Responses of the astroglia in sensory deprived olfactory bulb of developing rats

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Summary. The effects of unilateral olfactory deprivation on the glial population during the olfactory bulb development have been studied. The lack of sensory stimulation has been found to be related to an increase in glial fibrillary acidic protein (GFAP) in the three layers of the deprived bulbs. This increase is due to the higher number of astrocytes in the deprived bulb, which is much more noticeable in the plexiform layer than in the other two, together with a hypertrophy of the reactive astrocytes resulting in an increase in the number and thickness of their prolongations. Our results demonstrate that sensory olfactory deprivation acts as other noxious agents on the CNS, causing gliosis in the olfactory bulb. This gliosis is revealed by astrocytic hyperplasia and hypertrophy.

Key words: Olfactory deprivation. Olfactory bulb. Gliosis. Astrocytes. GFAP. Rats

Introduction

The lack of sensory stimuli in the early stages of growth has been found to be responsible, as numerous studies have indicated, for alterations in the development of some structures of the CNS. In order to demonstrate this, several simple experimental models have been designed, such as that described by Meisami (1976) to observe the effects of early olfactory deprivation. Unilateral anosmia in newborn animals produces, according to this author, a reduction in size and weight of the sensory deprived bulb.

The reduction in volume of the bulb has been attributed by several authors either to neuronal death (Skeen et. al. 1986) or to a reduction in the volume of the neurons and their processes (Meisami and Safari, 1981; Benson et al. 1984). Thus, Meisami and Safari (1981) and Meisami and Noshinfar (1986) described the loss of mitral and tufted cells and the reduction in volume of the surviving cells. Similarly, Meisami and Noshinfar (1985) found a reduction in the number of granular cells.

On the other hand, a glial reaction is known to be produced by diverse injuries in the CNS, such as hypoventilation (Becker and Takashima, 1985), degenerative illnesses such as the Creutzfeldt-Jacob disease (Neumann and Cohm, 1987), accumulation of metabolites as in the infantile neuronal lipofuscinosis (Pactau et al., 1985) and, of course, injuries (Cavanagh, 1970).

Our aim in this study has been to examine, by means of specific labelling with antibodies against the glial fibrillary acidic protein (GFAP), the astrocytic population after a period of sensory deprivation, in order to establish if this deprivation, and especially the subsequent loss of neuronal elements and their processes, acts as a noxious agent producing gliosis, either as a consequence of the death or cellular atrophy, or if the decrease in volume of the bulb is accompanied by a reduction in the glial population.

Materials and methods

Thirty Wistar rats from five litters were used for this research. Each rat was subjected to unilateral olfactory deprivation as described by Meisami (1976), by cauterization of the right external naris three days after birth. The animals were killed when 30 days old by intracardiac perfusion, after anesthesia. Both olfactory bulbs (OB) from animals belonging to three litters were fixed in a mixture of picric acid, paraformaldehyde and glutaraldehyde as described by Somogyi and Takagi (1982), embedded in paraffin and transversally cut in complete serial sections at 5, 10 and 15 μm. The bulbs of the remaining animals were embedded in epoxy resin and cut into 1 μm sections.
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Immunohistochemistry

After removal of paraffin and plastic, the sections were labelled with anti-GFAP serum (DAKO) following the PAP method described by Sternberger et al. (1970) and modified by Taylor (1978) and by Rodning et al. (1980) for embedding in paraffin and plastic respectively. All sera were diluted in tris buffer saline in the following proportions: anti-GFAP; 1:500; normal swine; 1:20 and swine anti-rabbit immunoglobulins and PAP complex; 1:100. 3,3′-diaminobenzidine (Sigma) was used as chromogen, and hematoxylin for counterstaining.

Numerical studies and statistical analysis

Only the GFAP-positive astrocytes exhibiting nucleus, cytoplasm and processes in the same high power field were counted. The counts were carried out across the entire control and deprived bulbs and at various levels. The numerical results obtained for both the layers and for the whole of the control and deprived bulbs were compared using the Wilcoxon signed rank test for paired samples with non-normal distribution. The results of the plexiform layer were also compared with the other two layers with the Wilcoxon signed rank test for independent samples.

Results

Both the paraffin-embedded and the plastic-embedded samples from control bulbs showed a homogeneous distribution of the glial population through the antero-posterior axis. However, differences between the different layers and between dorsal and ventral areas in comparison with the lateral and medial ones were observed.

Glomerular layer

Most of the GFAP-positive astrocytes were found with their somata located among the neuronal somata, their processes being directed towards the interior of the glomeruli. The rest of the GFAP-positive astrocytes surrounded the glomeruli, their processes not directed inwardly (Fig. 1A, B).

External plexiform layer

Abundant GFAP-positive astrocytes, with their processes spread out in several directions, were present here, the cells being more abundant in the medial and lateral zones than in the ventral and dorsal ones (Fig. 1C, D).

Granular layer

GFAP-positive astrocytes, with few short processes were found arranged concentrically. The distribution of these cells in this layer was similar to that observed in the plexiform layer; the most abundant being in the medial and lateral zone and the least in the ventral and dorsal ones (Fig. 1E, F).

The deprived bulbs showed a macroscopically observable reduction in size in comparison with the control ones. However, the histological study revealed that the astrocytic population of the deprived bulbs was increased compared to the control ones, as well as the number and the thickness of their cytoplasmic processes. Also the deprived bulb astrocytes appeared to have...
Table 1. Mean number of astrocytes per field in each layer in normal and sensory deprived bulbs.

<table>
<thead>
<tr>
<th>Layer</th>
<th>Normal Bulb</th>
<th>Deprived Bulb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular layer</td>
<td>8.84 ± 2.15</td>
<td>14.53 ± 3.00*</td>
</tr>
<tr>
<td>Plexiform layer</td>
<td>13.83 ± 3.83</td>
<td>24.64 ± 6.18*</td>
</tr>
<tr>
<td>Granular layer</td>
<td>15.53 ± 5.57</td>
<td>27.07 ± 3.68*</td>
</tr>
<tr>
<td>Average of layers</td>
<td>12.71 ± 4.94</td>
<td>22.15 ± 7.02</td>
</tr>
</tbody>
</table>

* Statistical significance at p < 0.01
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a more intense labelling with GFAP.

The average increases in the number of astrocytes per field in the plexiform and granular layers of the deprived bulbs, compared to controls, were 10.81 and 11.54 cells per field respectively, the differences being statistically significant (p < 0.01). Table 1.

Although there was an increase in the number of astrocytes in all the layers of the sensory deprived bulb in our samples it could be clearly observed that this increase was more marked in some cytoarchitecturally different regions. These regions were the periglomerular zones, the outer zone of the external plexiform layer, adjacent to the glomeruli (Fig. 1), and the zone nearest to the ependymal layer of the granular layer.

Discussion

This study demonstrates that in the experimental model of sensory deprivation designed by Meisami (1976), apart from the morphological (Meisami and Safari, 1981; Benson et al., 1984; Meisami and Noushinfar, 1985, 1986; Skeen et al., 1986) and functional (Kosaka et al., 1987) neuronal changes reported, an intense gliosis is also produced in the deprived bulb. This intense glial reaction seems to be caused, at least partly, by an astrocytic hyperplasia, since the number of these cells is significantly increased in the deprived bulbs.

The astrocytic population that proliferates most actively during gliosis, according to several authors, mainly a GFAP-negative one (Miyaake et al., 1988, 1989); although the same authors recognize that many GFAP proliferating cells seem to be microglial cells. In our samples of non-deprived bulbs, all the cells whose nucleus showed typical astrocytic features were GFAP-positive. That is why in our opinion, the existence of a GFAP-negative population in the olfactory bulb is unlikely. The bulb astrocytes would be more like the astrocytes of the grey matter which contain less intermediate filaments (Sokol, 1975) and which, if incorrectly fixed, could appear as GFAP-negative. In fact, in our experience, the demonstration of the presence of GFAP in astrocytes of early postnatal animals, when GFAP levels are still low, is unreliable and results depend on the fixatives used, especially aldehydes (Bullon et al., 1984). This is supported by Kitamura et al. (1987), who have demonstrated a different transcription rate of the GFAP gene in astrocytes of different brain regions; some regions show undetectable transcription despite having a certain amount of immunocytochemically demonstrable GFAP.

Although it appears that the gliosis in our experiment is caused by an astrocytic proliferation, the presence of thicker and more numerous processes in the deprived OB supports the idea that a typical hypertrophy of the reactive astrocytes may have also occurred.

Different stimuli have been proposed as the cause of the astrocytic proliferation, such as myelin breakdown (Osterberg and Wattenberg, 1962), influx of serum proteins (Katso, 1967), increased extracellular space (Cook and Wisniewski, 1975), nerve degeneration (Barret et al., 1981) and architectural disruption (Mathewson and Berry, 1985). Among these, the only ones likely to cause the gliosis observed in our experiment are the increased extracellular space and the architectural disruption, since the only changes observed were neuronal death and/or atrophy. Thus, in the plexiform layer, where the biggest increase of astrocytes occurs, olfactory deprivation causes the biggest decrease in the number of neurons. According to Shepherd (1974), the tufted cells, which decrease by 45% after deprivation (Meisami and Safari, 1981) are in this layer, as well as the dendritic branches of the mitral cells, which in the deprived bulb are shorter and with a lower number of processes (Meisami and Noushinfar, 1986) and the dendritic branches of the granule cells, which are also diminished in the deprived bulbs.

The increase in astrocytes observed in the glomerular and granular layers are very similar and may be caused by the decrease in the synaptic connections of the glomerular and periglomerular elements (Kosaka et al., 1987). The increased astrocytic response may also be due to the decrease in the tufted cells (Skeen et al., 1986) and the granule cells (Meisami and Noushinfar, 1985) and the synapses of the latter with the basal dendrites of the mitral cells (Meisami and Noushinfar, 1986).

In conclusion, this study has demonstrated that olfactory deprivation in newborn rats causes an increase in GFAP staining and that this increase is mainly due to astrocytic hyperplasia, occurring along with hypertrophy. Apparently, this hyperplasia is not enough to maintain the volume of the deprived bulb, which remains markedly reduced.

References


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