Review Article • DOI: 10.2478/s13380-013-0108-3 • Translational Neuroscience • 4(1) • 2013 • 34-45

VERSITA

Translational Neuroscience

THE OLFACTORY SYSTEM IN ALZHEIMER'S DISEASE: PATHOLOGY, PATHOPHYSIOLOGY AND PATHWAY FOR THERAPY

Tibor Kovács*

Department of Neurology, Semmelweis University, Budapest, Balassa u. 6., H-1083 Hungary

Abstract

Olfaction is frequently mentioned as a "neglected sense", although the olfactory system has several interesting and unique anatomical and physiological features. Olfactory involvement is present in several degenerative disorders, especially in Alzheimer's disease (AD). The peripheral and central parts of the olfactory system are damaged even in the early stages of AD, manifesting in profound olfactory deficits. Besides the early pathology, the olfactory system may be involved in the pathogenesis of AD by providing a route of entry for pathological agents still unknown. In contrast to this olfactory vector hypothesis, the olfactory system can be used to deliver therapeutic agents in AD, such as nerve growth factor and insulin, by decreasing the side-effects of the therapy or providing a non-invasive method of delivery.

Keywords

• Olfaction • Neurofibrillary tangles • Limbic system • Olfactory vector hypothesis • Alzheimer's disease

Received 15 January 2013
accepted 12 February 2013

Introduction

Alzheimer's disease (AD) is a devastating primary degenerative disorder of the nervous system. It is characterized neuropathologically by the accumulation of intraneuronal neurofibrillary tangles (NFT) and neuropil threads, together with extracellular senile plaques, and clinically by progressive cortical dementia with short term memory loss as an initial symptom. The pathomechanism of the disease is unknown and only modest symptomatic relief is provided by currently available treatments [1].

Olfaction is frequently mentioned as a "neglected sense", although it has several interesting and unique anatomical (Figure 1) and physiological features [2]. It is the only site of the central nervous system (CNS) where continuously replaced primary olfactory epithelial cells grow their axons into the CNS and form new functional synapses with the mitral cell processes in the olfactory bulb (OB) [3]. In addition, environmental factors such as toxins or pathogens can easily reach the CNS through the olfactory pathway. Moreover, the olfactory system is the main afferent of the limbic regions which are involved in memory formation and affected early in the course of AD pathology [4]. As shown by these peculiarities, the olfactory system has an exceptional role in AD. Neuropathological changes of the olfactory epithelium, bulb and cortical structures, olfactory dysfunction, and the role of the olfactory system in the pathogenesis of AD are summarized in this review, together with an outlook on the possible olfactory system-based therapeutic options.

Pathology of the olfactory epithelium

The olfactory epithelium in the postero-dorsal region of the nasal cavity contains primary olfactory receptor neurons (ORN) (about 6 million in every human nose) which send their axons through the cribriform plate into the OB [5].

Hyperphosphorylated tau filaments were found in the ORN of AD patients [6-8] which raises the possibility of *in vivo* histological diagnosis from biopsied tissue [9]. Unfortunately, these promising results were not verified by subsequent studies. Tau-immunoreactive filaments were detected even in non-demented young and aged adults [10-12], or the pathology was detected only in advanced stages of AD and not in its mild or moderate stages [13]. Tau-containing dystrophic neurites were present not only in AD, but also in other neurodegenerative diseases, as well as in normal aging; only fetal and neonatal cases were found to not possess them [10]. Recently, the pathology of the olfactory epithelium was studied in detail by Arnold et al. [14] in a large number of patients with AD and other neurodegenerative disorders, and in neuropathologically normal cases. β-Amyloid depositions were found in 71% of AD patients, compared to 22% of normal cases and 14% of patients with other diseases, such as α-synucleinopathies (dementia with Lewy bodies, multiple system atrophy and Parkinson's disease (PD)), tauopathies (corticobasal degeneration, progressive

* *E-mail: kovacs.tibor@med.semmelweis-univ.hu*

supranuclear palsy and frontotemporal lobar degeneration with tau inclusions) and TDP-43 proteinopathies (motoneuron disorder and frontotemporal lobar degeneration with ubiquitin inclusions). Tau-positive dystrophic neurites were seen in 55% of AD cases, 34% of normal, and 39% of other neurodegenerative cases. The amount of the β-amyloid and tau pathology was the highest in AD. α-Synuclein deposition was rarely seen, which is in contrast with the results of the same group published earlier, where almost all the AD cases and more than half of the normal controls had α-synuclein immunoreactive dystrophic neurites in the olfactory epithelium [15]. TDP-43-containing inclusions were not found. In spite of these non-specific findings, the authors again raised the suggestion of Tabaton et al. [9] to use the olfactory epithelium as a biomarker for AD. It should be mentioned that Crino et al. [16] found a higher percentage of AD patients (10 out of 12) bearing β-amyloid depositions in the olfactory epithelium.

Besides β-amyloid and tau pathology, other morphological abnormalities of the olfactory epithelium were also shown in AD. Apolipoprotein E (ApoE) was found in the Schwann cells of the olfactory nerve bundles and the number of ApoE-immunoreactive ORN was 3.5 times greater in AD than in controls with a similar number of ORN [17]. These features suggest that membrane transport processes might be altered in AD.

Increased activity of oxidative processes was also demonstrated in the olfactory epithelium. Immunoreactivity of 3-nitrotyrosine was localized in ORN, while in controls immunoreactivity occurred in vascular endothelial cells only [18]. Increased oxidative stress in the olfactory epithelium in AD was also suggested by macrophage infiltration, which was absent in controls [18]. The role of oxidative stress was also supported by the increased superoxide dismutase immunoreactivity seen in AD compared to controls, especially near the surface and in the basal region of the epithelium [19]. Similarly, the immunoreactivity of metallothionein (which is induced by oxidative stress) was increased in AD [20]. Oxidative damage in the olfactory epithelium was only partially similar to that seen in cortical

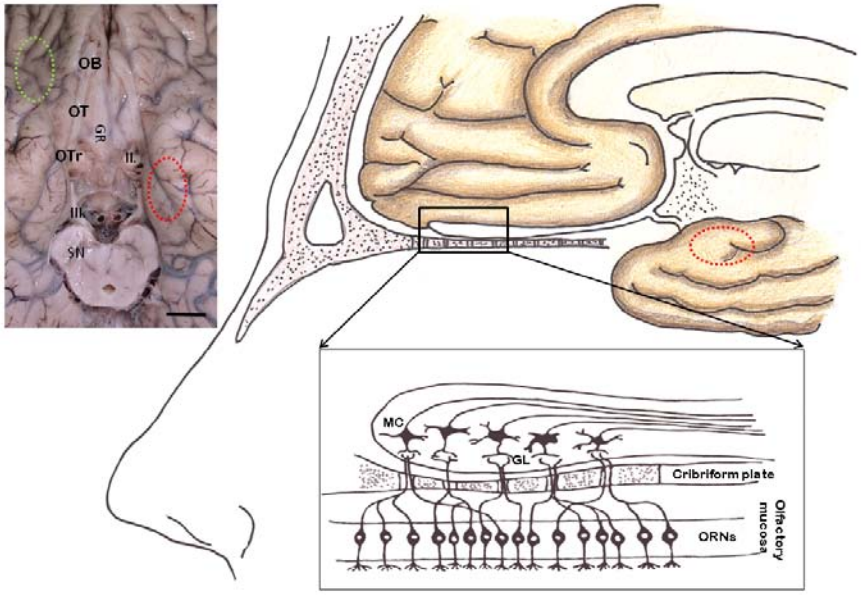

Figure 1. Anatomy of the olfactory system
Compared to macrosmatic animals, the human olfactory system is rudimentary in size. The small olfactory bulb (OB) in the olfactory sulcus bordered by the straight gyrus (gyrus rectus, GR) continues in the olfactory tract (OT) which ends in the olfactory trigone (OTr). The approximate locations of the primary (red dotted line) and the secondary (green dotted line) olfactory cortices are shown.
Microscopically, axons of the olfactory receptor neurons (ORN) enter the intracranial space through the cribriform plate and end in the OB forming glomeruli (GL) with the mitral cell (MC) processes. Bar represents 1 cm.
(SN: substantia nigra; II.: optic nerve; III.: oculomotor nerve).

regions in AD: nucleic acid or protein oxidation was not demonstrated in the olfactory system, while lipid peroxidation and heme oxygenase-1 activity were increased compared to the cortical regions [21]. The increased vulnerability of the olfactory epithelium in AD is suggested by a decreased number of ORN immunoreactive for calbindin-D28k, a calcium binding protein; although their number decreases with age as well, it was found significantly decreased in AD compared to age-matched controls [22].

Pathology of the olfactory bulb (Figure 2)

Axons of the ORN branch in the most superficial layer of the OB, forming the olfactory nerve layer (ONL), followed by the glomerular layer (GLL), where olfactory glomeruli are found. The glomeruli are complex arrays of synapses between axons of the ORN and dendrites of the mitral and tufted cells and several interneurons. The external plexiform layer (EPL) below the GLL is easily identified in the human OB, while the mitral cell layer (MCL) (formed by the somata of the mitral cells) is not well-defined in humans. The weakly developed internal plexiform layer (IPL) separates the MCL from the granule cell layer (GRL), the most voluminous layer of the human OB [23]. The caudal continuation of the OB is the olfactory tract (OT), containing the afferent and efferent axons of the OB. Within the GRL and in the OT, large multipolar neurons are embedded and form the anterior olfactory nucleus (AON). The AON is phylogenetically a part of the piriform cortex. The OB has a complex neurochemical organization, in which numerous transmitter systems are represented [5,24].

The organization of this complex neuronal structure is damaged during the course of AD. Olfactory nerve fibers enter the deeper parts of the OB (misrouted fibers) and form glomeruli outside the GLL (ectopic glomeruli), which can be hyperplastic [25-27]. Their biochemical character is distinct, as marked by the findings that, while in controls the greatest density of dopamine D2 receptors was found in the GLL

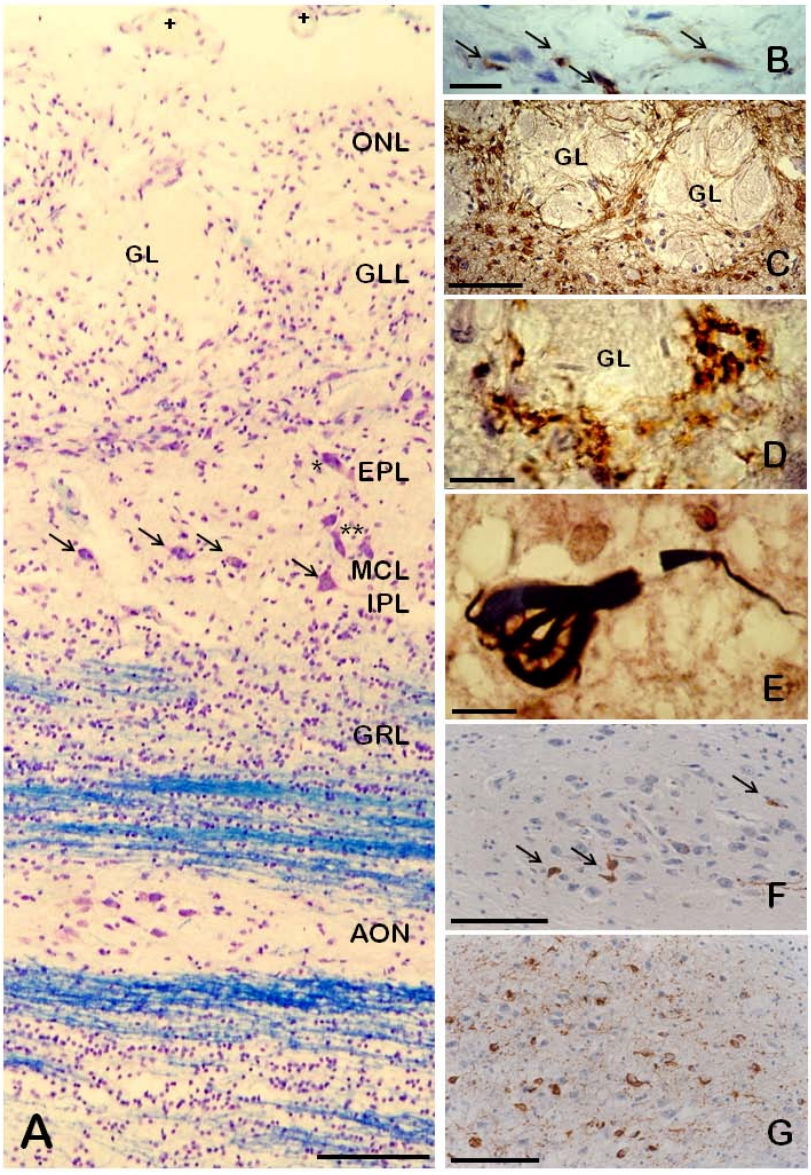

Figure 2. Pathology of the OB in AD and aging

A: Normal histology of the OB in human. Luxol fast blue/Nissl staining.
The outermost layer is the olfactory nerve layer (ONL), where axons from the ORN run. Leptomeningeal vessels are marked by +. Beneath the ONL, glomeruli (GL) (synaptic arrays of ORN axons with the mitral cell and processes and interneurones) are located in the glomerular layer (GLL) showing pronounced inter-individual variability. The external plexiform layer (EPL) separates the GLL from the mitral cell layer (MCL), formed by the large neuronal somata of the mitral cells (arrows). Some tufted cells (*) are seen in the EPL. The internal plexiform layer (IPL) is less clearly demarcated from the granule cell layer (GRL), where groups of large multipolar neurons are found forming the anterior olfactory nucleus (AON). Afferent and efferent projections of the OB run within the GRL and will form the optic tract. Myelinated axons are shown in turquoise.
B: ONL in AD. Tau immunohistochemistry.
Axons of the ORN (arrows) containing hyperphosphorylated tau, proving the tau pathology of the olfactory epithelium.
C: Large ectopic glomeruli (GL) in the EPL in AD with astroglial septa surrounded by astrocytosis. Glial fibrillary acidic protein (GFAP) immunohistochemistry with hematoxylin counterstaining.
D: Diffuse β-amyloid deposition around a glomerulus (GL) in AD. β-Amyloid immunohistochemistry with hematoxylin counterstaining.
E: NFT in a mitral cell in aging. Gallyas silver impregnation with nuclear red counterstaining.
F: AON with NFT (arrows) in a control patient in Braak stage 1. Tau immunohistochemistry with hematoxylin counterstaining.
G: AON with NFT and numerous neuropil threads in AD (Braak stage 6). Tau immunohistochemistry with hematoxylin counterstaining.
Bars represent 100 µm on A, F and G; 20 µm on B, D and E; and 50 µm on C.

of the bulb, in AD the labeling of the GLL was decreased so that glomerular and granular layers did not differ in labeling density [28].

There is robust data [29-43] in literature about amyloid and neurofibrillary pathology of the OB in AD and aging.

AD-type pathology is an early feature of the OB and there are cases in which neurofibrillary pathology appears in the OB earlier than in the entorhinal cortex [44], which is the earliest site of neurofibrillary degeneration according to the Braak staging [45]. However, some studies have found that neurofibrillary pathology appears in the OB parallel to the entorhinal cortex [38], or only in Braak stage 2 [40]. These differences can be explained by the different sampling protocols; in our study [44], the OB was sectioned in its whole width and every fifth section containing the AON was stained, in contrast to randomly selected sections only from parts of the OB.

In addition, Alzheimer-type pathology was detected in every AD patient in the majority of the studies [26,37,39-43], and in varying degree of the age-matched control cases, ranging from all of the cases [26] to none of the cases in Braak stage 0 and 1 [40].

Tau-immunoreactive fibers were found in the ONL, proving that the axons of the ORN are affected by neurofibrillary degeneration [26]. The majority of the NFT are located in the AON, although NFT-bearing neurons are found in every layer. Similarly, amyloid pathology was found in all AD cases. The distribution of senile plaques was different between control and AD cases: no classical (cored) and burnt-out plaques were detected in controls, while primitive plaques were more frequent in AD. The majority of senile plaques were of the diffuse type in both groups [26], which are regarded to be less toxic for the neurons in contrast to cored and primitive plaques [46].

In addition to tau and β-amyloid pathology, α-synuclein deposition and Lewy body pathology is common in the OB in AD [42,47,48], especially in Lewy-body variant AD [48]. The OB was found to be equally vulnerable to tau and α-synuclein pathology as the amygdala, which suggests linked neurodegeneration in these two anatomically remote regions [42].

There are data that show Alzheimer-type pathology is selective in the OB. For example, the number of somatostatin-containing neurons in the AON is reduced in AD and frequently shows co-localization with tau or β-amyloid [43]. Contrary to this parallel distribution, no correlation was found between cholinergic axons and AD-type pathology [37]. Recently, bipolar secretagogin-expressing neurons were described in the OT, which were selectively decreased in number in AD [49]. They expressed markers suggesting that their neuronal terminal differentiation is lacking [49], which raises the possibility that regenerative processes are damaged in the olfactory system in AD.

The extensive pathology of the OB in AD is highlighted by Mundinano et al. [50], who found that the volume of the OB was decreased only in patients with AD, while in PD and frontotemporal dementia it was similar to controls. In addition, the number of periglomerular dopaminergic cells was increased in AD and PD, which could reflect a compensatory mechanism created by the early degeneration of other neurotransmitter systems.

As a result of the extensive neuronal pathology both in the OB and in the olfactory cortical structures, the consequent axonal degeneration destroys the axons in the OT, which is manifested by loss of density and decreased cross-sectional areas of the OT [51,52].

These severe morphological alterations of the OB and OT described by histological methods were verified by imaging studies. Using magnetic resonance (MR) volumetric measurements, Thomann et al. [53,54] found that, compared to age-matched controls, the most severe atrophy of the OB and OT was seen in AD; but, atrophy was already found in mild cognitive impairment (MCI), suggesting early involvement of the olfactory system. The degree of atrophy correlated with the global cognitive performance as measured by the Mini Mental State Examination scores [54].

Pathology of the cortical olfactory structures

Axons of the mitral cells project to the medial surface of the temporal lobe in the piriform, periamygdaloid, and rostral entorhinal cortices, and to the anterior amygdala. The human primary olfactory cortex is located at the junction of the temporal and inferior frontal lobes (Figure 1), corresponding to the piriform cortex [55], while the secondary olfactory cortex lies only in the right orbitofrontal cortex. All of these cortical areas belong to the limbic system [4]. The entorhinal olfactory connection is the only part of the entorhinal cortex which receives direct sensory input, while other parts of it relay information from the cortical association areas towards the hippocampal formation [56].

Central olfactory areas are affected early during the formation of neurofibrillary pathology, as reflected by the Braak staging [45], where the initial pathology develops in the medial part of the entorhinal cortex. In the limbic stage, all cortical olfactory areas are affected by the pathology. In order to define the sequential pathology in central olfactory areas in AD, we examined them and found that cortical olfactory areas are not preferentially involved [44]. For example, the secondary olfactory cortex (Figure 1) with close olfactory connections is affected less severely than the medial orbitofrontal cortex without olfactory input. Significant correlation was found between the pathology of the OB, and olfactory and non-olfactory cortical areas. This suggests that the formation of NFT does not depend on synaptic connections and develops independently in different structures that have no structural relations [44].

Atrophy of cortical olfactory areas was found by imaging methods, even in patients with MCI who later converted to AD; in these patients, volume loss in the olfactory and polysynaptic hippocampal network was significantly greater than in the MCI patients who did not convert to AD [57]. Atrophy of the medial temporal lobe structures correlated with an olfactory deficit [58], and a decrease in olfactory cortical network clustering was a discriminative feature of AD [59]. Orbitofrontal cortical atrophy was detected even in incidental AD [60]. Atrophy in AD was also found in the medial subnucleus of the amygdala receiving olfactory afferents [61].

Olfactory dysfunction in AD

Olfaction is a composed function of olfactory threshold, odor identification and odor discrimination capabilities [2]. Olfactory threshold is the measure of the lowest concentration of an odorant which activates the olfactory receptor cells. Odor identification is the ability to identify and name odorants, while odor discrimination is the ability to differentiate between odorants. Olfactory threshold is likely to be influenced by the most peripheral part of the olfactory system, while discrimination and identification are partly cognitive tasks depending on central olfactory structures [62]. Olfactory threshold performance is significantly influenced by sniffing ability [63]. In PD, sniffing is altered [64], while in AD this deficit is not yet reported.

In a recent review by Rahyel et al. [65], 35 studies of AD regarding olfactory functions were analyzed and compared to 42 studies of those seen in PD. It was found that olfactory identification and recognition were affected more strongly than olfactory thresholds in AD (a finding which was not found in an earlier meta-analysis by Mesholam et al. [66], probably because of the smaller number of available studies), while the effect of PD was greater on olfactory threshold. This pattern shows that deficits in AD are more pronounced in higher-order processes, while PD seems to have an equal impact in all olfactory domains. These findings are in harmony with the pathological studies of McShane et al. [67] showing that anosmia was associated with Lewy bodies, rather than Alzheimer-type pathology in demented patients. Anosmia was found in 65% of patients with AD with concomitant Lewy-body pathology, while in pure AD it was found in only 23% [68]. It seems that olfactory identification ability is more severely damaged in dementia with Lewy bodies than in AD [69,70]. However, these findings are likely influenced by the frequent co-localization of Alzheimer-type and Lewy-body pathologies [71].

The effect of AD on olfactory function is complicated by the fact that impaired olfaction is an age-related process: the best odor performance is measured between 20 and 40 years of life, with steady decline after this age, which can result in complete anosmia in elderly people [72]. Similarly, during aging, Alzheimer-type changes appear in the brain. We have shown that 86% of normal aged subjects have NFT in the OB [26]. In subjects with olfactory dysfunction without neurological symptoms, Lewy bodies [73] and Alzheimer-type pathology [74,75] were detected in the brain. In addition to age-related changes of the olfactory epithelium [76,77] and the loss of mitral cells in the OB [23], these alterations might contribute to the age-related olfactory loss.

Despite the confounding effect of the loss of olfactory function with age, olfactory testing may be a useful tool in predicting cognitive decline in high-risk settings [78,79]. Olfactory identification deficit was found to predict the development of MCI [80], and subjective memory complaints were significantly associated with olfactory discrimination and identification deficits [81]. Olfactory deficit is present in MCI [82,83], independently from its subtype (amnestic or other) [84]. Conversion of MCI to AD was predicted by olfactory identification deficit alone [75] or in combination with other early markers such as hippocampal volume on MR imaging and memory tests [85,86]. The finding that MCI patients who were unaware of their deficit were more likely to convert to AD [87] was recently debated [88].

Interestingly, when MCI patients were grouped by the β-amyloid positron emission tomography (PET) results (Pittsburgh compound B (PiB) positive and PiB negative patients), olfactory identification scores did not differ between the groups, suggesting that olfactory deficit is independent from the β-amyloid burden [89].

An interesting olfactory 'stress' test was used by Schofield et al. [90] to show olfactory abnormalities in preclinical AD: based on the early damage of the OB and its cholinergic system, intranasal atropine was used to unmask incipient AD by measuring the change on the University of Pennsylvania Smell Identification Test (UPSIT) after atropine administration. The effect of the anticholinergic atropine correlated significantly with episodic memory score and left hippocampal volume; its effect in non-demented subjects on memory functions seemed more robust than the effect of the left hippocampal volume, raising the possibility that the effect of atropine might represent a proxy for a process more salient than hippocampal atrophy in the early stages of AD. Compared to conventional olfactory testing, olfactory stress tests show stronger correlations with other early markers of AD, giving them the possibility for being a simple and inexpensive screen for early AD in the future [90].

Olfactory dysfunction in AD can be measured with functional imaging and with electrophysiological methods, as well. Using functional MR imaging (fMRI) [91], the activation of the primary olfactory cortex was weaker in AD than in control subjects and the degree of activation significantly correlated with UPSIT scores. In addition, the intensity and volume of the activation in the primary olfactory cortex increased significantly as a function of odorant concentration in the AD group, but not in controls. Functional disruption of odor quality coding in the piriform cortex was also detected by fMRI in AD [92]. The activation of the primary olfactory cortex was decreased in AD patients on PET also [93].

ApoE polymorphism was shown to influence olfactory functions in MCI and in AD. Carrying the ε4 allele causes more pronounced olfactory deficit in MCI [94]. In addition to predementia and dementia cases, it was shown that the ε4 allele is associated with odor identification deficit in elderly people (75-80 years), independent from dementia conversion within 5 years [95]. Only olfactory identification, but not threshold levels, was affected by the ApoE ε4 carrier status [96]. However, it should be mentioned that, because of the long pre-clinical stage of AD [97], at least some of the ApoE ε4 carriers in these samples might have had AD-type pathology. This idea is supported by the findings of Handley at al. [98], who found that ApoE ε4 positive siblings with family history of AD had significantly higher olfactory identification deficit than ApoE ε4 positive cases without it. Odor identification deficit was also shown in members of families who are genetically at risk for developing AD, independent of the ApoE status [99]. Interestingly, in patients who were carrying the

presenilin 1 (*PSEN1*) mutation, olfactory tests were normal ten years before the development of dementia [100].

Olfactory dysfunction correlates to general cognitive functions [101-103] in AD and can be used to differentiate AD from major depression [104]. The differential diagnostic ability of olfactory dysfunction from other neurodegenerative disorders with dementia is poor because decreased olfaction is common among these disorders [2]; however, the degree of involvement is more severe in AD compared to frontotemporal lobar degeneration syndromes (frontotemporal dementia, semantic dementia and corticobasal degeneration) [105].

The role of the olfactory system in the pathogenesis of AD

Because of its special anatomical (close relation to the outer environment and the intracranial space through the cribriform plate) and physiological (neurogenesis in adulthood with regenerative capacity) features, the role of the olfactory system in the pathogenesis of degenerative disorders of the CNS has been frequently mentioned in the last 40 years. The olfactory connection in these disorders is highlighted by the fact that olfactory dysfunction is present in almost all cases in early, and even preclinical stages of AD and PD. The olfactory connection has become a popular topic recently in regards to PD, where the spreading of an unknown pathological agent is suspected through the olfactory pathways, in addition to the earlier hypothesis where this spreading happened only through the vagus nerve from the gastrointestinal system (the so called "dual hit" hypothesis) [106].

The olfactory vector hypothesis [107-109] suggests that environmental toxins, viruses, bacteria, or prions enter the central nervous system through the olfactory system, leading to pathological changes in the brain. However, the nature of these agents remains unknown, even after several propositions (reviewed by Honjo et al. [110]) which were all later debated. One of these agents is herpes simplex virus type 1 (HSV1); its role in the AD pathogenesis was recently reviewed by Speljko et al. [111]. Acute infection of the brain by HSV1 causes herpes encephalitis, where the affected regions [112] are in close similarity with the most severely involved areas in AD belonging to limbic structures, such as the medial temporal and orbitofrontal areas. Olfactory dysfunction is present in patients who survive herpes encephalitis [113]. In the early studies of Mann et al. [114], HSV1 antigens were detected in the olfactory tract. The idea of trans-olfactory spreading of HSV1 was proposed more than 30 years ago [115,116].

Among bacterial agents, the role of *Chlamydia pneumoniae* should be highlighted (see the review of Balin et al. [117]). *C. pneumoniae* is a ubiquitous organism and has been found in 90% of the brain samples of AD patients, in contrast to 5% positivity in non-AD cases [118]. Besides hematogenic spreading in macrophages, *C. pneumoniae* is known to attach to the nasal epithelial and olfactory neuroepithelial cells, and its genetic material was found in the OB, from which it can spread in the brain and induce pathology [117].

In addition to infectious agents, toxic substances, such as trace elements including zinc, iron, and aluminum, were also postulated in the pathogenesis of AD and were found to have accumulated in the olfactory areas of the brain in AD [119,120].

It should be emphasized that there are several arguments against the olfactory vector hypothesis; some of them listed by Doty [108], such as the existence of familiar forms of AD, the lack of olfactory dysfunction in some AD patients, and the more severe involvement of central olfactory structures than peripheral ones. A case report by Arriagada et al. [121] is also mentioned as an argument against this hypothesis: a 65-year-old patient is described with olfactory dysgenesis (the cribriform plate was imperforated and the olfactory bulbs were not developed) accompanied by NFT in the limbic cortex. However, it is curious that senile plaques were not found in the patient's brain, raising the possibility that this patient had a form of NFT pathology other than AD, such as frontotemporal degeneration.

The olfactory hypothesis was interpreted by Pearson et al. [122,123], in such a way that AD pathology begins in the olfactory centers of the medial temporal lobe and spreads in the retrograde direction via transneuronal pathways. However, this type of spreading was not found in our study [44]; for example, the olfactory neocortex was affected less severely than the medial orbitofrontal cortex. This suggests that the olfactory orbitofrontal cortex, with close connections to the primary olfactory cortex in the medial temporal lobe, was affected in later stages of AD than the medial orbitofrontal cortex, which is part of the limbic system and has no olfactory input. We found that the involvement of olfactory cortical areas and spreading of the pathology in the olfactory cortical structures are in agreement with the Braak staging (i.e. olfactory cortical areas are affected at least in the limbic stage). However, NFT were found in some patients in the OB, when the entorhinal cortex was free of them.

Furthermore, the neuropathological homogeneity of AD was abolished by the study of Murray et al. [124], which described two neuropathological subtypes of AD, other than the typical form. These are named limbic-predominant and hippocampal sparing subtypes, together accounting about 25% of AD cases. These results suggest that a redefinition of AD will be necessary in the near future.

Olfactory system as a therapeutic pathway in AD

Intranasal delivery of treatment is a feasible option in CNS diseases; it may decrease or abolish the side-effects seen after systemic administration and can substitute invasive methods of substance delivery.

Although recent results with amyloid vaccination in general are disappointing [125], nasal vaccination with β-amyloid for the treatment of AD was suggested [126]. It has been shown that β-amyloid peptide in rats is transported to the OB and frontal cortex after intranasal delivery [127].

Cholinesterase inhibitors are used to ameliorate the cholinergic hypofunction in AD [128]. The synaptic integrity and cholinergic phenotype of basal forebrain neurons is maintained by a neurotrophic factor, nerve growth factor (NGF) [129,130]. There were

promising trials using intracerebral NGF delivery for the treatment of AD [131-133] with limitations because of invasive methods of delivery. Intranasal NGF delivery, on the other hand, is an easy way of application, and it was found to be effective in animal experiments [129,134], even with higher bioavailability than after ocular administration [135].

The advantage of intranasal delivery of insulin compared to systemic administration is obvious. It is widely established that insulin has a substantial effect on synaptic functions of brain neurons [136] and facilitates memory formation [137]. In AD, insulin levels and insulin activity is decreased [138]. Because of this, AD is even mentioned as type 3 diabetes [139]. Owing to these features, one can hypothesize that normalization of brain insulin levels might improve the symptoms of AD. However, systemic administration of insulin in AD is problematic because the risk of hypoglycemia. Intranasally administered insulin avoids this unwanted systemic response and has no major side-effect [140,141]. Initially, the effect of intranasal insulin on memory functions in AD and in MCI in acute conditions (after 15 minutes of application) were studied and found to be improved depending on the ApoE ε4 status; only patients negative for apoE ε4 improved in verbal memory domains [142]. Verbal memory (together with attention and caregiver-related functional status) of AD patients also improved after 21 days of intranasal insulin treatment using 20 IU BID. Plasma levels of the shorter form of amyloid β (40 amino acid) were reduced without affecting the levels of the longer (42 amino acid) isoform [143]. Based on these results, a study with a longer treatment period was published recently [144]. One hundred and four patients with amnestic mild cognitive impairment or AD were treated for 4 months with placebo or intranasal insulin (10 or 20 IU BID). Treatment with 10 IU BID of insulin improved delayed memory and both doses of insulin (10 and 20 IU BID) preserved caregiver-rated functional ability and general cognition. Placebo-treated patients showed decreased glucose uptake in the parietotemporal, frontal, precuneal and cuneal regions on PET while patients on insulin treatment were stable. These results suggest that studies with longer treatment duration are necessary.

Because vitamin D regulates insulin receptor expression and enhances insulin action [145,146], a randomized controlled trial was performed with high-dose vitamin D2 treatment, followed by intranasal insulin application in AD patients [147]. The study did not find an effect of high-dose vitamin D2 treatment, but intranasal insulin was effective on the ADAS-Cog (Alzheimer's Disease Assessment Scale-cognitive subscale) scale [147].

In case of cholinesterase inhibitors, systemic side-effects are also limiting their use. Tacrine, the first cholinesterase inhibitor developed to treat AD, was found be transported into the brain from the nasal cavity. Intranasal administration resulted in higher bioavailability and reduced distribution into non-targeted organs in animal experiments [148,149], creating a possibility to decrease its systemic side-effects, which might be an option for other cholinesterase inhibitors too.

Besides providing a therapeutic pathway, olfactory system and olfactory function testing can also be used as a treatment response marker in AD. Keeping in mind that the OB and the cortical olfactory areas are rich in cholinergic innervation [150,151], the effect of cholinesterase inhibitors might be measured through the changes seen in olfactory tests. Velayudhan and Lovestone [152] found that after a three month treatment with the cholinesterase inhibitor donepezil, the change in smell identification function was the best predictor of the change seen in the Clinician Interview Based Impression of Change plus caregiver input (CIBIC-plus) scale.

Besides cognitive symptoms, behavioral and psychological symptoms of dementia (BPSD) are frequent in AD, and are especially important regarding caregiver stress. Aromatherapy could be one of the alternative therapeutic methods to control agitation in AD, but a study using Melissa officinalis oil found that aromatherapy was not superior to placebo or donepezil in the treatment of agitation in AD [153].

Conclusion

Olfactory system pathology is well described in AD, together with the consequent olfactory dysfunction. However, its possible role in the pathogenesis of AD is thrilling: through the olfactory epithelium, the intracranial space is accessible just through a few millimeters of tissue. This anatomical situation, together with the early involvement of the olfactory system in neurodegenerative disorders, while damage of other sensory qualities in these disorders is far less evident, is hardly coincidental. Although olfactory transneuronal spreading of the pathology is unlikely in AD, the predominantly limbic distribution of NFT in the early stages of the disease makes it likely that olfaction, as the only afferent pathway of the limbic system, is involved in the pathogenesis of AD, possibly using other pathways of spreading, such as the cerebrospinal fluid [154]. However, this gate of entry function can be used favorably for the treatment of AD as well, and a substantial part of future therapies will involve the olfactory system in degenerative disorders. Altogether olfaction will not be a neglected sense in AD in the future.

Acknowledgements

The technical assistance of Ms. Eszter Bíró and the artwork of Marianna Farsang, M.D., are thankfully acknowledged. The work of T. Kovács is supported by TÁMOP grant No: TÁMOP-4.2.1.B-09/1/KMR.

References

[1] Querfurth H.W., LaFerla F.M., Alzheimer's disease, N. Engl. J. Med., 2010, 362, 329-344
[2] Kovács T., Mechanism of olfactory dysfunction in aging and neurodegenerative disorders, Ageing Res. Rev., 2004, 3, 215-232
[3] Blanchart A., López-Mascaraque L., From the periphery to the brain: wiring the olfactory system, Transl. Neurosci., 2011, 2, 293-309
[4] Gloor P., The temporal lobe and limbic system, Oxford University Press, New York, 1997
[5] Halász N., The vertebrate olfactory system: chemical neuroanatomy, function and development, Akadémiai Kiadó, Budapest, 1990
[6] Talamo B.R., Rudel R., Kosik K.S., Lee V.M-Y., Neff S., Adelman L., et al., Pathological changes in olfactory neurons in patients with Alzheimer's disease, Nature, 1989, 337, 736-739
[7] Lee J.H., Goedert M., Hill W.D., Lee V.M., Trojanowski J.Q., Tau proteins are abnormally expressed in olfactory epithelium of Alzheimer patients and developmentally regulated in human fetal spinal cord, Exp. Neurol., 1993, 121, 93-105
[8] Yamagishi M., Ishizuka Y., Seki K., Pathology of olfactory mucosa in patients with Alzheimer's disease, Ann. Otol. Rhinol. Laryngol., 1994, 103, 421-427
[9] Tabaton M., Cammarata S., Mancardi G.L., Cordone G., Perry G., Loeb C., Abnormal tau-reactive filaments in olfactory mucosa in biopsy specimens of patients with probable Alzheimer's disease, Neurology, 1991, 41, 391-394
[10] Trojanowski J.Q., Newman P.D., Hill W.D., Lee V.M.Y., Human olfactory epithelium in normal aging, Alzheimer's disease, and other neurodegenerative disorders, J. Comp. Neurol., 1991, 310, 365-376
[11] Kaakkola S., Palo J., Malmberg H., Sulkava R., Virtanen I., Neurofilament profile in olfactory mucosa of patients with a clinical diagnosis of Alzheimer's disease, Virchows Arch., 1994, 424, 315-319
[12] Kishikawa M., Iseki M., Sakae M., Kawaguchi S., Fujii H., Early diagnosis of Alzheimer's?, Nature, 1994, 369, 365-366
[13] Hock C., Golombowski S., Mullerspahn F., Peschel O., Riederer A., Probst A., et al., Histological markers in nasal mucosa of patients with Alzheimer's disease, Eur. Neurol., 1998, 40, 31-36
[14] Arnold S.E., Lee E.B., Moberg P.J., Stutzbach L, Kazi H., Han L-Y., et al., Olfactory epithelium amyloid-beta and paired helical filaments-tau pathology in Alzheimer's disease, Ann. Neurol., 2010, 67, 462-469
[15] Duda J.E., Arnold S.E., Lee V.M.Y., Trojanowski J.Q., The expression of α-, β-, and γ-synucleins in olfactory mucosa from patients with and without neurodegenerative diseases, Exp. Neurol., 1999, 160, 515-522
[16] Crino P.B., Martin J.A., Hill W.D., Greenberg B., Lee V.M., Trojanowski J.Q., Beta-amyloid peptide and amyloid precursor proteins in olfactory mucosa of patients with Alzheimer's disease, Parkinson's disease, and Down syndrome, Ann. Otol. Rhinol. Laryngol., 1995, 104, 655-661
[17] Yamagishi M., Getchell M.L., Takami S., Getchell T.V., Increased density of olfactory receptor neurons immunoreactive for apolipoprotein E in patients with Alzheimer's disease, Ann. Otol. Rhinol. Laryngol., 1998, 107, 421-426
[18] Getchell M.L., Shah D.S., Buch S.K., Davis D.G., Getchell T.V., 3-Nitrotyrosine immunoreactivity in olfactory receptor neurons of patients with Alzheimer's disease: implications for impaired odor sensitivity, Neurobiol. Aging, 2003, 24, 663-673
[19] Kulkarni-Narla A., Getchell T.V., Schmitt F.A., Getchell M.L., Manganese and copper-zinc superoxide dismutases in the human olfactory mucosa: increased immunoreactivity in Alzheimer's disease, Exp. Neurol., 1996, 140, 115–125
[20] Chuah M.I., Getchell M.L., Metallothionein in olfactory mucosa of Alzheimer's disease patients and apoE-deficient mice, Neuroreport, 1999, 10, 1919-1924
[21] Perry G., Castellani R.J., Smith M.A., Harris P.L.R., Kubat Z., Ghanbari K., et al., Oxidative damage in the olfactory system in Alzheimer's disease, Acta Neuropathol., 2003, 106, 552-556
[22] Yamagishi M., Takami S., Getchell T.V., Ontogenetic expression of spot 35 protein (calbindin-D28k) in human olfactory receptor neurons and its decrease in Alzheimer's disease patients, Ann. Otol. Rhinol. Laryngol., 1996, 105, 132-139
[23] Bhatnagar K.P., Kennedy R.C., Baron G., Greenberg R.A., Number of mitral cells and the bulb volume in the aging human olfactory bulb: a quantitative morphological study, Anat. Rec., 1987, 218, 73–87
[24] Smith R.L., Baker H., Greer C.A., Immunohistochemical analysis of the human olfactory bulb, J. Comp. Neurol., 1993, 333, 519-530
[25] Struble R.G., Clark H.B., Olfactory bulb lesions in Alzheimer's disease, Neurobiol. Aging, 1991, 13, 469-473
[26] Kovács T., Cairns N.J., Lantos P.L., β-Amyloid deposition and neurofibrillary tangle formation in the olfactory bulb in ageing and Alzheimer's disease, Neuropathol. Appl. Neurobiol., 1999, 25, 481-491
[27] Hoogland P.V., van den Berg R., Huisman E., Misrouted olfactory fibres and ectopic olfactory glomeruli in normal humans and in Parkinson and Alzheimer patients, Neuropathol. Appl. Neurobiol., 2003, 29, 303-311
[28] Loopuijt L.D., Sebens J.B., Loss of dopamine receptors in the olfactory bulb of patients with Alzheimer's disease, Brain Res., 1990, 529, 239-244
[29] Averback P., Two new lesions in Alzheimer's disease, Lancet, 1983, 19, 1203
[30] Esiri M.M., Wilcock G.K., The olfactory bulbs in Alzheimer's disease, J. Neurol. Neurosurg. Psychiatry, 1984, 47, 56–60
[31] Mann D.M.A., Tucker C.M., Yates P.O., Alzheimer's disease: an olfactory connection?, Mech. Ageing Dev., 1988, 42, 1–15
[32] Ohm T.G., Braak H., Olfactory bulb changes in Alzheimer's disease, Acta Neuropathol., 1987, 73, 365–369
[33] Hyman B.T., Arriagada P.V., van Hoesen G.W., Pathologic changes in the olfactory bulb in ageing and Alzheimer's disease, Ann. N.Y. Acad. Sci., 1991, 640, 14–19

[34] Reyes P.F., Deems D.A., Suarez M.G., Olfactory-related changes in Alzheimer's disease: a quantitative neuropathologic study, Brain Res. Bull., 1993, 32, 1–5

[35] ter Laak H.J., Renkawek K., van Workum F.P.A., The olfactory bulb in Alzheimer disease: a morphologic study of neuron loss, tangles, and senile plaques in relation to olfaction, Alz. Dis. Assoc. Dis., 1994, 8, 38–48

[36] Arnold S.E., Smutzer G.S., Trojanowski J.Q., Moberg P.J., Cellular and molecular neuropathology of the olfactory epithelium and central olfactory pathways in Alzheimer's disease and schizophrenia, Ann. NY Acad. Sci., 1998, 855, 762-775

[37] Kovacs I., Török I., Zombori J., Kása P., Cholinergic structures and neuropathologic alterations in the olfactory bulb of Alzheimer's disease brain samples, Brain Res., 1998, 789, 167-170

[38] Christen-Zaech S., Kraftsik R., Pillevuit O., Kiraly M., Martins R., Khalili K., et al., Early olfactory involvement in Alzheimer's disease, Can. J. Neurol. Sci., 2003, 30, 20-25

[39] Tsuboi Y., Wszolek Z.K., Graff-Radford N.R., Cookson N., Dickson D.W., Tau pathology in the olfactory bulb correlates with Braak stage, Lewy body pathology and apolipoprotein ε4, Neuropathol. Appl. Neurobiol., 2003, 29, 503-510

[40] Attems J., Lintner F., Jellinger K.A., Olfactory involvement in aging and Alzheimer's disease: an autopsy study, J. Alzheimers Dis., 2005, 7, 149-157

[41] Attems J., Jellinger K.A., Olfactory tau pathology in Alzheimer disease and mild cognitive impairment, Clin. Neuropathol., 2006, 25, 265-271

[42] Fujishiro H., Tsuboi Y., Lin W.-L., Uchikado H., Dickson D.W., Co-localization of tau and α-synuclein in the olfactory bulb in Alzheimer's disease with amygdala Lewy bodies, Acta Neuropathol., 2008, 116, 17-24

[43] Saiz-Sanchez D., Ubeda-Bañon I., de la Rosa-Prieto C., Argandoña-Palacios L., Garcia-Muñozguren S., Insausti R., et al., Somatostatin, tau, and β-amyloid within the anterior olfactory nucleus in Alzheimer disease, Exp. Neurol., 2010, 223, 347-350

[44] Kovács T., Cairns N.J., Lantos P.L., Olfactory centres in Alzheimer's disease: olfactory bulb is involved in early Braak's stages, Neuroreport, 2001, 12, 285-288

[45] Braak H., Braak E., Neuropathological stageing of Alzheimer-related changes, Acta Neuropathol., 1991, 82, 239-259

[46] Wisniewski H.M., Weigel J., Kotula L., Some neuropathological aspects of Alzheimer's disease and its relevance to other disciplines, Neuropathol. Appl. Neurobiol., 1996, 22, 3-11

[47] Sengoku R., Saito Y., Ikemura M., Hatsuta H., Sakiyama Y., Kanemaru K., et al., Incidence and extent of Lewy body-related α-synucleinopathy in aging human olfactory bulb, J. Neuropathol. Exp. Neurol., 2008, 67, 1072-1083

[48] Beach T.G., White C.L. 3rd, Hladik C.L., Sabbagh M.N., Connor D.J., Shill H.A., et al., Olfactory bulb α-synucleinopathy has high specificity and sensitivity for Lewy body disorders, Acta Neuropathol., 2009, 117, 169-174

[49] Attems J., Alpar A., Spence L., McParland S., Heikenwalder M., Ehlén M., et al., Clusters of secretagogin-expressing neurons in the aged human olfactory tract lack terminal differentiation, Proc. Natl. Acad. Sci. USA, 2012, 109, 6259-6264

[50] Mundinano I-C., Caballero M-C., Ordónez C, Hernandez M., DiCaudo C., Marcilla I., et al., Increased dopaminergic cells and protein aggregates in the olfactory bulb of patients with neurodegenerative disorders, Acta Neuropathol., 2011, 122, 61-74

[51] Davies D.C., Brooks J.W., Lewis D.A., Axonal loss from the olfactory tracts in Alzheimer's disease, Neurobiol. Aging, 1993, 14, 353-357

[52] Armstrong R.A., Syed A.B., Smith C.U.M., Density and cross-sectional areas of axons in the olfactory tract in control subjects and Alzheimer's disease: an image analysis study, Neurol. Sci., 2008, 29, 23-27

[53] Thomann P.A., Dos Santos V., Seidl U., Toro P., Essig M., Schröder J., MRI-derived atrophy of the olfactory bulb and tract in mild cognitive impairment and Alzheimer's disease, J. Alzheimers Dis., 2009, 17, 213-221

[54] Thomann P.A., Dos Santos V., Toro P., Schönknecht P., Essig M., Schröder J., Reduced olfactory bulb and tract volume in early Alzheimer's disease - a MRI study, Neurobiol. Aging, 2009, 30, 838-841

[55] Zatorre R.J., Jones-Gotman M., Evans A.C., Meyer E., Functional localization and lateralization of human olfactory cortex, Nature, 1992, 360, 339-340

[56] Insausti R., Marcos P., Arroyo-Jiménez M.M., Blaizot X., Martínez-Marcos A., Comparative aspects of the olfactory portion of the entorhinal cortex and its projection to the hippocampus in rodents, nonhuman primates, and the human brain, Brain Res. Bull., 2002, 57, 557-560

[57] Prestia A., Drago V., Rasser P.E., Bonetti M., Thompson P.M., Frisoni G.B., Cortical changes in incipient Alzheimer's disease, J. Alzheimers Dis., 2010, 22, 1339-1349

[58] Murphy C, Jernigan T.L., Fennema-Notestine C., Left hippocampal volume loss in Alzheimer's disease is reflected in performance on odor identification: a structural MRI study, J. Int. Neuropsychol. Soc., 2003, 9, 459-471

[59] Li Y., Wang Y., Wu G., Shi F., Zhou L., Lin W., et al., Discriminant analysis of longitudinal cortical thickness changes in Alzheimer's disease using dynamic and network features, Neurobiol. Aging, 2012, 33, 427.e15-30

[60] Frisoni G.B., Prestia A., Rasser P.E., Bonetti M., Thompson P.M., In vivo mapping of incremental cortical atrophy from incipient to overt Alzheimer's disease, J. Neurol., 2009, 256, 916-924

[61] Cavedo E., Boccardi M., Ganzola R., Canu E., Beltramello A., Caltagirone C, et al., Local amygdala structural differences with 3T MRI in patients with Alzheimer disease, Neurology, 2011, 76, 727-733

[62] Hedner M., Larsson M., Arnold N., Zucco G.M., Hummel T., Cognitive factors in odor detection, odor discrimination, and odor identification tasks, J. Clin. Exp. Neuropsychol., 2010, 32, 1062–1067

[63] Laing D.G., Natural sniffing gives optimum odor perception for humans, Perception, 1983, 12, 99–107

[64] Sobel N., Thomason M.E., Stappen I., Tanner C.M., Tetrud J.W., Bower J.M., et al., An impairment in sniffing contributes to the olfactory impairment in Parkinson's disease, Proc. Natl. Acad. Sci. USA, 2001, 98, 4154–4159

[65] Rahayel S., Frasnelli J., Joubert S., The effect of Alzheimer's disease and Parkinson's disease on olfaction: a meta-analysis, Behav. Brain Res., 2012, 231, 60-74

[66] Mesholam R.I., Moberg P.J., Mahr R.N., Doty R.L., Olfaction in neurodegenerative disease: a meta-analysis of olfactory functioning in Alzheimer's and Parkinson's diseases, Arch. Neurol., 1998, 55, 84-90

[67] McShane R.H., Nagy Z., Esiri M.M., King E., Joachim C., Sullivan N. et al., Anosmia in dementia is associated with Lewy bodies rather than Alzheimer's pathology, J. Neurol. Neurosurg. Psychiatry, 2001, 70, 739-743

[68] Olichney J.M., Murphy C., Hofstetter C.R., Foster K., Hansen L.A., Thal J.L., et al., Anosmia is very common in the Lewy body variant of Alzheimer's disease, J. Neurol. Neurosurg. Psychiatry, 2005, 76, 1342-1347

[69] Williams S.S., Williams J., Combrick M., Christie S., Smith A.D., McShane R., Olfactory impairment is more marked in patients with mild dementia with Lewy bodies than those with mild Alzheimer disease, J. Neurol. Neurosurg. Psychiatry, 2009, 80, 667-670

[70] Sato T, Hanyu H, Kume K., Takada Y, Onuma T., Iwamoto T., Difference in olfactory dysfunction with dementia with Lewy bodies and Alzheimer's disease, J. Am. Geriat. Soc., 2011, 59, 947-948

[71] Duyckaerts C., Delatour B., Patier M.C., Classification and basic pathology of Alzheimer disease, Acta Neuropathol., 2009, 118, 5-36

[72] Doty R.L., Shaman P., Applebaum S.L., Giberson R., Siksorski L., Rosenberg L., Smell identification ability: changes with age, Science, 1984, 226, 1441–1443

[73] Wilson R.S., Yu L., Schneider J.A., Arnold S.E., Buchman A.S., Bennett D.A., Lewy bodies and olfactory dysfunction in old age, Chem. Senses, 2011, 36, 367-373

[74] Wilson R.S., Arnold S.E., Schneider J.A., Tang Y., Bennett D.A., The relationship between cerebral Alzheimer's disease pathology and odour identification in old age, J. Neurol. Neurosurg. Psychiatry, 2007, 78, 30-35

[75] Wilson R.S., Arnold S.E., Schneider J.A., Boyle P.A., Buchman A.S., Bennett D.A., Olfactory impairment in presymptomatic Alzheimer's disease, Ann. N.Y. Acad. Sci., 2009, 1170, 730-735

[76] Chen Y., Getchell T.V., Larry Sparks D., Getchell M.L., Patterns of adrenergic and peptidergic innervation in human olfactory mucosa: age-related trends, J. Comp. Neurol. 1993, 334, 104-116

[77] Paik S.I., Lehman M.N., Seiden A.M., Duncan H.J., Smith D.V., Human olfactory biopsy. The influence of age and receptor distribution, Arch. Otolaryngol. Head Neck Surg., 1992, 118, 731–738

[78] Schubert C.R., Carmichael L.L., Murphy C., Klein B.E.K., Klein R, Cruickshanks K.J., Olfaction and the 5-year incidence of cognitive impairment in an epidemiological study of older adults, J. Am. Geriatr. Soc., 2008, 56, 1517-1521

[79] Sohrabi H.R., Bates K.A., Weinborn M.G., Johnston A.N.B., Bahramian A., Taddei K., et al., Olfactory discrimination predicts cognitive decline among community-dwelling older adults, Transl. Psychiatry, 2012, 2, e118

[80] Wilson R.S., Schneider J.A., Arnold S.E., Tang Y., Boyle P.A., Bennett D.A., Olfactory identification and incidence of mild cognitive impairment in older age, Arch. Gen. Psychiatry, 2007, 67, 802-808

[81] Sohrabi H.R., Bates K.A., Rodrigues M., Taddei K., Laws S.M., Lautenschlager N.T., et al., Olfactory dysfunction is associated with subjective memory complaints in community-dwelling elderly individuals, J. Alzheimer Dis., 2009, 17, 135-142

[82] Royall D.R., Chiodo L.K., Polk M.J., Jaramillo C.J., Severe dysosmia is specifically associated with Alzheimer-like memory deficits in nondemented elderly retirees, Neuroepidemiology, 2002, 21, 68-73

[83] Djordjevic J., Jones-Gotman M., De Sousa K., Chertkow H., Olfaction in patients with mild cognitive impairment and Alzheimer's disease, Neurobiol. Aging, 2008, 29, 693-706

[84] Westervelt H.J., Bruce J.M., Coon W.G., Tremont G., Odor identification in mild cognitive impairment subtypes, J. Clin. Exp. Neuropsychol., 2008, 30, 151-156

[85] Devanand D.P., Liu X., Tabert M.H., Pradhaban G, Cuasay K., Bell K., et al., Combining early markers strongly predicts conversion from mild cognitive impairment to Alzheimer's disease, Biol. Psychiatry, 2008, 64, 871-879

[86] Lojkowska W., Eawicka B., Gugala M., Sienkiewicz-Jarosz H., Bochynska A., Scinska A., et al., Follow-up study of olfactory deficits, cognitive functions, and volume loss of medial temporal lobe structures in patients with mild cognitive impairment, Curr. Alzheimer Res., 2011, 8, 689-698

[87] Devanand D.P., Michaels-Marston K.S., Liu X., Pelton G.H., Padilla M., Marder K., et al., Olfactory deficits in patients with mild cognitive impairment predict Alzheimer's disease at follow-up, Am. J. Psychiatry, 2000, 157, 1399-1405

[88] Bahar-Fuchs A., Moss S., Rowe C., Savage G., Awareness of olfactory deficits in healthy aging, amnestic mild cognitive impairment and Alzheimer's disease, Int. Psychogeriatr., 2011, 23, 1097-1106

[89] Bahar-Fuchs A., Chételat G, Villemagne V.L., Moss S., Pike K., Masters C.L., et al., Olfactory deficits and amyloid-β burden in Alzheimer's disease, mild cognitive impairment, and healthy aging: a PiB PET study, J. Alzheimer Dis., 2010, 22, 1081-1087

[90] Schofield P.W., Ebrahimi H., Jones A.L., Bateman G.A., Murray S.R., An olfactory 'stress test' may detect preclinical Alzheimer's disease, BMC Neurol., 2012, 12, 24

[91] Wang J, Eslinger P.J., Doty R.L., Zimmerman E.K., Grunfeld R., Sun X., et al., Olfactory deficit detected by fMRI in early Alzheimer's disease, Brain Res., 2010, 1357, 184-194

[92] Li W., Howard J.D., Gottfried J.A., Disruption of odour quality coding in piriform cortex mediates olfactory deficits in Alzheimer's disease, Brain, 2010, 133, 2714-2726

[93] Kareken D.A., Doty R.L., Moberg P.J., Mosnik D, Chen SH, Farlow M.R., et al., Olfactory-evoked regional cerebral blood flow in Alzheimer's disease, Neuropsychology, 2001, 15, 18-29

[94] Wang Q-S., Tian L., Huang Y-L., Qin S, He L-Q., Zhou J-N., Olfactory identification and apolipoprotein ε4 allele in mild cognitive impairment, Brain Res., 2002, 951, 77-81

[95] Olofsson J.K., Nordin S., Wiens S, Hedner M., Nilsson L-G., Larsson M., Odor identification in carriers of ApoE-ε4 is independent of clinical dementia, Neurobiol. Aging, 2010, 31, 567-577

[96] Calhoun-Haney R., Murphy C., Apolipoprotein ε4 is associated with more rapid decline in odor identification than in odor threshold or Dementia Rating Scale scores, Brain Cogn., 2005, 58, 178-182

[97] Sperling R.A., Aisen P.S., Beckett L.A., Bennett D.A., Craft S., Fagan A.M., et al., Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease, Alzheimers Dement., 2011, 7, 280-292

[98] Handley O.J., Morrison C.M., Miles C., Bayer A.J., ApoE gene and familial risk of Alzheimer's disease as predictors of odour identification in older adults, Neurobiol. Aging, 2006, 27, 1425-1430

[99] Schiffmann S.S., Graham B.G., Sattely-Miller E.A., Zervakis J., Welsh-Bohmer K., Taste, smell and neuropsychological performance of individuals at familial risk for Alzheimer's disease, Neurobiol. Aging, 2002, 23, 397-404

[100] Nee L.E., Lippa C.F., Inherited Alzheimer's disease PS-1 olfactory function: a 10-year follow-up study, Am. J. Alzheimers Dis. Other Demen., 2001, 16, 83-84

[101] Larsson M., Semb H., Winblad B., Amberla K., Wahlund L.O., Bäckman L., Odor identification in normal aging and early Alzheimer's disease: effects of retrieval support, Neuropsychology, 1999, 13, 47-53

[102] Suzuki Y., Yamamoto S., Umegaki H., Onishi J., Mogi N., Fujishiro H. et al., Smell identification test as an indicator for cognitive impairment in Alzheimer's disease, Int. J. Geriatr. Psychiatry, 2004, 19, 727-733

[103] Tkalčić M., Spasić N., Ivanković M., Pokrajac-Bulian A., Bosanac D., Odor identification and cognitive abilities in Alzheimer's disease, Transl. Neurosci., 2011, 2, 233-240

[104] McCaffrey R.J., Duff K., Solomon G.S., Olfactory dysfunction discriminates probable Alzheimer's dementia from major depression: a cross-validation and extension, J. Neuropsychiatry Clin. Neurosci., 2000, 12, 29-33

[105] Luzzi S., Snowden J.S., Neary D., Coccia M., Provinciali L., Lambon Ralph M.A., Distinct patterns of olfactory impairment in Alzheimer's disease, semantic dementia, frontotemporal dementia, and corticobasal degeneration, Neuropsychologia, 2007, 45, 1823-1831

[106] Hawkes C.H., Del Tredici K., Braak H., Parkinson's disease: the dual hit theory revisited, Ann. NY Acad. Sci., 2009, 1170, 615-622

[107] Ferrera-Moyano H., Barragan E., The olfactory system and Alzheimer's disease, Int. J. Neurosci., 1989, 49, 157-197

[108] Doty R.L., The olfactory vector hypothesis of neurodegenerative disease: is it viable?, Ann. Neurol., 2008, 63, 7-15

[109] Tonelli L.H., Postolache T.T., Airborne inflammatory factors: "from the nose to the brain", Front. Biosci. (Schol. Ed.), 2010, 2, 135-152

[110] Honjo K., van Reekum R., Verhoeff N.P., Alzheimer's disease and infection: do infectious agents contribute to progression of Alzheimer's disease?, Alzheimers Dement., 2009, 5, 348-360

[111] Špeljko T, Jutric D, Šimić G., HSV1 in Alzheimer's disease: myth or reality?, Transl. Neurosci., 2011, 2, 61-68

[112] Esiri M.M., Herpes simplex encephalitis. An immunohistological study of the distribution of viral antigen within the brain, J. Neurol. Sci., 1982, 54, 209–226

[113] Landis B.N., Vodicka J., Hummel T., Olfactory dysfunction following herpetic meningoencephalitis, J. Neurol., 2010, 257, 439-443

[114] Mann D.M., Tinkler A.M., Yates P.O., Neurological disease and herpes simplex virus. An immunohistochemical study, Acta Neuropathol., 1983, 60, 24-28

[115] Twomey J.A., Barker C.M., Robinson G., Howell D.A., Olfactory mucosa in herpes simplex encephalitis, J. Neurol. Neurosurg. Psychiatry, 1979, 42, 983–987

[116] Dinn J.J., Transolfactory spread of virus in herpes simplex encephalitis, Br. Med. J., 1980, 281, 1392

[117] Balin B.J., Scott Little C., Hammond C.J., Appelt D.M., Whittum-Hudson J.A., Gérard H.C., et al., Chlamydophila pneumoniae and the etiology of late-onset Alzheimer's disease, J. Alzheimers Dis., 2008, 13, 371-380

[118] Balin B.J., Gerard H.C., Arking E.J., Appelt D.M., Branigan P.J., Abrams J.T., et al., Identification and localization of Chlamydia pneumoniae in the Alzheimer's brain, Med. Microbiol. Immunol., 1998, 187, 23–42

[119] Roberts E., Alzheimer's disease may begin in the nose and may be caused by aluminosilicates, Neurobiol. Aging, 1986, 7, 561-567

[120] Samudralwar D.L., Diprete C.C., Ni B-F., Ehmann W.D., Markesbery W.R., Elemental imbalances in the olfactory pathway in Alzheimer's disease, J. Neurol. Sci., 1995, 130, 139-145

[121] Arriagada P.V., Louis D.N., Hedley-Whyte E.T., Hyman B.T., Neurofibrillary tangles and olfactory dysgenesis, Lancet, 1991, 337, 559

[122] Pearson R.C., Esiri M.M., Hiorns R.W., Wilcock G.K., Powell T.P.S., Anatomical correlates of the distribution of the pathological changes in the neocortex in Alzheimer's disease, Proc. Natl. Acad. Sci. USA, 1985, 82, 4531-4534

[123] Pearson R.C., Cortical connections and the pathology of Alzheimer's disease, Neurodegeneration, 1996, 5, 429-434

[124] Murray M.E., Graff-Radford N.R., Ross O.A., Petersen R.C., Duara R., Dickson D.W., Neuropathologically defined subtypes of Alzheimer's disease with distinct clinical characteristics: a retrospective study, Lancet Neurol., 2011, 10, 785-796

[125] Castellani R.J., Perry G., Pathogenesis and disease-modifying therapy in Alzheimer's disease: the flat line of progress, Arch. Med. Res., 2012, 43, 694-698

[126] Lemere C.A., Maron R., Selkoe D.J., Weiner H.L., Nasal vaccination with beta-amyloid peptide for the treatment of Alzheimer's disease, DNA Cell Biol., 2001, 20, 705-711

[127] Sipos E., Kurunczi A., Fehér A., Penke Z., Fülöp L, Kasza A., et al., Intranasal delivery of human beta-amyloid peptide in rats: effective brain targeting, Cell. Mol. Neurobiol., 2010, 30, 405-413

[128] Pepeu G., Giovannini M.G., Cholinesterase inhibitors and beyond, Curr. Alzheimer Res., 2009, 6, 86-96

[129] Capsoni S., Giannotta S., Cattaneo A., Nerve growth factor and galantamine ameliorate early signs of neurodegeneration in anti-nerve growth factor mice, Proc. Natl. Acad. Sci. USA, 2002, 99, 12432-12437

[130] Cuello A.C., Bruno M.A., Allard S., Leon W., Iulta M.F., Cholinergic involvement in Alzheimer's disease. A link with NGF maturation and degradation, J. Mol. Neurosci., 2010, 40, 230-235

[131] Tuszinsky M.H., Thal L., Pay M., Salmon D.P., U H.S., Bakay R., A phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer disease, Nat. Med., 2005, 11, 551-555

[132] Mandel R.J., CERE-110, an adeno-associated virus-based gene delivery vector expressing human nerve growth factor for the treatment of Alzheimer's disease, Curr. Opin. Mol. Ther., 2010, 12, 240-247

[133] Eriksdotter-Jönhagen M., Linderoth B., Lind G., Aladellie L, Almkvist O, Andreasen N., et al., Encapsulated cell biodelivery of nerve growth factor to the basal forebrain in patients with Alzheimer's disease, Dement. Geriatr. Cogn. Disord., 2012, 33, 18-28

[134] Chen X.Q., Fawcett J.R., Rahman Y.E., Ala T.A., Frey W.H., Delivery of nerve growth factor to the brain via the olfactory pathway, J. Alzheimers Dis., 1998, 1, 35-44

[135] Capsoni S., Covaveuszach S., Ugolini G., Spirito F., Vignone D., Stefanini B., et al., Delivery of NGF to the brain: intranasal versus ocular administration in anti-NGF transgenic mice, J. Alzheimers Dis. 2009, 16, 371-388

[136] Chiu S.L., Chen C.M., Cline H.T., Insulin receptor signaling regulates synapse number, dendritic plasticity, and circuit function in vivo, Neuron, 2008, 58, 708-719

[137] McNay E.C., Ong C.T., McCrimmon R.J., Cresswell J., Bogan J.S., Sherwin R.S., Hippocampal memory processes are modulated by insulin and high-fat-induced insulin resistance, Neurobiol. Learn. Mem., 2010, 93, 546-553

[138] Bosco D., Fava A., Plastino M., Montalcini T., Pujia A., Possible implications of insulin-resistance and glucose metabolism in Alzheimer's disease pathogenesis, J. Cell. Mol. Med., 2011, 15, 1807-1821

[139] Steen E., Terry B.M., Rivera E.J., Cannon J.L., Neely T.R., Tavares R., et al., Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease - is this type 3 diabetes?, J. Alzheimers Dis., 2005, 7, 63-80

[140] Shemesh E., Rudich A., Harman-Boehm I, Cukiernab-Yaffe T., Effect of intranasal insulin on cognitive function: a systematic review, J. Clin. Endocrinol. Metab., 2012, 97, 366-376

[141] Ott V., Benedict C., Schultes B., Born J., Hallschmid M., Intranasal administration of insulin to the brain impacts cognitive function and peripheral metabolism, Diabetes Obes. Metab., 2012, 14, 214-221

[142] Reger M.A., Watson G.S., Green P.S., Baker L.D., Cholerton B., Fishel M.A., et al., Intranasal insulin administration dose-dependently modulates verbal memory and plasma amyloid-beta in memory-impaired older adults, J. Alzheimers Dis., 2008, 13, 323-331

[143] Reger M.A., Watson G.S., Green P.S., Wilkinson C.W., Baker L.D., Cholerton B., et al., Intranasal insulin improves cognition and modulates beta-amyloid in early Alzheimer's disease, Neurology, 2008, 70, 440-448

[144] Craft S., Baker L.D., Montine T.J., Minoshima S., Watson G.S., Claxton A., et al., Intranasal insulin therapy for Alzheimer disease and amnestic mild cognitive impairment: a pilot clinical trial, Arch. Neurol., 2012, 69, 29-38

[145] Maestro B., Davila N., Carranza M.C., Calle C., Identification of a vitamin D response element in the human insulin receptor gene promoter, J. Steroid Biochem. Mol. Biol., 2003, 84, 223-230

[146] von Hurst P.R., Stonehouse W., Coad J., Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient - a randomised, placebo-controlled trial, Br. J. Nutr., 2010, 103, 549-555

[147] Stein M.S., Scherer S.C., Ladd K.S., Harrison L.C., A randomized controlled trial of high-dose vitamin D2 followed by intranasal insulin in Alzheimer's disease, J. Alzheimers Dis., 2011, 26, 477-484

[148] Jogani V.V., Shah P.J., Mishra P., Mishra A.K., Misra A.R., Nose-to-brain delivery of tacrine, J. Pharm. Pharmacol., 2007, 59, 1199-1205

[149] Jogani V.V., Shah P.J., Mishra A.K., Misra A.R., Intranasal mucoadhesive microemulsion of tacrine to improve brain targeting, Alzheimer Dis. Assoc. Disord., 2008, 22, 116-124

[150] Durand M., Coronas V., Jourdan F., Quirion R., Developmental and aging aspects of the cholinergic innervation of the olfactory bulb, Int. J. Dev. Neurosci., 1998, 16, 777-785

[151] Kása P., Rakonczay Z., Gulya K., The cholinergic system in Alzheimer's disease, Prog. Neurobiol., 1997, 52, 511-535

[152] Velayudhan L., Lovestone S., Smell identification test as a treatment response marker in patients with Alzheimer disease receiving donepezil, J. Clin. Psychopharmacol., 2009, 29, 387-390

[153] Burns A., Perry E., Holmes C., Francis P., Morris J., Howes M.J., et al., A double-blind placebo-controlled randomized trial of Melissa officinalis oil and donepezil for the treatment of agitation in Alzheimer's disease, Dement. Geriatr. Cogn. Disord., 2011, 31, 158-164

[154] Papp M.I., Komoly S., Szirmai I.G., Kovács T., Similarities between CSF-brain extracellular transfer and neurofibrillary tangle invasion in Alzheimer's disease, Neurobiol. Aging, 2006, 27, 402-412