ELSEVIER

Developmental Brain Research 122 (2000) 35–46

DEVELOPMENTAL BRAIN RESEARCH

www.elsevier.com/locate/bres

Research report

Effects of unilateral deprivation in postnatal development of the olfactory bulb in an altricial rodent, the gerbil (Meriones unguiculatus)

L.A. Vallejo[a], M. Garrosa[b], A. Al-Majdalawi[b], A. Mayo[c], M.J. Gayoso[b,*]

[a]*Department of Otorhinolaryngology, Rio Hortega Hospital, University of Valladolid, Institute of Neuroscience of Castilla y León (INCYL), Av. Ramón y Cajal 7, 47005 Valladolid, Spain*

[b]*Department of Cell Biology, School of Medicine, University of Valladolid, Institute of Neuroscience of Castilla y León (INCYL), Av. Ramón y Cajal 7, 47005 Valladolid, Spain*

[c]*Department of Statistics and Operations Research, University of Valladolid, Av. Ramón y Cajal 7, 47005 Valladolid, Spain*

Accepted 18 April 2000

Abstract

To establish if olfactory bulb sensitivity to functional deprivation is related to the degree of development at birth, we studied the effects of surgical closure of one naris in the gerbil olfactory bulb development. The naris closure was performed at three different ages: at birth, P7 and P14 and maintained for 30 or 60 days. In coronal sections we measured total bulbar surface area and surface area of the different bulbar layers establishing an estimate multiple regression model for the percentage of surface area decrease in the deprived bulb related to non deprived one. The internal and external plexiform layers are the most sensitive layers to deprivation and age and duration of deprivation were factors in their mathematical models. The glomerular layer showed a surface reduction of about 25% without dependence either on age or duration. The deprived glomerular layer showed a much lower tyrosine hydroxylase-immunoreactivity and immunoreactive cell density than those in the non deprived one. However, differences in calbindin-immunoreactive and NADPH-diaphorase positive cell density between deprived and non deprived glomerular layer were not significant. Our results indicate that olfactory bulb sensitivity to functional deprivation is not related to the degree of precocity and changes in age and duration of deprivation cause different effects on the olfactory bulb layers.

Theme: Development and regeneration

Topic: Sensory systems

Keywords: Sensory deprivation; Neural plasticity

1. Introduction

The unilateral naris closure in neonatal macrosmatic animals causes significant changes in functional activity of the olfactory bulbs [26,39,40] and when maintained for 20 or more days produces substantial changes in volume, layer thickness and cell number and size.

In laboratory rats, after 20 days of neonatal olfactory deprivation, the volume of the ipsilateral main olfactory bulb decreases approximately 25% when compared with control or contralateral non-deprived olfactory bulb [19,30,31,49]. The total protein content, RNA and DNA decrease in the deprived bulb [52]. Volume and weight reduction are caused by decrease in both number and size of the olfactory bulb neurons and affects all bulbar layers except the periventricular core [57,58]. Decrease in the number of tufted and granule cells has been consistently reported [30,49–51,54,61,62]. However, the decrease in the number of mitral cells remains controversial since some authors have reported it [50,54], whereas others have not found such a decrease [25,30]. More agreement exists on the reduction in size of soma and glomerular tufts of mitral cells after neonatal olfactory deprivation [14,53]. The hyperplasia and hypertrophy of reactive astrocytes does not compensate this neuron volume decrease [47].

The dramatic loss of tyrosine hydroxylase (TH) immunoreactivity is the most characteristic change in neurotransmitter expression in this experimental model [6,7,9–

*Corresponding author. Tel.: +34-983-423-081; fax: +34-983-423-022.

E-mail address: gayoso@med.uva.es (M.J. Gayoso)

0165-3806/00/$ – see front matter
PII: S0165-3806(00)00050-X

11,42,64,65]. TH loss correlates well with dopamine depletion [20]. TH is expressed by olfactory bulb dopaminergic neurons which are mainly periglomerular and tufted cells. The periglomerular cells are an heterogeneous group in which several subgroups can be defined by their contents in calcium-binding proteins (calretinin or calbindin), neurotransmitters and neuropeptides [41,44,66] and NADPH-diaphorase or NO synthase activity [2]. In TH-immunoreactive periglomerular cells, dopamine co-localizes with GABA [41,43] and, after olfactory deprivation, these cells lose their TH-immunoreactivity but show a non reduced GABA- [12,42], glutamic acid decarboxylase- and aromatic L-acid decarboxylase-immunoreactivity [6,65]. This indicates that TH expression is down-regulated but that periglomerular cells do not disappear [6].

The sensitivity of different olfactory bulb neuronal types to deprivation seems to be related to postnatal neurogenesis time, since the most sensitive neurons are granule and tufted cells which show the most delayed neurogenesis time [3,13,34,35,]. The decrease in cell number is thought to depend more on increased neuronal death than on the reduction of postnatal proliferation and differentiation [31].

If olfactory bulb plasticity were linked to the degree of development, one may suppose that the more precocial the animal, ie. born with more developed bulbs, the less sensitive to olfactory deprivation it would be. The spiny mouse (Acomys cahirinus) is a precocial rodent born with an olfactory bulb structure that has a development similar to that of the rat at P14 [46]. In this rodent, Brunjes [17] observed a greater and more persistent sensitivity to olfactory deprivation than in the rat. Therefore, the study of olfactory bulb plasticity in more altricial rodents is of considerable interest to understand the relation between maturity and plasticity in the olfactory system. The Mongolian gerbil (Meriones unguiculatus) is an altricial rodent in which the olfactory bulb takes 21 days after birth to reach a fully developed structure similar to that reached by the rat at P14 [24] and by the spiny mouse at P1 [46].

We studied the effects of surgical closure of one naris on olfactory bulb development in the gerbil in order to test whether the olfactory bulb is more or less sensitive to functional deprivation in altricial than in precocial species. Likewise, we tested the influence of age and duration of deprivation in the size variation of the different olfactory bulb layers, using TH- and calbindin-immunoreactivity and NADPH-diaphorase activity to gain further insight into the participation of the different olfactory bulb neurons in functional deprivation response.

2. Materials and methods

Thirty nine newborn gerbils (Meriones unguiculatus) of both sexes were used for the experiments. All of them were maintained under air-conditioning at 20–22°C, 50% humidity and 12–12 h light–dark cycle. Pups remained with their mothers up to 30 days of age, when they were moved to independent cages. Care and manipulation of the animals followed the guidelines of the European Communities Council (86/609/EEC) for laboratory animal care and experimentation.

Unilateral olfactory deprivation was accomplished at three ages: newborn, 7 days and 14 days. This olfactory deprivation was maintained for 30 or 60 days and then the animals were sacrificed. The six groups obtained were called 1/30, 1/60, 7/30, 7/60, 14/30 and 14/60; the numerator meaning the day of deprivation and the denominator its duration. A group of non-deprived animals 60 days old were used as control. Each group contained six animals except for the 14/60 group which had only three. In all the experimental animals the right naris was cauterized in order to occlude it [50].The newborn gerbils were previously anesthetized with hypothermia and the others with Equithesin.

At the end of the deprivation period, animals were anesthetized with Equithesin (2.5 ml/kg) and transcardially perfused with 4% paraformaldehyde and 0.2% picric acid in 0.1 M Millonig's phosphate buffer. The animals were then decapitated, the cranium opened and the whole head left for 24 h at 4°C in the fixative. Afterwards, the brain was removed, washed and left in 30% sucrose at 4°C until it sunk. Brains were frozen in dry ice and the olfactory bulbs were sliced, in a sliding microtome at 40 μm, in five series. Sections were stored in vials with 30% sucrose, refrozen and kept in a freezer at −30°C until staining. Once defrosted, two series of olfactory bulb sections were mounted onto glass-slides and stained with cresyl violet for measurement of surfaces and the other three series were free-floating processed for TH or calbindin (CB) immunostaining or for NADPH-diaphorase histochemistry.

For immunohistochemistry the avidin-biotin-immunoperoxidase method was used. The sections were sequentially incubated in (1) 0.2% Triton X-100 in Phosphate buffer saline (PBS) for 15 min; (2) 20% normal goat serum (for TH) or normal horse serum (for CB) in Triton–PBS for 1 h; (3) rabbit polyclonal antibody to TH (Eugene Tech International, Inc; 1:1000) or mouse monoclonal anti-Calbindin-D (Sigma Chemical Company; 1:200) in Triton–PBS overnight at 4°C; (4) Triton–PBS for 10 min, three times; (5) biotinylated anti-rabbit IgG (TH) or anti-mouse IgG (CB) (Vectastain ABC Kit, Vector Laboratories) for 1 h; (6) Vectastain ABC Reagent in Triton–PBS for 1 h; (7) PBS for 10 min; (8) 0.1 M Tris–HCl buffer (pH 7.6) 10 min; (9) 0.05% diaminobenzidine and 0.003% hydrogen peroxide in Tris-buffer. NADPH-diaphorase activity was detected incubating the sections for 60 min at 37°C in a 0.1 M Tris–HCl buffer (pH 8) solution containing: 1 mM β-NADPH (Sigma Chemical Company), 0.8 mM nitro blue tetrazolium (Sigma Chemical Company) and 0.08% Triton X-100 [1]. The immuno- and histochemically reacted sections were mounted onto

gelatinizated slides, air-dried and dehydrated and cover-slipped.

In order to search for regional differences in deprivation response, coronal sections at six different levels in each olfactory bulb were studied. In the non-deprived bulbs, each level was separated from the next by about 400 μm. In the deprived bulbs, morphological characteristics, general shape of sections, shape of glomerular layer, aspect of accessory olfactory bulb and anterior olfactory nucleus were used to select appropriate sections for comparison.

Selected images of olfactory bulbs were taken with an Axiophot Zeiss photomicroscope and analyzed with the 1.55 NIH Image program in a 660 AV Macintosh computer coupled to a digitizer tablet (KURTA IS/ONE) to measure surfaces and cell densities. The same person delineated and measured all the sections.

To quantify the effects of deprivation on the olfactory bulb surface we compared a section of the deprived olfactory bulb with the corresponding section of the non-deprived one at each level and in each animal calculated the percentage reduction of deprived olfactory bulb surface (DOB) related to the non-deprived (NDOB), using the formula: $100\times(NDOB-DOB)/NDOB$. We obtained, from representative sections of each one of the six bulbar levels, the mean of each animal and from them the mean and standard deviation of each experimental group. In this way we studied the total surface, the surface of each bulbar layer, the TH and calbindin-immunoreactivity and the NADPH-diaphorase activity in the glomerular layer. Since the olfactory nerve layer had great variability due to the histological procedures, we did not measure this layer and the total surface of each section was measured without the olfactory nerve layer. To quantify the TH and calbindin-immunoreactivity and NADPH-diaphorase activity, the number of positive cells was counted using the 1.55 NIH Image program. The density of positive cells was calculated and its percentage reduction in the same way as that for the surface reduction.

The paired *t*-test and an estimate multiple regression model [60] was used in order to estimate the percentage reduction according to the age and duration of deprivation. The model will only contain significant factors selected by a stepwise procedure. The significance level was placed at 0.05. The SAS statistical Package (SAS Institute Inc.) was used to perform these analysis.

3. Results

A complete closure of the operated naris was obtained in 75% of experimental animals and only completely occluded animals were used in this study. The non-deprived olfactory bulbs did not show significant qualitative or quantitative differences compared to the control ones. All deprived olfactory bulbs had an evident decrease in size with reduction in their three diameters.

The histological organization of the control and experimental olfactory bulbs of the gerbil does not differ from that of other mammals (Fig. 1A). Despite the size decrease caused by neonatal functional deprivation, the general structure and the different histological layers are basically maintained (Fig. 1B).

The section total surface area of the deprived olfactory bulbs compared to the non-deprived ones decreased $36.7\pm2.07\%$ (mean±standard error) in the 1/60 group and $25.71\pm1.38\%$ in the 14/30 group (Fig. 2A). The mean decrease in the total section surface area of deprived related to non-deprived bulbs in all experimental groups was $32.81\pm0.78\%$ ($P<0.001$). Both factors, age (A) and duration (D) of deprivation are significant for this effect. The mathematical model obtained for the percentage surface decrease (SD) is described by the formula: $SD=30.59-0.45\ A+0.09\ D$. Age and duration are expressed in days. In this model the age has a negative and relatively high coefficient; the earlier the deprivation age the higher the reduction in surface. The duration of deprivation has a positive and five times smaller coefficient. The differences in total surface area between deprived and non-deprived sections in all the six levels were significant but were greater and more significant in levels 3 to 5. To illustrate this effect, we have represented only the reduction in total section surface area in the six levels for the 1/60 and 1/30 groups in which the effect of deprivation was more pronounced (Fig. 3A).

Each olfactory bulb layer has a different response to functional deprivation. Thus, the surface area of the glomerular layer (GL) decreased in the deprived bulbs of all the experimental groups a mean percentage of $24.93\pm1.12\%$ ($P<0.001$) of non-deprived ones and the differences among groups of olfactory deprivation (Fig. 2B) were not statistically significant. Neither age nor duration fulfilled the significance level to enter in a mathematical model. In the glomerular layer, the largest section surface area and the highest differences between deprived and non-deprived bulbs were also found in the central levels (Fig. 3B).

We have also studied the number of glomeruli and the mean glomerular surface area by section. The number of glomeruli by section suffer, in the deprived bulbs, a mean decrease of $6.3\pm1\%$ ($P<0.001$) and the differences among the different experimental groups were not statistically significant. The number of glomeruli is also higher in the central bulbar levels. The mean glomerular surface area in deprived bulbs decreased a mean percentage of $19.69\pm1.07\%$ ($P<0.001$) with no significant differences among groups. The central levels also showed the highest glomerular surface area and differences between deprived and non-deprived bulbs. In these two parameters no variable fulfilled the significance level to enter a mathematical model.

The surface area of the external plexiform layer (EPL) in the deprived bulbs decreased in relation to non-deprived

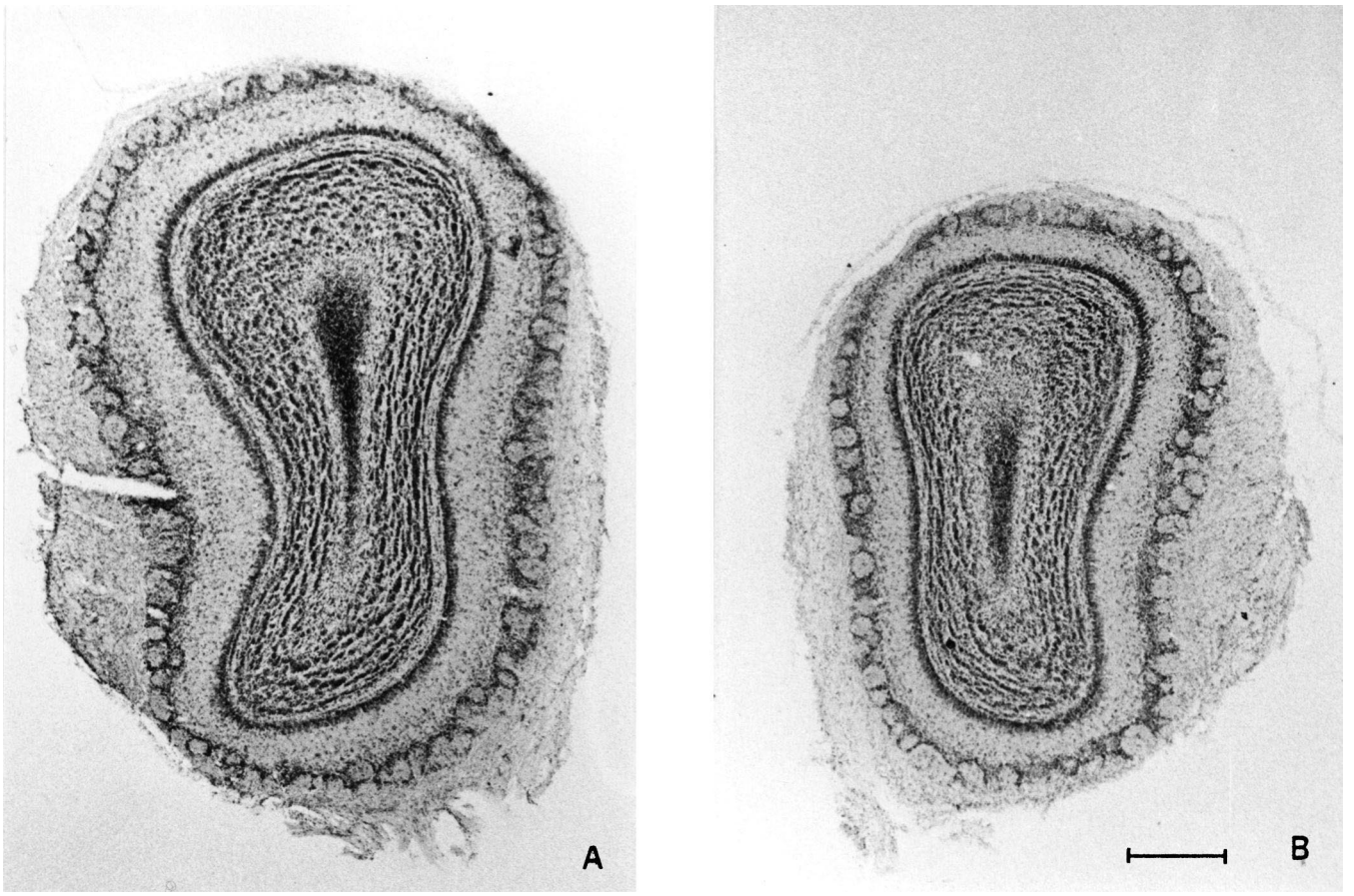

Fig. 1. Coronal sections of non-deprived (A) and deprived (B) gerbil olfactory bulb (1/60 group) stained with Cresyl violet. In the deprived bulb the histological structure is maintained in spite of its reduced size. Scale bar: 500 μm.

bulbs, between 44.94±1.52% (1/60 group) and 31.12±1.63% (14/30 group). The graphic representation of this layer surface reduction (Fig. 2C) gives a similar pattern to those of the total surface. The mean decrease was 39.37 ±1.02% ($P<0.001$). The surface area decrease model for this layer is: SD=35.92−0.55 A+0.14 D. As well as for the total surface area, age and duration of deprivation are significant factors and also they have similar coefficients. Consequently, surface area reduction was higher in the neonatally deprived groups than in the other groups. Longer periods of deprivation also lead to higher percentages of reduction. The differences in the surface area of this layer between deprived and non-deprived bulb were significant in all the studied levels (Fig. 3C) and higher in the central levels, as they were in general for the parameters studied.

The mitral cell layer (MCL) underwent the smallest decrease in surface area of the studied bulbar layers (Fig. 2D). The percentage of surface area reduction in the deprived side varied from 24.36±2.17% (1/60 group) to 11.33±1.77% (14/30 group) with a mean of 18.25 ±1.27 ($P<0.001$). The surface area of this layer and the differences were also higher in the middle sections of the olfactory bulb (Fig. 2D). Only age of deprivation was a significant factor for the mathematical model of surface area reduction of this layer, SD=22.05−0.61 A. Thus, surface reduction is about 24% if deprivation is made at birth and decreases as age of deprivation increases.

The internal plexiform layer (IPL) was the most sensitive layer to functional deprivation in the gerbil olfactory bulb, since the IPL surface of the deprived bulbs suffers the highest reduction related to the non-deprived ones (Fig. 2E). This surface reduction varied between 49.03±1.68% (1/60 group) to 35.46±2.18% (14/30 group). The mean of all experimental groups was 42.19±1.27% ($P<0.001$). The model formula for IPL is: SD=34.39−0.47 A+0.21 D. In this layer the effect of deprivation duration seemed to be more significant than in other layers due to its higher coefficient. Despite its small surface this layer had the most significant surface reduction in all the studied levels (Fig. 3E).

The deprived granule cell layer (GCL) showed a surface decrease from 31.74±2.25% (1/30 group) to 21.42±1.78% (14/30 group) (Fig. 2F) with a mean of 28.88 ±1.07% ($P<0.001$). Only the age of deprivation is a significant factor for the model of this layer: SD=31.49−0.43A. Decrease is more significant in the central levels where the layer surface was also higher (Fig. 3F).

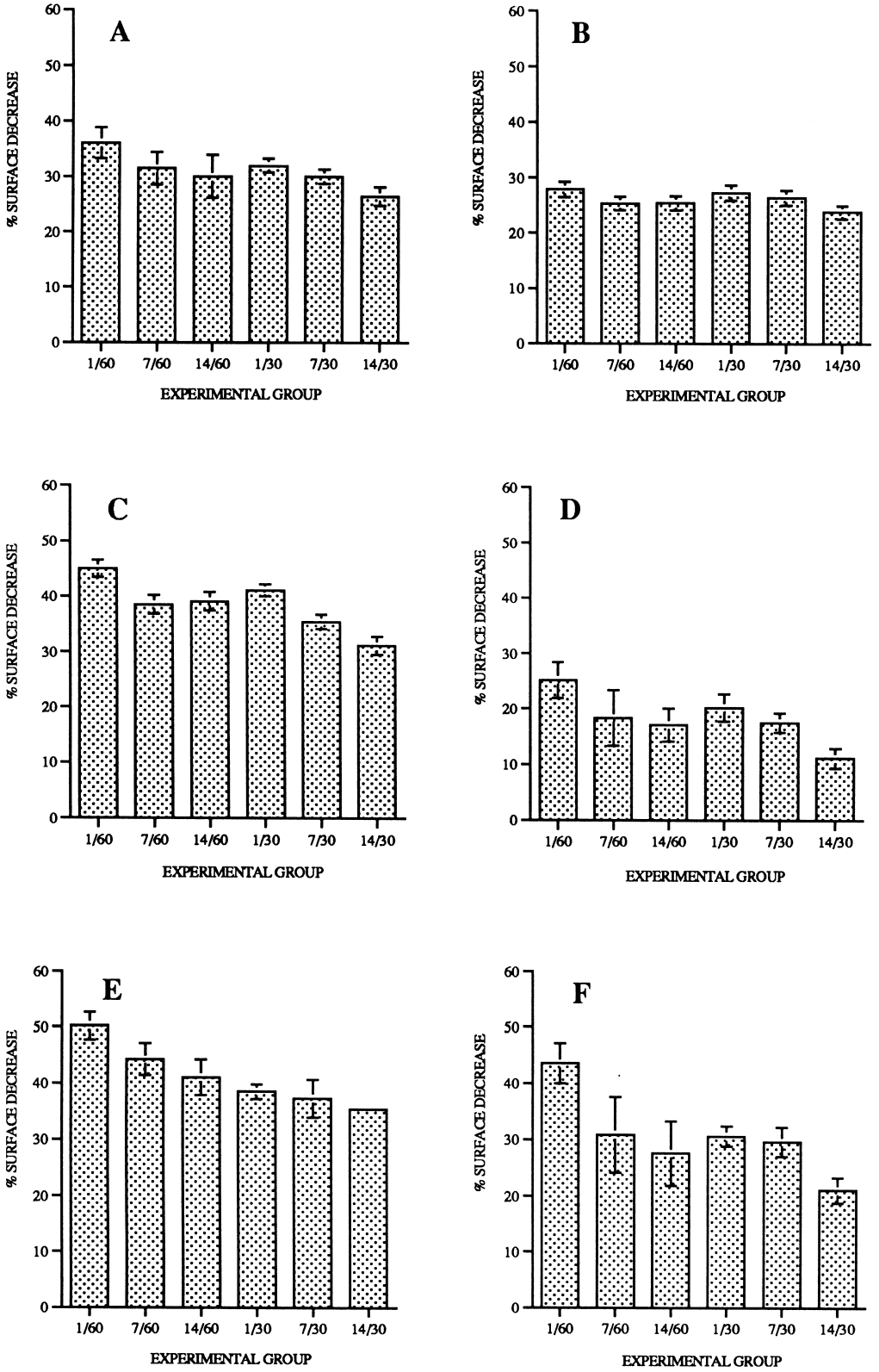

Fig. 2. Percentage of surface area decrease of total surface area (A) and surface area of different layers (B to F) of deprived olfactory bulbs related to non-deprived ones in the different experimental groups. (B): Glomerular layer. (C): External plexiform layer. (D): Mitral cell layer. (E): Internal plexiform layer. (F): Granular cell layer.

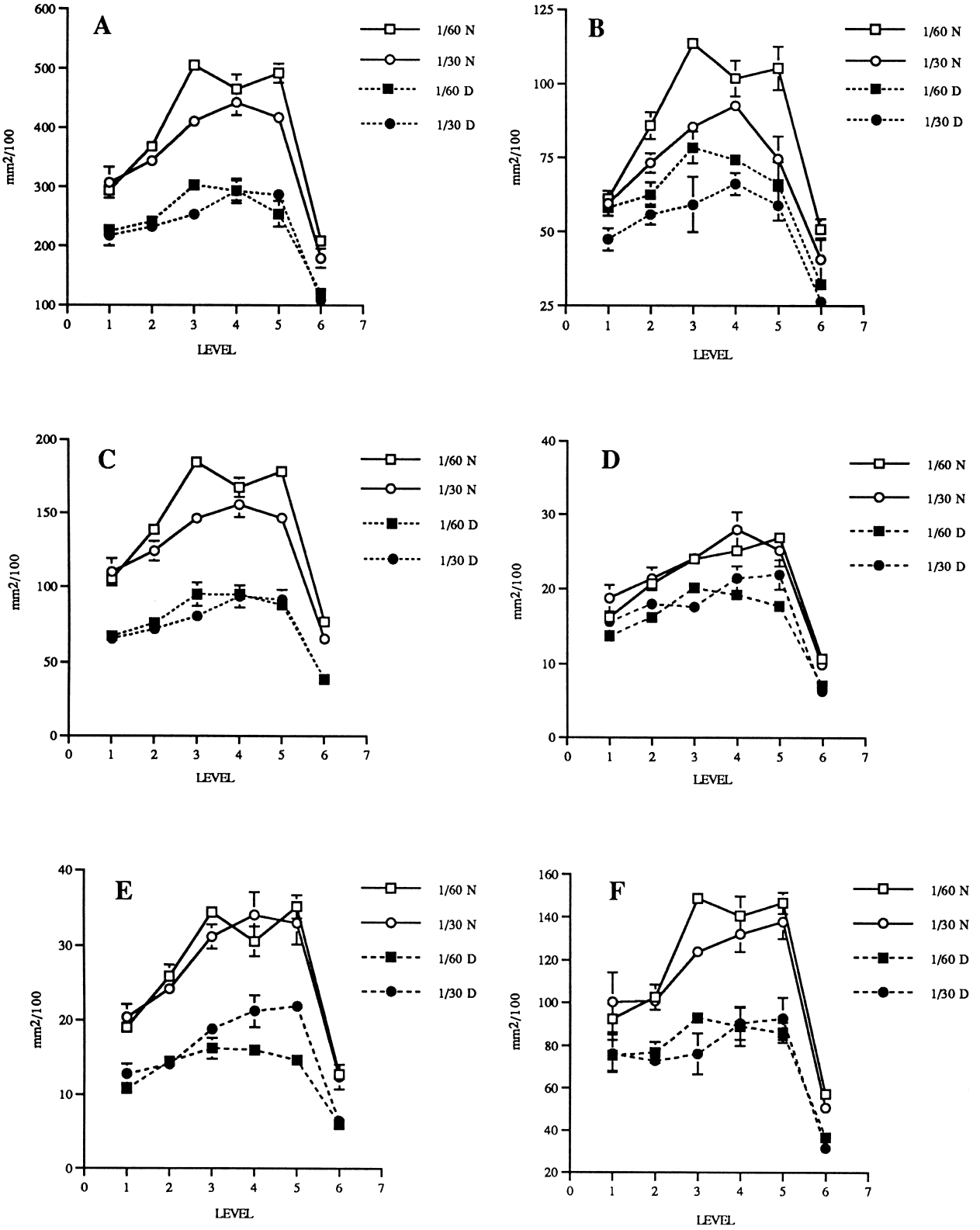

Fig. 3. Comparison of total surface area (A) and surface area of different layers (B to F) of gerbil deprived (D) and non-deprived (N) olfactory bulb of 1/60 and 1/30 groups in the different studied levels. (B): Glomerular layer. (C): External plexiform layer. (D): Mitral cell layer. (E): Internal plexiform layer. (F): Granular cell layer.

The subependymal layer (SEL) suffered the smallest surface reduction and was also very variable. For these reasons we decided not to make further analyses of this layer.

In control and non-deprived gerbil olfactory bulbs the tyrosine hydroxylase-immunoreactivity (TH-IR) is, as in other species, mainly located in the GL with some scattered immunoreactive cells in the EPL, MCL and GCL. The periglomerular immunoreactive cells have their bodies stained as well as dendritic processes and some of their axons (Fig. 4A). These periglomerular cells comprise different morphological types with neuronal bodies ranging between 8 and 12 μm in diameter although some positive neurons were larger. In the GL-EPL relatively abundant TH-IR cells were observed. In this zone the neuronal bodies were larger, some of them reaching 20 μm or more

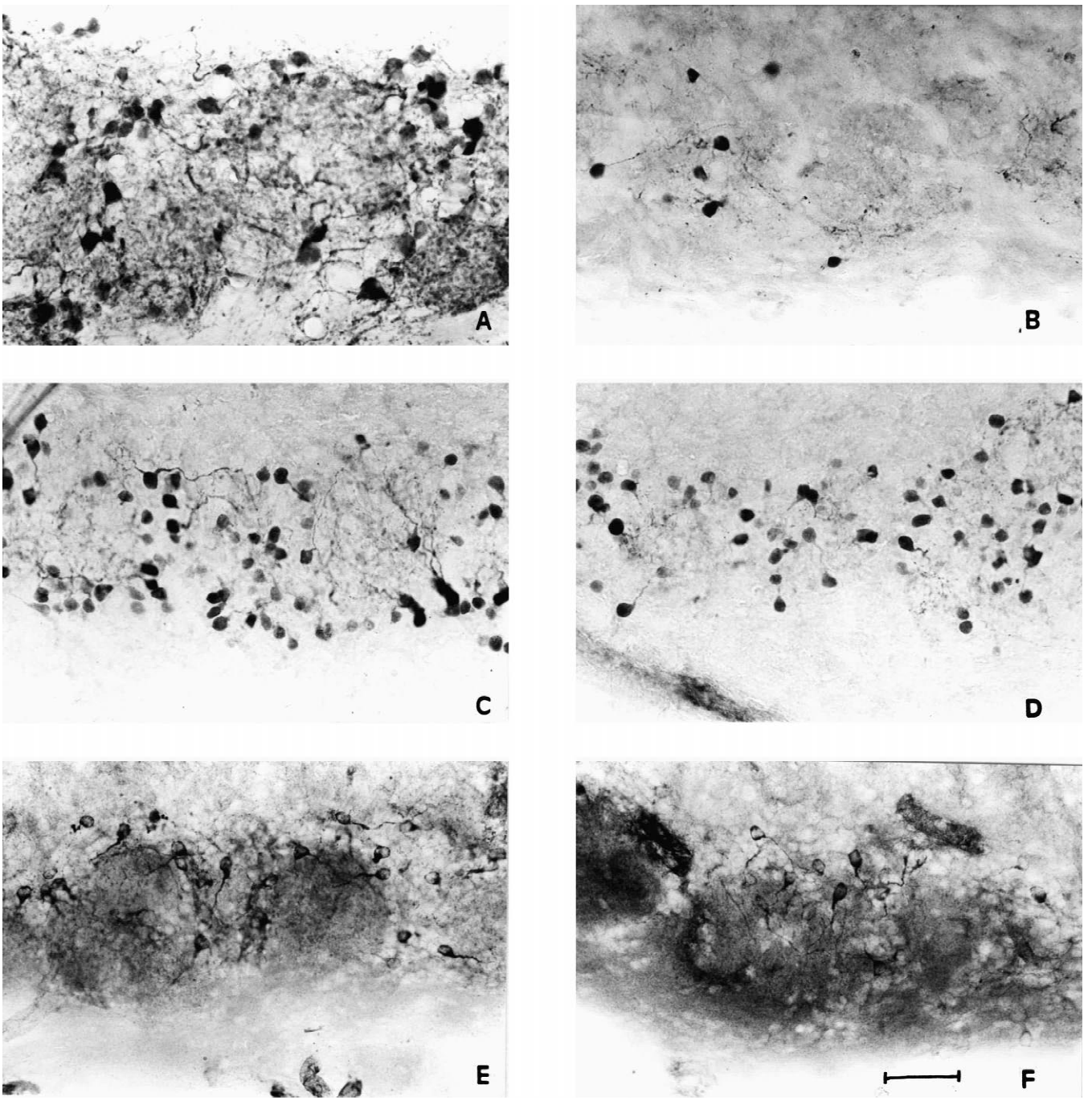

Fig. 4. Coronal sections of gerbil non-deprived (A, C and E) and deprived (B, D and F) olfactory bulbs immunostained for TH (A and B) and calbindin (C and D) and histochemically reacted for NADPH-diaphorase activity (E and F). The deprived bulbs show a greatly reduced reactivity for TH (B) but not for either calbindin (D) or NADPH-diaphorase (F). Scale bar: 50 μm.

of body diameter. Due to their intraglomerular ramifications some of these neurons could be external tufted cells whereas others bearing horizontally or verticatlly oriented dendrites could be different types of external short axon cells. Very few positive cells were distinguished in the EPL, MCL and GCL. After olfactory deprivation, TH-IR drastically decreased in all experimental groups. In deprived olfactory bulbs, immunoreactivity is lost mainly in the finest dendritic ramifications and axons with immunoreactivity decreased to some sparse neuronal bodies (Fig. 4B). The deprived glomeruli did not lose their TH-IR homogeneously since some of them are almost completely depleted while other adjacent glomeruli maintained some TH-IR. We counted the positive cells and the GCL surface by section and calculated the immunoreactive cell density as well as the percentage decrease in TH-IR cell density. The percentage of TH positive cell density decrease in the deprived bulbs was significant ($P<0.001$) in all the studied groups with a mean reduction of $76.11\pm6.59\%$ and non-significant differences among groups.

Calbindin-immunoreactivity (CB-IR) in the control and non-deprived gerbil olfactory bulb was also found mainly in the periglomerular cell layer with sparse immunoreactive cells in the other layers. In the GL, immunoreactive cells were periglomerular with neuronal bodies between 8 and 10 μm in diameter and a relatively morphological variety including cells bearing biglomerular dendritic arborizations (Fig. 4C). In the GL-EPL border there are some CB-IR cells with extraglomerular dendritic arborization in vertical or in horizontal arrangement. In the internal bulbar layers positive cells were very scarce. In the deprived olfactory bulb the CB-IR cells (Fig. 4D) were similar to those of the non-deprived one but their immunostaining was weaker. The differences in CB-immunoreactive cell density between deprived and non-deprived olfactory bulbs were non-significant in all the experimental groups studied. CB-immunoreactive cell density was higher in the central part sections, levels 3 and 4, of the deprived and non-deprived olfactory bulb than in the more rostral or more caudal levels.

The histochemical reaction for NADPH-diaphorase activity, in the control and non-deprived bulb sections, stains the GL and GCL strongly, the EPL weakly and MCL and SEL were not stained. In the GL, glomeruli and periglomerular cell were stained (Fig. 4E). Some glomeruli were deeply stained in the dorsomedial and dorsolateral parts whereas other glomeruli are faintly or not stained. The soma of the NADPH-diaphorase positive periglomerular cells is rounded and small (8–10 μm in diameter) and their dendrites, without labeled spines, are ramified into both stained and non stained glomeruli. Some superficial short axon cells are stained in the GL-EPL border. We also found some faintly positive cells in the EPL. In the GCL, in addition to strongly stained neuropile and fibers, there was a relatively high number of strongly NADPH-diaphorase positive cells, some of them close to the white matter layer. These cells had different morphology, generally stellate. Their soma size was medium or large, some of them being over 25 μm in diameter. They had few stained dendrites which were usually very long. Functional deprivation did not alter the morphology of the diaphorase positive cells (Fig. 4F) and the density of these neurons in the GL did not show statistically significant differences after olfactory deprivation.

4. Discussion

The macroscopically observable hypotrophy in the deprived gerbil olfactory bulb is more constant and reproducible than we have observed in rats [47]. The total surface decrease obtained in our experiments, ranging between 25 and 36%, could represent a volume reduction between 43 and 59%, considering the olfactory bulb as a regular cylinder whose diameters decreased proportionally. This reduction in size is greater than that obtained in the rat which was about 25% [50,51], about 26% in the mouse [14] and similar to that of a precocial rodent, the spiny mouse, about 40% [17]. As the gerbil olfactory bulb develops more slowly than those of the rat and mouse and all of them more slowly than the spiny mouse olfactory bulb [46,48], it does not seem to be any correlation between sensitivity to functional olfactory deprivation and developmental precocity in rodents.

The mathematical model obtained from our experiments for the percentage of total surface decrease after deprivation may describe any particular case within our experimental conditions, deprivation age (1–14 days) and duration (30–60 days). This model quantifies the previously stated observations in other species that the earlier the age or longer the duration of deprivation the higher the surface or size reduction [18,27]. Although this formula is only appropriate for our experimental conditions, the negative and high age coefficient suggests that if deprivation were performed in adult animals (30 days old) the size reduction would be around 19%. This hypothetical decrease is similar to the 20% size reduction obtained in adult spiny mouse deprived on P30 [17] and higher than the 12.9% obtained in adult rats after 60 days of deprivation [23]. Deprivation duration is the other factor in this mathematical model. In the gerbil, longer periods of deprivation lead to larger size reduction, the maximum being reached after 60-day deprivation. This period is longer than that described in the rat where the highest effect is reached after 30 days of deprivation [27,51] and decreases a little after 60 days [51]. The more pronounced total surface reduction in the bulbar central levels in gerbil could be explained by the fact that these sections have more surface and hence all functional effects can be more pronounced.

To compare different bulbar layer plasticity under our

experimental conditions we can consider, for example, the 1/60 group. The layers ordered from the most to the least sensitive are IPL, EPL, GCL, GL, MCL and SEL. Both plexiform layers are more sensitive than the total surface (32.47±1.06%) whereas GL, MCL and SEL are less sensitive and the differences between the surface percentage decrease of these layers and that of total surface were statistically significant. The GCL did not show significant differences with the total surface.

In the GL we obtained a surface reduction of about 25% with no significant differences among groups and neither age nor deprivation duration are factors for this reduction. We can infer that whatever the factors may be, functional deprivation will cause approximately the same effect in this layer. Since, in our experiments, the decrease of GL surface is larger than that of glomerulus mean surface, the interglomerular neuropile must be more sensitive to deprivation than the glomeruli and more similar to the plexiform layers. The reduction in glomerulus mean surface of deprived gerbil may correspond to a simplified synaptic organization subsequent to reduction of intraglomerular dendrites as observed in deprived mouse olfactory bulb [14]. However, Meisami's group [50,51,54] did not find any significant size variation in deprived rat olfactory glomeruli. The reduction observed in number of glomeruli by section in deprived gerbil olfactory bulb is statistically significant, whereas in the rat a decrease has been reported but without statistical significance [50,51,54]. The reduction in glomeruli number by section together with the decrease in section number due to bulb hypoplasia could indicate that the number of glomeruli is actually reduced by deprivation, although the reduction in glomerular surface may account for part of the reduction in number of glomeruli.

In our experiments, the layer which undergoes the most significant surface decrease is the IPL. Similar results have been obtained in rat and mouse [16,50,51,54]. This layer is made up of secondary dendrites of mitral and tufted cells, granule cell processes and different types of short axon cells. Surface decrease of this layer could be due to an alteration in development producing a lower size, number and extension of neuronal processes mainly of mitral, tufted and granule cells. The high sensitivity of EPL to functional deprivation is concordant with the observation that in rats deprived at P20 the EPL is the only layer which undergoes some degree of size decrease [12].

In the MCL we also observed a significant surface decrease, about 18%. The results in deprived rat MCL are controversial since some authors have found a decrease of 25 and 45% in mitral and tufted cell number respectively after functional deprivation [50,51,54] but Benson et al. [14] did not observe any decrease in number of principal cells except for their size and dendritic ramifications. As we did not count the mitral and tufted cells, our results could be explained by either or both processes. Taking into account that these neurons are differentiated in the prenatal period [34,35] a decrease in their number must be due to neuronal death.

The GCL has consistently been found to shrink after functional deprivation in the rat [30], mouse [14,25] and spiny mouse [17]. This size decrease has been related to delayed postnatal granule cell development [13] and also to a decrease in the number of reciprocal synapses between mitral and granule cells in deprived mouse [14]. By contrast, the paraventricular zone of the olfactory bulb has been reported not to show any changes after functional deprivation [14,50]. This area appeared to decrease a little in our study, although no morphometric analysis was performed on it.

The general arrangement of TH-IR cells observed in the non-deprived gerbil olfactory bulb is similar to that described in other rodent species [29] but we find less types of interneurons than have been described in the rat and mouse [8,33,44].

The reduction in TH-IR that we obtained in the gerbil deprived olfactory bulbs was very high. However, our quantitative results do not evidence the magnitude of immunoreactivity loss since we only counted the number of TH-IR and our semi-automatic counting system considered a cell as positive cell even if it only showed immunoreactivity in the cellular body. TH-IR decrease has been shown to be a consequence of a down-regulation of dopamine synthesizing pathway [9,41,45]. The TH and GABA (or GAD) are co-localized in some of the periglomerular cells in rat olfactory bulb [32,41,43]. After functional deprivation most of the TH-IR disappeared but GAD-IR remained [6,42].

After olfactory deprivation in rodents some degree of variability in TH-IR loss has consistently been observed [5,6,42]. The deprived gerbil olfactory bulb showed a very extensive and constant loss of TH-IR although there were some glomeruli which maintained a small TH-IR. These results have been considered to be due to a septal window between both nasal fossae which could allow stimulation in some areas of the deprived olfactory mucosa [4,37,40] and to retronasal stimulation through the choanae [21]. These possibilities might explain the detection of odors in rats with an occluded naris and contralateral bulbectomy [63]. Since we did not find any inter-septal communication in gerbil nose we consider that our experiments support possible olfactory stimulation through the posterior nasal openings.

Calbindin-immunoreactivity was found in non-deprived gerbil olfactory bulb almost exclusively in periglomerular cells and we did not find either horizontal or vertical oriented cells described in the rat IPL-GCL border [15,22] and in hedgehog olfactory bulb [1]. In fact we found very few positive cells in GCL. These observations indicate that regarding CB-positive interneurons, gerbil olfactory bulb organization could be simpler than in other mammals such as rat and hedgehog.

After olfactory deprivation, CB-IR cell density in

glomerular layer of gerbil olfactory bulb did not change significantly in any of our experimental groups. Since glomerular surface decreased in about 24%, the number of CB-IR cells must decrease in a similar proportion to maintain density. This decrease in CB-IR is much less than in TH-IR. A possible explanation for this difference could be based on the different synaptic connections of these two types of periglomerular cells [44]. The TH-IR periglomerular cells in the rat olfactory bulb receive asymmetrical synapses from olfactory axon terminals [44] whereas the CB-IR periglomerular neurons have almost no synaptic contact of this type [66]. Functional deprivation might only have an indirect effect on CB-IR periglomerular cells. The different response to olfactory epithelium lesion in BALB mice and other mice strains [12] can also be interpreted in this way. In these experiments the BALB mice do not suffer the severe decrease in TH-IR that other mice strains do. The periglomerular cells in BALB mice have no direct synaptic connections with olfactory axon terminals [68] and it has been suggested that this could explain the different response in TH-IR of periglomerular cells to deafferentation [12]. Nevertheless, Philpot et al. [56] have found after naris occlusion from P1 to P30 in the rat a 25–30% reduction in CB-IR cell density in the glomerular layer, a 64% of reduction in parvalbumin-IR cell density in the external plexiform layer and no reduction in calretinin-IR. These authors considered that, as with TH-IR, the decrease in calcium binding protein content reflects a shift in neuronal phenotype rather than cell death. These decreases were independent of deprivation age [56]. The role of calcium and calcium-binding proteins in functional deprivation is not well understood but is probably crucial given the pivotal activity of this ion in the cascade of intracellular activation events [55].

The arrangement of NADPH-diaphorase positive glomeruli preferentially observed in the dorsomedial region of non-deprived gerbil bulbs were coincident with other rodents [2,28,36,38,67] and also with other mammals [1]. Likewise, the positive periglomerular and external short axon cells are similar to those described in other rodents [2,28,38]. The NADPH-diaphorase positive cells that we found in GCL and SEL border may correspond to those described as Blanes and Golgi cells in the rat [2,28,59] and hedgehog [1]. As the NADPH-diaphorase positive cells did not change either in morphology or in GL cell density after deprivation, we may consider that, as with calbindin containing cells, they may not be directly regulated by olfactory receptor activity.

Acknowledgements

This work was supported by FIS grant 94/5478 and Junta de Castilla y León grant VA-44/98. We thank Luis Santiago, Teresa Rodríguez and M[a] Jesús Diéguez for their technical assistance.

References

[1] J.R. Alonso, R. Arévalo, E. García-Ojeda, A. Porteros, J.G. Briñón, J. Aijón, Active and calbindin D-28k-immunoreactive neurons and fibers in the olfactory bulb of the hedgehog (erinaceus europeaus), J. Comp. Neurol. 349 (1995) 1–21.
[2] J.R. Alonso, R. Arévalo, A. Porteros, J.G. Briñón, J. Lara, J. Aijón, Calbindin D-28K and NADPH-Diaforase activity are localized in different populations of periglomerular cells in the rat olfactory bulb, J. Chem. Neuroanat. 6 (1993) 1–6.
[3] J. Altman, Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb, J. Comp. Neur. 137 (1969) 433–458.
[4] M.J. Baer, J.F. Bosma, J.L. Ackerman, The postnatal development of the rat skull, The University of Michigan Press, Michigan, 1983, 272–276.
[5] H. Baker, Neurotransmitter plasticity in the juxtaglomerular cells of the olfactory bulb, in: F.L. Margolis, T.V. Getchell (Eds.), Molecular Neurobiology of the Olfactory System, Plenum Press, New York, 1988, pp. 185–212.
[6] H. Baker, Unilateral neonatal olfactory deprivation alters tyrosine hydroxylase expression but not aromatic amino acid decarboxylase or GABA immunoreactivity, Neuroscience 11 (1990) 605–615.
[7] H. Baker, A.I. Farbman, Olfactory afferent regulation of the dopamine phenotype in the fetal rat olfactory system, Neuroscience 52 (1993) 115–134.
[8] H. Baker, L. Franzen, D. Stone, J.Y. Cho, F.L. Margolis, Expression of tyrosine hydroxylase in the aging, rodent olfactory system, Neurobiol. Aging 16 (1995) 119–128.
[9] H. Baker, T. Kawano, V. Albert, T.H. Joh, D.J. Reis, F.L. Margolis, Olfactory bulb dopamine neurons survive deafferentation-induced loss of tyrosine hydroxylase, Neuroscience 11 (1984) 605–615.
[10] H. Baker, T. Kawano, F.L. Margolis, T.H. Joh, Transneuronal regulation of tyrosine hydroxylase expression in olfactory bulb of mouse and rat, J. Neurosci. 3 (1983) 69–78.
[11] H. Baker, K. Morel, D.M. Stone, J.A. Maruniak, Adult naris closure profoundly reduces tyrosine hydroxylase expression in mouse olfactory bulb, Brain Res. 614 (1993) 109–116.
[12] H. Baker, A.C. Towle, F.L. Margolis, Differential afferent regulation of dopaminergic and GABAergic neurons in the mouse main olfactory bulb, Brain Res. 450 (1988) 69–80.
[13] S.A. Bayer, 3-H-Thymidine-radiographic studies of neurogenesis in the rat olfactory bulb, Exp. Brain Res. 5 (1983) 329–340.
[14] T.E. Benson, D.K. Ryugo, J.W. Hinds, Effects of sensory deprivation on the developing mouse olfactory system: A ligth and electron microscopic morphometric analysis, J. Neurosci. 4 (1984) 638–653.
[15] J.G. Briñón, J.R. Alonso, R. Arévalo, E. García-Ojeda, J. Lara, J. Aijón, Calbindin D-28k-positive neurons in the rat olfactory bulb. An immunohistochemical study, Cell Tissue Res. 269 (1992) 289–297.
[16] P.C. Brunjes, Unilateral odor deprivation: Time course of changes in laminar volume, Brain. Res. Bull. 14 (1985) 233–237.
[17] P.C. Brunjes, Precocity and Plasticity: Odor deprivation and brain development in the precocial mouse Acomys Cahirinus, Neuroscience 24 (1988) 579–582.
[18] P.C. Brunjes, Unilateral naris closure and olfactory system development, Brain Res. Rev. 19 (1994) 146–160.
[19] P.C. Brunjes, L.L. Frazier, Maturation and plasticity in the olfactory system of vertebrates, Brain Res. Rev. 396 (1986) 1–45.
[20] P.C. Brunjes, L.K. Smith-Crafts, R. McCarty, Unilateral odor deprivation: Effects on the development of olfactory bulb catecholamines and behavior, Dev. Brain. Res. 22 (1985) 1–6.
[21] K.J. Burdach, R.L. Doty, The effects of mouth movements, swallowing, and spitting on retronasal odor perception, Physiol. Behav. 41 (1987) 353–356.
[22] M. R Celio, Calbindin D-28k and parvalbumin in the rat nervous system, Neuroscience 35 (1990) 375–475.

[23] J. Y Cho, N. Min, L. Franzen, H. Baker, Rapid down-regulation of tyrosine hydroxylase expression in the olfactory bulb of naris-occluded adult rats, J. Comp. Neurol. 369 (1996) 264–276.
[24] S. Coca-Menchero, M. Garrosa, M. Sánchez-Gutiérrez, L. García-Puente, Light and electron microscopic study on the developing olfactory bulb, Trab. Inst. Cajal 72 (1981) 131–145.
[25] F.S. Corotto, J.R. Henegar, J.A. Maruniak, Odor deprivation leads to reduced neurogenesis and reduced neuronal survival in the olfactory bulb of the adult mouse, Neuroscience 61 (1994) 739–744.
[26] W.E. Cullinan, P.C. Brunjes, Unilateral odor deprivation: effects on the development of staining for olfactory bulb succinate dehydrogenase and cytochrome oxidase, Dev. Brain Res. 35 (1987) 35–42.
[27] D.M. Cummings, P.C. Brunjes, The effects of variable periods of functional deprivation on olfactory bulb development in rats, Exp. Neurol. 148 (1997) 360–366.
[28] B.J. Davis, NADPH-diaphorase activity in the olfactory system of the hamster and rat, J. Comp. Neurol. 314 (1991) 493–511.
[29] B.J. Davis, F. Macrides, Tyrosine hydroxylase immunoreactive neurons and fibers in the olfactory system of the hamster, J. Comp. Neurol. 214 (1983) 427–440.
[30] L.L. Frazier, P.C. Brunjes, Unilateral odor deprivation: early postnatal changes in olfactory bulb cell density and number, J. Comp. Neurol. 269 (1988) 355–370.
[31] L. Frazier-Cierpial, P.C. Brunjes, Early postnatal cellular proliferation and survival in the olfactory bulb and rostral migratory stream of normal and unilaterally odor-deprived rats, J. Comp. Neurol. 289 (1989) 481–492.
[32] C.M. Gall, S.H.C. Hendry, K.B. Seroogy, E.G. Jones, J.W. Haycock, Evidence for coexistence of GABA and dopamine in neurons of the rat olfactory bulb, J. Comp. Neurol. 266 (1987) 307–318.
[33] N. Halász, O. Johansson, T. Hökfelt, A. Ljungdahl, M. Goldstein, Inmunohistochemical identification of two types of dopamine neurons in the rat olfactory bulb as seen by serial sectioning, J. Neurocytol. 10 (1981) 251–259.
[34] J.W. Hinds, Autoradiographic study of histogenesis in the mouse olfactory bulb. I. Time of origin of neurons and neuroglia, J. Comp. Neurol. 134 (1968) 287–304.
[35] J.W. Hinds, Autoradiographic study of histogenesis in the mouse olfactory bulb. II. Cell proliferation and migration, J. Comp. Neurol. 134 (1968) 305–322.
[36] D.A. Hopkins, H.W.M. Steinbusch, M. Markerink-van Ittersum, J. de Vente, Nitric oxide synthase, cGMP, and NO-mediated cGMP production in the olffactory bulb of the rat, J. Comp. Neurol. 375 (1996) 641–658.
[37] N. L Hunt, B.M. Slotnick, Functional capacity of the rat olfactory bulb after neonatal naris occlusion, Chem. Sens. 16 (1991) 131–142.
[38] J. Kishimoto, E.B. Keverne, J. Hardwick, P.C. Emson, Localization of nitric oxide synthase in the mouse olfactory and vomeronasal system: A hystochemical, immunological and in situ hybridazation study, Europ. J. Neurosci. 5 (1993) 1684–1694.
[39] A.Y. Klintsova, B.D. Philpot, P.C. Brunjes, Fos protein immunoreactivity in the developing olfactory bulbs of normal and naris-occluded rats, Dev. Brain Res. 86 (1995) 114–122.
[40] D.L. Korol, P.C. Brunjes, Rapid changes in 2-deoxyglucose uptake and amino acid incorporation following unilateral odor deprivation: A laminar analysis, Dev. Brain. Res. 5 (1990) 75–84.
[41] K. Kosaka, Y. Aika, K. Toida, C.W. Heizmann, W. Hunziker, D.M. Jacobowitz, I. Nagatsu, P. Streit, T.J. Visser, T. Kosaka, Chemically defined neuron groups and their subpopulations in the glomerular layer of the rat main olfactory bulb, Neurosci. Res. 23 (1995) 73–88.
[42] K. Kosaka, K. Hama, I. Nagatsu, J.Y. Wu, O. P Ottersen, J. Storm-Mathisen, T. Kosaka, Postnatal development of neurons containing both catecholaminergic and GABAergic traits in the rat main olfactory bulb, Brain Res. 403 (1987) 355–360.
[43] K. Kosaka, Y. Hataguchi, K. Hama, I. Nagatsu, J.Y. Wu, Coexistence of immunoreactivities for glutamate decarboxylase and tyrosine hydroxylase in some neurons in the periglomerular region of the rat main olfactory bulb: Possible coexistence of gamma-aminobutyric acid (GABA) and dopamine, Brain Res. 343 (1985) 166–171.
[44] K. Kosaka, K. Toida, F.L. Margolis, T. Kosaka, Chemically defined neuron groups and their subpopulations in the glomerular layer of the rat main olfactory bulb-II. Prominent differences in the intraglomerular dendritic arborization and their relationship to olfactory nerve terminals, Neuroscience 76 (1997) 775–786.
[45] T. Kosaka, K. Kosaka, K. Hama, J.Y. Wu, I. Nagatsu, Differential effect of functional olfactory deprivation on the GABAergic and catecholaminergic traits in the rat main olfactory bulbs, Brain Res. 413 (1987) 197–203.
[46] M. Leon, R. Coopersmith, C. Ulibarri, R.H. Porter, J.B. Powell, Development of olfactory bulb organization in precocial and altricial rodents, Dev. Brain Res. 12 (1984) 45–53.
[47] M.C. Martínez García, M. Cuervas-Mons Finat, E. Latorre Macarrón, M.M. Bullón Sopelana, M.J. Gayoso Rodríguez, Responses of astroglia in sensory deprived olfactory bulb of developing rats, Histol. Histopathol. 6 (1991) 235–239.
[48] J.J. McManus, Early postnatal growth and the development of temperature regulation in the Mongolian Gerbil (Meriones Unguiculatus), J. Mammal. 52 (1971) 782–792.
[49] E. Meisami, Effects of olfactory deprivation on postnatal growth of the rat olfactory bulb utilizing a new method for production of neonatal unilateral anosmia, Brain Res. 107 (1976) 437–444.
[50] E. Meisami, Influence of early anosmia on the developing olfactory bulb, Prog. Brain Res. 48 (1978) 211–230.
[51] E. Meisami, The developing rat olfactory bulb: prospects of a new model system in developmental neurobiology, in: E. Meisami, M.A.B. Brazier (Eds.), Neural Growth and Differentiation, Raven Press, New York, 1979, pp. 183–206.
[52] E. Meisami, R. Moussavi, Lasting effects of early olfactory deprivation on the growth, DNA, RNA, and protein content, and Na+-K+ ATPase and AchE activity of the rat olfactory bulb, Brain Res. 254 (1982) 217–229.
[53] E. Meisami, E. Noushinfar, Early olfactory deprivation and the mitral cells of the olfactory bulb: A Golgi study, Int. J. Dev. Neurosci. 4 (1986) 431–444.
[54] E. Meisami, L. Safari, A quantitative study of the effects of early unilateral olfactory deprivation on the number and distribution of mitral and tufted cell and of glomeruli in the rat olfactory bulb, Brain Res. 221 (1981) 81–107.
[55] A. Posada, P.G. Clarke, Fast retrograde effects on neuronal death and dendritic organization in development: The role of calcium influx, Neuroscience 89 (1999) 399–408.
[56] B.D. Philpot, J.H. Limand, P.C. Brunjes, Activity-dependent regulation of calcium-binding proteins in the developing rat olfactory bulb, J. Comp. Neurol. 387 (1997) 12–26.
[57] J.P. Royet, F. Jourdan, H. Ploye, Morphometric modifications associated with early sensory experience in the rat olfactory bulb – I. Volumetric study of the bulbar layers, J. Comp. Neurol. 289 (1989) 586–593.
[58] J.P. Royet, F. Jourdan, H. Ploye, C. Souchier, Morphometric modifications associated with early sensory experience in the rat olfactory bulb- II. Stereological study of the population of olfactory glomeruli, J. Comp. Neurol. 289 (1989) 594–609.
[59] J.W. Scott, J.K. McDonald, J.L. Pemberton, Short axon cells of the rat olfactory bulb display NADPH-diaphorase activity, neuropeptide Y-like immunoreactivity and somatostatin-like immunoreactivity, J. Comp. Neurol. 260 (1997) 378–391.
[60] G.A.F. Seber, Linear regression analysis, Wiley and Sons, New York, 1977.
[61] L.C. Skeen, B.R. Due, F.E. Douglas, Effects of early anosmia on two classes of granule cells in developing mouse olfactory bulb, Neurosci. Lett. 54 (1985) 301–306.
[62] L.C. Skeen, B.R. Due, F.E. Douglas, Neonatal sensory deprivation

reduces tufted cell number in mouse olfactory bulb, Neurosci. Lett. 63 (1986) 5–10.
[63] B.M. Slotnick, A.J. Pazos, Rats with one olfactory bulb removed and the contralateral naris closed can detect odors, Physiol. Behav. 48 (1990) 37–40.
[64] D.M. Stone, M. Grillo, F.L. Margolis, T.H. Joh, H. Baker, Differential effect of functional olfactory bulb deafferentation on tyrosine hydroxylase and glutamic acid decarboxylase messenger-RNA levels in rodent juxtaglomerular neurons, J. Comp. Neurol. 311 (1991) 223–233.
[65] D.M. Stone, T. Wessel, T.H. Joh, H. Baker, Decrease in tyrosine hydroxylase, but not aromatic L-amino acid decarboxylase, messenger RNA in rat olfactory bulb following neonatal unilateral odor deprivation, Brain Res. 8 (1990) 291–300.
[66] K. Toida, K. Kosaka, C.W. Heizmann, T. Kosaka, Chemically defined neuron groups and their subpopulations in the glomerular layer of the rat main olfactory bulb- III. Structural features of calbindin D28K-immunoreactive neurons, J. Comp. Neurol. 392 (1998) 179–198.
[67] S.R. Vincent, H. Kimura, Histochemical mapping of nitric oxide synthase in the rat brain, Neuroscience 46 (1992) 615–624.
[68] E.L. White, Synaptic organization of the mammalian olfactory glomerulus: New findings including an intraspecific variation, Brain Res. 60 (1973) 299–313.