CHANGES IN THE POSTNATAL EXPRESSION OF CALCIUM BINDING PROTEINS IN NEURONS OF THE SUPERIOR COLLICULUS AFTER NEONATAL ENUCLEATION.

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Parvalbumin (PV), calbindin (CB) and calretinin (CR) are three members of the calcium binding family of proteins (CBP's) expressed throughout the central nervous system (CNS) of mammals (Baimbridge et al, 1992). Their Ca^{2+}-buffering property underlies many of their functions. During postnatal development these proteins are expressed by neurons when they make synaptic contacts which integrate them into the appropiate neural circuits. Superficial layers of superior colliculus receive two visual projections coming from the retina and visual cortex respectively (Huerta et al., 1984). Most of the fibres of both visual pathways arrive to the superior colliculus during first postnatal days and then presumably modulate the expression of CBP's in neurons located in superficial collicular strata. Experimental suppression of one of the two visual afferent pathways should modify the pattern of CBP expression in the superior colliculus. To test this hypothesis unilateral ocular enucleation was carried out under cryoanaesthesia in newborn Sprague-Dawley rat pups. At the stage of young adult (50 days), their CNS was fixed by percardial perfusion with a mixture of 4% paraformaldehyde, 0'1% glutaraldehyde and 15% picric acid in 0'1M phosphate buffer (pH 7'4). 60 micron cryotome sections of cryoprotected superior colliculus were sequentially incubated in: primary polyclonal antibodies (Swant) at different concentrations (anti-PV 1:5000, anti-CB 1:10000, anti-CR 1:2000) for two days, secondary biotinylated goat anti-rabbit (1:200) for two hours, HRP conjugated avidin-biotin complex (1:200) for two hours and finally DAB (0'05%) with H_2O_2 (0'04%) for ten minutes.

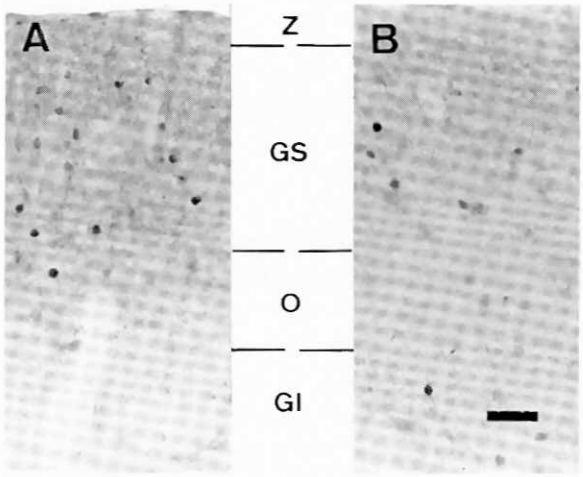

Figure 1: Parvalbumin immunopositive neurons in the superior colliculus of control (A) and enucleated (B) cases. In A the immunopositive neurons are scattered mainly in the stratum griseum superficiale (GS). Contralateral enucleation causes a considerable reduction of immunostained neurons in the GS. Z: Stratum zonale, O: Stratum opticum, GI: Stratum griseum intermedium. Scale bar: 50 µm.

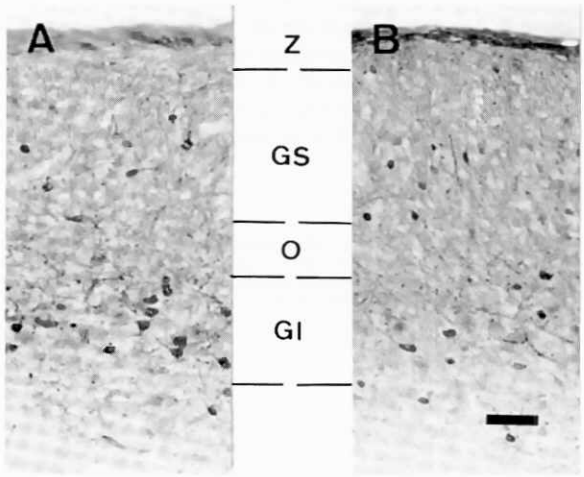

Figure 2: A Calbindin immunopositive neurons of a normal case located in the three superficial layers of superor colliculus. They are more abundant in the stratum griseum intermedium (GI). B After neonatal enucleation immunopositive neurons are mainly reduced in GI. Z: Stratum zonale, O: Stratum opticum, GS: Stratum griseum superficiale. Scale bar: 50 µm.

PV immunopositive (PVIP) neurons in the superior colliculus of control animals were observed scattered in the stratum griseum superficiale (GS), were scarce in the stratum opticum (O) and in the stratum griseum intermedium (GI) and were absent in the sratum zonale (Z) (Fig. 1A). Contralateral enucleation caused a considerable reduction (60%) of PVIP neurons in the GS (Fig. 1B). Calbindin immunopositive (CBIP) neurons in normal cases were seen in all three superficial layers of superior colliculus, being more abundant in the GI (Fig. 2A). In enucleated animals the number of CBIP cells diminished slightly (10%) in the GS, whereas in the GI their number was severely reduced (70%) (Fig. 2B). In the superior colliculus of control cases, calretinin immunopositive (CRIP) neurons were observed scattered in the lower half of the GS and GI and were very scarce in the Z (Fig 3A).

Suppression of afferents from the retina was followed by a clear increase in number (60-80%) of CRIP cells in all superficial layers of deafferentiated colliculus (Fig. 3B). This increase was more considerable in Z. Thus, unilateral enucleation carried out at the neonatal stage differentially affected the postnatal expression of CBP's in the superior colliculus. In contrast to the decrease in number of PVIP and CBIP neurons, the subpopulation of CRIP cells clearly increased in superficial layers after neonatal enucleation.

Suppression of retinal inputs eliminates the retinal stimuli and the anterograde trophic support to collicular neurons. In fact, neonatal enucleation causes apoptosis of some neurons in superficial collicular layers (unpublished results). One can hypothesize that the degree of retinal inputs to the different subpopulations of CBP-containing neurons is variable and in consecuence, the lack of these afferents affects with variable intensity the expression of CBP's. Besides, after unilateral enucleation an increase of microglial cells in the superior colliculus has been observed (Echevarria-Aza et al., 1993). These cells produce cytokynes which might modify the expression of CBP's.

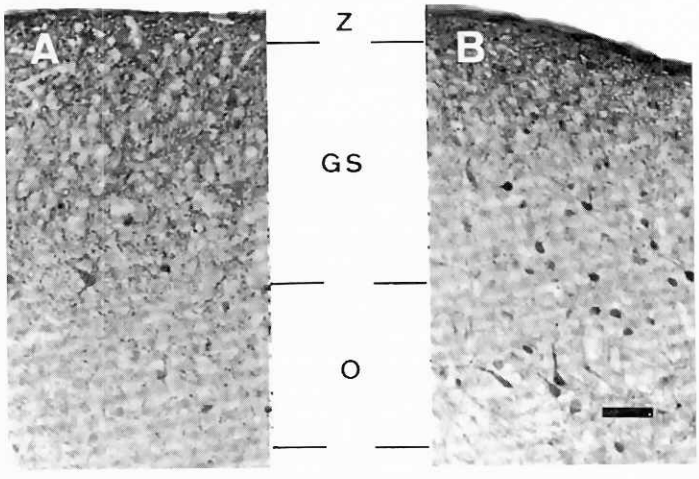

Figure 3: A Calretinin immunostained neurons of a normal case are located in the lower half of the GS and GI. B In an enucleated case immunopositive neurons were notably increase in all superficial collicular layers. Z: Stratum zonale, O: Stratum opticum. Scale bar: 50 µm.

Thus interruption of retinal afferents to the superior colliculus leads to differential changes in the expression of CBP's in superficial layers of the superior coliculus.

References

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