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The Anterior Olfactory Nucleus: Quantitative Study of Dendritic Morphology

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Abstract

The anterior olfactory nucleus (AON) occupies a crucial position within the olfactory circuit as it is able to influence function in nearly every major synaptic processing stage of both the ipsilateral and contralateral pathway. Nevertheless, very little is known about the region's internal organization and circuitry. The present study provides basic quantitative and qualitative data on the morphology of several cell types within the two major regions of the AON: *pars externa* and *pars principalis*. In *pars externa* two types of cells are analyzed, the "classical" cell (Type I), containing only apically-directed dendrites with large spines, and a previously unreported cell with basilar dendrites and complex, spiny apical processes (Type II). In *pars principalis* the characteristic pyramidal cell is described both on the basis of the depth of the cell bodies in the cell layer comprising the structure and on the basis of their radial location. Several other non-pyramidal neurons are also described. The findings provide useful basic information necessary for understanding and modeling the circuitry of the AON.

Keywords

olfactory cortex; olfactory peduncle; *pars externa*; *par principalis*; pyramidal cells

Odorant stimuli in the nasal cavity are detected by olfactory sensory neurons residing in the olfactory mucosa. Axons from these cells enter the brain and innervate the glomeruli of the olfactory bulb. In the glomeruli the information is transferred to mitral and tufted cells whose axons form the lateral olfactory tract (LOT). The LOT carries odor information first to the anterior olfactory nucleus (AON; also referred to as the "anterior olfactory cortex"; Haberly, 2001; Brunjes, Meyer and Illig, 2005) and then to the piriform cortex (PC). The location of the AON in the olfactory circuit ensures that it plays a key role in information processing. Ipsilaterally, it receives and projects information from both the bulb and the PC, and thus can regulate activity in either structure. It also has contralateral projections to the bulb, AON and anterior PC, suggesting that it has a key role in integrating the interhemispheric processing of olfactory information.

The AON is the largest component of the olfactory peduncle and is comprised of two regions: *pars externa* and *pars principalis* (Fig 1a). "Layer I" of the AON is the plexiform region found just under the pial surface. It is divided into two subzones. Layer 1A, containing fibers from the LOT and the distal dendrites of AON neurons, is thickest on the dorsolateral to ventromedial surface of the peduncle. The deeper Layer 1B contains communicating fibers interacting with proximal dendrites of AON cells (Price, 1973). Embedded in Layer I is "*pars externa*", a thin band of cells that lines the caudal boundary of

the olfactory bulb. Under the plexiform region is a dense layer of cells (“Layer II”) known as *pars principalis*. Researchers have subdivided *pars principalis* in several ways (Brunjes et al.; 2005); the present paper employs the system described by Haberly and Price (1978) who used cytoarchitectonic differences to parcel the region into *pars medialis*, *pars dorsalis*, *pars lateralis* and *pars ventroposterior* (Fig 1b). The deep core of the peduncle contains the subependymal layer (the remnant of the olfactory ventricle which forms the support of the “rostral migratory stream”) and a surrounding plexiform layer comprised of axons from the anterior limb of the anterior commissure and the medial forebrain bundle (Brunjes et al.; 2005).

The organization and function of the AON is just beginning to be understood. Characterizing the constituent neurons of the region is an important step in this effort. To date, a few qualitative Golgi studies of cell morphology in the AON have been reported (Valverde, et al., 1989 summarizes much of the early work, see also Haberly and Price 1978; Scott et al., 1985; Reyher et al., 1988; Brown and Brunjes 1990, Brunjes et al., 2005). *Pars externa* has been characterized as having only a single cell type with two apically-directed dendrites containing large spines (see below). Pyramidal cells are the most numerous cellular variety found in *pars principalis* although several less common species have also been described (see discussion below). The present study is a quantitative analysis of the main cell classes stainable with the Golgi-Cox method. At the outset it should be remembered that the Golgi method has several limitations, including the fact that it selectively stains only a small proportion of cells and may not impregnate them in their entirety. Importantly, however, it a) provides an overview of many different kinds of neurons in a brain region b) facilitates the collection of a relatively large sample of cells, and c) is a good first step in establishing basic neuronal features and allowing information processing in the region is to be modeled and compared with that of other areas (Peters and Jones, 1984).

MATERIALS AND METHODS

All procedures were performed according to NIH guidelines and protocols approved by the University of Virginia IACUC. Tissue from 8 juvenile (mean weight = 48 gm) male Long-Evans hooded rats (Harlan) was employed. The subjects were killed with an overdose of barbiturate anesthetic (Euthasol, 0.39 mg drug/gm body weight) and their brains placed in Golgi-Cox solution using procedures previously described (Glaser and Van Der Loos '81; Brunjes et al., 1982; Brown and Brunjes, 1990). Test sections from neocortical regions near the AON were examined regularly to determine optimal impregnation. By varying the staining time to accommodate *each* subject, complete staining was obtained in all samples, minimizing possible artifacts. The tissue was then embedded in Parlodion, sectioned at 120µm, counterstained with methylene blue, dehydrated, mounted and coverslipped with DPX.

Every attempt was made to select and reconstruct cells that were well-stained and centered in the section such that the bulk of the dendritic field was not truncated or obscured. Cells were traced at 400X using a computer-controlled microscope system (Neurolucida: Microbrightfield). Two standard methods of dendritic field analysis were used to provide a general estimate of the total amount and distribution of dendritic material and the number and extent of the dendritic arborizations (Fig 1c). The first (“Branch” analysis) determined the length and number of branches at successive orders of bifurcation from the soma (Brown and Brunjes, 1990). The second (“Sholl” analysis, Sholl, 1956) quantified the extent of dendritic branching by placing a series of concentric spheres around the soma and counting the number of intersections between dendrites and each sphere and the total length of

dendrites contained within each shell. The initial ring was 20 μm from the soma, with each subsequent one placed at 10 μm intervals.

Two populations of cells were quantified in *pars externa* (Fig 2). “Type I” cells were characterized by approximately two apically directed primary dendrites that extend into Layer 1B and no basilar dendrites. Fifty of these cells were reconstructed. A rarer, previously undescribed cell type was also encountered. “Type II” cells often had basilar dendrites usually constrained to the cell body layer, and one to three primary apical dendrites that exhibit considerable complexity. A sample of 24 of these cells was obtained.

“Pyramidal” cells were the only variety quantified in *pars principalis*. In order to be included in the sample cells needed to have a single dendrite at least as long as the cell body extending from the apical side of the soma as well as several basilar processes (Fig 1c). Basilar dendrites were quantified separately from the apical trees. Cells were geographically categorized in two ways: by the position of their soma (in the superficial, middle or deep thirds of Layer II) and radially, using the nomenclature of Haberly and Price (1978; Fig 1b). Table 1 lists the distribution of the cells sampled. Fewer cells were available for study in *pars medialis* and *pars ventroposterior* due to the relatively small size of these areas. Several other varieties of neurons were observed but in such small numbers that accurate estimates of variance could not be made, and are qualitatively described below. Furthermore, images made from slides obtained during the work of a previous study (Meyer, Illig and Brunjes, 2005) were also used in the discussion of data below. Data were analyzed via ANOVA using SPSS 16.0. Data for all cells are available at <http://neuromorpho.org/>. Images were acquired and minimally adjusted for brightness and contrast with Adobe Photoshop CS2; plates were constructed with Adobe Illustrator (San Jose, CA, USA).

Results

1) Pars Externa

Previous examinations of neuronal morphology in *pars externa* uniformly reported a single cell type with a) apical dendrites sparsely covered with long thin spines b) no basilar dendrites and c) an axon extending from the basal pole of the cell (e.g., Haberly and Price, 1978; Scott et al., 1985; Reyher et al., 1988; Brunjes et al., 2005). In the sample examined in the present study, we defined neurons such as these “Type I” cells (Fig 2). Typical of most neural populations, Type I cells exhibit considerable variation. Most have 2 large apical dendrites emerging as thick stalks from the soma, though occasionally cells with one or three primary processes were observed. A limited number of higher order branches are apparent, each becoming progressively thinner as they branch from primary processes. The dendrites often branch within the cell layer of *pars externa*, and were observed to extend into the superficial region of Layer 1b, and occasionally into the LOT or the granule cell layer of the olfactory bulb. The spines are generally longer than those seen on cells in *pars principalis*.

“Type II” neurons (Fig 2) were less frequently encountered, were not localized to a particular region of *pars externa*, and did not differ in the projected area of their cell bodies from Type I neurons ($t = 0.116$). The cells contained one or more spiny dendrites emerging from the basal half of the soma and extending into the deep portion of the cellular layer and only rarely into the plexiform region that separates *pars externa* and *pars principalis*. The average total dendritic length of the basilar dendritic trees of the 24 cells sampled was 278 μm (SEM=34.66). The number of basal dendrites extending from the soma was small; 12 of the cells had one (Fig 2 depicts one example), 10 had two and the remaining cells had either 3 or 4. The primary basilar dendrites exhibited considerable complexity as 58% had at least 2 higher branch orders and 25% had 3 or more. Examining the total dendritic length at each

branch order revealed that the primary dendrites comprised 22% of the dendritic mass, the secondary dendrites 45%, and the tertiary processes 16%. Fifty-four percent of the population of cells had basilar dendrites extending more than 100 μm away from the cell body, and a quarter of the sample exhibited processes that extended past 130 μm .

Type II cells also exhibited longer and more complex apical dendritic processes. Type II cells had longer total apical dendritic length (Mean= 898.322, SEM=47.5642 for Type I, 1098.96 and 77.39 for Type II, $F_{1,72}=5.312$, $p=0.024$); more total apical branches (Mean= 20.20, SEM=1.277 for Type I, 25.58 and 2.383 for Type II. $F_{1,72}=4.744$, $p=0.033$), and crossed more Sholl spheres (Mean= 62.90, SEM=3.144 for Type I, 77.167 and 5.369 for Type II. $F_{1,72}=6.004$, $p=0.033$) than the “classical” *pars externa* cell. Type II cells consistently had more and longer branches at every order higher than 3 (Fig 2 C, E.) as well as more Sholl shell crossings and dendritic length per shell at nearly every point of examination (Fig 2 D and F). Finally, note that Type I cells had more and longer apical primaries and, probably as a direct result, second order branches (Bonferroni tests: p 's = 0.001–0.007).

Pars principalis

Nearly every study of *pars principalis* has recognized pyramidal cells as the most numerous cell type. Therefore, these cells will be described first, followed by observations of the other neuron types encountered.

Pyramidal cells

The pyramidal cells in *pars principalis* are morphologically similar to those from many cortical regions, with an apical dendrite extending towards the pial surface, multiple basal dendrites, many dendritic spines, and a deeply directed axon. AON pyramidal neurons can be visualized with a number of techniques. For example, Nissl sections (Fig 3a) reveal profiles with a characteristic large dendrite extending from the apical pole of roughly triangular cell bodies. Figs 3 b and c depict cells immunostained for the calcium binding proteins calbindin and parvalbumin with similar morphologies; apical primaries that are thick proximally and thin as they extend, numerous branches that form a large dendritic tree and a family of basilar processes.

Considerable variation in the length of primary dendrites was observed in the Golgi-stained tissue (Fig 3d). Some cells had an obvious central primary extending through layer II with smaller caliber fibers emerging occasionally along the length. Others were very short and then gave rise to 2–3 processes with similar sizes (Valverde et al, 1989). Deep and intermediate cells fell into two morphological classes: one had thin dendritic branches with few spines while the other had thicker processes and more numerous spines. In all cells once dendrites entered Layer I they rarely branched except near their most distal ends (Fig 3f).

Identifying an apical primary dendrite was often complicated in the superficial cells of the sample. Since cells in this zone are near to Layer I, little space exists for long primary dendrites, so processes ramify very near to the somata. As a result, superficial cells tend to have a more multipolar morphology, though they might still have the same functional dendritic structure as a more typical pyramidal cell (Valverde et al., 1989; Migliore and Shepherd, 2005). Nevertheless, the present sample was limited to cells with a single primary dendrite as long as the cell body.

As in many other brain regions (e.g., Feldman, 1984; Furtak et al, 2007), occasional cells were observed with obliquely or even laterally-oriented apical dendritic trees. In superficial layer cells, different orientations resulted in unexpected situations such as basilar dendrites projecting directly into Layer I. Apical dendrites were often observed to bend around

overlying obstacles in Layer I (e.g., the granule cell layer of the olfactory bulb or *pars externa*) in order to gain access to regions closer to the fibers from the LOT. Dendrites were observed to penetrate completely through *pars externa*. No evidence for the bundling of apical dendrites was observed as had been observed in the cerebral cortex (Jones, 2000). This might reflect the different paths by which the primary afferent information enters the neuropil: in the AON it comes from the surface through the LOT rather than from the deep thalamic radiations.

Most cortical regions have inputs from different sources organized in stratified zones. Several observations suggest that inputs to cells in *pars principalis* might also be stratified. For example, most of the dendrites of superficial cells extended well into Layer I, even into the fibers of the LOT, suggesting their primary inputs were from sensory fibers in the LOT. Pyramidal cells found in the deep regions often had apical dendritic fields that were contained primarily within Layer II or perhaps the deeper regions of Layer I, suggesting less substantial LOT input. Second, one variant of deep pyramidal cells (often with very thin dendrites and relatively few spines), had stratified branches, with several small caliber processes in the region just above the soma, very few branches in the intermediate zone of Layer II, and then a larger dendritic mass in superficial regions (Fig 3d).

In pyramidal cells from the cerebral cortex, hippocampus and PC (Haberly, 2001; Furtak et al., 2007; Spruston, 2008), basilar dendrites receive different inputs than the apical dendritic tree. In *pars principalis* cells basilar dendrites were observed to arise from both lateral and ventral sides of cell body (Fig 3e). In the deepest of the cells the processes extended into the cell poor region under layer II. Basilar dendrites often extended apically to overlap with regions containing the lower apical branches. Two morphological variants of primary basilar dendrites were observed: large caliber processes that often branched and exhibited spines and dendrites that were very thin, mostly smooth and only rarely branched.

Quantification

Several of the measures made yielded very similar results. For example, the total number and total length of dendritic branches was highly correlated within both apical and basilar fields (r^2 's = 0.84 – 0.87). Similarly, the total dendritic length was highly correlated to the total number of Sholl shell crossings (r^2 's = 0.97 – 0.99). However, correlations were much lower when measures were compared between the apical and basilar dendrites (r^2 's = 0.05 – 0.08), suggesting that the morphology of the two dendritic fields is regulated independently.

The data set was analyzed by the location of the neuronal cell bodies in two ways: by their depth in Layer II (divided into deep, intermediate and superficial thirds, Figs 4 and 5), and by their radial location in *pars principalis* (dividing the zone into *pars medialis*, *pars dorsalis*, *pars lateralis*, and *pars ventroposterior*, Figs 6 and 7). An ANOVA was performed with these two independent variables and using total dendritic length, total branch number, and total number of Sholl shell crossings as dependent variables, each divided into apical and basilar dendritic domains. For both apical and basilar data, depth and depth/ radial location interaction effects were not significant (all p 's > 0.05). The location effect was not significant for the apical data (p = 0.072 for both total branch number and Sholl crossings), but was significant for the basilar data (branch: p = 0.024; Sholl, p = 0.026). Therefore, only the location factor was retained and a simple linear regression model constructed. Residual diagnostics confirmed normality and homogeneity of variance assumptions. Under the simple linear regression model, the location factor remained significant (branch: p = 0.018; Sholl: p = 0.019). Tukey's HSD test revealed that the mean basilar dendritic length was greater in *pars ventroposterior* than in *pars lateralis* (branch: p = 0.012; Sholl: p = 0.013). The right columns of Fig. 7 demonstrate that for both branch and Sholl measures *pars ventroposterior* processes often exceeded those of the other three regions.

Since statistical analyses indicated that cells from all locations were similar, all data were pooled in order to describe the morphology of the average AON pyramidal cell. For apical dendrites, the mean total number of branches was 19 (SEM: 0.60), and their mean total length was 922 μm (SEM: 27.7). The median number of branches was 17 (1st quartile: 13, 3rd quartile 25) and length was 845 μm (1st quartile: 640 μm , 3rd quartile 1118 μm). For the overall population, 25% of the apical dendritic tree was found within 70 μm of the soma, while 75% was found within 180 μm . Fig 3D depicts the cells from the sample that most closely matched the average total apical dendritic lengths for the three depth regions measured.

The average number of primary basilar dendrites extending from the cell body was 4.40 (SEM: 0.11). For the entire basilar dendritic tree, the mean total number of branches was 18 (SEM: 0.62), and their mean total length was 550 μm (SEM: 27.7). The median number of branches was 16 (1st quartile: 12, 3rd quartile 22) and length was 441 μm (1st quartile: 22 μm , 3rd quartile 701 μm). A quarter of the basilar dendritic tree was found within 25 μm of the soma, while 75% was found within 55 μm .

Non-pyramidal Cells

A variety of cell types have been previously described in *pars principalis* (see Discussion). The present sample allowed the description of three types of cells, all of which were found almost exclusively in the dorsolateral region of *pars principalis*, at the junction of *pars dorsalis* and *pars lateralis*. The most commonly observed was a tangential bipolar cell reliably found at the junction between Layer I and II (Fig 3g, h). These cells have a fusiform cell body giving rise to thick dendrites that are initially smooth but then exhibit evenly distributed spines. Branches were more likely to extend into Layer I than deep into the tissue. Various other morphologies were observed in the same region at the dorsalis/lateralis border, including cells with stellate (Fig 3i) or X shaped (Fig 3j) dendritic trees.

Discussion

Knowledge of the resident neurons is an essential first step in understanding and modeling the circuitry of any brain region. The data presented here comprise the most comprehensive quantitative study of AON cells to date. The morphology of the major neuron types is analyzed and several new varieties of cells are described for both *pars externa* and *pars principalis*. The limitations of the Golgi technique are well known and include the unknown basis for the selectivity of the stain and the difficulty in filling neurons completely (e.g., Haberly, 1983). The method also makes secondary characterization (e.g., counterstaining) of cells quite difficult. However, Golgi-staining has a long history as the preferred technique for providing a rigorous initial overview of cell populations (Peters and Jones; 1984).

Two cell types were examined in *pars externa* (Fig 2). “Type I” cells typically have 1–3 primary apical dendrites with large spines and no basilar dendrites, and have been observed in many studies (Haberly and Price, 1978; Scott et al., 1985; Reyher et al., 1988; Brunjes et al, 2005). In addition, a novel cell type was described (Type II cell, Fig 2). These neurons have complex and spiny apical dendrites and prominent basilar processes. The existence of cells with basilar dendrites is important as it indicates that the neurons probably receive input from sources other than the LOT fibers that are known to innervate the apical dendritic domain. Further research will need to sort out these circuits, but possibilities include inputs from *pars principalis*, the contralateral AON (Illig and Eudy, 2009), other brain regions, or intrinsic processing of information within *pars externa*. Nevertheless, the findings suggest that synaptic relations are more complicated in *pars externa* than previously believed.

The present study also described novel cell types in *pars principalis* (Fig 3g-j). There have been several reports indicating that the region has a diverse neuronal population (Reyher et al, 1988; Valverde et al., 1989; Brunjes et al., 2005), and indeed the wealth of neurotransmitter phenotypes found in the area (e.g., Brunjes et al., 2005; Meyer et al., 2006) suggests that the region is richly varied. Reyher et al. (1988), examining tissue from rats, observed bipolar or fusiform cells in all four of the major radial regions of *pars principalis*. Valverde et al. (1989) described 4 classes of non-pyramidal cells in hedgehog tissue, each with oval cell bodies and long, smooth and straight dendrites. One variety, found ubiquitously, had coextensive dendritic and axonal fields. A second type, found only deep in the tissue, was multipolar or bitufted, with axons traveling horizontally and collaterals extending towards the pial surface. A third class, found at the deep border of Layer I, had a tangential orientation and elaborate axons that extended parallel to the pial surface. The final variety was oriented in a radial fashion and was likened to a chandelier cell in the neocortex.

We observed several non-pyramidal cells. A spiny bipolar neuron was reliably observed at the Layer I/II border that is morphologically different from those reported by Valverde et al. (1989); the dendrites are oriented towards the pial surface rather than the cell layer. These cells also appear to be different than the tangentially-oriented, horizontal spiny cells and moderately spiny cells observed in the PC by Haberly (1983) as those cells reside more superficially in Layer I. The PC also contains tangentially-oriented semilunar cells at the Layer I/II border, but these have been reported to have only apically-oriented dendrites and non-uniform spine distribution (Haberly, 1983; Wilson et al, 2000). Two other relatively rare variants were also reported (Figs 3 h,i) to emphasize the potential complexity of *pars principalis*. Interestingly, all of the cells observed in the present study were confined to the transition region between *pars dorsalis* and *pars lateralis*. While the function of the cells remains to be determined, the dorsal and lateral AON are known to have different contralateral projections (Eudy and Illig, 2008) and segregated projections to the anterior PC (Haberly, 2001, Haberly and Price 1978). Several factors, including the variants of the Golgi stains employed (Haberly 1983) and species/strain differences, probably explain why investigators examining the region report different types of non-pyramidal cells.

There are a wealth of studies examining the morphology and regional variability of pyramidal neurons as they are the primary neurons of the paleo-, archi-, and neocortex (e.g., Feldman, 1984; DeFelipe and Fariñas 1992; Elston and Rockland, 2002; Furtak et al. 2007; Spruston, 2008). Cells found in *pars principalis* share the common morphology, although the immunostaining data in Figure 3 b,c suggests that they may be subpopulations of pyramidal cells in the region, perhaps with inhibitory neurotransmitter phenotypes. The present data was analyzed with two geographical measures as factors: the “radial location” of the cells (whether they resided in *pars medialis*, *pars dorsalis*, *pars lateralis* or *pars ventroposterior*) or by depth (deep, middle or superficial). Only one significant difference was observed when pyramidal cell size was compared across the four radial locations: the basilar dendritic fields of cells in *pars ventroposterior* were larger than those in *pars lateralis*. More will be needed to be known about the circuitry of *pars principalis* before this finding can be interpreted, but it does indeed strengthen the conclusion that there are regional differences in the structure, and thus probably the function, of *pars principalis* (Meyer, et al, 2005).

In most cortical structures pyramidal cell shape and size varies considerably depending on their deep-to-superficial location (e.g., Spruston 2008; Haberly 1983). Examining the possibility of similar differences due to cell location the AON was important for several reasons. First, evidence of stratification would bolster claims that the AON is simply a less rigorously laminated “cortical” structure, and not a “nucleus” (Brunjes et al., 2005). Indeed, Valverde et al (1989) suggested that Layer II in the hedgehog AON was not uniform and

recognized 2 subdivisions, a superficial zone “contain(ing) extraverted cells with several ascending dendrites that reach layer I and few or no basal dendrites” (p 589) and a deep layer comprised primarily of pyramidal cells. They also reported two separate cell zones under the main body of Layer II. Such lamination would seem to suggest that the AON was indeed “cortical”. Second, since in caudal regions the AON transforms into the PC, it is possible that the regions share a general plan, with Layer II in the AON separating into Layers II and III of the PC.

No deep-superficial effects were observed in the morphological characteristics of AON pyramidal cells. One difficulty encountered is that superficial pyramidal cells are difficult to define (Haberly 1983, Valverde et al., 1989) since there is little space in which to develop a characteristic long apical dendrite. Thus, these cells may assume a multipolar morphology while still maintaining the functional properties of a pyramidal neuron. Nevertheless, some provocative evidence of differential dendritic organization was observed. For example, deep cells were observed with apical dendritic tufts that stratified primarily in Layer II, thus not extending into the region of direct influence of LOT fibers. The fact that they do not extend into Layer IA suggests that these neurons are involved in the secondary processing of olfactory information within the AON. Evidence for deep-to-superficial gradients in neurochemical features of the AON (e.g., calbindin-immunoreactive cells are more numerous in the deep portion of Layer II while parvalbumin-immunoreactive cells are found superficially; Meyer et al., 2006) suggest that this difference in dendritic organization may reflect a functional organization of inputs.

A second observation was the stratification of apical dendritic branches. Deep pyramidal cells were observed with one set of dendritic branches in the region of the cell body and relatively few others until the superficial region of Layer II (Fig 3d). Once again, the results suggest that the neurons receive differential input along their apical dendritic trees. Indeed, one ubiquitous feature of pyramidal cells is the existence of domains with distinct synaptic inputs (Spruston, 2008). Typically, proximal synapses on the apical dendrite arise from local sources while distal contacts are from more distant locations. Basilar dendritic domains usually have an entirely separate source of inputs from the apical trees. A number of inputs from widely different areas are known for cells in *pars principalis* including fibers from olfactory (e.g., the contralateral AON, ipsilateral and contralateral PC) and non-olfactory (e.g., orbitofrontal cortex, hippocampus, subiculum, septum, amygdala, hypothalamus, monoamine fibers, see Table 2 in Brunjes, et al. 2005) regions. Developing stratified input might be simplified in *pars principalis* by the spatial segregation of inputs: LOT fibers provide synapses from above Layer II while many of the centrifugal fibers enter the olfactory peduncle through the deeply-situated anterior limb of the anterior commissure. One simple strategy would be to limit deep fibers to basal synapses, reserve proximal apical sites for recurrent and internal connections, and focus the distal dendritic field for LOT connections. Obviously much more work is needed before the organization of the area is fully understood, but the present data affords the first close characterization of some of the resident cells of the region, and thus should provide useful background for more closely defining the structure and function of the AON.

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Figure 1.

A: Photomicrographs of rostral-to-caudal, coronal, Nissl-stained sections through the rat anterior olfactory nucleus. p= *pars principalis*, e= *pars externa*. B: Photomicrograph of Golgi and Nissl counter-stained section through the anterior olfactory nucleus showing method used to classify cell location. Each cell was assigned two values: whether it was found in the deep (D), intermediate (M) or superficial third of Layer II, and whether it resided in *pars medialis* (1), *pars dorsalis* (2) *pars lateralis* (3) or *pars ventroposterior* (4). * = cell poor zone. C. Summary of Golgi measures. Left = “Branch” method. Dendrites extending from the cell body are classified as “primary” or first order processes. At bifurcations, each daughter branch is assigned the next higher branch order. Both numbers and length of dendritic processes at each branch order were recorded. Right = “Sholl” method. A series of concentric sphere are centered on the cell body. The first had a diameter of 20 μ m with each subsequent shell 10 μ m larger. The number of times the shells were crossed and the total length of dendritic material found within each sphere was recorded. Scale bars = 400 μ m.

Figure 2.

Pars externa. Top: camera lucida drawings of two cell types observed in *pars externa*. Type I cell represents the “classical” cell with only apical dendrites. Type II cells have basal dendrites and complex apical fields. Scale bar = 40 μm . Bottom: results of quantitative measurements of apical dendritic fields. Black bars = Type I cells, Gray bars = Type II cells. Left column: results of “branch” analysis, right column: results of “Sholl” analysis (see Fig 1b). Top row shows the percentage of the cells observed with processes at each branch order (left) or Sholl sphere (right). By the 7th branch and by 220 μm from the soma only about a quarter of the sample continued to contribute data. Therefore, the more distal data (grayed region) was not included in subsequent graphs to avoid large numbers of empty cells.

Second row: mean number of branches at each order (left) and Sholl sphere crossings (right). Bottom: Mean length of processes at each branch order (left) or within the Sholl sphere (right).

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Figure 3.

Cell types in *pars principalis*. A) Glycol methacrylate (2 μ m) Nissl-stained section demonstrating abundant triangular-shaped cell bodies with large apical dendrites characteristic of pyramidal cells. Scale bar = 50 μ m. B, C) cells immunostained for calbindin (b) and parvalbumin (c). Each exhibits a cell with a single apical dendrite, triangular soma and plentiful basilar dendrites. Scale bar = 40 μ m. Tissue from Meyer et al., 2006. D). Camera lucida drawings of Golgi-stained pyramidal cells from deep (left), intermediate and superficial (right) regions of *pars principalis*. These cells were chosen as their total apical dendritic length was closest to the mean for each of the three regions. Dotted lines represent the Layer I/II boundary. Arrow on deep cell depicts long region of apical dendrite in Layer

II devoid of branches. Scale bar = 40 μm . E). Camera lucida drawing of basilar dendrites of a cell from the intermediate layer of *pars dorsalis*. This cell was chosen as its total basilar dendritic length closely approximated the average for the entire sample. Scale bar = 40 μm . Arrowhead depicts axon. F) Drawings of pyramidal cell dendrites in Layer I. The drawing at top was taken from *pars dorsalis*, the bottom two from *pars lateralis*. Dotted line at left represents Layer II/I border. Dotted line at right for two bottom drawings represents Layer Ib/Ia border. All three of these processes extended to within 20 μm of the pial surface of the AON. Scale bar = 10 μm G). Tangentially-oriented non-pyramidal cells found at the border of *pars dorsalis* and *pars lateralis*. Scale bar = 40 μm . H) Photomicrograph of the region containing the region containing the cells shown in (f). Arrowhead depicts three laterally oriented cells. (the arrow on the left points to bottom cell in (f), the arrow on the right corresponds to the cell on the top in (f). I, J) Other examples of Golgi-stained, non-pyramidal cells with radially oriented (h) and X shaped (i) apical dendrites.

Figure 4.

Quantitative analysis of apical dendritic fields of *pars principalis* pyramidal cells by depth of cell bodies in Layer II. Left and right columns report results for the “branch” and “Sholl” analyses, respectively (Fig 1b). As in Figure 2, the top row shows the percentage of the cells observed with processes at each branch order (left) or Sholl sphere (right). Grayed area represent portion of the sample in which approximately only a quarter of the sample continued to contribute data. Therefore, the more distal data (grayed region) were not included in the subsequent graphs to avoid spurious estimates. Second row: mean number of branches at each order (left) and Sholl sphere crossings (right). Bottom: mean length of processes at each branch order (left) or within each Sholl sphere (right).

Figure 5. Quantitative analysis of the basilar dendritic fields of *pars principalis* pyramidal cells by depth of cell bodies in Layer II. See Figure 4.

Figure 6.
Quantitative analysis of the apical dendritic fields of *pars principalis* pyramidal cells by radial location of cell bodies of cell in Layer II. See Figure 4

Figure 7.
Quantitative analysis of the basilar dendritic fields of *pars principalis* pyramidal cells by radial location of cell bodies of cell in Layer II. See Figure 4

Table I

Number of pyramidal cells sampled in each location of *pars principalis* of the AON.

Radial Zone	Deep	Middle	Superficial	Sum
<i>pars medialis</i>	6	7	6	19
<i>pars dorsalis</i>	22	29	22	73
<i>pars lateralis</i>	31	27	28	86
<i>pars ventroposterior</i>	9	8	11	28
Sum	68	71	67	206