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Differential effect of enucleation on two populations of layer V pyramidal cells

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A number of recent investigations demonstrate that the layer V pyramidal cells of striate cortex are sensitive to alterations in visual input. Enucleation in the newborn rabbit produces significant decrements in spine density along central portions of pyramidal cell apical dendrites[8]. In mice, enucleation results in more selective spine reductions limited to the region of apical dendrite traversing layer IV and adjacent portions of layer III in visual cortex. This region roughly corresponds to the zone of specific thalamic input, as determined by anterograde degeneration techniques[17]. Likewise, dark-rearing results in loss or deformation of spines along the apical shafts of pyramidal cell dendrites[9,16].

Evidence derived from electron microscopy suggests that spine reductions may reflect alterations in synaptic input. A number of investigations have demonstrated that the dendritic spines of cortical pyramidal cells are postsynaptic specializations, and phagocytosis of spines has been observed in deafferented cells[1,2,10,11,19]. Furthermore, alterations in the density and size of synaptic profiles within visual cortex have been demonstrated following light deprivation[5,6].

It thus appears that an analysis of spine alterations may be valuable in assessing the sites of synaptic termination of particular afferent systems. In the present investigation we have employed this technique to explore further the pattern of inputs to the visual cortex. While previous investigators have treated the layer V pyramidal cells as a homogeneous population, it has long been recognized that this layer may be divided into distinct sublaminae. Lorente de Nó distinguished pyramidal cells in layer Va from those in Vb on the basis of cell body size and axonal projections. He suggested that the smaller pyramidal cells of layer Va give rise to 'association' fibers, while the large cells of layer Vb give rise to descending 'projection' fibers[13]. These anatomical differences suggest the possibility that distinct groups of layer V pyramidal cells may also receive different types of synaptic input. In order to explore this possibility, we have examined the effects of visual deafferentation in these two populations of cells.

Fourteen Sprague–Dawley rats from 3 litters were employed as subjects. Seven served as control subjects, and the remaining 7 were enucleated bilaterally one day

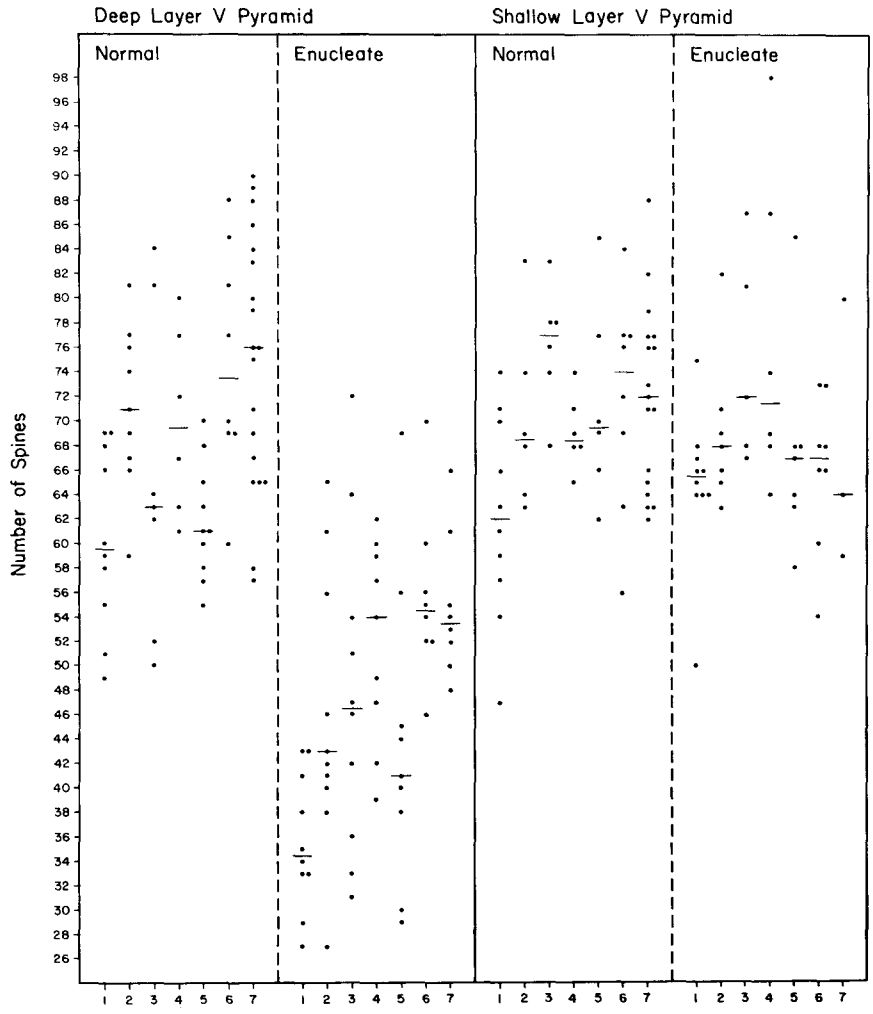

Fig. 1. Spine counts on 75 μm segments of layer V pyramidal cells in visual cortex. Each black dot (·) represents a single pyramidal cell count and the solid line (—) represents the median for a single subject.

after birth. Subjects were sacrificed at 25 days of age. Coronal slabs of visual cortex were stained by the rapid Golgi method[8], sectioned at 80 μm, embedded in mounting material, and coded to prevent a counting bias. Resolvable spines were counted at $\times$ 500 magnification. For the purpose of counting, layer V pyramidal cells were divided into a superficial group with cell bodies located 500–600 μm below the cortical surface, and a deep group with cell bodies located at depths of 650–850 μm. Only completely impregnated cells whose dendrites extended to the molecular layer were included. Spines were counted on the portion of the apical dendrite located 400–475 μm below the cortical surface. This corresponds to the region of specific thalamic afferent termination as determined by anterograde degeneration techniques (Killackey and Winslow, unpublished observations).

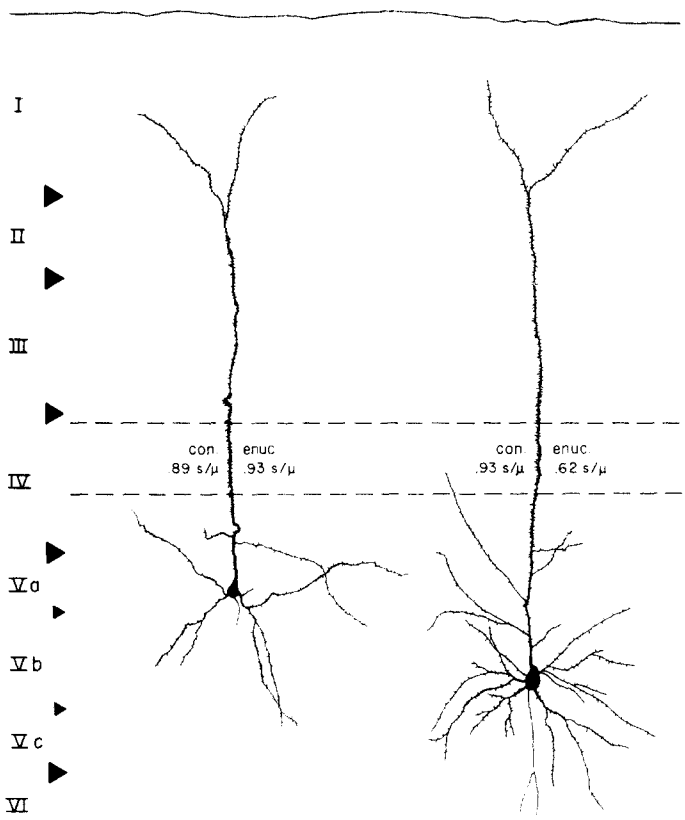

Fig. 2. The superficial and deep pyramidal cells of layer V of visual cortex shown in relation to the spine counting zone (dashed lines). The median spine counts for each condition are expressed in spines/μm (s/μ).

Fig. 1 presents individual spine counts and median values for each subject. (The median was chosen as the best indicator of central tendency because fewer assumptions are required concerning the distribution of the data.) In deep layer V pyramidal cells, a significant reduction in spine density is noted in enucleated animals as compared with control animals (Mann–Whitney U-test, two-tailed analysis, $P < 0.002$)[15]. The median spine densities, expressed in spines/μm, are 0.62 and 0.93 for enucleated and control subjects (see Fig. 2). This suggests a spine loss of approximately 30% following enucleation, a figure consistent with previous reports[8]. However, comparison of spine densities in superficial pyramidal cells reveals no significant differences between enucleation and control groups (Mann–Whitney U-test, two-tailed analysis). The median spine densities are 0.93 spines/μm for the enucleation group and 0.89 spines/μm for the control group (see Fig. 2).

These results suggest that visual deafferentation has differential effects on deep and superficial layer V pyramidal cells within visual cortex. Following enucleation, the deeper cells exhibit a dramatic reduction in spine density along the portion of the apical dendritic shaft which traverses the region of specific thalamocortical input,

while spine density in the same portion of the apical dendrite remains unchanged in more superficial cells. On the basis of comparison of Nissl and Golgi material, we feel that our superficial cells are roughly comparable to the smaller cells of layer Va and the deep cells equivalent to those of layer Vb.

Previous investigators have suggested on the basis of such spine loss data that the geniculostriate projections terminate directly on the apical shafts of pyramidal cells[7,8]. However, it is not possible to determine whether reductions in spine density are the direct result of geniculostriate deafferentation, or whether the loss is mediated through other affected cortical neurons. The phenomenon of transneuronal degeneration is particularly robust in the visual system, where deafferentation has been shown to result in the progressive degeneration of the postsynaptic element[2–4,12,14]. This transneuronal effect is not restricted to the first postsynaptic site, but can involve successive neuronal links in the visual pathway. The sensitivity of spines to such transneuronal influences is probably dependent on such variables as age, species, severity of manipulation, and the time course of experimental observations. It is also unclear whether denervation effects truly represent spine losses, or whether reductions in spine density reflect a failure of normal spine development during early postnatal maturation.

Despite these interpretational difficulties, some tentative conclusions may be drawn from the present data. Specifically, two points deserve discussion: the differential response of the deep and superficial pyramidal cells to enucleation, and the failure of visual deafferentation to completely denude the layer IV portion of the apical dendrite in the affected deeper cells. Both results suggest that the layer V pyramidal cells receive major projections other than (or in addition to) direct thalamic inputs, and that these inputs are responsible for the maintenance of spine populations within layer IV. The most probable source of this input is the layer IV stellate cell. Valverde has reported results which indicate that these cells receive direct thalamic inputs[17]. He observed that stellate cell dendrites 'migrate' away from the zone of deafferentation following enucleation and suggested that this migration might reflect the establishment of new synaptic connections by the deafferented cells. These alterations in dendritic organization were present in 48-day-old animals, but not in animals 24 days of age. Valverde proposed that this difference may be attributable to the fact that stellate cells are not completely mature in the 24-day-old mouse. This suggestion is supported by our observations of growth cones at the tips of many stellate cell dendrites in the 25-day-old rat. It is possible that these immature cells may not transmit the effects of deafferentation to the pyramidal cells at this early stage of development. In this context, it is also significant that Valverde noted more diffuse spine losses on pyramidal cell apical dendrites in 48-day-old animals as compared with younger subjects[17].

In a more recent study, Valverde has explored the development of pyramidal cell spine populations[18]. He compared spine counts in normally reared mice, dark-reared mice, and mice raised in darkness for 20 days and returned to a normal light cycle. The results of this study suggest that spine growth occurs in two phases: one which precedes eye opening, and one which follows this event. The first phase of

growth is apparently independent of visual input, but light stimulation is necessary for an accelerated second phase of spine growth. While the distinction between direct sensory deafferentation and dark-rearing should be kept in mind, these results nonetheless suggest that different inputs to the pyramidal cells may develop at different times.

In conclusion, we suggest that the spine loss noted in deeper cells indicates that they may receive direct thalamocortical input. There is strong presumptive evidence that layer IV stellate cells also receive such input and in turn project to both classes of layer V pyramidal cells. This interpretation implies that there are two channels for the processing of specific thalamocortical input which have different developmental time courses. The first is a direct channel to the deep layer V pyramidal cells, which develops relatively early. The second channel reaches maturity later and is mediated through the layer IV stellate cells. Each of these channels may subserve different aspects of visual processing. Finally, the relatively late development of the stellate cell system suggests that these connections may be more sensitive to postnatal environmental influences.

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