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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 gliofibrillary acid 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 noxius 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 Noushinfar (1986) described the loss of mitral and tufted cells and the reduction in volume of the surviving cells. Similarly, Meisami and Noushinfar (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 Cohn, 1987), accumulation of metabolites as in the infantile neuronal lipofuscinosis (Paetau 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 gliofibrillary 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 noxius 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).

Fig. 1. GFAP in the glomerular layer (A,B), the plexiform layer (C,D) and the granular layer (E,F) of normal (A, C, E) and sensory deprived bulbs (B, D, 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.

	Normal Bulb	Deprived Bulb
Glomerular layer	8.84 ± 2.15	14.53 ± 3.00*
Plexiform layer	13.83 ± 3.83	24.64 ± 6.18*
Granular layer	15.53 ± 5.57	27.07 ± 3.68*
Average of layers	12.71 ± 4.94	22.15 ± 7.02

* Statistical significance at $p < 0.01$

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 ependimal 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 (Miyake 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 (Skoff, 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 (Bullón 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 (Klatzo, 1967), increased extracellular space (Cook and Wisniewski, 1973), 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

Barret C.P., Guth L., Donati E.J. and Krikorian J.G. (1981). Astroglial reaction in the grey matter of lumbar segments after mid-thoracic transection of the adult rat spinal cord. Exp. Neurol. 73, 365-377.

Becker L.E. and Takashima S. (1985). Chronic hypoventilation and development of brain stem gliosis. Neuropediatrics 16, 19-23.

Benson T.E., Ryugo D.K. and Hinds J.W. (1984). Effects of sensory deprivation on the developing mouse olfactory system: a light and electron microscopic, morphometric analysis. J. Neurosci. 4, 638-653.

Bullón M.M., Alvarez-Gago T., Fernández-Ruiz B. and Aguirre C. (1984). Glial fibrillary acidic (GFA) protein in rat spinal cord. An immunoperoxidase study in semithin sections. Brain Res. 309, 79-83.

Cavanagh J.B. (1970). The proliferation of astrocytes around a needle wound in the rat brain. J. Anat. 106, 471-487.

Cook R.D. and Wisniewski H.M. (1973). The role of oligodendroglia and astroglia in Wallerian degeneration of the optic nerve. Brain Res. 61, 191-206.

Kitamura T., Nakanishi K., Watanabe S., Endo Y. and Fujita S. (1987). GFA-protein gene expression on the astroglia in cow and rat brains. Brain Res. 423, 189-195.

Kosaka T., Kosaka K., Hama K., Wu J. and Nagatsu I. (1987). Differential effect of functional olfactory deprivation on the GABAergic and catecholaminergic traits in the rat main olfactory bulb. Brain Res. 413, 197-203.

Klatzo I. (1967). Neuropathological aspects of brain oedema. J. Neuropathol Exp. Neurol. 26, 1-14.

Mathewson A.J. and Berry M. (1985). Observations on the astrocyte response to a cerebral stab wound in adult rats. Brain Res. 327, 61-69.

Meisami E. (1976). 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, 437-444.

Meisami E. and Safari L. (1981). A quantitative study of the effects of early unilateral olfactory deprivation on the number and distribution of mitral and tufted cells and of glomeruli in the rat olfactory bulb. Brain Res. 221, 81-107.

Meisami E. and Noushinfar E. (1985). Effects of early olfactory deprivation on the internal granular cells of the olfactory bulb. Neuroscience 11, 447 (Abstract).

Meisami E. and Noushinfar E. (1986). Early olfactory deprivation and the mitral cells of the olfactory bulb: a Golgi study. Int. J. Dev. Neurosci. 4, 431-444.

Miyake T., Hattori T., Fukuda M., Kitamura T. and Fujita S. (1988). Quantitative studies on proliferative changes of reactive astrocytes in mouse cerebral cortex. Brain Res. 451, 133-138.

Miyake T., Hattori T., Fukuda M. and Kitamura T. (1989). Reactions of S-100-positive glia after injury of mouse cerebral cortex. Brain Res. 489, 31-40.

Neumann M.A. and Cohn R. (1987). Long duration Jakob-Creutzfeldt disease. Arch. Gerontol. Geriatr. 6, 279-287.

Osterberg K.A. and Wattenberg L.W. (1962). Oxidative histochemistry of reactive astrocytes. Arch. Neurol. 7, 211-218.

Paetau A., Elovaara I., Paasivuo R., Virtanen I., Palo J. and Haltia M. (1985). Glial filaments are a major brain fraction in infantile neuronal ceroid-lipofuscinosis. Acta Neuropathol. (Berl) 65, 190-194.

Rodning CH.B., Erlandsen S.L., Coulter H.D. and Wilson I.D. (1980). Immunohistochemical localization of IgA antigens in sections embedded in epoxy resin. J. Histochem. Cytochem. 28, 199-205.

Shepherd G. (1974). The synaptic organization of the brain. Oxford University Press. New York. pp. 111.

Skeen L.C, Due B.R. and Douglas F.E. (1986). Neonatal sensory deprivation reduces tufted cell number in mouse olfactory bulbs. Neurosci. Lett. 63, 5-10.

Skoff R. (1975). The fine structure of pulse labeled (H^3-thymidine) cells in degenerating rat optic nerve. J. Comp. Neurol. 161, 595-612.

Somogyi P. and Takagi H.A. (1982). A note on the use of picric acid paraformaldehyde glutaraldehyde fixative for correlated light and electron microscopic immunocytochemistry. Neuroscience 7, 1779-1783.

Sternberger L.A., Hardy P.H.J., Cuculis J.J. and Meyer H.G. (1970). The unlabelled antibody-method of immunohistochemistry. Preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antihorseradish peroxidase) and its use in identification of spirochetes. J. Histochem. Cytochem. 18, 315-333.

Taylor C.R. (1978). Immunoperoxidase techniques. Practical and theoretical aspects. Arch. Pathol. Lab. Med. 102, 113-121.

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