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Gender and age related changes in number of dopaminergic neurons in adult human olfactory bulb

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ABSTRACT

Introduction: Dopamine is one of the major brain neurotransmitters, and the loss of dopaminergic neurons in basal ganglia cause motor deficits in Parkinson's disease. We proposed that the difficulty in olfaction observed in the elderly may be due to an alteration in the number of dopaminergic neurons.
Materials and methods: Sections were taken from olfactory bulbs of post-mortem tissue specimens of 13 humans, males and females, aged from 19 to 63 years ($\leq$35 and $\geq$50 years), with no history of neurological disorders. The tissues were fixed, embedded, cut on a freezing microtome, and prepared for immunohistochemical analysis using tyrosine hydroxylase (TH) and aromatic L-amino acid decarboxylase (AADC) antibodies. The number of positive neurons was counted.
Results: TH- and AADC-positive cells were present in the glomerular layer. There was no significant difference between the numbers of TH- and AADC-positive cells, in males and females, and in young and elderly individuals. The quantitative analysis revealed that the number of TH- and AADC-positive neurons were significantly higher in males than in females ($P < 0.05$). Moreover, there was a significant increase in the number of TH- and AADC-positive neurons in the olfactory bulbs of the elderly compared with young individuals ($P < 0.05$).
Conclusion: Factors such as gender and age may affect the number of dopaminergic neurons, and there is a correlation between increased dopaminergic neurons and olfactory performance. Moreover, the increase in dopaminergic cells in the olfactory bulb of the elderly may indicate the existence of rostral migratory stream in adult humans.

1. Introduction

Dopamine may have an important role in vertebrate olfaction. A considerable number of dopaminergic cells have been demonstrated in the olfactory bulbs (OB) of all vertebrate classes, including humans (Smeets, 1994; Hoogland and Huisman, 1999). Subpopulations of periglomerular neurons (in the glomerular layer) and tufted cells (in the superficial external OB plexiform layers) express aromatic L-amino acid decarboxylase (AADC) and tyrosine hydroxylase (TH), which is the rate-limiting enzyme of the dopamine synthesis (Halasz et al., 1981; Baker et al., 1991; Davila et al., 2003; Weihe et al., 2006). Dopaminergic neurons were mostly characterized in the glomerular layer of the human OB (Smith et al., 1991). It appears that the number of dopaminergic neurons in the OB is part of a dynamic process,

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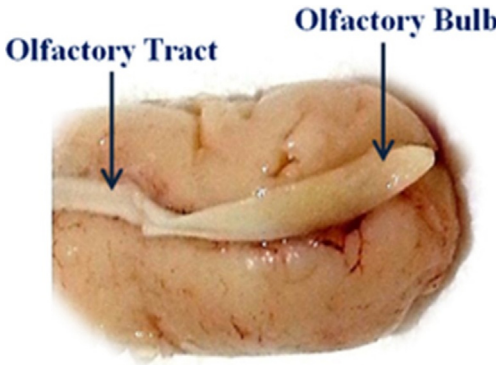

Fig. 1. Human olfactory bulb and tract.

and depends on olfactory input. Furthermore, it has been reported that odor deprivation considerably decreases TH-positive neurons in the OB glomerular layer (Baker et al., 1993).

Although many aspects of the human OB dopaminergic neurons have been recognized in the past few decades (Smith et al., 1991; Hoogland and Huisman, 1999; Bedard and Parent, 2004; Huisman et al., 2008), there are important fundamental questions regarding the difference in the number of neurons expressing the dopaminergic phenotype in humans.

Before investigating whether the TH/AADC distribution in the OB of patients with a neurodegenerative disorder are comparable to age-matched controls, knowledge regarding the number of TH- and AADC-immunoreactive neurons in healthy individuals is necessary. Therefore, this study evaluated the effect of age and gender on the variability of TH and AADC immunoreactivity in the OB of healthy individuals. Providing information regarding healthy people can help establish the extent of hyposmia observed in neurodegenerative disorders is related to an OB dopaminergic dysfunction. Because neurodegenerative disorders, like Parkinson's, usually manifest in the elderly, we focused on the number of dopaminergic neurons in individuals >50 years, and compared them with young individuals (<30 years of age). Moreover, gender differences were considered.

2. Materials and methods

2.1. Tissue preparation

The present data derived from the analysis of sections taken from OB of post-mortem tissue specimens (Fig. 1) of both genders. The tissues were received from Iranian Tissue Bank (ITB) and Research Center (14158-868), Tehran University of Medical Sciences (Permission number: 8265). The OB from 13 cases, aged from 19 to 63 years (age ≤35 years and age ≥50years were grouped as young and old, respectively). The cases had no history of neurological disease, and in particular, no hyposmia had been reported. For better defining the specimens as healthy individuals, alpha-synuclein immunostaining was carried out to exclude Lewy body pathology. All OB were immune-negative for alpha-synuclein (Fig. 2), indicating that the cases in this study did not suffer from Parkinson's disease. The post-mortem delay varied between 5 and 25 h (Table 1). Cases were grouped as females ($n = 6$) or males ($n = 7$), to compare the TH- and AADC-positive cells between them. The average age of males and females was 42.2 and 41.5, respectively.

OB were dissected, immersion-fixed for 6–10 days in phosphate-buffered 4% paraformaldehyde (pH 7.4), and stored at 4 °C (Hoogland and Huisman, 1999). After fixation, the bulbs were cryoprotected in solutions of 10, 15, and 30% sucrose in 0.1 M phosphate buffer (pH 7.4), for three days at room temperature (Huisman et al., 2008). Then, the specimens were embedded in OCT, and 10 μm thickness sections in a frontal plane were cut using a freezing microtome. These sections were stored by freezing in a cryoprotective solution.

2.2. Immunohistochemical (IHC) staining

Every 10th frontal section was stained immunohistochemically for tyrosine hydroxylase, using a standardized protocol previously

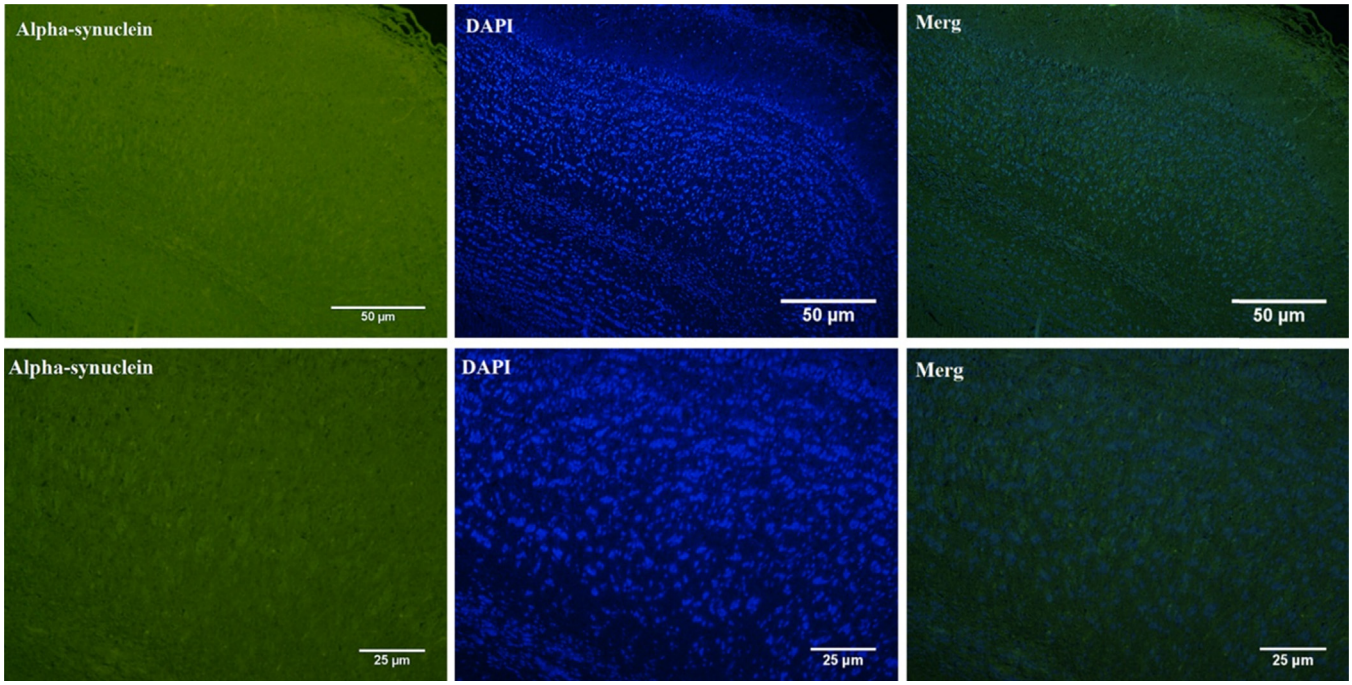

Fig. 2. Photomicrograph of alpha-synuclein immune-negative staining in human olfactory bulb to show Lewy bodies. All olfactory bulbs were immune-negative for alpha-synuclein (phospho-synuclein). Cell nuclei were counterstained with DAPI. Upper row: 100× and lower raw: 200×.