasma levels for early diagnosis. Nevertheless, the changes in OT and AVP should be investigated for later stages of AD. Although we evaluated the OB and EC volumes, we did not run a smell acuity and odor recognition test for the AD patients, which was a weakness of this study. Thus,future studies should consider filling this gap. Due to the difficulty of reaching AD patients, the low number of patients was another limitation of the study.

Conflict of interests
The authors declare that they have no competing interests.

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There are no financial supports

Ethical approval
This study was approved by Hatay Mustafa Kemal University Clinical Research Medical Ethics Committee (Ethical permission no:2018/19).

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