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Original article

Histological observations on the anterior olfactory nucleus in human

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KEYWORDS

Anterior olfactory nucleus,
Histomorphology, Localization.

ABSTRACT

Aim: Localization of isolated clusters of anterior olfactory nucleus (AON) in a human olfactory bulb and tract. *Materials and methods*: This investigation was done on human olfactory bulbs and their tracts, collected from the freshly donated cadavers, before embalming, in the Department of Anatomy, IPGMER, Kolkata. H&E stained histological slides were prepared along the whole length of specimens and examined under a Leica DM 2000 microscope and with a Leica Quin image analyzer. *Results*: The anterior olfactory nucleus was detected in the form of a major cluster and in two smaller clusters of neurons. The major cluster was located at the caudal pole of the bulb and was composed of medium-sized triangular cells which had an average diameter of 13.92 ± 3.43 µm. Out of the two minor clusters, one was detected at the beginning and another at the middle of the olfactory tract. Here neurons were little larger in size and their diameter ranged approximately 15–17 µm. Olfactory striae also accommodated some neurons in a scattered manner. *Conclusion*: This observation will be helpful in exploration of the complex role of AON in the organization and function of the olfactory system and its clinical significance in human.

1. Introduction

The olfactory system of microsmatic mammal such as human is poorly developed, unlike those of other macrosmatic mammals (dog, cat, etc.). The olfactory pathways comprise the olfactory epithelium, olfactory nerves, olfactory bulb, olfactory tract, anterior olfactory nucleus (AON), olfactory stria, portions of the amygdaloid complex, and pyriform cortex. In human, the functions of emotional behavior, sexual behavior, etc. are not solely dependent on this system due to better development of neocortex and activities of other sensory modalities such as taste, vision, hearing, etc. Even then, the importance of this system is no less in human beings. The olfactory system undoubtedly enhances the charm of living and an appreciation of the wonders of nature and its countless aberrations, both natural and man-made.

Observations on gross anatomical and histological structure of different parts of olfactory system have been recorded in different vertebrates by several workers from time to time. Parent stated that structurally the human olfactory bulb has a laminar organization of the olfactory nerve fiber layer, layer of olfactory glomeruli, the mitral cell layer, granule cell layer, and fibers of olfactory tracts.[1] Further he mentioned that the zone just caudal to the bulb accommodates a scattered group of neurons which form the anterior nucleus. He also stated that some cells of this loosely organized nucleus were found along the tract near the base of the hemisphere. Jenkins mentioned that the olfactory bulb was proportionately quite large in dogs when compared with that of human.[2] He also recorded the disposition of olfactory glomeruli, mitral cells, and granule cells. Williams et al stated that the AON was formed by some scattered multipolar neurons of medium size.[3]

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The fibers of the olfactory tract are mostly composed of the centripetal axons of mitral and tufted cells. Some of these fibers synapse with the cells of the AON and may give collaterals to them. The AON probably serves the purpose of a relay station in the olfactory pathway.

The available literature indicates that the cells of AON are distributed in scattered groups throughout the length of the tract. However, while working on the anatomical aspect of the tract, it is observed that these cells are localized mainly in a particular zone. The present study was undertaken to make methodical records of the location of the nucleus, cellular dimensions, and their distribution pattern in detail.

2. Materials and methods

Eight fresh adult cadavers of either sex, donated to the Anatomy Department of IPGME & R, Kolkata, had been selected for this study before embalming. Only those brought shortly after their death and without any obvious pathology affecting the brain tissue were considered. The skulls were opened by a circumferential saw cut passing just above the nasion and the inion. The brains along with the olfactory bulbs and tracts of both sides were removed from cranial cavity. Much care was taken while separating the bulbs from their firm attachment with the olfactory nerve fibers, which entered the bulb after passing through the cribriform plate of the ethmoid bone. Few specimens were destroyed during the process of collection. A slice of tissue from the anterior aspect of the base of the brain along with the olfactory bulb and tract was separated by a sharp knife and preserved in 10% buffered neutral formalin. The solution was freshly prepared before tissue collection. The tissues were preserved for 48 hours with intermittent changes of fresh solution. After 48 hours, the bulbs along with the tracts were separated from the rest of the brain tissue. The length, breadth, and thickness of the bulbs were measured with the help of sliding calipers and again placed in fresh fixative for histological processing.

After dehydration through graded alcohol solutions and clearing by xylene, wax impregnation was done by keeping the tissues in molten paraffin (with melting point 58–60°C) as per the standard method. Since the tissues were thin and delicate, care was taken to embed them along their length. This enabled us to get a complete section of the olfactory bulb and tract together along its length. Five to six micron thick sections were cut with the help of a rotary microtome and slides were prepared as per the routine method. After deparaffinization, the slides were stained with hematoxylin and eosin and mounted, following standard histological methods. Our observations were made irrespective of the side (right or left) of the tissues taken. All the histological sections were examined with a Leica DM 2000 microscope and the measurements were done with a Leica Quin image analyzer.

3. Results

The olfactory bulb was found to be a small, flattened, oval, elongated structure placed on the cribriform plate of the ethmoid bone and situated inferior to the anterior end of the olfactory sulcus on the orbital surface of the frontal lobe of brain. The shape and size of both the bulbs were almost similar. The olfactory tract was in the form of a thick tape-like structure which extended from the bulb to the anterior per-

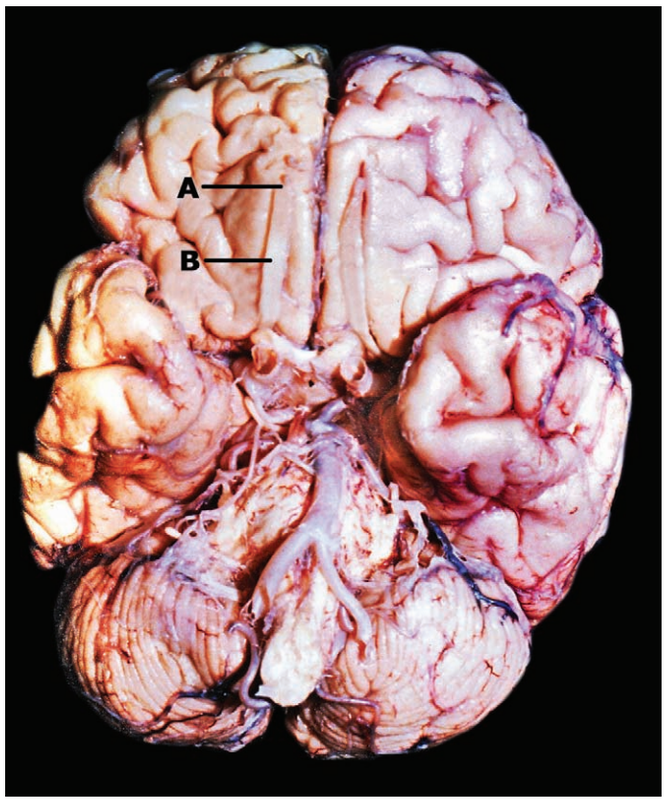

Fig. 1 – Photograph of the ventral surface of the human brain showing the disposition of the olfactory bulb (A) and tract (B).

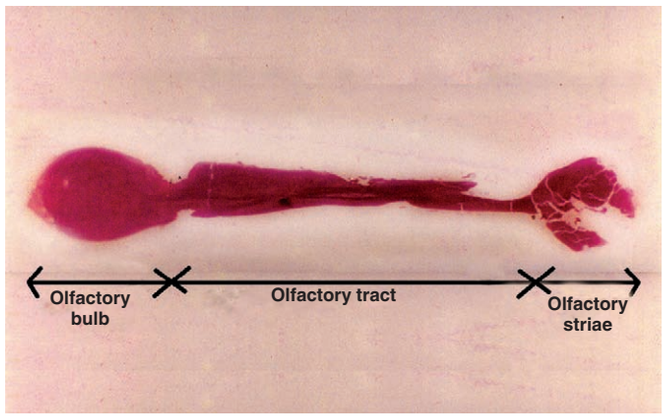

Fig. 2 – Contact print of a section of the olfactory bulb along with the tract and striae in human (×6.6).

forated substance and divided into an inner (medial) and outer (lateral) olfactory striae (Figs. 1 and 2).

A sharp constriction at the junction of the bulb and the tract was noticed (Fig. 2). The point of this constriction was considered to be the posterior pole of the bulb. The anterior end of the bulb was found to be narrow. Existence of AON in the form of a major cluster of cells within the olfactory bulb and few smaller clusters in the olfactory tract was clearly visible in the histological sections.

3.1 *Anterior olfactory nucleus (major cluster)*

Location: It was situated close to the caudal pole of the olfactory bulb (Fig. 3). The anterior end of the AON was placed at a distance of 4.009 ± 0.063 mm from the tip of the olfactory bulb. The average distance between the posterior end of the AON and the caudal pole of the bulb was 1.630 ± 0.032 mm. The average depth from the periphery of the bulb to the outer light zone of the AON was 1.660 ± 0.106 mm.

Shape and size: It was an oval-shaped zone having a light outer layer and a dark inner layer (Fig. 4). The average long and short diameters of the AON were found to be 2.432 ± 0.007 mm and 1.629 ± 0.126 mm, respectively.

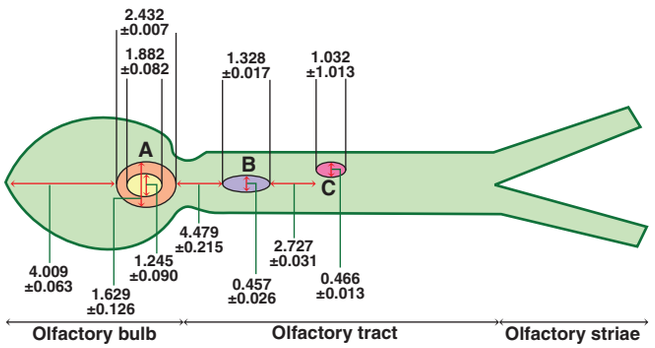

Fig. 3 – Line diagram of olfactory bulb, olfactory tract, and striae, showing different zones of cell clusters of anterior olfactory nucleus (AON) with their locations (A – Major cluster, B – first minor cluster, C – second minor cluster). All measurements are taken in millimeter.

Histomorphology: The population of the neuronal cells in the peripheral lighter zone was scanty. Occasionally blood vessels were detected in this area (Fig. 4). The deeper dark area had the average long and short diameters of 1.882 ± 0.082 mm and 1.245 ± 0.090 mm, respectively. The deeper dark area was composed of a considerable number of medium-sized triangular cells. The average diameter of these cells was 13.92 ± 3.43 μm (Fig. 5). The average density of the cells in the deep zone was 584± 29.25/mm² (Table 1). However, all these cells were unevenly distributed within these zones. Most of the cells were found to be triangular with a centrally placed nucleus.

Table 1 – Average cell diameters and cell densities in different groups of anterior olfactory nucleus.

Neuronal groups	Cell diameter (micrometers)	Cell density (per square millimeters)
AON within the olfactory bulb	13.92 ± 3.43	584 ± 29.52
First cluster within the tract	16.60 ± 1.75	532.20 ± 44.80
Second cluster within the tract	15.12 ± 1.68	472 ± 29.28
Cells within the striae	13.44 ± 1.27	433.25 ± 20.25

AON: Anterior olfactory nucleus.

3.2 *Minor clusters of neurons within the olfactory tract*

Besides this oval-shaped major cluster of AON within the olfactory bulb, two small clusters of neurons were detected within the tract. The first cluster was found at the beginning of the tract (Figs. 6a and 6b); the average distance between the anterior end of the first minor cluster and the posterior end of the major cluster of AON was 4.479 ± 0.215 mm. The long and short diameters of this zone were 1.328 ± 0.017 mm

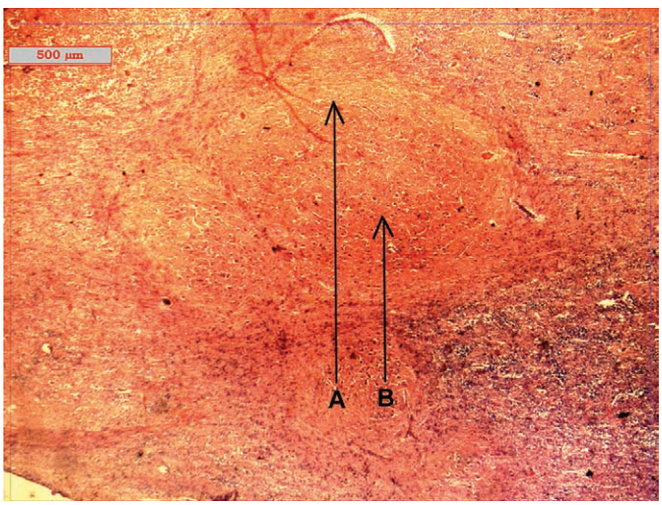

Fig. 4 – Photomicrograph of the AON within the olfactory bulb. A lighter peripheral zone (A) surrounding an oval darker zone (B) is clearly visible (40×).

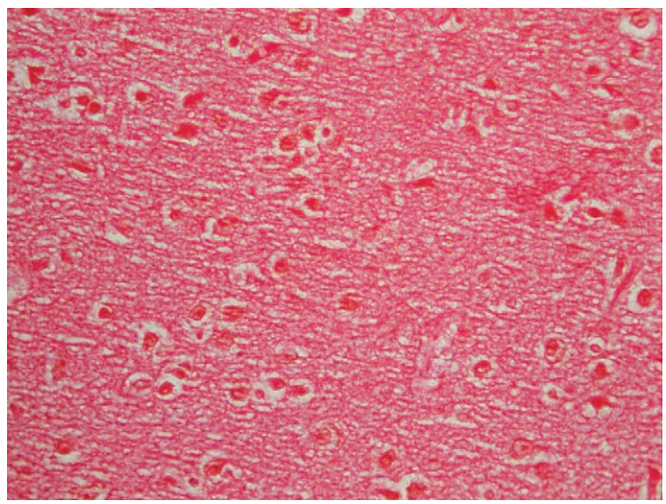

Fig. 5 – Photomicrograph of the darker zone of AON within the olfactory bulb showing the distribution of medium–sized neurons (200×).

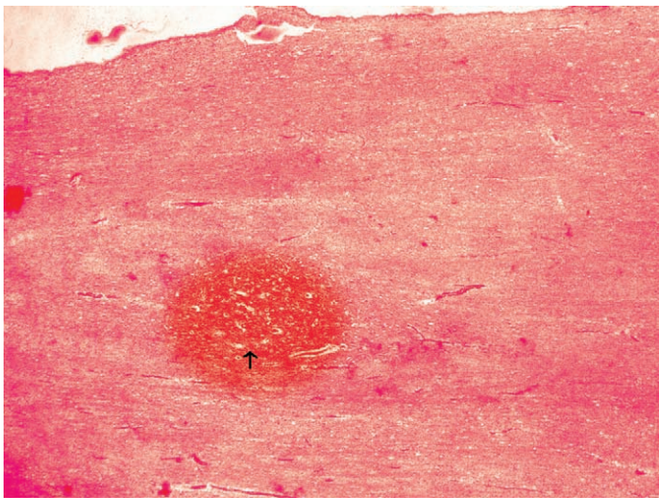

Fig. 6a – Photomicrograph of a cluster (arrow) of neurons of AON (first minor cluster) situated close to the beginning of the olfactory tract (40×).

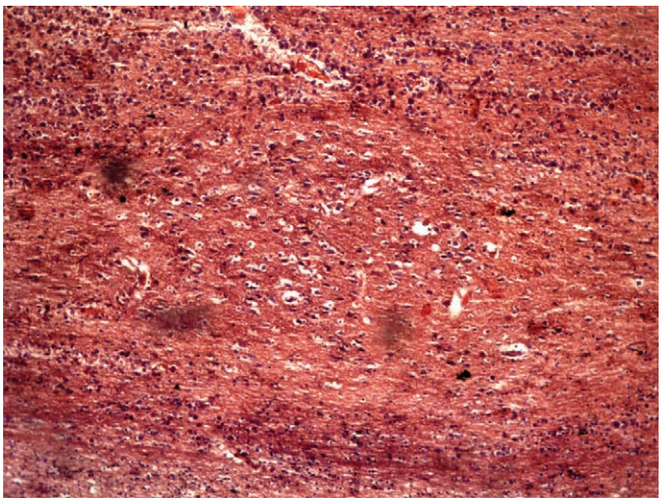

Fig. 6b – Photomicrograph of first minor cluster in higher magnification (200×).

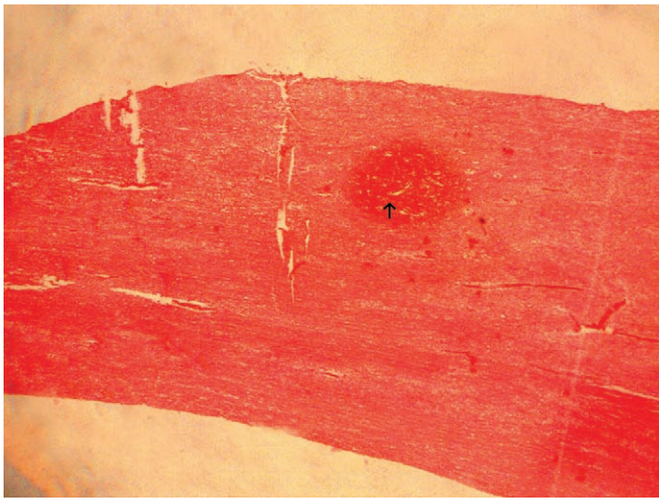

Fig. 7a – Photomicrograph of a cluster (arrow) of neurons of AON (second minor cluster) situated at the middle of the olfactory tract (40×).

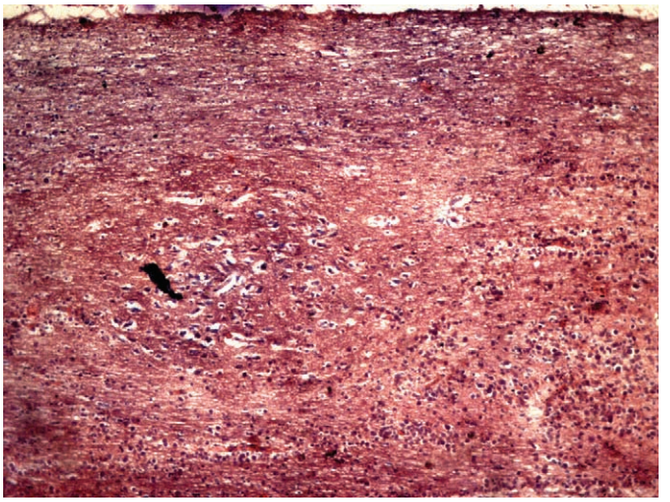

Fig. 7b – Photomicrograph of second minor cluster in higher magnification (200×).

and 0.457 ± 0.026 mm, respectively. The average diameter of these cells was 16.60 ± 1.75 μm. The density of these cells was 535.20 ± 44.80 cells/mm² (Table 1). The second cluster was detected almost at the middle of the tract but was placed close to the periphery (Figs. 7a and 7b). It was placed 2.727 ± 0.031 mm behind the first cluster of cells in the olfactory tract. The average long and short diameters of this zone were 1.032 ± 0.013 mm and 0.466 ± 0.013 mm, respectively. The average diameter of these cells was 15.12 ± 1.68 μm and the density was 472 ± 29.28 cells/mm² (Table 1).

Other than these neurons found in clusters, a considerable number of neurons were found scattered within the inner and outer olfactory striae (Fig. 8). These cells were also triangular in shape. The average diameter and density of the cells were 13.44 ± 1.27 μm and 433.25 ± 20.25/mm², respectively (Table 1).

4. Discussion

An oval area packed with a cluster of moderately large neurons encircled by a lighter zone within the caudal end of the olfactory bulb was detected which represented a part of the AON. Since two other small clusters of cells were also found in the olfactory tract, the first group of cells was considered to be the major cluster of the AON. The peripheral lighter zone was devoid of large neuronal cells and represented the pars externa. The average long and short diameters of this major cluster were 2.432 mm and 1.629 mm, respectively. It was located on an average 4.009 mm behind the anterior pole of the bulb and 1.630 mm in front of the posterior pole. A few blood vessels were found around the periphery of this zone.

Brunjes et al stated that in rodents, the pars externa is a thin band of tightly packed neuronal cells oriented in an ob-

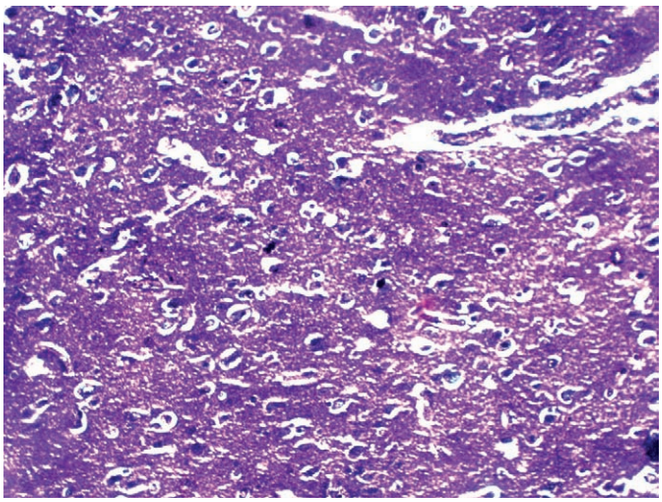

Fig. 8 – Photomicrograph of neurons distributed along a portion of the olfactory striae (400×).

All photomicrographs were taken with the help of a Leica DM 2000 – Microscope and Leica Quin image analyzer.

lique plane and lies over a portion of the large population of cells that comprises the pars principalis.[4] Herrick speculated that these neuroglial cells are arranged to insulate the AON from the rest of the parenchyma of olfactory bulb.[5] Davis and Macrides mentioned that in the lateral aspect of the hamster brain, the cellular layer of pars externa merges with the granule cell layer of the bulb.[6] However, Crosby and Humphrey reported that the pars externa is absent in adult humans.[7] Reyher et al claimed that it is present in the marmoset.[8] Carmichael et al noted that the pars externa is present in macaque monkeys in a less organized manner.[9]

The pars principalis or the deeper zone was also oval in shape which had average long and short diameters of 1.882 mm and 1.245 mm, respectively. The neurons were unevenly distributed throughout this zone. The nerve cells were almost triangular in shape. Parent stated that in the caudal part of the olfactory bulb, groups of neurons, intermediate in size between mitral and granule cells, form the AON.[1] While reporting on cell morphology in the AON, Brunjes stated that the AON is often characterized as a region populated primarily by pyramidal cells which are similar to the neocortical pyramidal cells with a thick apical dendrite.[4] Valverde et al reported that the deep pyramidal cells have a standard pyramidal shape but the superficial cells appear polymorphic due to extroversion of their basal dendrites.[10] Reyher et al mentioned that there are fusiform cells with radial dendrites extending toward the pia mater.[8]

Besides the major cluster, other two small clusters of cells were also detected and presumed to be components of the AON. One of these small clusters was located at the beginning of the olfactory tract. This was also oval in shape but had no peripheral lighter zone. Another small cluster was detected in the olfactory tract near its middle but close to the periphery. A good number of scattered neurons were detected within the inner and outer olfactory striae and also considered to be components of the AON. In each section, we have measured the average cell diameters and cell densities in all the clusters of AON.

Parent stated that posterior to the olfactory bulb are scattered groups of neurons intermediate in size, between mitral and granule cells, that form the AON.[1] He also mentioned that some cells of this loosely organized nucleus are found along the olfactory tract near the base of the hemisphere. Williams et al recorded that various characteristic cell layers disappear at the posterior end of the bulb, but deeply placed granule cell layers are replaced throughout the length of the olfactory tract by scattered groups of medium-sized multipolar neurons which constitute the AON.[3] Posteriorly, these groups of neurons continue into the olfactory striae and trigone to abut on or become continuous with the gray matter of the prepiriform cortex, the anterior perforated substance, and precommissural septal areas. They also stated that the AON is the most rostral of the structures innervated by the olfactory bulb. It is a poorly laminated cortical structure, having a superficial plexiform layer (layer I), a compact pyramidal cell layer (layer II), and a polymorphic cell layer (layer III). Brunjes also indicated that in rodents the location of AON is between the olfactory bulb and piriform cortex.[4]

Our observation on the major cluster of AON, having a laminar pattern with an outer light zone and a deeper dark zone, partly conforms to the description of the AON found in the literature. The existence of various isolated clusters of neurons of the AON within the specific locations of the olfactory bulb and tract as detected in this study has few references in the medical literature. Although some authors have mentioned the presence of medium-sized neurons, the literature with numerical values in terms of various dimensions of the clusters, cell diameters, and cell densities could not be detected.

Pearce et al recorded the presence of Lewy bodies associated with neuronal loss in the AON in patients of impaired olfaction with idiopathic Parkinson's disease.[11] They also found a strong correlation of neuronal loss with disease duration.

5. Conclusion

The present observations on neuronal diameter and their population density in the different clusters of anterior olfactory nucleus in human might be helpful to the future workers to establish their functional and clinical correlation.

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