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"The Formation of a Cortical Somatotopic Map"

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THE FORMATION OF A CORTICAL SOMATOTOPIC MAP

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The primary somatosensory cortex of small rodents is a homomorphic representation of the body surface. Similar representations are characteristic of the subcortical pathways leading from the periphery to cortex and these representations develop in a sequence beginning at the periphery and ending in the cortex. Further, central representations at all levels of the neural axis are altered by perinatal pertubations of the peripheral surface. This has lead to the hypothesis that the periphery plays an instructional role in the formation of central neuronal structures. The morphology of this discrete organization has been thoroughly examined during the development of the thalamocortical projections. The mechanism (s) which underlie the formation of these representations remains unclear although some recent evidence suggests the involvement of activity-dependent processes.

Ordered connections are a fundamental feature of cortical organization. They are characteristic of thalamic inputs to cortical areas, the connections between cortical areas and the output from cortex to subcortical targets. Such connections are said to be topographic in the sense that there is a detectable and orderly relation between a set of projection neurons and their target neurons. Further, in primary sensory corticies, there is a detectable relation between a peripheral receptor sheet and the related cortical area that, depending on the sensory system, is referred to as retinotopy, tonotopy or somatotopy. In this review, we focus on the emergence of topography and somatotopy in the rodent somatosensory system. Since the initial description of Woolsey and Van der Loos[1] of the isomorphic relation between the distribution of vibrissae on the mouse face and discrete cytoarchitectonic units in layer IV of somatosensory cortex that they called "barrels", the rodent somatosensory system has been the subject of intense study.

The borders of rat somatosensory cortex form a map which is a caricature of the body surface reflecting the differential innervation of the skin surface (see figure 1.). This map has been demonstrated by a number of anatomical techniques including the Nissl stain[2]; succinic dehydrogenase[3] , cytochrome oxidase[4] and acetlcholinesterase (AChE) histochemistry[5,6] and serotonin (5-HT) immunocytochemistry[7]. Most importantly for the present review, the distribution of thalamocortical afferent terminals arising in the ventral posterior nucleus forms this same map[8,9]. Within this overall map, there is a great deal of specific detail. For example, the size of discrete clusters of thalamocortical afferents in rat somatosensory cortex varies in direct relationship to the position and innervation density of vibrissae

on the snout. Rostral vibrissae are smaller and less densely innervated than more caudal vibrissae[10]. Similarly, in somatosensory cortex, the size of individual thalamocortical axon terminals associated with the rostral vibrissae is smaller than that of those associated with the more caudal vibrissae[8] (see figure 2).This implies a particularly close relation between the periphery and a cortical map. This map is both topographic in terms of relations between the thalamus and cortex and somatotopic in terms of its relation to the body surface.

Before turning to the development of thalamocortical projections in this system and potential mechanisms that may guide their discrete organization, it should be noted that the map which characterizes the rat somatosensory cortex is also demonstrable in subcortical synaptic stations between the periphery and cortex[11]. During development, these maps emerge sequentially beginning at the periphery and ending in cortex[12,13]. Further, peripheral damage during the perinatal period (extending from approximately six days before birth, E 15, in the rat to four days after birth, P 3) alters both cortical and subcortial maps. This has been taken as strong evidence that the periphery has the major organizing influence on the somatosenory cortical map[13,14]. Early peripheral damage can result in both the shrinking of the portions of the cortical representation related to the damaged body surface and expansion of adjacent intact portions of the cortical map[15,16]. Later peripheral damage produces local map changes without affecting overall boundaries[17].

Map and Thalamocortical Arbor Formation

A recent experiment[18] which utilized ACHE histochemistry as an early marker of thalamocortical terminals found that the rat somatosensory cortex map begins to emerge shortly before birth. A patternless distribution of dense AChE-reactive afferents can be detected on E 20. This is followed by a pattern which can be related to the body surface (E 21), then more discrete partitioning which, in the case of the face representation, is first into the major rows of vibrissae (early P0) and then into clusters related to individual vibrissae (late P 0). This is strong evidence that thalamocortical afferents, a source extrinsic to the cortex, are the first elements to exhibit a peripherally-related pattern in the cortex. This is a point worth stressing as others have postulated that glial elements and extracellular matrix molecules intrinsic to the cortex play the primary role in pattern formation[19,20].

Study of the ingrowth of thalamocortical afferents to somatosensory cortex of the rodent has been greatly facilitated by the introduction of liphophilic dyes which diffuse along axons in aldehyde fixed tissues[21]. This has allowed the study of both the distribution of populations of thalamocortical axons [22,23,24] and the morphology of individual thalamocortical axons during the period they form terminal arbors[25] (see figure 2). One major finding from the study of the ingrowth of the thalamic afferent population to rat somatosensory cortex[23] has been that this process is continuous and not punctuated by a "waiting period" as previously suggested[26]. A second major finding has emerged from retrograde labeling of thalamocortical projections first with horseradish peroxidase and more recently with liphophilic dyes. This is the high

degree of topographic order which characterizes the developing thalamocortical projection as it is establishing initial relations with the neocortex [27,28]. A high degree of topographic order is detectable in thalamic afferents at E 16 when they are leaving the thalamus and before reaching the cortex [25]. This suggests that topography is an intrinsic property of thalamic projections which is imposed on the cortex during thalamic afferent ingrowth.

The ingrowth and morphology of individual thalamic afferents to the somatosensory cortex of the rat has been followed from the time at which they leave the thalamus (E 16) until well after cortical pattern formation is complete (P 7). This process can be subdivided into three phases. First, topographic outgrowth from the thalamus resulting in a tangential distribution of thalamic fibers within the intermediate zone beneath the cortical plate. In the second, these axons invade the cortex without detectable waiting. The fibers that enter the cortex are radial branches of tangentially oriented parent fibers apparently formed by interstitial budding. In the third phase, restricted terminal arbors form in layer IV without initial overgrowth (see figure 2). An incipient and simple terminal arbor can be dedected on individual thalamic afferents on P 1 and on this day, afferents are in the location of their layer IV target neurons. Thus, the formation of terminal arbors by individual thalamic afferents correlates closely with the formation of the discrete map as detected by AChE histochemistry and it is during this third phase that somatotopy emerges.

Infraorbital nerve section on P 0 results in both a disordered map and abnormally large and widely branched thalamic terminal arbors in the adult[29]. On P 3 thalamic afferent terminals in rats whose infraorbital nerves have been severed on the

day of birth are wider than normal afferents at this age[30]. This is further evidence of the instructional role of the periphery on somatosensory cortex map formation and it implies that the chief effect is exerted both quite early and during the process of arbor formation.

Potential Mechanisms Involved in Cortical Pattern Formation and Maintenance

The findings reviewed to this point provide strong circumstantial evidence for the involvement of the periphery in cortical map formation. However, the mechanism(s) by which the peripheral map is imposed on the cortex is not at all understood. We will also address this question within the context of thalmocortical arbor formation.

In the visual system, the development of ocular dominance columns is dependent on neuronal activity for the parcellation of the primary visual cortex into territories related to one or the other of the two eyes [31,32,33]. However, several studies of the rodent somatosensory system have failed to demonstrate an equivalent role for neuronal activity in map formation. Neither tetrodotoxin (TTX) blockade of primary afferent[34] nor cortical activity[35,36,37] has a detectable effect on cortical map formation. Similarly, application of APV, an antagonist of N-methyl-D-aspartate (NMDA) receptors, to the somatosensory cortex does not alter the cortical somatotopic map[38].

There are several potential reasons for this difference between results obtained in rat somatosensory cortex and the findings in cat visual cortex. First, both spontaneous and stimulus-evoked activity in the visual system of newborn cats

appears to be much higher than in newborn rodents[39,40,41]. Thus, depression or blockade of activity in a system in which there is normally very little may have different effects than such interference in a system in which activity levels are much higher. Secondly, ocular dominance columns in the feline visual cortex develop over a period of several postnatal weeks while rat cortical somatotopy develops much earlier and over a much shorter time period.. Thus, the postnatal activity-blockade in the rodent has relatively little time to influence cortical development. Three recent studies have provided data addressing this issue. First, two experiments assayed the effects of manipulations designed to disrupt normal cortical activity in an altrical rodent, the hamster, where the map only becomes apparent at a longer interval after birth. In these experiments, application of TTX to the somatosensory cortex [37] or the NMDA antagonist MK-801 to the visual cortex [42] failed to alter map formation. On the other hand, vibrissae-related patterns fail to develop in the brainstem of mice lacking functional NMDA receptors [43]. Unfortunately, these mice die at birth so effects upon the cortical map cannot be assessed.

In summary, the role that impulse activity may play in the development of the rodent's primary somatosensory cortex is not clear and resolution of this question may require approaches which differ substantially from those employed thus far to evaluate its influence in the visual system. Its role may be a more subtle one which could not be detected in most of the experimental paradigms employed to date in the rat somatosensory system.

Recent studies of the role of the biogenic amine, 5-HT, in cortical development have provided results which may reflect the involvement of activity-

dependent processes in rat somatosensory cortical development. Serotoninergic axons reach the cortex prior to birth[44] and form a pattern mimicking that of thalamocortical axons [7,45,46]. This pattern appears about 1 day after that for thalamocortical axons and disappears by the end of the second postnatal week[7]. Depletion of 5-HT from the developing cortex by subcutaneous injection of the toxin 5,7-dihydroxytryptamine (5,7-DHT) results in an approximately one-third reduction in the area of the discrete clusters of thalamocortical afferent terminations corresponding to the vibrissae[47]. This reduction occurs without any corresponding decrease in cortical weight, the area of the vibrissae representation, or that of the thalamic barreloids whose cells give rise to the thalamocortical projection.

While this effect of 5-HT reduction might be explained by a direct effect of 5,7-DHT or reduced 5-HT on growing axons[48], it is also possible that the changes might involve altered cortical activity. Developing thalamocortical axons transiently express 5-HT_{1B} receptors[49] and these receptors mediate strong presynaptic inhibitory effects of 5-HT upon thalamocortical transmission during the first two weeks of life[50] (see figure 3). Thus, depletion of 5-HT might be expected to enhance thalamocortical transmission and perhaps also activity-dependent processes involved in shaping the arbors of thalamocortical axons.

This reduction in size of thalamocortical axon clusters in 5,7-DHT-treated rats might then reflect processes similar to those postulated in studies of the development of frog retinotectal projections[51,52]. Interference with retinotectal activity by application of NMDA receptor antagonists to the tectum of "three-eyed" frogs disrupts the segregation of axons into eye-specific domains. In addition, the

application of exogenous NMDA actually sharpens boundaries between tectal regions innervated by one or the other eye and reduces the size and branching of retinotectal axons. The smaller size and decreased branching of retinotectal axons in NMDA-treated tecta may reflect an augmentation of the normal process in which co-active axon branches are selectively stabilized.

It seems reasonable to suggest that similar processes occur during terminal arbor formation in the rat somatosensory cortex. We would hypothesize that the control of arbor formation is in a dynamic balance between a tendency for individual axons to extend their arbors widely and an opposing tendency for axons arising from the same thalamic aggregate, a barreloid[53], to form clustered terminations. We suggest that the tendency toward clustering and the formation of compact high-density arbors is promoted both by the activity of aggregates of thalamic neurons and by the ability of their axons to control the activity of postsynaptic cells in the cortex. A reduction in either of these two variables reduces clustering and increases the extent, but lowers the branch density, of individual thalamocortical axons.

Does this simple proposal fit the existing data? In the developing rodent, thalamic input to cortex appears highly damped and these afferents provide a very weak excitatory drive to cortical neurons[41]. The likely source of this dampening is the 5-HT mediated presynaptic inhibition of thalamocortical afferents[50] (see figure 3). This inhibition means that the primary stimulus for the clustering of thalamocortical axon terminations is the activity of thalamic barreloid neurons *independent of the influence of their axons on cortical cells.* Given this proposal, it follows that the application of TTX or APV directly to cortex would have relatively little influence on

axonal clustering[35,36,37,38]. since it would affect a mechanism that is only a very weak promoter of this process in normal animals. In contrast, depletion of cortical 5-HT might be expected to enhance clustering[47] because of the concomitant reduction in presynaptic inhibition and a markedly increased influence of thalamic axons on the activity of cortical cells.

Two results that would appear to be difficult to reconcile with our proposal are the different effects of neonatal infraorbital nerve section and TTX blockade of this same nerve on the patterning of thalamocortical afferents. The former manipulation increases the spread of individual arbors by P 3[30] and, at least in the adult, decreases clustering[29] (see figure 2). The later would appear to have no effect upon the clustering of thalamocortical afferents[34]. While it seems reasonable to expect that these two manipulations would have similar effects on primary afferent activity; they may not have the same effects on the intrinsic activity of second- and thus higher order trigeminal neurons. Neonatal transection of the infraorbital nerve causes a dramatic upregualtion in the galinin content of the central arbors of damaged primary afferents[54]. Galinin has strong inhibitory effects upon brainstem neurons and might be expected to decrease the intrinsic activity of these neurons and thus their excitation of thalamic cells. Our preliminary results suggest that Galinin is not upregulated after TTX application. The net effect of these changes would be a greater reduction in thalamic activity after nerve cut than TTX application and a reduced tendency of thalamocortical axon arbors to cluster after the former manipulation. While this is speculative, it is supported by one other result. Vinblastine application to the infraorbital nerve does not block afferent activity but it, like nerve cut, produces an

upregulation of primary afferent galinin and results in a cortical pattern similar to that seen after nerve transection[55].

Conclusions

At present, we can define the time course of the development of the somatotopic map in rat somatosensory cortex and closely correlate map formation with the development of thalamocortical afferent terminals. It is also clear that the periphery plays a major role in guiding map formation. Further, we are beginning to elucidate mechanisms which modulate the process of map formation. However, a major question remains. That is: is the "signal" by which the periphery guides the cortex, indeed, an activity-dependent one as we hypothesize?

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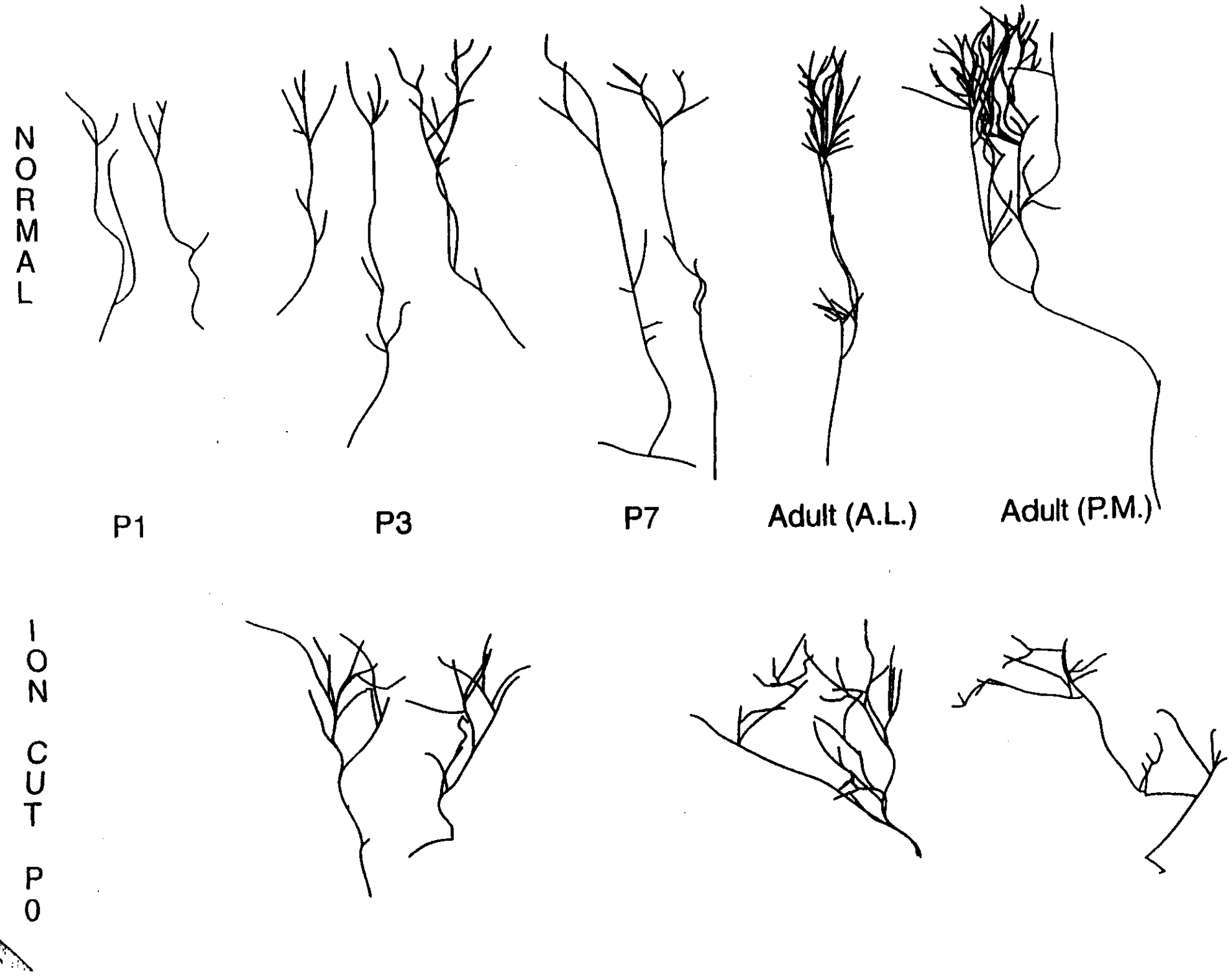
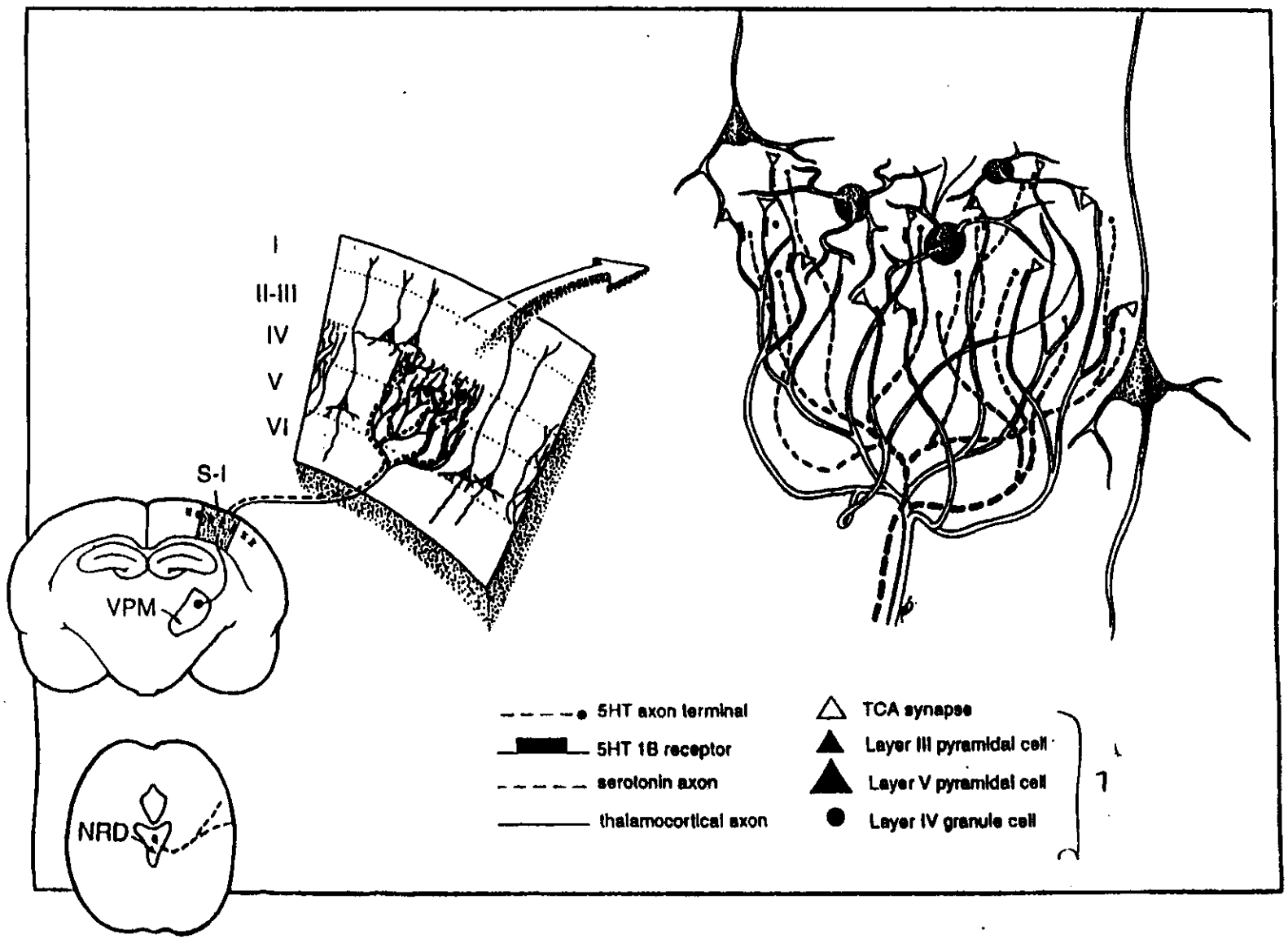
Figure Legends

Figure 1. Photomicrograph of a section through layer IV of the neonatal rat somatosensory cortex immunostained for serotonin. *FP*, forepaw; *Lj*, lower jaw; *T*, trunk; *V*, mystacial vibrissae; *17*, primary visual cortex. The scale bar equals 0.25 mm and the arrow labeled *a* points anteriorly.

Figure 2. Schematic drawings of individually labeled thalamocortical afferent terminals at different ages. Normal afferents are illustrated in the top row and afferents from rats subjected to day of birth infraorbital nerve section are illustrated below. Based on references 8, 25, 29 and 30.

Figure 3. Schematic representation of the thalamocortical and serotonin circuitry in neonatal rat somatosensory cortex. Based on references 7, 8, 49 and 50.


NORMAL
P1
P3
P7
Adult (A.L.)
Adult (P.M.)
ION CUT
P0


I
II-III
IV
V
VI
S-I
VPM
NRD
5HT axon terminal
5HT 1B receptor
serotonin axon
thalamocortical axon
TCA synapse
Layer III pyramidal cell
Layer V pyramidal cell
Layer IV granule cell