*Brain Research*, 96 (1975) 82–87


# Changes in pyramidal cell density consequent to vibrissae removal in the newborn rat

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(Accepted May 28th, 1975)

The phenomenon of transneuronal degeneration is a well-known consequence of sensory system damage. The effects of peripheral receptor destruction have been demonstrated in a number of central nervous system structures including the lateral geniculate nucleus[2,5,16], olfactory bulb[17] and cochlear nucleus[19]. However, studies of such transneuronal effects at the cortical level have largely been limited to the visual system. Reductions in the number of spines on pyramidal cell apical dendrites and alterations in the dendritic organization of layer IV stellate cells have been noted within visual cortex following enucleation[7,22,28]. A number of recent reviews have summarized these transneuronal effects along with evidence which suggests that spine losses may reflect alterations in synaptic organization[3,6,23]. In the present experiment we have explored the possibility that similar reductions in dendritic spine density may occur in somatic sensory cortex following receptor damage.

In certain rodents, portions of the primary somatic sensory cortex exhibit an elaborate cytoarchitectonic organization which replicates the organization of the peripheral receptors, the mystacial vibrissae and sinus hairs[31,32,34]. This pattern of organization is also mirrored in the pattern of specific thalamic input to this region[11,12]. Furthermore, damage to the mystacial vibrissae soon after birth disrupts the cytoarchitectonic organization of one portion of this field, the posteromedial barrel subfield[30,33]. Accordingly, this region appears to provide an ideal system in which to study the effects of peripheral damage on cortical dendritic morphology.

In a recent experiment we demonstrated that eye enucleation has a differential effect on the dendritic spine populations of the deep and superficial layer V pyramidal cells of visual cortex[22]. Hence, the present experiment poses two questions: first, does vibrissae damage in newborn rats produce spine losses on layer V pyramidal cells of the posteromedial barrel subfield and second, does this receptor damage affect deep and superficial cells equally?

Fourteen Sprague–Dawley rats from 3 litters were employed in the present experiment. Seven animals served as control subjects. In the remaining 7 subjects bilateral electrocauterization of individual vibrissae follicles (roughly 50/animal) was performed with the aid of an operating microscope one day after birth. This proce-

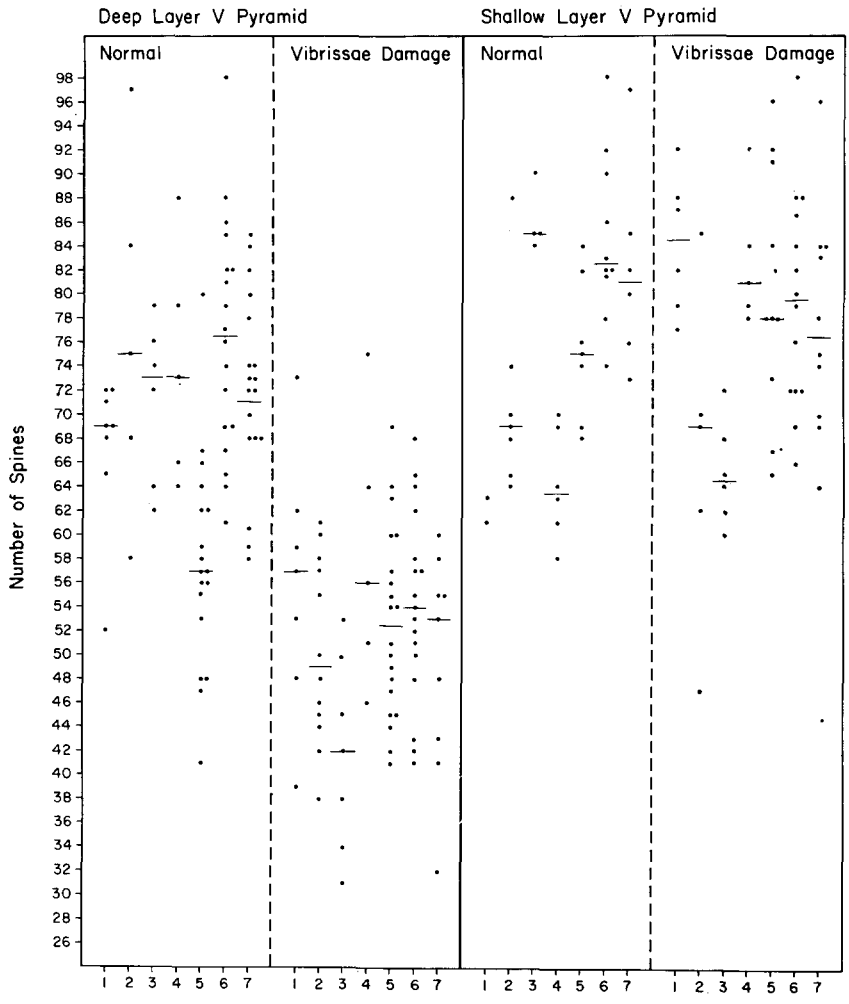


Fig. 1. Spine counts on 75 $\mu$m segments of layer V pyramidal cells in somatic sensory cortex. Each black dot (.) represents a single pyramidal cell count and the solid lines (–) represent the medians for a given subject.

dure did not result in permanent removal of the mystacial vibrissae. At 15 days of age these subjects possessed a variable number of misshapened vibrissae which tended to be clumped in a 'moustache' rather than grouped in a discrete array. All subjects were sacrificed at 25 days of age. Coronal slabs of somatic sensory cortex were stained by the rapid Golgi technique[7], sectioned at 80 $\mu$m, embedded in mounting material and counted at $\times$ 500 magnification (numerical aperture of objective 0.75). The slides were coded to prevent a counting bias. Layer V pyramidal cells were divided into two groups: a superficial group with cell bodies located 550–650 $\mu$m below the cortical surface and a deep group with cell bodies located at depths of 700–850 $\mu$m. Only completely impregnated cells with apical dendrites extending to the molecular layer were

included. Pyramidal cells were chosen only from the approximate location of the posteromedial barrel subfield (the portion of somatic sensory cortex with the greatest medial to lateral curvature). Spines were counted on the portion of apical dendrite located between 425–500 $\mu$m below the cortical surface, a region corresponding to the zone of specific thalamic afferent termination as determined by anterograde degeneration techniques[11].

Fig. 1 presents individual spine counts and median values for each subject. The deep layer V pyramidal cells of the experimental group exhibit a significant reduction in spine density as compared with those of control subjects (Mann–Whitney U-test, two-tailed analysis, $P < 0.002$)[25]. Median spine densities are 0.98 spines/$\mu$m for control subjects and 0.69 spines/$\mu$m for experimental subjects. Comparison of spine densities in superficial layer V pyramidal cells, however, reveals no significant difference between experimental and control groups (Mann–Whitney U-test, two-tailed analysis). The median spine density is 1.0 spine/$\mu$m for both control and experimental groups. The spine loss noted in deep layer V pyramidal cells is particularly dramatic in light of the fact that the experimental procedure did not result in a permanent loss of the vibrissae. Furthermore, the findings of Welker and Woolsey[32] suggest that a region previously referred to by one of us (H.P.K.) as a portion of the posteromedial barrel subfield[12] lies slightly rostral to this area *sensu stricto* and corresponds to the cortical representation of the subnasal sinus hairs rather than the mystacial vibrissae (compare Fig. 1B of Killackey and Leshin[12] with Fig. 2C of Welker and Woolsey[32]). In the present study dendritic spines were counted over an area which encompasses both the posteromedial barrel subfield and this more rostral cortical region. Accordingly, our estimates of spine loss may be on the low side, since cells were examined in both afflicted and normal cortical areas.

The results of the present experiment suggest that the layer V pyramidal cells of somatic sensory cortex respond to peripheral receptor damage in a manner that is markedly similar to that noted in visual cortex[22]. In both the visual and somatic sensory systems, receptor damage results in a spine loss restricted to the deeper layer V pyramidal cells, and in both cases the magnitude of the loss is approximately the same (somatic sensory cortex 26%; visual cortex 30%). In addition, both cortical areas contain a second cell type which responds to peripheral receptor damage. The layer IV stellate cells of mouse visual cortex exhibit a dramatic modification in the pattern of their dendritic orientation following visual deafferentation. In the 48-day-old mouse enucleated at birth, the afflicted stellate cells apparently direct their dendrites into layers V and III of neocortex and away from layer IV, the zone of specific thalamic afferent termination[28]. An alteration which is perhaps comparable has been reported following receptor damage in the somatic sensory system. Vibrissae removal in the newborn mouse results in a disruption of the normal aggregation of layer IV cells into multicellular units known as 'barrels'[30,33]. While this effect is observed in Nissl-stained material, evidence from Golgi preparations suggests that many of the cells which compose the barrels are stellate cells[12,13,18]. Therefore, it is not unreasonable to interpret this lack of aggregation as an anomaly of the stellate cells. The demonstration of transneuronal changes in both major cell types of sensory neocortex

following receptor damage complicates the interpretation of pyramidal cell spine loss data, particularly if such losses are regarded as having hodologic value.

In this context we would like to briefly consider the question of which neocortical cell type is the recipient of specific thalamocortical afferents. The classical view of both Ramón y Cajal and Lorente de Nó was that specific thalamocortical projections terminate primarily, but not exclusively, on stellate cells[14,21]. This view was based on the observation that large numbers of stellate cells are located in the same cortical lamina (layer IV) which receives the bulk of the specific thalamic input. Alternatively, Globus and Scheibel have suggested that cortical pyramidal cells are the major recipients of specific thalamocortical afferents. This view is based on observations of pyramidal cell spine loss in visual cortex following enucleation or lateral geniculate lesions[7]. However, at present it is unclear whether such alterations result from removal of direct thalamocortical afferents or whether this spine loss is a secondary effect consequent to changes in the stellate cells[22,27,28]. The complexity of the cortical neuropil has thus far prevented a direct answer to this question by electron microscopy. While electron microscopic evidence suggests that specific thalamic afferents terminate on dendritic spines[1,4,10], the identity of the type of cell which bears these spines is still equivocal. Although many investigators, including Ramon-Moliner[20], Sholl[24], and Globus and Scheibel[8] have regarded stellate cells as being essentially spine free, the classical drawings of Ramón y Cajal and Lorente de Nó depict stellate cells as bearing many spines (for example, see Fig. 387 of Ramón y Cajal[21] and Fig. 9 of Lorente de Nó[13]). A number of recent investigators have also called attention to the fact that many stellate cells are invested with spines[9,12,15,29]. In this regard it should be noted that only one electron microscopic study has clearly identified the cortical element on which thalamic fibers terminate. In cat motor cortex thalamic fibers terminate largely on the spines of pyramidal cell apical dendrites[26]. However, motor cortex lacks a stellate cell layer, in contrast to primary sensory cortex which possesses a well-defined layer IV rich in stellate cells. How thalamic afferents terminate in the absence of stellate cells may have little or no bearing on the mode of termination of thalamic afferents in primary sensory cortex.

In light of these considerations, we tentatively suggest that both deep layer V pyramidal cells and layer IV stellate cells receive direct thalamocortical input in primary visual and somatic sensory cortex. This notion is based on evidence which suggests that early receptor damage has at least two effects on the development of sensory cortex, both of which are observed in the region of specific thalamic afferent termination: first, layer IV stellate cells may undergo alterations in organization or dendritic morphology, and second, the deeper layer V pyramidal cells undergo reductions in spine density along the portion of apical dendrite which crosses the zone of thalamic input. Available evidence suggests that this spine loss is not entirely an effect secondary to stellate cell alterations[22]. Undoubtedly, these two different effects are part of an integrated response to peripheral receptor damage. However, both the interrelationship between these two effects and what the effects tell us about the organizations of thalamocortical projections are at present unclear.

This research was supported by N.S.F. Grant GB-41294.

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