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·Review·

Hyposmia: a possible biomarker of Parkinson's disease

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Hyposmia, identified as reduced sensitivity to odor, is a common non-motor symptom of Parkinson's disease (PD) that antedates the typical motor symptoms by several years. It occurs in ~90% of early-stage cases of PD. In addition to the high prevalence, the occurrence of hyposmia may also predict a higher risk of PD. Investigations into hyposmia and its relationship with PD may help elucidate the underlying pathogenic mechanisms. This review provides an update of olfactory dysfunction in PD and its potential as a biomarker for this devastating disease.

Keywords: hyposmia; Parkinson's disease; olfaction; biomarker

Introduction

In 1817, James Parkinson first noted people in London exhibiting particular symptoms including involuntary tremulous motion, lessened muscular power, a propensity to bend the trunk forward, and a running pace. He found that individuals with this disease, later called Parkinson's disease (PD), had difficulty planning and controlling movements[1]. Today, 1–2% of the population >65 years old suffer from this disease and the rate increases to 3–5% at 85 years and older[2]. James Parkinson in this thesis stated "the senses and intellects being uninjured"[1], but in 1975 Ansari and Johnson first reported olfactory dysfunction in PD patients[3] and today, hyposmia is widely accepted as a major symptom. In fact, it is hypothesized that this olfactory dysfunction is not simply a benign symptom, but rather a potential culprit behind PD[4]. This article therefore discusses the current research in this area and explores the relationship between hyposmia and PD.

Anatomy of the Olfactory System

Olfactory information-processing is a complicated multi-step process. There are many places in the olfactory system where PD-related pathology could occur; therefore, we first overview the anatomy of this system (Fig. 1).

First, odorant molecules are directed with the air into the nasal cavity and contact the olfactory epithelium. This epithelium is a sheet of cells that lines the dorsal surface of the nasal cavity. The epithelium contains four main cell-types: bipolar olfactory receptor cells, basal cells, supporting cells, and microvillus cells[5], of which the olfactory receptor cells are the most important because they detect odorant molecules. These receptor cells are abundant in the epithelium (~ 6 000 000 per individual), occurring at 3–5 μm intervals across the surface[5]. Each receptor cell sends 10–30 cilia towards the epithelial surface and a long unmyelinated axon through the cribriform plate to the olfactory bulb[5]. Once odorants bind to their receptors, they activate GTP-binding proteins, which in turn trigger the synthesis of the second messenger 3',5'-cyclic monophosphate (cAMP). cAMP opens a cyclic nucleotide-gated channel, resulting in an influx of Na^+ and Ca^{2+} ions and depolarization of the receptor cell[6]. Each receptor neuron expresses only one kind of receptor among approximately a thousand receptor proteins. Sensory neurons expressing the same receptor

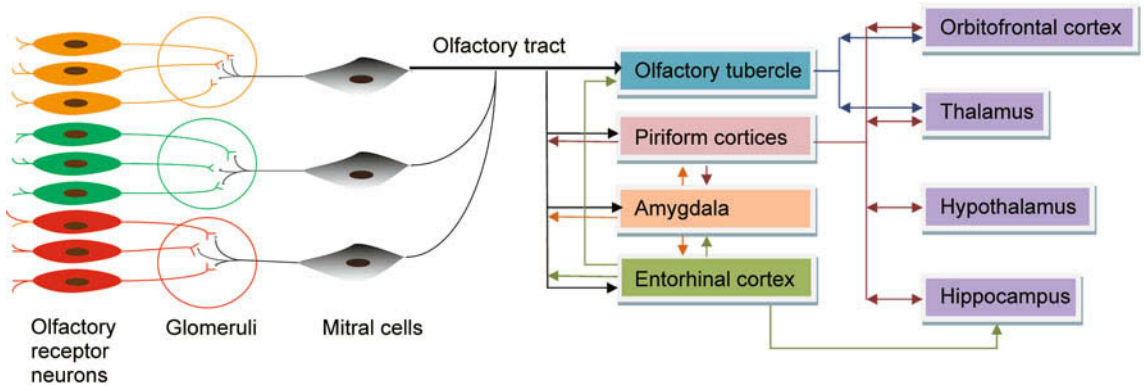

Fig. 1. Schematic of the olfactory system. Each olfactory sensory neuron expresses only one type of receptor. Neurons expressing the same receptor project into specific glomeruli within the olfactory bulb and form synapses with mitral cells and tufted cells (not shown) in the glomeruli. The axons of mitral/tufted cells form the olfactory tract and project to many central olfactory structures including the olfactory tubercle, piriform cortices, amygdala, and lateral entorhinal cortex. Connections are extensive among these structures and other brain regions such as the orbitofrontal cortex, thalamus, hypothalamus, and hippocampus. The olfactory bulb also receives projections from secondary olfactory structures other than the olfactory tubercle. Directionality is denoted by arrows showing the projections among these brain structures.

are arranged in overlapping but distinct bands across the olfactory epithelium and converge by projecting into specific glomeruli within the olfactory bulb[7,8]. There is evidence that the responses to hundreds of pure odorants are arranged systematically across the olfactory bulb in a manner closely related to odorant chemical structure[8].

The olfactory bulb consists of six layers based on cell type and spatial distribution: the olfactory nerve layer, glomerular layer, external plexiform layer, mitral cell layer, internal plexiform layer, and granular layer[7,9-11]. The olfactory nerve layer, consisting of axons of olfactory sensory neurons, covers the outer region of the olfactory bulb. The axons then enter the glomeruli, where they synapse with the apical dendrites of mitral cells and tufted cells[7,9-11]. Periglomerular interneurons also send neurites into the glomeruli, where they release dopamine and gamma-aminobutyric acid to modulate synaptic transmission[11]. Below the glomerular layer, the external plexiform layer is composed of the dendrites of mitral cells and the somata of tufted cells, and the somata of mitral cells constitute the mitral cell layer deep to the external plexiform layer[7,9-11]. The axons of mitral cells and tufted cells go through the internal plexiform layer, making up the major output of the olfactory bulb together with the axons of granule cells in the granular layer[7,9-11].

The olfactory tract then projects to many central (secondary) olfactory structures, including the anterior olfactory nucleus, olfactory tubercle, anterior and posterior piriform cortices and endopiriform nucleus, transitional periamygdaloid cortex and anterior cortical nucleus of the amygdala, and lateral entorhinal cortex[12]. Connections among these structures and other brain regions are extensive, and most of the secondary olfactory structures also send direct fibers back to the olfactory bulb[12].

Pathology of the Olfactory System in PD

The typical motor symptoms of PD arise from dopamine deficiency in the striatum[13]. The mesostriatal dopamine pathway which projects from the substantia nigra to the dorsal striatum plays a crucial role in motor control, and significant loss of dopaminergic neurons in the substantia nigra gives rise to the movement disorders[14]. In PD, dopaminergic neuron loss is continuous, and most motor symptoms become apparent only after extensive cell death, when mechanisms cannot compensate for the dopamine decline[15]. In addition, abnormal cytoplasmic proteinaceous inclusions (Lewy bodies/Lewy neurites), the primary structural component of which is alpha-synuclein, are also present in many surviving cells in the substantia nigra[16]. In a pathological study, Braak *et al.* proposed a neuropathological staging procedure based

on topographic changes in the Lewy bodies and Lewy neurites[17]. According to Braak staging, the pathological process progresses in six stages. In stage 1, the inclusion body pathology initially occurs in the olfactory bulb and/or the dorsal motor nucleus of the glossopharyngeal and vagal nerves. In stage 2, the pathology occurs in the medulla oblongata and pontine tegmentum. In stage 3, the amygdala and substantia nigra become the foci of pathology and the illness reaches the symptomatic phase. In stage 4, the pathology further deteriorates and the temporal cortex is involved. In the final stages 5 and 6, the pathological process appears in the mature neocortex and the disease manifests itself globally, resulting in cognitive problems associated with advanced PD[17, 18]. The staging theory suggests an upward course of brain pathology, beginning in the olfactory bulb, the dorsal motor nucleus IX and the adjoining intermediate reticular zone, then reaching the lower brain stem nuclei and eventually extending into the cerebral cortex. This is consistent with the clinical features; non-motor symptoms such as hyposmia and autonomic dysfunction precede the motor symptoms by several years[19].

According to Braak's report, the pathological involvement of olfactory structures is mainly in the anterior olfactory nucleus[20], which contains large multipolar neurons and is embedded in the olfactory bulb and olfactory tract[10]. At the very beginning, only a few Lewy neurites are visible; as the pathology progresses, more Lewy neurites and also Lewy bodies emerge[20]. However, the pathology shows no tendency to advance into non-olfactory regions[20]. In addition to the Lewy pathology, neuronal loss is also seen in the anterior olfactory nucleus from PD patients postmortem and has a strong correlation with disease duration[21]. Moreover, the total number of tyrosine hydroxylase-immunoreactive neurons in the olfactory bulb of PD patients is twice as high as that of controls, indicating that an increase in dopaminergic neurons contributes to hyposmia in PD[22]. Outside the olfactory bulb, Lewy bodies have also been identified throughout the amygdala in PD, but concentrated in the cortical and basolateral nuclei[23]. Particularly, the cortical nucleus that has major olfactory connections exhibits a constant significant loss of volume and neuron number, indicating that its degeneration contributes to hyposmia in PD[23]. Besides, the olfactory nerves from PD patients also exhibit gross atrophy and abnormal architecture under microscopic examination[21]. But no specific changes have been reported in the olfactory epithelium, suggesting that hyposmia does not result from epithelial abnormalities[24].

Role of the Olfactory System in the Pathogenesis of PD

Anatomically, the axons of olfactory receptor cells enter the brain through the cribriform plate. This direct projection from the nasal cavity effectively bypasses the blood-brain barrier; so the olfactory system might be a portal through which exogenous agents can enter the brain[25,26]. Extensive studies have shown that xenobiotic-like viruses, aerosolized metals, and toxins, which have been implicated as risk factors for PD, can enter the brain *via* the olfactory system[26,27]. Under normal circumstances, immune cells and detoxifying enzymes in the olfactory system prevent xenobiotics from entering and damaging the brain[26,28].

It is well accepted that the olfactory system is among the earliest structures affected in PD and hyposmia is one of the earliest non-motor symptoms commonly seen in PD patients[17-19,29]. These, together with the unique anatomy of this system (bypassing the blood-brain barrier), lead to the hypothesis that PD may be a primary olfactory disorder[4,26]. Some hold that the olfactory system is the path through which certain pathogens enter the brain and initiate the disease pathology[26]. This "olfactory vector hypothesis" was supported by Prediger e*t al.*, who found that rats given 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP) intranasally exhibit loss of smell and motor deficits[30]. However, other studies have raised doubts. For example, some patients with PD do not have olfactory dysfunction[26, 31]. Recently, Kuo *et al.* found that transgenic mice expressing mutant alpha-synuclein (A53T) have abnormal motor behavior but a normal sense of smell[32]. However, Lewy pathology is absent from the mouse brain, revealing the limitations of animal models of PD[32]. In Lewy pathology staging, Braak considers that pathology in the anterior olfactory nucleus tends to be confined and the dorsal motor nucleus is more likely to be the starting point[20], but so far no direct evidence rules out the possibility that the olfactory system is the origin of PD pathology, because connections exist between the anterior olfactory nucleus and the dorsal

motor nucleus, as well as the substantia nigra[26]. It is likely that the olfactory system plays a role in the pathogenesis of PD in cooperation with other factors[26].

Hyposmia as A Possible Biomarker of PD

PD is a multisystem disorder, as it also affects systems other than the motor system[19]. Hyposmia is one of the cardinal non-motor symptoms in PD and is present in up to 90% of PD patients[31, 33]. Hyposmia precedes the motor features by several years[34], and is thus considered as a candidate biomarker for PD because of its high prevalence in PD patients and also the convenience of testing compared with other putative biomarkers such as neuroimaging of the dopamine system[35]. Bohnen *et al.* compared the accuracy of various clinical motor and non-motor tests for the diagnosis of PD and found that odor identification is the most accurate predictor of PD[36]. Deeb *et al.* further evaluated the diagnostic accuracy of odor identification using the University of Pennsylvania Smell Identification Test (UPSIT) and the dopamine transporter scan (DaTSCAN). They found that UPSIT is as sensitive as DaTSCAN, both having ~90% sensitivity, and more importantly, UPSIT is cheaper and more convenient than DaTSCAN[35].

In spite of the high sensitivity, the relatively low specificity limits the diagnostic application of hyposmia, because olfactory dysfunction exists not only in PD, but also in other neurological diseases[37]. Research has demonstrated that, compared to PD patients, patients with mild Alzheimer's disease and the parkinsonism-dementia complex of Guam exhibit equivalent odor identification and detection deficits, suggesting that olfactory testing cannot be used to distinguish these three diseases[37]. In contrast, patients with progressive supranuclear palsy[37, 38], MPTP-induced parkinsonism[37], multiple system atrophy[38], corticobasal degeneration[38], or essential tremor[39] have normal or nearly normal olfactory function. Therefore, olfactory tests can be used to differentiate idiopathic PD from these diseases.

Olfactory dysfunction is also a predictive marker for the development of PD. In Ponsen's study, olfaction was tested in 361 asymptomatic relatives (parents, siblings, or children) of patients with PD. Forty hyposmic and 39 normosmic individuals were followed up for a 2-year clinical evaluation with single-photon emission computed tomography to assess nigrostriatal dopaminergic function. Two years from baseline, 10% of the individuals with idiopathic hyposmia, who also had significantly reduced [^{123}I]-βCIT (a dopamine transporter ligand) binding at baseline, had developed clinical PD as opposed to none of the other relatives in the cohort, indicating that idiopathic olfactory dysfunction is associated with an increased risk of developing PD of at least 10%[40]. In the longitudinal Honolulu-Asia Aging Study, olfaction was assessed in 2 267 men aged 71 to 95 years without clinical PD and dementia at the time of testing. In the 8-year follow-up, 35 men were diagnosed with PD. The odds ratios for PD in the lowest quartile of odor identification was 5.2 (95% confidence interval, 1.5–25.6) compared with the top two quartiles, implying that impaired olfaction may be a useful screening tool to detect people at high risk for developing PD in later life[34].

Olfaction Assessment Methods

Several methods are available to diagnose hyposmia, including psychophysical tests, electrophysiological methods, psychophysiological tests, and neuroimaging. Psychophysical tests include tests of odor sensitivity (threshold), identification, discrimination, and memory. In these tests, the participant is required to perceive an odor and answer a question about it. Currently, the most widely used instrument to assess olfactory identification is the UPSIT[41]. The Cross-Cultural Smell Identification Test (CC-SIT) was developed to be valid in most cultural settings[42]. 'Sniffin' sticks' uses a pen-like device to test the odor detection threshold, odor discrimination, and odor identification[43]. It is worth noting that cultural differences may cause some bias in the results, especially when analyzing data from different research centers all over the world in large-scale experiments[44]. Kobayashi *et al.* used a new olfactory test developed for Japanese, the Odor Stick Identification Test for Japanese (OSIT-J), in 52 US and 50 Japanese participants and found the average score for the former was significantly lower than that of the latter, reflecting cultural differences in odor experience[44].

The odor event-related potential (OERP) can be recorded in response to olfactory stimuli, reflecting sequential activation of the stations in the olfactory pathway. The latencies to these responses are prolonged in

PD patients[45]. PD may impair sniff airflow rate and volume, which may confound the results of psychophysical olfactory tests[46]. The advantage of the OREP is that it can quantify olfaction independent of sniff volume or odor memory. However, the clinical use of the OREP is restricted because of its dependence on complicated equipment[9].

Psychophysiological tests mainly record the physiological responses to odors, most notably heart rate, respiration, and blood pressure[9]. The Sniff Magnitude Test quantifies the decrease of inhalation when a bad odor is encountered. Under such circumstances, a participant with a normal sense of smell immediately ends the sniff, whereas a person suffering from anosmia or hyposmia continues a normal or nearly normal sniff[9, 47]. This test measures the reflex-like response to odors, and minimizes the cognitive demands of other olfactory tests. Therefore it is a potential clinical tool to assess olfactory function in PD patients with cognitive impairment[47]. However it is worth noting that the Sniff Magnitude Test probably assesses suprathreshold hedonics, a component of olfaction distinct from odor identification, detection, and short-term memory, and it can be influenced by prior experience and cognitive factors[48].

Functional imaging studies reveal the metabolism in different brain regions. With this method, metabolic reduction in PD patients has been visualized within various areas including the medial prefrontal cortex, the dorsolateral prefrontal cortex, the medial occipital cortex, and the lateral parieto-temporo-occipital area[49]. In addition to the wide decline in metabolism, PD patients with severe hyposmia exhibit broader occipital hypometabolism[49]. Functional imaging methods can also be used to detect neurotransmitter deficiency in PD, conceivably helping narrow the at-risk population after screening for hyposmia[34, 40].

Conclusions

PD is typically diagnosed by the pivotal motor symptoms of the disease such as rigidity, akinesia, bradykinesia, tremor, and postural instability. Unfortunately these symptoms do not become noticeable until ~80% of the dopaminergic neurons in the substantia nigra have died. Therefore, early diagnosis is crucial for early intervention in PD, especially for those at a high risk such as first-degree relatives of patients with the disease. Hyposmia could be used as a biomarker for PD to screen high-risk populations and discriminate it from other Parkinsonian syndromes, but the low specificity limits broad clinical application. In combination with other non-motor symptoms in PD such as autonomic dysfunction, depression, visual symptoms, and REM sleep behavior disorder, the specificity of hyposmia as a biomarker for PD may be enhanced[50]. Currently, several large-scale longitudinal studies are under way, such as PARS (Parkinson Associated Risk Study) and TREND (Tübinger evaluation of Risk factors for the Early detection of NeuroDegeneration)[50, 51]. These may help to establish an effective screening protocol for the early diagnosis of PD at the population level.

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