Original Communication

THREE-DIMENSIONAL RECONSTRUCTION OF THE ANTERIOR OLFACTORY NUCLEUS IN THE HUMAN OLFACTORY BULB AND PEDUNCLE

Steven Berendsen[1], Jocelyne VM Peters[1], Evelien Huisman[1], Willie Vorster[2], Piet Hoogland[2]

[1]Department of Anatomy and Neuroscience, VUmc University Medical Center, Amsterdam, The Netherlands
[2]Department of Anatomy, School of Medicine, University of Namibia, Windhoek, Namibia

RESUMEN

El bulbo y pedúnculo olfatorio humano contienen muchos grupos celulares más o menos separados que habitualmente son considerados como parte del núcleo olfatorio anterior retro-bulbar (AON). La presunción que estos grupos celulares sean considerados como extensión rostral del AON en el hemisferio rostral retrocede a la descripción de un único caso por Crosby y Humphrey (1941). Para mejorar nuestra comprensión de la anatomía del AON bulbar y peduncular humano investigamos la morfología, forma y tamaño de estas partes en este núcleo en tejido post-mortem de individuos de edades conocidas. Se obtuvieron seis bulbos y pedúnculos olfatorios, incluyendo la sustancia perforada anterior (SPA), de cerebros donados; se realizaron cortes seriales horizontales a 40µm y se tiñó con substancia de Nissl. Las neuronas de tamaño mediano a grande de esta parte del AON se tiñeron intensamente y tenían un diámetro promedio de 16µm. La reconstruc-ción tridimensional demostró que en todos los casos, excepto uno, el AON bulbar y peduncular consistieron en una cadena discontinua de grupos celulares conectados por puentes de neuropilas pobres o libres de células. El número de grupos celulares y de puentes conectores difiere en cada individuo. Concluimos que las porciones bulbar y peduncular del AON humano debería ser considerado como una especialización humana más que como una extensión rostral del área AON retro-bulbar. Esto es acorde con las propiedades neuro-clínicas previamente publicadas y la degeneración temprana selectiva, pre-clínica, de estos nichos celulares en la enfermedad neuro-degenerativa.

Palabras clave: *Disfunción olfatoria, puentes de neuropila, enfermedad de Parkinson.*

ABSTRACT

The human olfactory bulb and peduncle contain several more or less separated cell groups that are usually regarded to be part of the retrobulbar anterior olfactory nucleus (AON). The assumption that these cell groups are to be considered as the rostral extension of the AON in the rostral hemisphere goes back to the description of one single case by Crosby and Humphrey (1941). To improve our understanding of the anatomy of the human bulbar and peduncular AON, we investigated the morphology, size and shape of these parts of this nucleus in postmortem tissue of aged individuals. Six olfactory bulbs and peduncles including the substantia perforata anterior (SPA) were obtained from donor brains and 40µm horizontal serial sections were cut and stained with Nissl substance. The medium to large sized neurons of these parts of the AON were intensely stained and had an average diameter of 16µm. Three dimensional reconstruction demonstrated that in all but one of the cases the bulbar and peduncular AON consisted on a discontinuous chain of cell groups connected by cell poor to cell free bridges of neuropile. The number of cell groups and the connecting bridges differ in every individual. We arrived at the conclusion that the bulbar and peduncular parts of the human AON should be regarded a human specialization rather than just being rostral extensions of the retrobulbar AON area. This is in line with previously published neurochemical properties and the selective early, preclinical degener-ation of these cell clusters in neurodegenerative diseases.

Key words*: Olfactory dysfunction, neuropile bridges, Parkinson's disease.*

***** *Correspondence to*: **Prof. Dr. Willie Vorster**. Department of Anatomy, University of Namibia, School of Medicine, Windhoek, Namibia. wvorster@unam.na

Received: 15 October, 2014. **Revised**: 17 November, 2014. **Accepted:** 3 December, 2014.

INTRODUCTION

Olfaction is usually not regarded to play a major role in human life compared to other mammals. However, humans have a unique olfactory system. Although the size of the olfactory bulb and peduncle should be regarded as modest if compared with other terrestrial mammals, the human olfactory bulb and olfactory tract are characterized by the presence of a unique series of cell clusters that are known as the bulbar and peduncular part of the anterior olfactory nucleus (AON). These particular well circumscribed cell masses have never been observed in any other mammalian species. The most detailed descr-iption of these cell groups can be found in an article by Crosby and Humphrey (1941). However, this description was based on only one specimen. These authors regarded the peduncular and bulbar AON as more or less interrupted extensions of the AON that is located in the rostral most part of the hemispheres. Arguments other than a rostro-caudal continuity were not given. There is no experimental or other evidence for this assumption. Later reports either ignored the bulbar AON (Allison, 1954; Hubbard et al, 2007) or mainly referred to the anatomy of the AON of rats when discussing the 'mysterious but complex role' of the AON in olfactory information processing (Brunjes et al, 2005). The reason why these cell groups in the olfactory bulb and peduncle have received much attention in the recent literature is that these cell groups become severely affected in very early, preclinical stages of neurodegenerative diseases. The bulbar and peduncular AON have been shown to be selectively vulnerable to protein aggregation and neuronal loss in aged individuals and patients with Alzheimer's disease (AD) (Esiri and Wilcock, 1984; Ohm and Braak, 1987; Hyman et al, 1991; Kovacs et al, 1999), Parkinson's disease (PD) (Pearce et al, 1995; Hubbard et al, 2007; Ubeda Bañon et al, 2010), multisystem atrophy (MSA) (Kovacs et al, 2003) and Pick's disease (Yoshimura, 1988). These neuropathological changes are thought to contribute to the olfactory dysfunction, which is a highly prevalent feature of AD, PD and many related disorders (Berendse and Ponsen, 2006; Sato et al, 2011; Doty, 2012). In none of these studies, the AON and its subdivisions were clearly defined and often the results were confusing where the AON was mentioned in the results. Therefore, we thought that a more precise description of the bulbar and peduncular parts of the human AON is necessary.

In the present study, we aim to provide a morphologic definition and an accurate anatomical description of the presumed bulbar and peduncular parts of the human AON based on 3D-reconstructions of consecutive Nissl-stained sections in a serious of six olfactory bulbs and their belonging peduncles. This anatomical description will address the following questions:

1) Do the distinct cell groups of the AON, which are visible in Nissl-stained sections, form a continuity in the OB, peduncle and rostral part of the SPA in the aged human brain?

2) Is the morphology of the distinct cell groups of the AON in the different parts of the olfactory system similar?

3) Does the reconstruction help us to define whether the human bulbar and peduncular AON should be regarded to be separate entities or just rostral extensions of the retro bulbar AON? Because PD and AD are usually regarded as degenerative diseases related to the process of aging we restricted ourselves to the specimen obtained from people over 65 years old.

MATERIALS AND METHODS

Post-mortem human tissue.

Post-mortem human brain tissue of non-demented controls was obtained from the department of Anatomy and Neurosciences, VU University medical center (VUmc, Amsterdam, The Netherlands). All donors gave written informed consent for brain autopsy and use of brain tissue for assessing the neuropathological and clinical information for scientific research, in compliance with ethical and legal guidelines. Records including a short summary of medical history were available for all six donors included in this study. Brains from donors with known neurological disorders were excluded from this study. The demographic data, including age, gender, and causes of death, of all donors used in this study are listed in Table1.

Case	*Age*	*Gender*	*Cause of death*
351	81	Female	Melanoma
352	90	Female	Cardiac failure
469	90	Male	Cardiac failure
445	80	Female	Cardiac failure
1	94	Female	Cardiac failure
474	86	Female	Cardiac failure

Table 1.- Demographic data

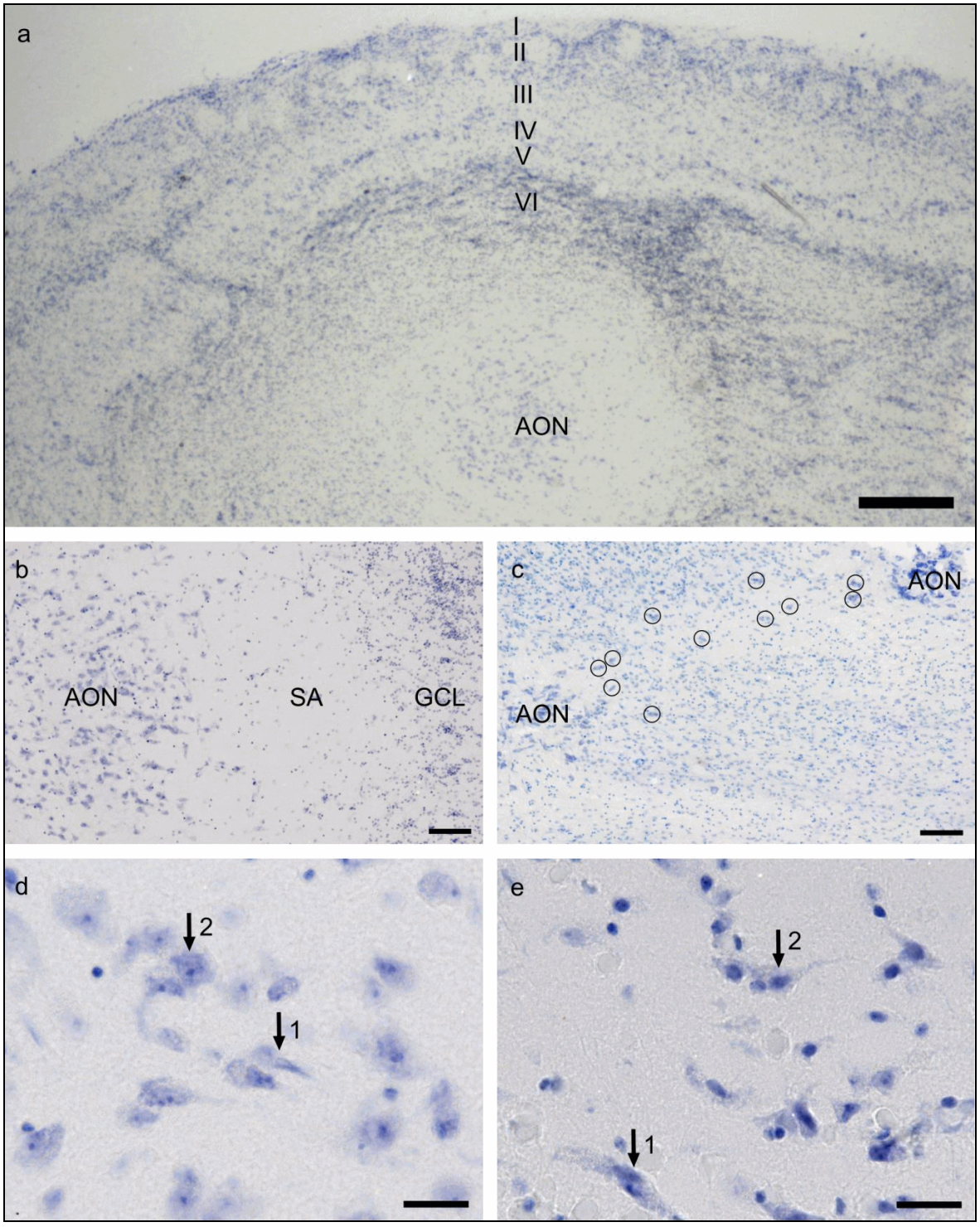

Figure 1.- a. Photomicrograph showing the lamination pattern of the OB in a Nissl staining; the olfactory nerve layer (I), the glomerular layer (II), the external plexiform layer (III), the mitral cell layer (IV), the internal plexiform layer (V), the granule cell layer (VI) and the AON. Scale bar 100um. **b.** Higher magnification showing the relation between the AON and the surrounding stratum album (SA) and the granule cell layer (GCL). **c.** A cell poor zone between two cell clusters. The AON neurons in the cell poor zone are indicated by circles.**d and e**. Different types of neurons in the bulbar AON and the peduncular part respectively. Arrows demonstrate multipolar neurons.

Tissue processing and 3D reconstructions. Donated bodies were embalmed within 48 hours after death. The bodies were embalmed by injecting 6 litres of an embalming fluid into the femoral artery. One litre of the embalming fluid contained 4% paraformaldehyde, 1.5% ethanol,

40g salicylic acid, 2g thymol, 10g chloral hydrate, 26g NA2SO4, 1g K2SO4, 12g NaCl and 20g Na2CO. One day after embalming, the brains including the olfactory bulbs were removed from the skull. The olfactory bulbs and peduncles and the adjacent substantia perforata anterior (SPA) were dissected and immersed in 4% para-formaldehyde in 0.1M phosphate buffer at 4°C for 4-10 days. After post fixation, the tissue was embedded in gelatin, cryoprotected using 30% sucrose in phosphate buffer (pH 7.6), frozen and subsequently cut into 40µm horizontal sections on a freezing microtome. The free-floating sections were mounted on glass-slides and stained for Nissl substance by the routine method. In the OB and peduncle the various parts of the AON were delineated using a computer-assisted morphometry system consisting of a Leica DMR photomicroscope with a CCD microfire (Optronics) color video camera and a LEP XY motorized stage controller for automatic sampling equipped with the Neurolucida software version 9.0 (MicroBrightfield Inc., Colchester, VT). The AON was defined using the peripheral neurons of this nucleus by two investigators (SB, JP). Limits of the inter-observer variability were set on a maximum of 10%. The inter-observer variability was between 3-5%.

RESULTS

Localization of the bulbar and peduncular AON.

The OB consists of six different layers that can easily be recognized in Nissl-stained sections as previously described by Smith et al (1993): the olfactory nerve layer (I), the glomerular layer (II), the external plexiform layer (III), the mitral cell layer (IV), the internal plexiform layer (V) and the granule cell layer (VI) (Fig.1a). The stratum album is a cell poor area within the granule cell layer of the OB, in which de AON neurons are localized (Figs.1b and c) (Hoogland and Huisman, 1999). The medium- to large-sized pyramidal neurons of the AON could easily be identified in the Nissl-stained sections based on size, staining intensity and defined darkly stained nucleolus (Figs.1d-e). The average diameter of the large pyramidal neurons in the AON in the OB was 16µm (range 14-18µm). In addition, the AON neurons were darker stained than the neurons of the granule cell layer in the OB. Neurons in the AON of the OB and peduncle were identified as multipolar (Figs.1d-e).

The various groups of AON neurons had an elongated shape in the OB while a fusiform shape was observed in the peduncle. The location of the AON within the peduncle was highly variable. The AON was found at the periphery of the peduncle, as well as in the middle.

3D reconstructions of the AON

Three dimensional reconstructions of the AON from consecutive sections throughout the entire OB, peduncle and most rostral part of the SPA showed that, in five out of six cases, the peduncular and bulbar AON formed an interrupted chain of cell clusters from the SPA until the rostral end of the bulb. These clusters were connected by bridges of neuropile that contained very few neurons. In general, the AON was situated in the center of the OB, but its location was variable in the peduncle. The size and shape of the AON was highly variable as illustrated in figure 2. In one case (474) the bulbar and peduncular AON were not interrupted and formed a continuous cellular structure that was connected to the retrobulbar AON (Fig.2a). In the OB and peduncle of donors 351 and 352, small interruptions in the AON within the OB and peduncle were observed (Figs.2b and c). In donor 445, the AON seemed to consist of three completely separated islands in the OB (Fig.2d), but more detailed analyses showed that few AON neurons were visible between these islands (Fig.1c).

In donors 469 and 1, more variability was present in the anatomical localization of the AON in the OB and peduncle. Again, separated islands of the AON were observed in these donors. The distance between the separate islands (Figs.2e and f) was larger than in the above cases. Between these groups of AON, white stretches of cell poor to cell free neuropile areas were observed, these stretches were recognized as stratum album.

Continuity of the peduncular AON with retrobulbar parts in the telencephalon was clear in the majority of the cases but in two cases (445 and 474) this connection was very thin (Figs.2a and d).

DISCUSSION

In the present study, we presented the first detailed 3D reconstruction of the human bulbar and peduncular AON. Our results provide evidence that these parts of the AON can be regarded as an irregular continuum from the rostral part of the SPA to the OB in the individuals used in the present study: Compact, circumscribed cell areas connected by cell poor zones. The well-defined islands of multipolar

AON neurons were connected by narrow bridges containing only a few of the same cell types as in the compact islands. A few of these bridges appeared to be cell free. It was also shown that the size and shape of the islands that constitute the bulbar and peduncular AON are highly variable just as the cell free and cell poor bridges formed by the neuropile of the stratum album.

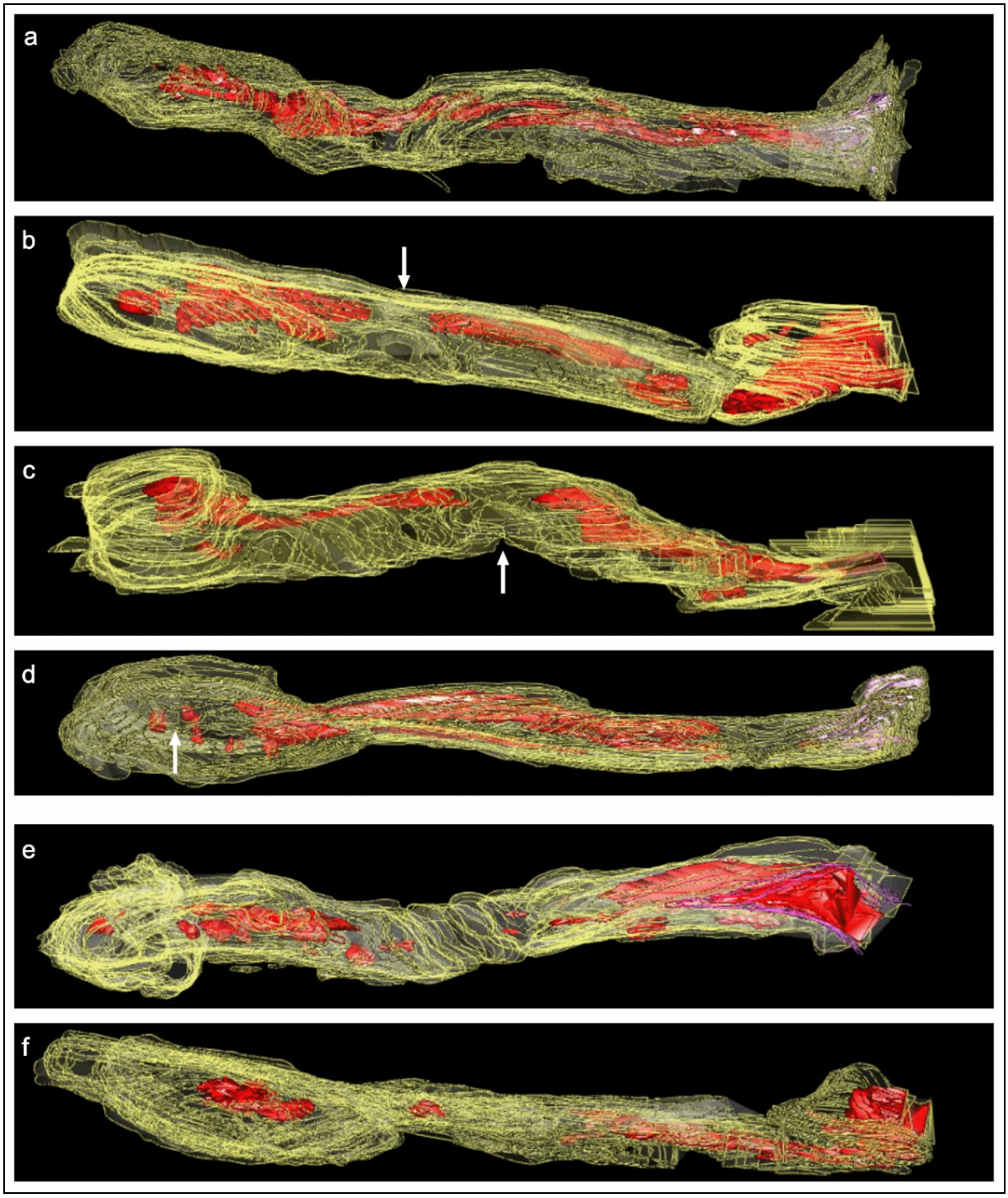

Figure 2.- 3D images of the OB (yellow) and the AON (red): a 474, b 351, c 352, d 445 e 469 and f 1. The OB is on the left side, the SPA at the right. White arrows demonstrate the interruptions of the AON. **E** and **f** show the widest interruptions in the AON.

The AON has been described as an ensemble of several separated groups of neurons within the OB and peduncle in most previous studies (Crosby and Humphrey, 1941; Allison, 1954; Bhatnagar et al, 1987; Pearce et al, 1995; Hoogland and Huisman, 1999). Our data, however, indicated that only if we had included the cell poor neuropile zones around the cell clusters, the AON would be a continuum from the SPA to the OB, albeit interrupted by cell poor bridges. A possible reason for the existence of the neuropile bridges may be the neuronal cell loss in the OB during aging (Bhatnagar et al, 2005). If so, the rostral AON should show fewer interruptions between the cell clusters in children. To our knowledge no such data are available in the literature. The present study only concerned the olfactory bulbs and peduncle of aged human subjects.

Indication that the bulbar and peduncular parts of the human AON are different from any part of the AON as described in rats, mice, cat and monkey can be deducted from both cytoarchitectonic (Crosby and Humprey, 1941; Brunjes et al, 2005). Crosby and Humphrey (1941) separated the bulbar and peduncular parts from the more caudal parts of the AON on the basis of cell size, form and staining intensity. Histochemical properties of the AON as reviewed by Brunjes and coworkers (2005) showed that the bulbar and peduncular AON in humans have little neurohistochemical properties in common with any part of the non-human AON. This must, however, be interpreted with care since many interspecies differences are present and also because the techniques used in the various studies differ considerably. Although these immunohistochemical characteristics need more detailed studies, the differences found until now may be used as arguments to consider the peduncular and bulbar parts of the human AON different from the bordering retrobulbar parts that can be considered to be homologous to the AON in other mammals. Together with the unique location, the characteristic appearance in humans and the very early involvement in Alzheimer's and Parkinson's disease, these immunohistochemical differences form a strong indication that the peduncular and bulbar parts of the AON should not be regarded as just a rostral extension of the retrobulbar part. Rather these rostral parts should be regarded separate entities that are only recognized in humans, albeit that they border the retrobulbar AON.

In conclusion the human peduncular and bulbar AON constitute an ensemble of cell groups that are separated by cell poor zones. Three dimensional reconstructions showed that the anatomy of these cell groups is very variable. These rostral parts of the human AON seem to differ neurohistochemically from the retrobulbar parts of the human AON and the AON in other mammals (Brunjes et al, 2005). These rostral parts are particularly vulnerable in the very early stages of neurodegenerative diseases. The specific shape, location and neurohistochemistry indicate that the bulbar and peduncular AON in humans must be regarded as separate entities, instead of only being rostral extensions of the retrobulbar AON.

Conflict of Interests
None

Funding
None

Ethical Approval
Not necessary

Informed consent
Not necessary

Contributions
Equal contributions by all the authors

REFERENCES

Allison AC. 1954. The secondary olfactory areas in the human brain. J Anat 88: 481-88.

Berendse HW, Ponsen MM. 2006. Detection of preclinical Parkinson's disease along the olfactory tract. J Neural Transm Suppl 70: 321-25.

Bhatnagar KP, Kennedy RC, Baron G, Greenberg RA. 1987. Number of mitral cells and the bulb volume in the aging human olfactory bulb: a quantitative morphological study. Anat Rec 218: 73-87.

Brunjes PC, Illig KR, Meyer EA. 2005. A field guide to the anterior olfactory nucleus (cortex). Brain Res Brain Res Rev 50: 305-35.

Crosby EC, Humphrey T. 1941. Studies of the vertebrate telencephalon. The nuclear pattern of the anterior olfactory nucleus, tuberculum olfactorium and the amygdaloid complex in adult man. J Comp Neurol 74: 309-52.

Doty RL. 2012. Olfactory dysfunction in Parkinson disease. Nat Rev Neurol 15: 329-39.

Esiri MM, Wilcock GK. 1984. The olfactory bulbs in Alzheimer's disease. J Neurol Neurosurg Psychiatry 47: 56-60.

Hoogland PV, Huisman E. 1999. Tyrosine hydroxylase immunoreactive structures in the

aged human olfactory bulb and olfactory peduncle. J Chem Neuroanat 17: 153-61.

Hubbard PS, Esiri MM, Reading M, McShane R, Nagy Z. 2007. Alpha-synuclein pathology in the olfactory pathways of dementia patients. J Anat 211: 117-24.

Hyman BT, Arriagada PV, Van Hoesen GW. 1991. Pathologic changes in the olfactory system in aging and Alzheimer's disease. Ann N Y Acad Sci 640: 14-19.

Kovacs T, Cairns NJ, Lantos PL. 1999. Beta-amyloid deposition and neurofibrillary tangle formation in the olfactory bulb in ageing and Alzheimer's disease. Neuropathol Appl Neurobiol 25: 481-91.

Kovacs T, Papp MI, Cairns NJ, Khan MN, Lantos PL. 2003. Olfactory bulb in multiple system atrophy. Mov Disord 18: 938-42.

Ohm TG, Braak H. 1987. Olfactory bulb changes in Alzheimer's disease. Acta Neuropathol 73: 365-69.

Pearce RK, Hawkes CH, Daniel SE. 1995. The anterior olfactory nucleus in Parkinson's disease. Mov Disord 10: 283-87.

Sato T, Hanyu H, Kume K, Takada Y, Onuma T, Iwamoto T. 2011. Difference in olfactory dysfunction with dementia with Lewy bodies and Alzheimer's disease. J Am Geriatr Soc 59: 947-48.

Smith RL, Baker H, Greer CA. 1993. Immunohistochemical analyses of the human olfactory bulb. J Comp Neurol 333: 519-30.

Ubeda-Bañon I, Saiz-Sanchez D, de la Rosa-Prieto C, Argañdona Palacios L, Garcia Muñozguren S Martinez-Marcos A. 2010. α-Synuclein in the human olfactory system in Parkinson's disease: involvement of calcium-binding protein- and substance P-positive cells. Acta Neuropathol 119: 723-35.

Yoshimura N. 1988. Olfactory bulb involvement in Pick's disease. Acta Neuropathol 77: 202-05.

ACKNOWLEDGEMENTS

The authors wish to acknowledge Dr W.D.J. van de Berg for advice and help with the manuscript.

