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Volumetric Changes in the Anterior Olfactory Nucleus of the Rat after Neonatal Olfactory Deprivation[1]

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The effect of olfactory deprivation in the postnatal development of the anterior olfactory nucleus (AON) was studied in 60-day-old rats which underwent unilateral naris closure after birth (postnatal day 1). Volumetric and morphometric analyses of the AON ipsilateral and contralateral to the closed naris were performed and data were statistically compared among them and with those of control animals. The volumes of the AONs and those of their subdivisions were calculated by the Cavalieri method and the area of the subdivisions was measured at seven established rostrocaudal levels. Whereas no statistically significant differences were detected between the ipsilateral and the contralateral AONs, comparison of these with controls revealed significant reductions in the volumes and dimensions of most AON subdivisions. The reduction was larger in the ipsilateral than in the contralateral AON and more pronounced in the rostralmost subdivisions (external and lateral) than in the caudal ones, the dorsal subdivision not being affected. These data demonstrate that the disruption of the normal afferent activity to one olfactory bulb has effects on the postnatal development of both the ipsilateral and the contralateral AONs. In addition, the most affected subdivisions were those that develop later and that receive the bulk of projections from the olfactory bulb, suggesting that the degree of maturity is an important factor in susceptibility to changes induced by reduced afferent activity. Finally, the results indicate that, contrary to the olfactory bulb, the contralateral AON cannot be used as a control structure in deprivation studies.

Key Words: **anterior olfactory nucleus; naris closure; olfactory system; postnatal development; quantitative neuroanatomy; sensory deprivation.**

INTRODUCTION

In macrosmatic mammals, the olfactory system is crucial for behavioral responses, such as territory marking, classic predator–prey interactions, and identification of conspecifics (13, 34). The information received by the olfactory mucosa is relayed in an ipsilateral way to the main olfactory bulb (MOB) and then, through the lateral olfactory tract, to more caudal olfactory structures including the anterior olfactory nucleus (AON), taenia tecta, pyriform cortex, olfactory tubercle, entorhinal cortex, and indusium griseum (2, 33). All these structures receiving direct afferents from the MOB have been referred to as the primary olfactory cortex (12, 22, 23, 34). The rostralmost region of the primary olfactory cortex is the AON (12, 34), which constitutes the first bilaterally innervated structure of the olfactory pathway, receiving direct input from the ipsilateral MOB and indirect input from the contralateral AON through the anterior commissure (2, 7, 33). This projection between both AONs constitutes the interbulbar commissural pathway and it enables the cross-coordination of the olfactory afferences in the primary olfactory cortex (29, 34).

The AON is a bilaminated structure, consisting of an external plexiform layer and a rather homogeneous cellular layer constituted by tightly packed cells (7, 16, 34). Five different subdivisions have been established in the AON on the basis of topological criteria: external (AONe), lateral (AONl), dorsal (AONd), medial (AONm), and ventroposterior (AONvp) (12, 15–17, 29). Although the cytoarchitectonic homogeneity of the AON makes it difficult to establish accurately the boundaries between subdivisions, the regions defined in this way demonstrate differences in their connectivity (16, 29).

Neurogenesis in the rat AON occurs during embryonic days 15–21 with two apparent developmental gradients: caudal to rostral and superficial to deep (5). Peripheral inputs to the AON are constituted by axons from the mitral and tufted cells of the MOB which convey the olfactory information to the AON, and they

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also show a gradient of innervation during development which is parallel with the neurogenetic gradient. Thereby, the first axons arising from the MOB innervate the caudalmost regions of the AON (6, 31). Electrophysiological studies have demonstrated that projections to the AON arising from the contralateral one exist by birth although considerable postnatal maturation occurs (25). During the maturation period, the AON, just like many other brain regions, exhibits a phase of increase from postnatal day 2 (P2) to P30 followed by a process of reduction in volume (P30–P60), and it shows changes of both metabolic activity and dendritic and neuronal maturation from birth to P60 (2, 7).

Given the different rostrocaudal rate of development of this structure and the high degree of postnatal maturation, the AON constitutes an ideal region to study the influence of the neural activity elicited by new sensory conditions in developmental processes. An experimental approach such as deprivation, directed to limit the entry of stimuli into a given sensory system, allows the comparison between normal and experimental situations, providing insights into the development and plasticity of the system (9). In the olfactory system, unilateral naris occlusion performed on neonatal rats produces a variety of morphological, histochemical, and metabolic changes in the MOB ipsilateral to the occluded naris, including a reduction in its total size (8, 14, 26), a decrease in the expression of some enzymes such as tyrosine hydroxylase (TH) (3) and of calcium-binding proteins such as calbindin D-28k and parvalbumin (27), and a reduced utilization of glucose (20). Although the alterations observed in the MOB could be reflected in more central olfactory structures such as the AON, only a few and subtle changes have been reported in these structures after deprivation (9). Nevertheless, although the only study available on the effects of deprivation in the AON (7) did not reveal significant size changes comparing the ipsilateral and the contralateral AON, it was performed on 30-day-deprived animals that had not reached adulthood. Given the high degree of postnatal maturation of the AON, which extends until P60, it is possible, therefore, that no evident changes are observable until the AON has completely finished its development. On the other hand, it is also possible that given the convergence of bilateral olfactory information on each AON, the contralateral AON could also be affected, thus showing nonsignificant changes compared with the ipsilateral one. In this context, the aim of the present study was to investigate the effects that olfactory deprivation has on the development of the AON from birth to adulthood, taking into account not only the effects on the ipsilateral AON but also the possible affectation of the contralateral side.

MATERIALS AND METHODS

Three timed pregnant female Wistar rats were housed singly under constant temperature conditions (22°C) on a 12/12-h light/dark cycle with food and water available *ad libitum*. Cages were checked daily to determine the date of birth.

On the day of birth (P1), two-thirds of the pups of each litter ($n = 6–8$) underwent unilateral naris closure. They were anesthetized by hypothermia, and electrocautery was applied briefly to the right external naris ("deprived" condition). In order to ensure the naris closure, the injured area was sewn up. One-third of the pups of each litter ($n = 2–3$) were similarly treated except that the electrocautery was placed just above the naris (control condition). After surgery, xylocaine and antibiotic cream were applied to the wound area to alleviate pain and to prevent infection, respectively. Normal body temperature was then restored by placing the pups on a heating pad. When the pups were moving freely, they were returned to their home cage to recover with their mother. As there was no blood and little apparent pain, the mothers readily accepted the pups. The lesion was examined daily under a magnifying glass, and animals in which naris closure was complete from P1 to P60 were selected. All experimental procedures conformed to NIH guidelines and were also in accordance with the guidelines of the European Communities Council Directive (86/609/EEC) and current Spanish legislation for the use and care of laboratory animals (BOE 68/8509-12, 1988).

Eight unilaterally deprived Wistar rats (four males and four females) and four control animals (two males and two females) with similar weight (200–215 g) were perfused when they reached P60. They were deeply anesthetized by an intramuscular injection of a mixture of ketamine chlorhydrate (Ketolar; Parke–Davis, Barcelona, Spain) and tiacine chlorhydrate (Rompún; Bayer, Leverkussen, Germany), 1 ml/kg body weight. They were intracardially perfused with 100 ml Ringer's solution followed by 400 ml fixative solution made up of 4% (w/v) paraformaldehyde and 0.2% (w/v) picric acid in 0.1 M phosphate buffer, pH 7.3 (PB). The brains were removed and tissue blocks containing both the MOBs and the AONs were separated using a rodent brain matrix (RBM-2000c; ASI Instruments, Warren, MI) and were postfixed in the same fixative solution at 4°C for 2 additional hours and then cryoprotected by immersion in 30% sucrose in PB at 4°C until they sank. These tissue blocks, eight control (from both right and left sides of four control animals) and eight ipsilateral and eight contralateral to the occluded naris from eight deprived animals were cut at the coronal plane at 30 μm thickness by using a cryostat (Leica Jung, Nussloch, Germany). From each tissue block six series were obtained.

Sections containing the MOB were used to assess correct deprivation by immunohistochemical detection of TH, whereas all the sections containing the AON were Nissl stained with 0.25% thionin to perform the volumetric and morphometric analysis.

Methods for Assessing Correct Deprivation

According to a large number of works reporting the involvement of afferent activity to the MOB in the regulation of the expression of TH (see, among others, 3, 4, 11), the loss of this marker in the MOB ipsilateral to the closed naris was used as a control for correct deprivation. Then, one series of each MOB of both deprived and control animals was processed for immunohistochemical detection of TH following the avidin–biotin immunoperoxidase method (19).

The immunohistochemical detection of TH was carried out as follows. Free-floating sections were washed in PB and incubated for 30 min in 10% normal horse serum and 0.05% Triton X-100 in PB. After being washed in PB, they were incubated for 48 h at 4°C in anti-TH primary antibody (KTHM888; Incstar Corp., Stillwater, MN) raised in mouse against rat brain TH diluted 1:10,000 in 0.05% Triton X-100 in PB. This primary antibody has been fully characterized by Western blot and does not cross-react with other proteins from the same biosynthetic pathway. The sections were washed (3 × 10 min) in PB and incubated for 1 h at room temperature in biotinylated horse anti-mouse immunogammaglobulins (Vectastain, Vector Laboratories, Burlingame, CA) diluted 1:200 in PB. After being washed in PB (3 × 10 min), sections were transferred to Vectastain ABC reagent (Vector Laboratories) diluted 1:200 in PB for 2 additional hours. Tissue-bound peroxidase was visualized by using 0.05% 3,3′-diaminobenzidine tetrahydrochloride and 0.003% hydrogen peroxide in 0.2 M Tris–HCl buffer, pH 7.6, for 10–15 min, until the desired staining intensity was reached.

Controls for the immunohistochemical procedure were performed by incubating sections in the same medium omitting: (1) the primary antibody, (2) the biotinylated immunogammaglobulin, or (3) the avidin–peroxidase complex. No residual reaction was observed.

Analysis

Nissl-stained sections through the whole length of the AON were digitized on a photomicroscope (Olympus AX-80) with a digital camera (Apogee Instruments) connected to a computer with the appropriate software (Adobe PhotoShop 5.5; Adobe Systems, Inc.). In each image, the boundaries of the entire AON as well as of each subdivision were plotted on the images and their areas were measured by using NIH Image software.

The Cavalieri method for estimating the volume was performed as described (18). First, and in order to determine the proper sampling interval, the volume of each AON subdivision and the whole AON was calculated by measuring the area of each subdivision in all sections of the AON from one animal. The sum of the areas was multiplied by the section thickness (30 μm), thus obtaining the total volume. A sampling ratio was determined for each subdivision by determining the smallest number of sections which could be measured while still remaining within 95% of the tissue volume calculated by using all sections. This criterion was satisfied, for all subdivisions, by measuring every third section. Then, for each AON, the volume was estimated using the formula $V_{est} = \Sigma a \times t$, where a is the area of the structure under analysis and t is the distance between the measured sections. In our study, since we measured every third section, their thickness being 30 μm, $t = 90$.

The volumes obtained in this way were gathered as three groups: two experimental groups from deprived animals (AON ipsilateral and contralateral to the closed naris) and an additional group composed of the AONs of control animals. The right and left AONs from control animals were used without distinction in the statistical analysis as they did not demonstrate right/left differences. The total volumes of the entire AONs and of each subdivision were compared between the ipsilateral and the contralateral AONs from deprived animals by means of the Wilcoxon rank test for paired data. To compare each of both experimental groups with the respective control values, the Mann–Whitney U test was employed. For all tests, values of $P < 0.01$ were considered highly significant, and $0.01 < P < 0.05$ was considered significant for the differences.

In addition, in order to detect rostrocaudal differences in the affectation degree, statistical analysis was performed comparing the area of the subdivisions at seven defined rostrocaudal levels along the AON. These selected levels (see criteria under Results) were comparable in each experimental group. At these levels, the boundaries of each subdivision were established, and their areas were calculated and statistically analyzed as previously described.

RESULTS

Control of Olfactory Deprivation

After the brain was removed, the MOBs ipsilateral to the closed naris from deprived animals showed a remarkable reduction in size, compared with the contralateral ones, that was macroscopically distinguishable. The observation of Nissl-stained sections demonstrated a clear reduction in the dimensions of the layers of the ipsilateral MOB compared to the contralateral one.

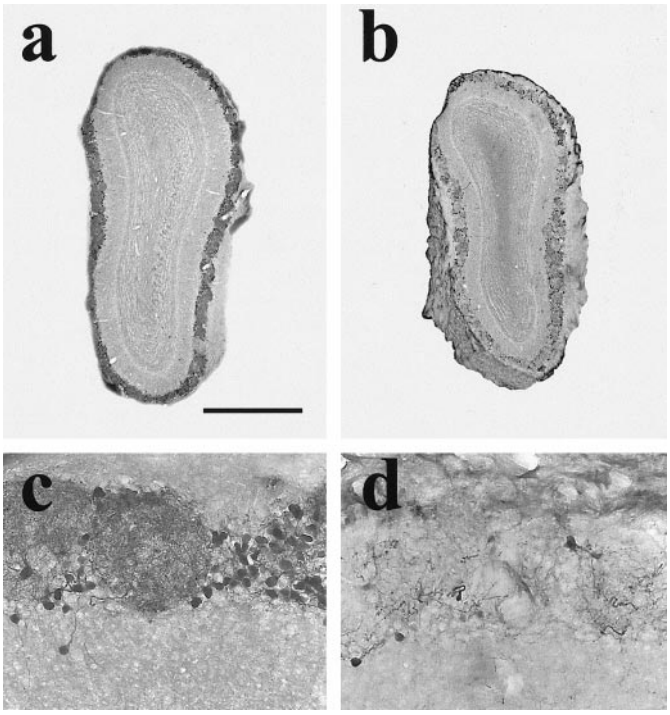


FIG. 1. Photomicrographs of tyrosine hydroxylase-immunostained coronal sections of the main olfactory bulb of 60-day-old rats which underwent unilateral naris closure at birth. The olfactory bulb contralateral to the closed naris shows normal density of immunoreactive juxtaglomerular cells (a), whereas there is a striking decrease of these elements in the ipsilateral one (b). High magnifications of (a) and (b) are shown in (c) and (d), respectively. Scale bar for a and b, 1 mm. Scale bar for c and d, 125 μm.

In addition, the immunohistochemical detection of TH revealed that, whereas similar numbers and distribution of TH-immunostained cells were observed in the MOBs of control animals and in the contralateral MOBs of deprived animals (Figs. 1a and 1c), there was an evident decrease in the number of TH-immunostained juxtaglomerular cells in the MOBs ipsilateral to the occluded naris (Figs. 1b and 1d). These observations confirmed that all experimental animals had been accurately deprived.

Delimitation of the AON Subdivisions

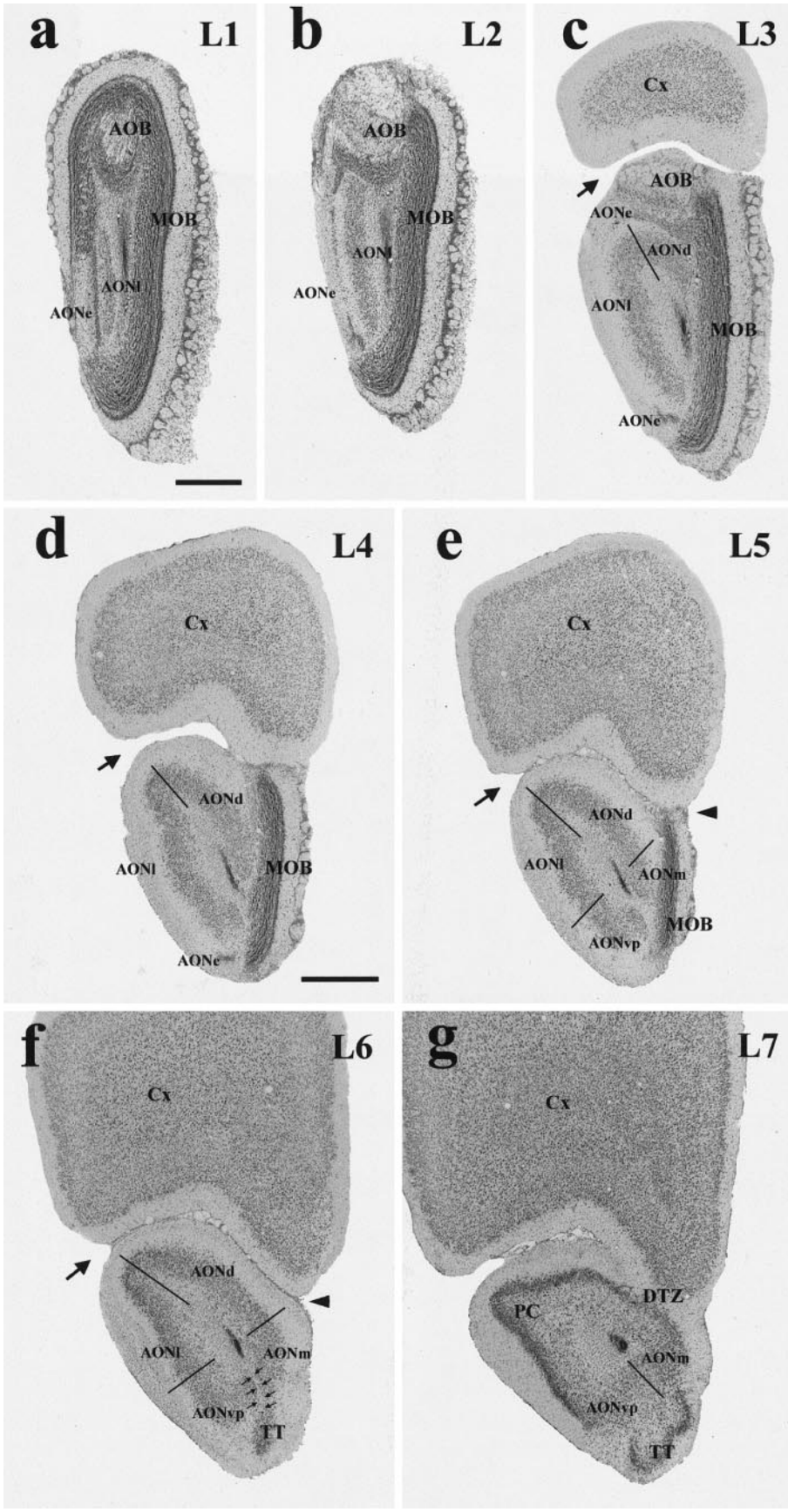
The subdivisions of the AON have been defined and named on the basis of their relative location to the anterior commissure (1, 2, 5, 7, 12, 15–17, 29). In this study, we have followed the nomenclature proposed by Haberly and Price (16) according to which the AON consist of five subdivisions: AONe, AONl, AONd, AONm, and AONvp. Nevertheless, given the cytoarchitectonical homogeneity of the cellular layer, the exact boundaries between these subdivisions have not been precisely defined and discrepancies exist concerning their exact location. Since the present study requires homogeneous criteria for the location of the boundaries of each subdivision, we have established them helped by anatomical landmarks. It is possible that our criteria do not match exactly those of Haberly and Price (16); however, they allow a more homogeneous comparison between different animals (Fig. 2). Thus, the AONe is the only subdivision that can be clearly delineated regarding the histological characteristics, as it is clearly separated from the remaining ones. The boundary between the AONl and the AONd was established by a line traced from the dorsalmost region of the commissural bundle to the "fissura rhinalis" (Figs. 2c–2f). Between the AONd and the AONm, the boundary was established by the line traced from the center of the ependymal region to the "fissura circularis rhinencephali" (Figs. 2e and 2f), and the AONl–AONvp boundary was defined as a ventral prolongation of the former line. The boundary between the AONm and the AONvp is easy to distinguish since these subdivisions are separated by a narrow, but evident, fiber tract (Fig. 2f).

Volumetric Study

The analysis of the AON volume demonstrated the existence of differences between the three experimental groups (AON ipsilateral, AON contralateral, and AON control), although these differences were not as evident as those detected in the MOB.

The mean total volume of the AON and those of each subdivision of each experimental group are graphically represented in Fig. 3. The results indicated that the total volume of the AON, as well as those of its subdivisions, was reduced in both hemispheres of deprived animals. The statistical analysis did not detect significant differences between the total volumes of both AONs of deprived animals, whereas it did ($P < 0.05$) when the total volume of any of the AONs of deprived animals was compared to control volume (Fig. 3).

FIG. 2. Photomicrographs of coronal Nissl-stained sections of the AON along the rostrocaudal axis. The seven levels shown have been selected according to morphological and anatomical features and have been used as reference levels in this study. (a) Level 1, (b) level 2, (c) level 3, (d) level 4, (e) level 5, (f) level 6, and (g) level 7. The boundaries between subdivisions are marked with lines. Arrows point to the "fissura rhinalis" and arrowheads to the "fissura circularis rhinencephali." Small arrows in f point to the narrow fiber tract that establishes the boundary between the AONm and the AONvp. AOB, accessory olfactory bulb; AONd, dorsal subdivision of the AON; AONe, external subdivision of the AON; AONl, lateral subdivision of the AON; AONm, medial subdivision of the AON; AONvp, ventroposterior subdivision of the AON; Cx, cerebral cortex; DTZ, dorsal transitional zone; MOB, main olfactory bulb; PC, pyriform cortex; TT, taenia tecta. Scale bar in a is for a–c, 1 mm. Scale bar in d is for d–g, 1 mm.


a
L1
AOB
MOB
AONl
AONe
b
L2
AOB
MOB
AONl
AONe
c
L3
Cx
AOB
AONe
AONd
AONl
MOB
AONe
d
L4
Cx
AONd
AONl
MOB
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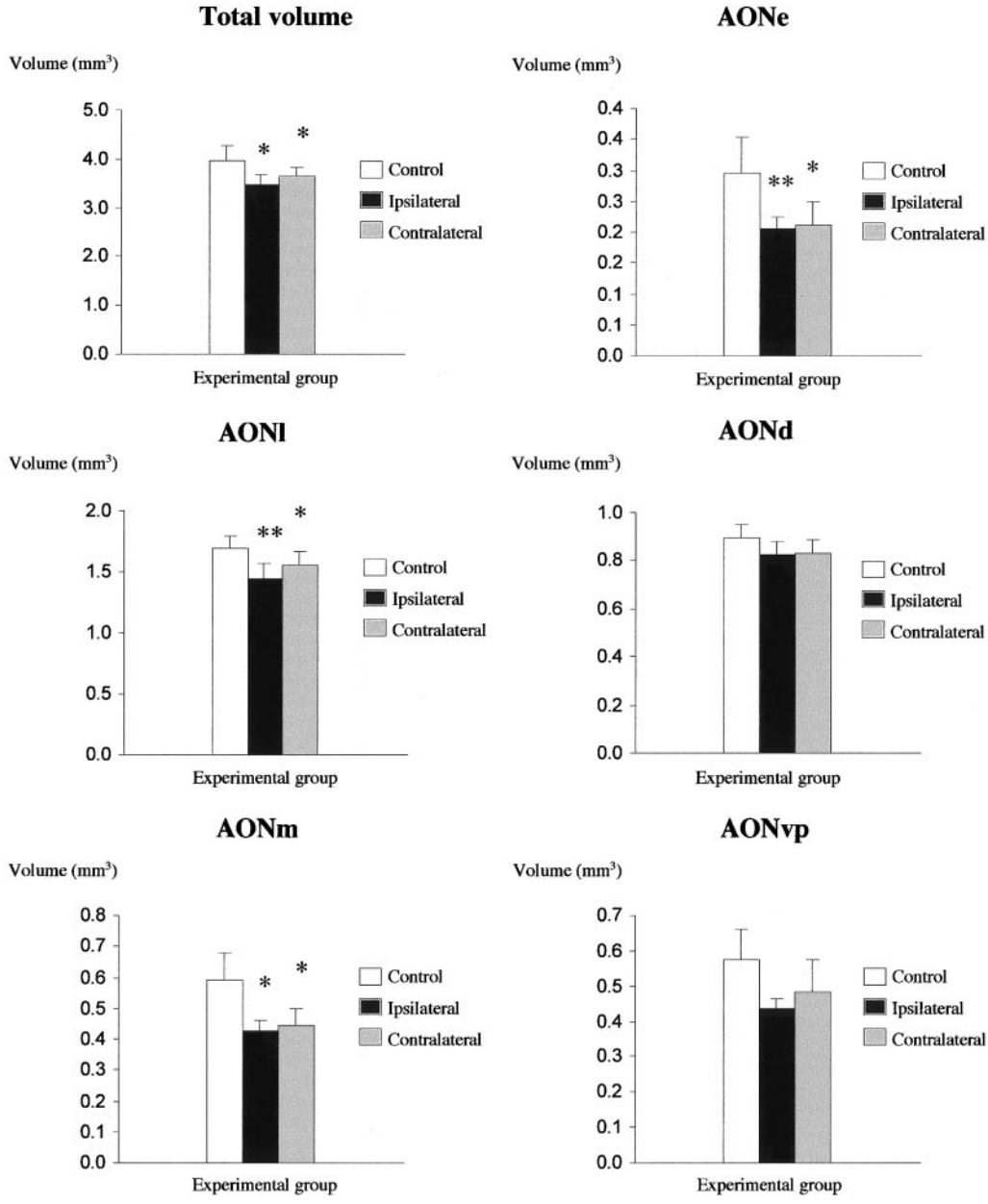

FIG. 3. Graphic representations of the mean volumes ± SEM of the whole AON and of each subdivision. Asterisks indicate statistically significant differences between either the ipsilateral or the contralateral AON of deprived animals and the controls. *Significant difference ($0.01 < P < 0.05$). **Highly significant difference ($P < 0.01$).

The volume reduction did not affect all subdivisions in the same way, and whereas the rostralmost subdivisions (AONe and AONl) demonstrated the largest reduction, others such as the AONd did not demonstrate significant differences (Fig. 3). In this sense, both the AONe and the AONl demonstrated statistically significant volume reduction compared with their respective controls. These reductions were highly significant ($P < 0.01$) in the case of the ipsilateral hemisphere and significant ($P < 0.05$) in the case of the contralateral one (Fig. 3).

In the caudalmost subdivisions the volume differences were lower. Thus, the AONm of both hemispheres of deprived animals demonstrated a significant volume reduction ($P < 0.05$) compared to controls. In the AONvp the statistically significant reduction was detected only in the AON ipsilateral, whereas no significant differences were detected in the AONd (Fig. 3).

These results indicated that olfactory deprivation produced a decrease in volume of the deprived AONs that was larger in the ipsilateral AON to the closed

naris than in the contralateral one. In addition, all subdivisions were not affected in the same degree. The reduction in volume was larger in the AONe and the AONl, whereas the AONd seemed to be scarcely affected.

Morphometric Study

Regional differences in the maturation rates have been observed in the AON. Developmental studies demonstrated that this olfactory structure exhibits a caudal-to-rostral neurogenetic gradient. Thus, at birth, the rostralmost subdivisions (AONe and AONl) have a lower maturation degree than the caudal ones and, within a given subdivision, the rostralmost regions are also less mature than the caudalmost (2, 6, 7). The different maturation stage could induce distinct degrees of susceptibility to the effects of certain experimental conditions as suggested (2, 21). Therefore, to check whether olfactory deprivation affects distinct regions differentially, we analyzed the dimensions of the AON subdivisions at seven reference levels along the rostrocaudal axis.

Selection of Reference Levels

The seven levels are representative of the whole of the AON and, in addition, they were selected according to morphological and anatomical features in such a way that the same level can be unequivocally compared in different animals. Description of the selected levels is as follows:

First level (L1) (Fig. 2a): it is the section of the series next to that in which the AON appears for the first time. The dorsal region in this section is occupied by the accessory olfactory bulb and the medial region by the caudal region of the MOB.

Second level (L2) (Fig. 2b): it is the section in which the AONl and the AONe reach their maximum length in the dorsoventral axis. It is, in addition, the last section in which the AONe is observed as a continuous cellular row.

Third level (L3) (Fig. 2c): at this level AONe is separated into two cellular subgroups, ventral and dorsal, being the first section in which the dorsal subgroup of the AONe is placed dorsal to the AONd. In the dorsal region of the section, the granule cell layer of the accessory olfactory bulb is reduced to a triangular zone whose ventral limit is separated from the AON by its plexiform layer, and the AONd appears as a dorsal extension of the AONl.

Fourth level (L4) (Fig. 2d): it is the first section in which the accessory olfactory bulb does not appear, and only the ventral subgroup of the AONe is distinguishable.

Fifth level (L5) (Fig. 2e): it is the last section in which glomeruli of the MOB are observable. The cellular layers of the different subdivisions of the AON have extended, forming a practically closed ring. At this level, the AONm and the AONvp can already be distinguished.

Sixth level (L6) (Fig. 2f): it is the section following that in which the MOB can be no longer observed.

Seventh level (L7) (Fig. 2g): it is the first section in which the olfactory peduncle has clear continuity with the cortex through the dorsal transitional zone. At this level, the AONm and the AONvp are the only subdivisions that can be differentiated, whereas the AONl and the AONd have been replaced by the pyriform cortex.

Morphometric Analysis

The mean areas of the AON subdivisions at each selected level are graphically represented in Fig. 4. The statistical analysis was performed as in the case of the volumetric study, searching for significant differences in each subdivision at each selected level between: (a) ipsilateral vs contralateral AONs from deprived animals and (b) either ipsilateral or contralateral vs control.

Ipsilateral vs Contralateral AONs from Deprived Animals

After checking that the data from the ipsilateral and the contralateral AONs of deprived animals could be considered homogeneous samples, we performed the statistical analysis by using the Wilcoxon rank test for paired data. As in the case of the volumetric analysis, the statistical test did not demonstrate significant differences between the two hemispheres of deprived animals in any AON subdivision at any of the analyzed levels.

Deprived Animals vs Control Animals

In most cases, the dimensions of the AON of deprived animals were clearly reduced compared to their respective controls, these reductions being larger in the ipsilateral AON than in the contralateral. Nevertheless, the statistical significance of the reduction varied at different levels within a given subdivision.

AONe. The dimensions of the AONe ipsilateral to the closed naris were significantly smaller, at all studied levels, than in control animals. This reduction was highly significant ($P < 0.01$) at the rostral levels, reaching a maximum at L2 (Figs. 4, 5a, and 5b). Significant differences were not detected at L3 when both subgroups (ventral and dorsal) of the AONe were analyzed together, whereas analyzing them separately, the test detected significant differences in the ventral subgroup. This significant reduction was maintained at the caudalmost level (L4) where only the ventral subgroup of this subdivision appeared (Figs. 4, 5c, and 5d).

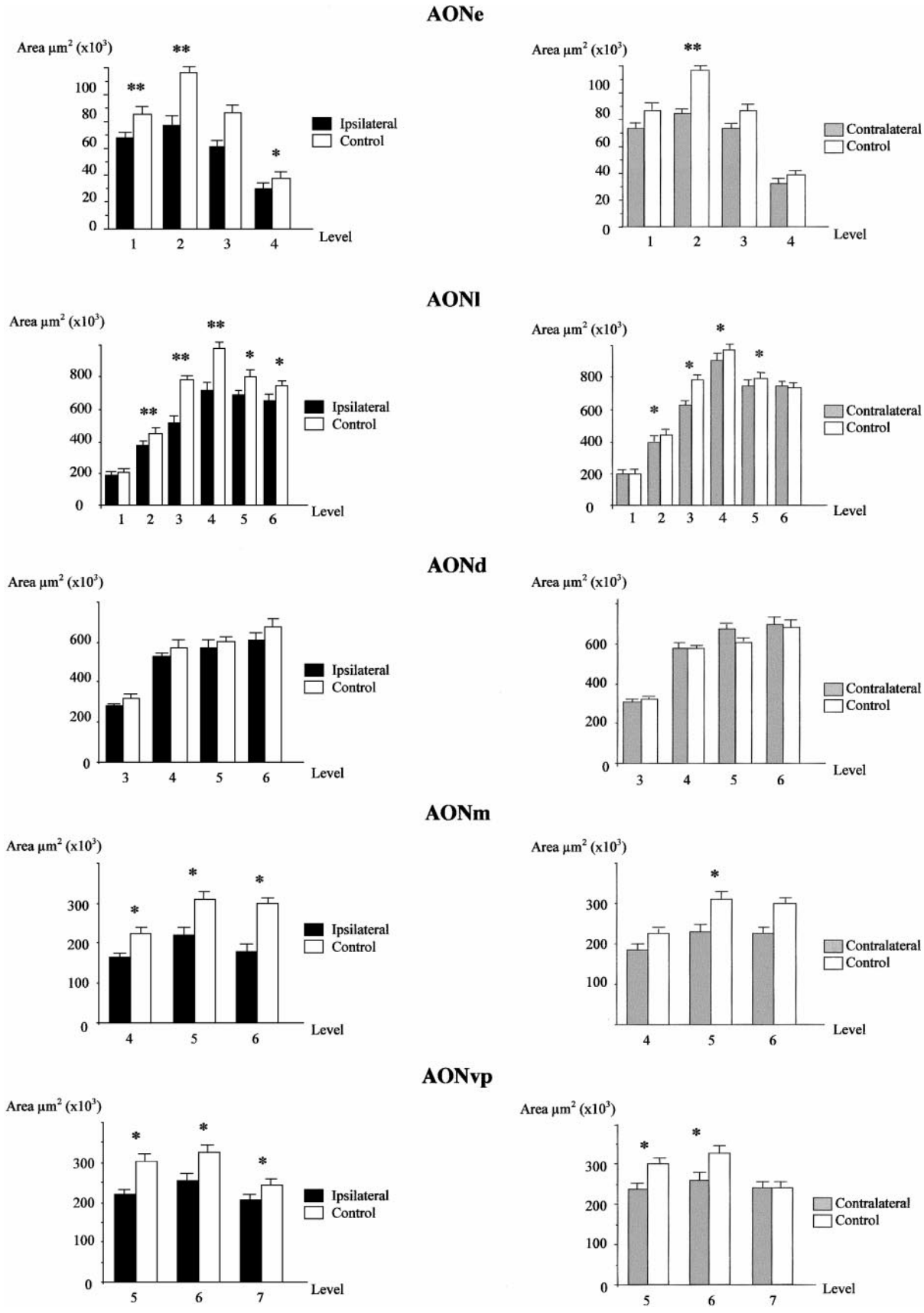

FIG. 4. Mean area ± SEM of each subdivision of the AON at each reference level. In the left column charts include the data from control and the AON ipsilateral to the occluded naris of deprived animals. In the right column charts include the data of the AON of control animals and the AON contralateral to the occluded naris. Asterisks indicate statistically significant differences between either the ipsilateral or the contralateral AON of deprived animals and the controls. *Significant difference ($0.01 < P < 0.05$). **Highly significant difference ($P < 0.01$).

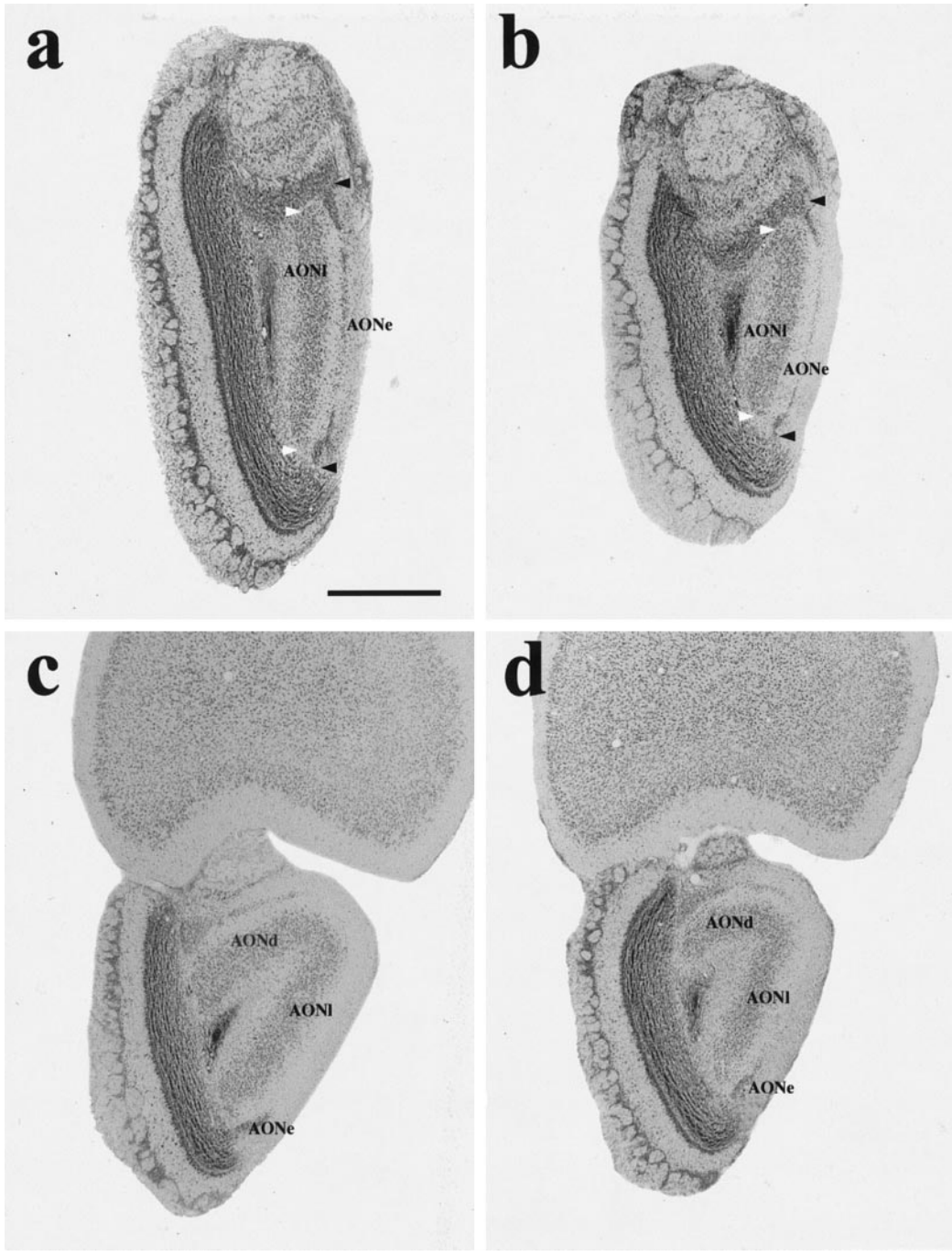

FIG. 5. Pairs of photomicrographs of coronal Nissl-stained sections of the AON at two different levels from control and deprived animals. (a, b) Pair of sections at level 2. The AONe and the AONl of a control (a) and the ipsilateral side of a deprived animal (b) are shown. The dorsal and ventral boundaries of the AONe are marked with white arrowheads and those of the AONl with black arrowheads. Note the differences in the dimensions of these subdivisions. (c, d) Pair of sections at level 4 of the AON of a control rat (c) and the AON ipsilateral to the occluded naris (d). Note the reduction in size in the deprived AON (d) that is especially evident in the AONl. Scale bar, 1 mm.

The dimensions of the contralateral AONe were also smaller than those of the control AONe at all levels; however, only at L2 was this difference highly significant ($P < 0.01$).

AONl. The statistical analysis demonstrated significant differences at most levels comparing both AONs from deprived animals with controls (Figs. 4 and 5). The ipsilateral AONl demonstrated a highly significant reduction ($P < 0.01$) in the rostral levels (L2–L4) and significant reduction ($P < 0.05$) at the caudal levels (L5–L6). The contralateral AONl showed a significant reduction in size ($P < 0.05$) in L2–L5,

whereas no differences were found at both the rostralmost (L1) and the caudalmost (L6) levels.

AONd. This subdivision seemed to be not affected by the olfactory deprivation. The test used did not detect significant differences at any of the analyzed levels in any of the comparison groups.

AONm and AONvp. Both subdivisions, which appeared only in the three caudalmost levels, were similarly affected by the naris occlusion. At all levels of the ipsilateral hemisphere the test detected significant differences from controls ($P < 0.05$), whereas in the contralateral side, the differences were significant only at the medial level (L5) in the AONm and at the two rostralmost levels (L5–L6) in the AONvp (Fig. 4).

DISCUSSION

In the present report we analyze the effects that unilateral neonatal olfactory deprivation has on the postnatal development of the AON and if this effect is homogeneous along the rostrocaudal axis and in all subdivisions. One of the main findings is that both AONs ipsilateral and contralateral to the closed naris were affected by the deprivation, with a significant reduction in their dimensions compared to controls. Unilateral olfactory deprivation produces very few alterations in the MOB contralateral to the injured naris and, in fact, it is indistinguishable from those of animals which did not undergo naris occlusion (9). In this context, the contralateral MOB has been used as a control to compare the effects that deprivation has on the ipsilateral one (4, 9, 11). By contrast, our results demonstrate that in the case of the AON, both the ipsilateral and the contralateral structures are affected as a consequence of the deprivation, showing, in several subdivisions and at some levels, statistically significant size reduction. This indicates that the contralateral AON cannot be used as a control to compare the effects observed in the ipsilateral structure after deprivation.

A previous work focusing on the development of the AON in unilaterally deprived rats concluded that the procedure had little effect on the postnatal development of the AON, despite the evident changes observed in the MOB (7). Although this finding seems to be contradictory to our data, there are some differences in the design of the study that may explain this discrepancy. First, in the study by Brown and Brunjes (7) the AONs of deprived rats were examined at P30, whereas we studied them at P60. The development of the AON is not complete until P60 and the P30–P60 period is a phase during which the AON exhibits significant developmental reductions in size (7). Therefore, differences could be not discernible until the final development of the structure has been reached. Second, the analyses of the different developmental patterns after deprivation were performed by comparing left/right AONs from deprived animals. Our study confirms these results since no differences were observed between the ipsilateral and the contralateral AONs in our material. However, compared to controls, both experimental groups demonstrate significant differences, therefore indicating that modifications in the development of the AON occur after unilateral deprivation.

The reduction in size of the ipsilateral MOB of deprived animals has been mainly related to enhanced cell death, with a particular decrease in the number of the last neuronal elements to be generated (e.g. tufted and granule cells), their immaturity perhaps contributing to their susceptibility to deprivation (9). Cell death or cell shrinkage as a consequence of reduced activity and/or loss of connections could also account for the reduction in size of the AON. A decreased cell production is not probable because the neurogenesis in the AON takes place only during the embryonic period and proliferation does not continue into adulthood as it does in the MOB (5, 6, 9). The size reduction could be the result of less dendritic development, the cell number not being affected. An examination of dendrogenesis within the AON has shown robust changes during the normal development, and this development of the dendritic fields seemed to be sensitive to manipulations of the afferent stimulation (7).

The postnatal growth and development of the AON takes a biphasic pattern according to which first (P1–P30), a volumetric expansion takes place, followed by a regression from P30 to P60 (7). The restriction of olfactory stimuli, which we have maintained during the period P1–P60, could affect both developmental phases and thus, the reduction in size of the AON may arise through either a decreased expansion in the first postnatal period or an increased regression in the second phase.

The reduction of the total volume of the AON was not homogeneous, but distinct AON subdivisions were differentially affected, and even within a given subdivision, distinct degrees of affectation could be observed at different rostrocaudal levels. A general pattern can be concluded: rostral subdivisions (AONe and AONl) and rostral levels within them were more severely affected by unilateral deprivation than caudal levels and subdivisions. This supports the existence of anatomical, developmental, and physiological singularities between subdivisions and within subdivisions as suggested (21–23, 28).

There are some explanations to justify the heterogeneous affectation of the AON, being that some AON subdivisions and regions are more susceptible than others to the changes induced by deprivation. We hypothesize that this particular susceptibility to deprivation is related to the developmental gradient of the AON. The rostralmost subdivisions exhibit a smaller

growth ratio during early development than the caudal regions (7). The same gradient appears in neurogenesis; the rostral regions are developed later than the caudal ones (5), and the synapsing axons arising from the MOB appear earlier in the caudal regions (6). In agreement with these data, the rostral portion of the AON might be more susceptible to deprivation-induced changes since it is the last portion to undergo cellular proliferation and to receive the inputs from the MOB.

The peculiarities in the connections among subdivisions of the AON could also explain, at least in part, why they are not affected in the same manner. The axons of the lateral olfactory tract arising from the MOB arrive ipsilateral to both the entire AONe and some regions of the AONl (32). The development of the ipsilateral AONe and the AONl could be more affected because they receive directly the information from the deprived MOB. Additionally, since the bulk of projections from most subdivisions of the AON arrives to both the contralateral AONe and the AONl (16), the reduced activity in the ipsilateral AON as a consequence of the lack of activity in its corresponding MOB could modify the degree of induced activity in these subdivisions of the contralateral AON. In fact, these data are in accordance with those reported by Brown and Brunjes (7), who described a decrease in 2-deoxyglucose uptake in the AONl after deprivation, suggesting that naris closure does indeed affect the metabolic activity of this structure.

Different affectation degree may also be due to the differential innervation of the AON by particular neuronal populations of the MOB (33, 34). The tufted cells of the MOB mainly project to the rostral portion of the AON (AONe and AONl) (16, 24, 28, 30). This cell population is the most severely affected by naris occlusion, with a large reduction in its cell number (10, 14). By contrast, the caudal regions of the AON are innervated by the mitral cells (6, 10) whose numbers are relatively unaffected by deprivation (9). Therefore the larger affectation of the AONe and the AONl may also result from a lower number of fibers arising from tufted cells and/or the attenuation of the activity of the remaining ones.

In the present paper we demonstrate that the reduction of the olfactory inputs to one MOB affects the postnatal development of both AONs, observed as a size reduction. The affectation degree is clearly larger in the AON ipsilateral to the occluded naris than in the contralateral one. Within each AON, the most affected subdivisions are the rostralmost. Although several factors can be responsible for the differential degree of affectation among subdivisions, the lower maturity at the moment of deprivation and being the target of the most direct olfactory inputs would account for higher susceptibility to deprivation.

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