

## Effect of pre- and postnatal retinal deprivation on the striate-peristriate cortical connections in the rat

HERMES BRAVO and OSCAR INZUNZA

Departamento de Anatomía, Escuela de Medicina,  
Pontificia Universidad Católica de Chile, Santiago, Chile

*The tangential distribution of the striate-peristriate cortical connections in normal, postnatally eye enucleated and congenitally anophthalmic rats, was studied after a single injection of wheat germ agglutinin conjugated with horseradish peroxidase into the striate cortex. The typical normal pattern of separate fields in the peristriate cortex is altered in eye enucleated animals, in such a way that their areal distribution in the cerebral cortex is increased and each field tends to fuse with the adjacent one. This process is more marked in anophthalmic animals, a finding that is in agreement with the notion that ganglion cells exert their influence before the visual pathway is functional.*

**Key words:** *anophthalmic rats; cortical connections; eye enucleation; retinal deprivation; striate-peristriate connections*

### INTRODUCTION

During development of striate-peristriate cortical connections, infragranular layers precede supragranular layers in the process of connectivity. This phenomenon has been associated with the pattern in which cortical neurons are formed. In other words, axons which arise in different cortical layers arrive at their cortical targets in the order in which the corresponding cortical neurons were generated (inside-outside sequence) (2, 13). These cortico-cortical connections appear genetically determined and they have a laminar pattern with two basic projection types: one including layer IV (*e.g.*, striate-peristriate pathway) and the other one including layer I and avoiding layer IV (*e.g.*, peristriate-striate pathway) (3).

Manipulation of the afferents produced some modifications in the cortical representation pattern of the periphery. For example, the hindpaw representation in the

primary somatosensory cortex is enlarged after removal of the fetal forelimb (11). Similarly, the interhemispheric visual connections are also expanded when afferents from the retina are interrupted (19, 20). On the other hand, Koralek and Killackey (12) have demonstrated that prenatal somatosensory deprivation induces a reduction of the callosal somatosensory connections to the second somatosensory area (SII). Although this difference on the effects of somato-sensory and visual deprivation is not well understood, one possible explanation could be that sensory deprivation has a differential effect on the connectivity of secondary cortical areas that process different sensory information.

In the present study we have used the wheat germ agglutinin-horseradish peroxidase (WGA-HRP) tracing technique to compare the pattern of striate-peristriate connections of normal rats with that of pre- and postnatally eye deprived animals, in

order to ascertain the modifications in cortical connectivity induced by retinal deafferentation during development.

#### MATERIALS AND METHODS

Three groups of adults albino rats of the Sprague-Dawley strain were studied: 5 normal ones, 5 rats subjected to bilateral eye enucleation on the 3rd postnatal day (P3) and 5 congenitally anophthalmic rats of the KV strain (equivalent to prenatally enucleated rats) (9, 10, 20).

When weighing 250-300 g, the animals were anaesthetized with ketamine 34 mg i.p., supplemented with sodium pentobarbital. The head was fixed in a stereotaxic apparatus, the skull was opened, and 0.05-0.08  $\mu$ l of a solution of 5% WGA-HRP was injected into the visual cortex (area 17), using the stereotaxic coordinates of Montero *et al* (16), and later confirmed by determining its myeloarchitecture and the criteria of labeling in the thalamus (21).

After 24 h of survival, the animals were deeply anaesthetized with sodium pentobarbital and perfused through the left ventricle with 250 ml saline followed by 250 ml freshly prepared 3% paraformaldehyde in phosphate buffer (PB) 0.1 M pH 7.4. After 1 h of fixation, 200 ml 10% sucrose was perfused. The brain was removed and kept in 30% sucrose in PB; the cerebral cortex was flattened and prepared for serial tangential sectioning (17) at 40  $\mu$ m thickness. Every other section was reacted for HRP using the tetramethyl benzidine (TMB) protocol of Gibson *et al* (5); the remaining sections were stained for myelin with a modification of the Gallyas silver stain (6).

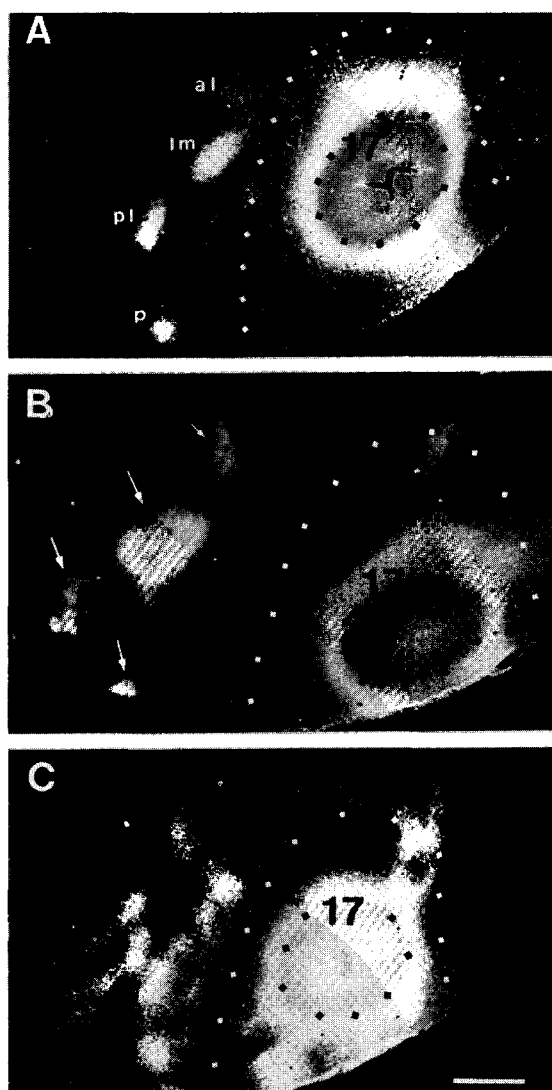
The pattern of striate-peristriate cortico-cortical connections was correlated with the location of the striate cortex revealed in the myelin stained sections. Excluding the halo which is known to represent a reactive expansion of the tracer where the uptake and transport are not detectable (7, 8), the injection site and the lateral peristriate fields of projections were drawn under the microscope with the aid of a camera lucida. Using a digitizing tablet and a microcomputer, the injection site and the area of

projections to the lateral peristriate cortex (area 18a) were measured throughout the thickness of the cortex. The projection fields in normal and visually deprived animals with similar injection sites (same stereotaxic coordinates and tangential sectional areas) were compared.

#### RESULTS AND DISCUSSION

Figure 1 shows low power photomicrographs of typical tangential sections reacted for HRP from three different experiments. The section in Figure 1A is from a normal rat in which the lateral peristriate projection areas are seen as separate fields (al, lm, pl and p), as described previously (14, 16, 17). The section in Figure 1B, from a postnatally enucleated (P3) rat, shows the same projection fields, but peristriate areas are closer to each other, occupying a tangential sectional surface in the cerebral cortex almost 3-fold larger than that of the normal rat. The section in Figure 1C, from a congenitally anophthalmic rat, shows that the projection fields to the lateral peristriate cortex are almost fused, occupying an even larger tangential sectional area (4-fold larger than that of a normal rat; see also Fig 2). All these sections were taken from the same depth of the cerebral cortex (300-350  $\mu$ m from the pial surface).

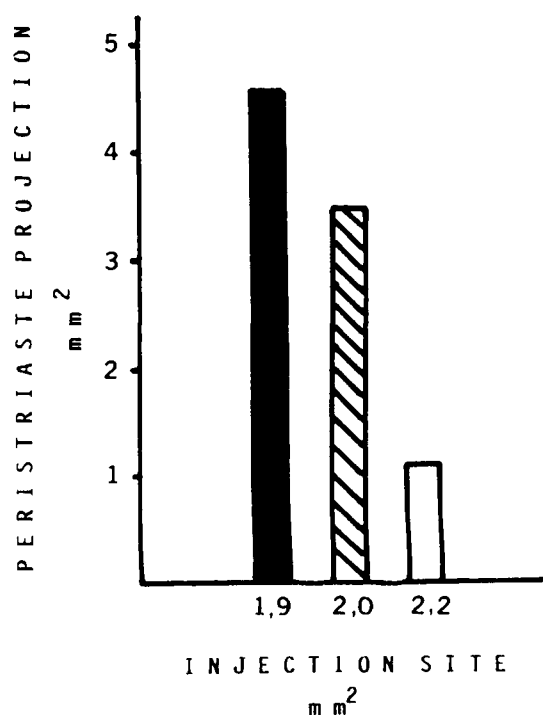
The quantitative data shown in Figure 2 represent the means of the tangential sectional areas from the lateral peristriate cortex that receive projections from area 17, and include all the serial sections from layers I to VI, taken from 1 rat of each of the 3 different series. The significant differences in the extent of the projections cannot be explained by variations in the size of the injection sites, nor by their topographical locations. The previously described findings were confirmed by additional experiments in 4 rats of each of the 3 different series, allowing comparisons between the quotients between sizes of projection fields and sizes of injection sites (see Table I). Analysis of those data, by a Kruskal-Wallis test for several independent samples ranked on a completely randomized design, reveals statistically significant differences (overall p



**Fig 1:** Dark field low magnification photomicrographs of tangential sections of the cerebral cortex processed for HRP. Borders of striate cortex (area 17) taken from adjacent sections stained for myelin (white dots). White areas in the lateral peristriate (left of the figure) are projections fields from area 17, named as *al*, *lm*, *ll*, *pl*, *p*, or pointed with white arrows. The area delimited by black dots represents the actual injection site in area 17. A, normal rat; B, postnatally eye enucleated rat; C, congenitally anophthalmic rat. Scale bars (A to C) = 1 mm.

< 0.01). Thus, the quotient was higher for congenitally anophthalmic rats compared to postnatally enucleated rats ( $p < 0.001$ ) and normal rats ( $p < 0.001$ ); otherwise, the quotient of postnatally enucleated rats was also higher than that of normal animals ( $p < 0.01$ ).

The above results suggest that the normal pattern of ipsilateral cortico-cortical connections of area 17 in the rat is modified



**Fig 2:** Quantitative estimates of tangential sectional areas of lateral peristriate fields that receive projections from striate cortex (shown in Fig 1). Extent of injection site and projections to peristriate cortex are averaged from all sections measured from layer I to VI in each experiment. Open bar, normal rat; slanted bar, postnatally eye enucleated rat; solid bar, congenitally anophthalmic rat

when the afferents from the retina are removed. In normal animals, these connections form an elaborate pattern in which several peristriate visual areas are recognized (1, 4, 15, 16, 17). These multiple visual areas remain invariant, in spite of changes in the injection sites of neuronal markers into the striate cortex, and they are consistently clustered around a number of fixed projection areas in the peristriate cortex (14). Moreover, these striate-peristriate connections have a remarkable complementarity with the distribution of the callosal connections (17, 23). In the absence (congenital) of retinal afferents or after their removal during early postnatal development, a clear expansion of the striate-peristriate connections occurs, similar to that previously described for the callosal projections (20). This expansion of the cortico-cortical connections can be attributed to the persistence of exuberant projections along development. For example, Olavarría *et al*

TABLE I  
Quotient between sizes of projection fields / injection sites

Rats	Normal	Postnatally enucleated	Congenitally anophthalmic
	0.5*	1.6*	2.4*
	1.0	1.3	2.6
	1.1	1.4	2.6
	1.3	1.6	2.7
	1.3	1.7	2.8
Means	1.04	1.52	2.62
$\Sigma$ ranks	16	39	65

\* Same data as in Figure 2.

Kruskall-Wallis test:  $s^2 = 19.79$ ;  $t = 12.15$ ; overall  $p < 0.01$ .

Normal vs postnatally enucleated rats:  $p < 0.01$ .

Normal vs congenitally anophthalmic rats:  $p < 0.001$ .

Postnatally enucleated vs congenitally anophthalmic rats:  $p < 0.001$ .

(18) have demonstrated that between the 4th and 7th postnatal day of normal development, the callosal projections change from a continuous distribution to another in which all the features of the adult pattern are clearly recognized. This adult-like pattern is complete by day 12th. The transformation of the callosal pattern has been attributed to the withdrawal of axons and disappearance of a number of callosal neurons, due to the influence exerted by the retinal afferents. Recently, we have described that the retinal afferents are necessary for the fine tuning of the distribution of callosal connections during development (20). Ipsilateral striate-peristriate axons penetrate the gray matter before the callosal axons (2). Consequently, it is likely that the influence of retinal efferents (*e.g.*, ganglion cell spontaneous activity) (22) on striate-peristriate connections is exerted from earlier periods of development. If this were the case, one would expect that prenatally eye deprived rats should have a more significant change in the cortico-cortical projections when compared to postnatally eye enucleated

animals. Our results agree with this assumption, since we found a quantitative difference in the areal distribution of the striate-peristriate projections between postnatally enucleated and congenitally anophthalmic animals. It remains to be determined whether the difference is due to a larger arborization of the striate-peristriate axon terminals or to a more numerous population of cortical neurons that maintain their axons connected to the lateral peristriate cortex.

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