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Rapid alteration of thalamocortical axon morphology follows peripheral damage in the neonatal rat

(development/somatosensory/initial connectivity/arbor formation/neocortex)

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ABSTRACT **The effect of day of birth (postnatal day 0; P0) infraorbital nerve section on the morphology of individual thalamocortical axons in rat somatosensory cortex was examined on P3. Thalamic fibers were labeled in fixed brains with the carbocyanine dye 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate, and individual photoconverted thalamocortical fibers were reconstructed. In normal animals on P3, axon arbor terminal formation within layer IV has commenced and terminal arbor width is comparable to that of a cortical "barrel." After infraorbital nerve section, the average width of thalamocortical terminal arbors is significantly greater than is the average arbor width of normal rats of the same age; however, neither the number of branches per terminal arbor nor total arbor length differs between groups. These observations suggest that the role of the periphery in guiding terminal arbor formation is exerted both very rapidly and at the level of the single thalamic axon. Further, these results indicate a close association between individual axon terminal arbor morphology and pattern formation in the rat somatosensory cortex.**

The rodent's primary somatosensory cortex (SI) contains a pattern, demonstrable by a number of anatomical methods, that is homomorphic with the distribution of cutaneous receptors on the rodent's body surface (for recent reviews, see refs. 1 and 2). This pattern is observable either as aggregations of cortical neurons, which form "barrels," or as discrete clusters of thalamocortical axons, which match the distribution of barrels (3, 4). When the pattern of peripheral receptors is altered during the course of early development, the cortical pattern is altered concomitantly. For example, when a row of mystacial vibrissae on the face of a newborn rodent is removed by cautery, the resulting cortical pattern displays a fused band rather than a row of discrete clusters of neural elements related to individual vibrissae (5, 6). The altered cortical patterns appear to develop with the same time course as normal cortical patterns (7, 8).

The correlation between peripheral manipulations and cortical alterations have been taken as evidence that cortical pattern formation is guided by information from the periphery. It has also been suggested that thalamocortical terminal arbor morphology may underlie the peripherally dependent characteristics of the cortical pattern (9, 10). This suggestion is based on two observations. First, in the normal adult rat, individual terminal arbor size closely matches the size of the corresponding barrel, and both of these vary in size according to their position in the overall representation. Second, when the infraorbital nerve (ION) is cut on the day of birth, both the cortical pattern and the morphology of thalamocortical terminal arbors appear profoundly altered when examined in adults. Axon arbors extend over wider distances and have a lower branching density than normal.

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While the effects of neonatally inflicted peripheral damage appear clear in the adult, no information is currently available regarding the development of individual thalamocortical axons after peripheral manipulation. The close relationship between individual thalamic terminations and the cortical pattern in the adult rat would predict that peripheral manipulations would alter the initial development of individual thalamocortical terminal arbors. We tested this hypothesis by examining the morphology of individual axons on postnatal day 3 (P3) in rats subjected to ION section on the day of birth (P0) and comparing them to axons of the same age from normal rats. On P0, thalamocortical axons have reached the vicinity of their presumptive target cells but have not yet commenced terminal arbor formation; by P3 arbor formation is well underway.

METHODS

On the day of birth (P0), Sprague–Dawley rat pups were cryoanesthetized, and the left ION was cut ~10 hr after birth. The cut skin was sealed with cyanoacrilate; the pups were warmed on a heating pad and then returned to their mother. On P3, ~82 hr after birth, pups were deeply anesthetized with Nembutal and perfused transcardially with 4% or 10% (wt/vol) paraformaldehyde. Brains were removed and dissected, and the brain rostral to the mesencephalon was postfixed overnight. Brain stems were placed in 0.1 M phosphate buffer and then sectioned and stained for cytochrome oxidase activity (11). Absence of the cytochrome oxidase-stained somatotopic pattern in the brain stem trigeminal nuclei *ipsilateral* to the lesion was taken as evidence of a complete ION section. The brains of both normal and ION-sectioned animals were processed for 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI; Molecular Probes) studies as described below.

DiI was dissolved in dimethylformamide (20 mg/ml; Sigma) and then pressure injected into the ventral posterior nucleus of the thalamus through a glass micropipette (tip diameter, 40 μm). Brains were stored in fixative at room temperature or at 37°C for up to 5 weeks. Brains were then embedded in 2% agarose, and 150-μm serial sections were cut in the coronal plane with a Vibratome (Technical Products International) and collected in 0.1 M phosphate buffer. Tissue sections were counterstained with bisbenzimide (0.01% bisbenzimide in distilled water, final concentration of 0.00025%) to visualize cortical cytoarchitecture. Wet-mounted sections were examined under epifluorescence illumination (DiI excitation wavelength, 547 nm), and sections containing labeled thalamocortical axons were selected for photoconversion. Individual sections were photoconverted according to the protocol of Sandell and Masland (12) as modified by Catalano *et al.* (13).

Out of a total of seven normal and seven ION-sectioned animals that were processed, brains from four normal and

Abbreviations: SI, somatosensory cortex; ION, infraorbital nerve; Px, postnatal day *x*; DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate.

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three ION-sectioned animals had sufficiently high quality labeling to be selected for detailed analysis. Labeled thalamocortical axons in SI were drawn using a camera lucida attachment to a Leitz Orthoplan microscope using a ×40 oil-immersion lens. SI was identified by a combination of gross anatomical landmarks, including positional relationship to the striatum and hippocampal formation, and by extrapolation from heavily labeled cases in which a vibrissae-related pattern was evident. The region of SI from which our sample of labeled fibers was drawn is shown in Fig. 2. Only the most complete axons (those with growth cones at most of their branch ends) were drawn. The observer was aware of the experimental status of the tissue. Axon arbors were measured from the camera lucida drawings. For quantification purposes, the axon arbor was defined as the portion of the axon within layer IV. Arbor width was defined as the maximum medial-lateral extent of an axon's branches, measured parallel to the pia in layers IV and III. The number of axons measured in each animal at P3 was 2, 4, 6, and 12 axons for normal animals and 2, 3, and 22 axons for ION-sectioned animals. Average arbor width was calculated for each animal; these means were then averaged to obtain the group mean width for each condition (normal or experimental). The same was done for the number of branches and total arbor length per axon arbor. Arbor width, number of branches, and total branch length in normal and experimental animals were compared with a Mann–Whitney U test using the SYSTAT software package.

RESULTS

Section of the ION on the day of birth results in an absence of a vibrissae-related pattern in the brain stem, which confirms previous reports (14). In the case illustrated in Fig. 1, the left ION was sectioned at P0. The normal punctate vibrissae-related pattern seen in the right trigeminal nucleus is absent on the left side, ipsilateral to the ION section.

The camera lucida drawings in Fig. 2 present examples of individual thalamocortical axons taken from primary SI of a normal P3 animal. At P3, normal thalamocortical axons consist of a radially oriented parent fiber that gives rise to one or two branches within layers V and VI. Within layer IV, the main fiber breaks up into a cluster of branches that form the terminal arbor (Fig. 2). The medial-lateral extent of the normal terminal arbor is restricted at this age. The width of normal axon arbors within layer IV ranged between 75 and 265 μm, with a group mean of 144.0 ± 6.4 μm. The group mean arbor width is similar to the dimensions of the large vibrissae-related

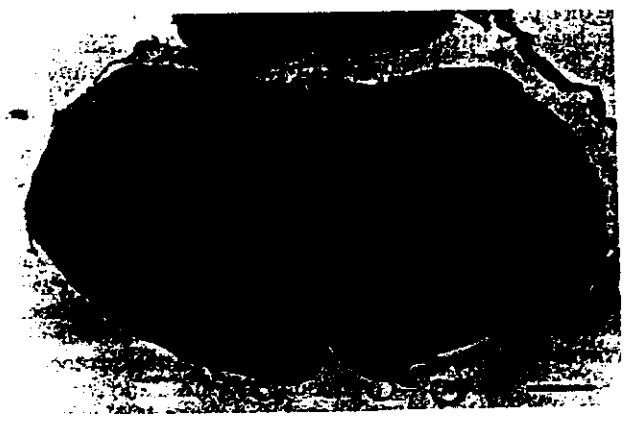

FIG. 1. Coronal section through the brain stem trigeminal complex at the level of subnucleus interpolaris of a P3 rat in which the left ION was cut on the day of birth. The section is stained for cytochrome oxidase activity to visualize the vibrissae-related pattern. The pattern contralateral to the nerve section (right side) is normal, while that on the left side ipsilateral to the nerve section is aberrant. (Bar = 500 μm.)

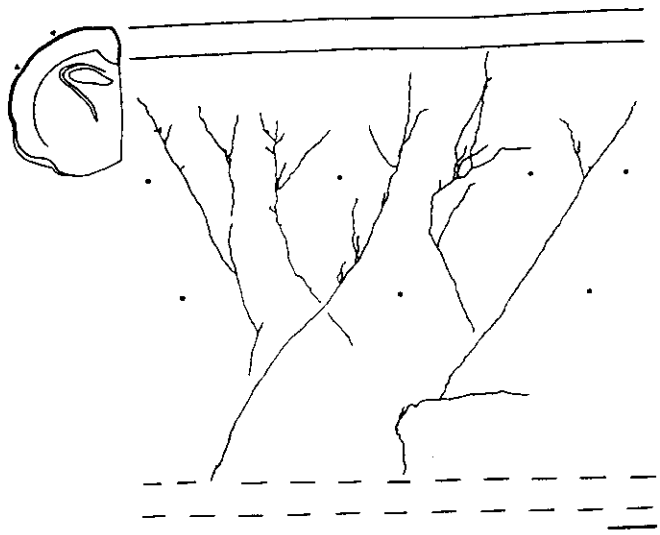

FIG. 2. Camera lucida drawings of normal P3 thalamocortical axons. (*Inset*) The drawing shows the location within somatosensory cortex from which axons in this study were drawn (area between arrowheads). The circles indicate the border between cortical layers IV and V. The squares indicate the border between cortical layers V and VI. Solid lines limit extent of layer I. Broken lines delimit layer VIB. (Bar = 100 μm.)

clusters at P2 (150–200 μm) as measured from Fig. 2 of Erzurumlu and Jhaveri (15). Axon terminal arbors within layer IV had a range of 6–22 branches, with a group mean of 10.4 ± 1.4 branches and a total terminal arbor length of 505.6 ± 84.6 μm.

The morphology of axon terminal arbors in the SI of the hemisphere affected by ION section was quite variable. At a qualitative level, some axons appeared normal, whereas the terminal arbors of other axons appeared much larger than normal and to extend over a wider area (Fig. 3). The width of axon arbors within layer IV in the ION-sectioned rats ranged between 75 and 410 μm, with a group mean of 249.2 ± 33.2 μm. Thus, mean thalamocortical terminal arbor width was nearly twice as large in hemispheres contralateral to the ION sections as it was in the normal P3 rats ($U = 0.000$, $P = 0.034$; see Table 1). Although both the number of axon branches in the terminal arbor and the total length of the terminal arbor in ION-sectioned cases were somewhat larger than in normal controls, these differences were not statistically significant (see Table 1). Thus, ION section on the day of birth affects the morphology of thalamocortical axons, not by affecting the number of branches but by affecting the areal distribution of those branches. Further, this morphological difference is detected within 3 days of the ION section—that is, by P3.

DISCUSSION

The present study provides further evidence that a peripheral "signal" plays a role in guiding pattern formation in rat somatosensory cortex and provides definitive evidence that this signal exerts its influence on initial thalamocortical arbor formation. This influence is exerted very early in development, soon after projections between subcortical stations are in place (16, 17). Further, the morphological manifestation of this influence after ION section is a wider than normal terminal arbor without any significant change in terminal branching or total length during this early time period. It is noteworthy that neither the number of branches nor total axon length is affected by the experimental manipulation. This indicates that the axon-building machinery of the thalamocortical projection neurons is relatively normal. From this perspective, the likely

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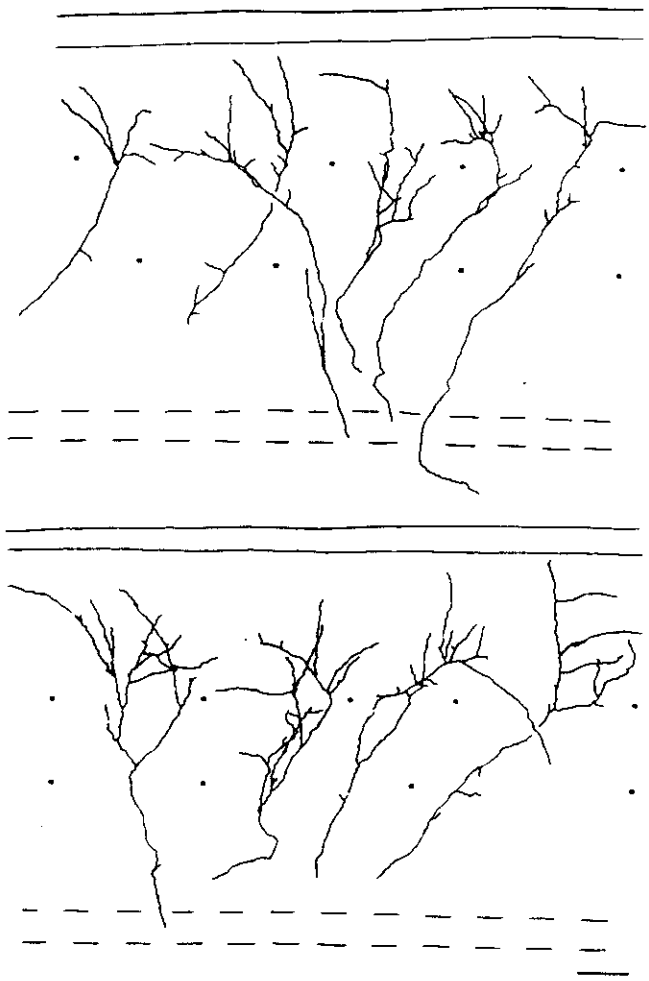

FIG. 3. Camera lucida drawing of P3 thalamocortical axons from somatosensory cortices in which the contralateral ION was sectioned on the day of birth. Axon arbors within layer IV in such hemispheres exhibited a range of morphologies, from normal (*Upper*) to clearly altered (*Lower*). Average axon arbor widths in layer IV of these experimental hemispheres are larger than normal, although they have a normal number of branches. (Bar = 100 μm.) Lines, circles, and squares are as indicated in Fig. 2.

role of the peripheral signal is to restrict the spatial distribution of the terminal arbor and perhaps promote clustering among terminal arbors associated with the same vibrissae. It should be noted that the increase in terminal arbor width in the ION cases was also noted in the one study that examined arbor size in the adult rat after neonatal ION section (10).

Several recent studies have suggested that pattern formation in rat SI is best characterized as a progressive developmental event rather than as a regressive refinement of an initially crude organization (13, 15, 18, 19). This appears to be true both at the level of the overall pattern and at the level of individual thalamocortical axons. Upon initial ingrowth into neocortex, the population of thalamocortical axons exhibits a high degree of topographic organization (20). This is the context within which the peripheral signal must be operating.

While the nature of the peripheral signal is not known, it does not appear to be neuronal impulse activity in any straightforward sense. Henderson *et al.* (21) have reported that tetrodotoxin blockade of the ION from birth does not prevent vibrissae-related pattern formation in either the brain stem or cortex. Further, Chiaia *et al.* (22) have reported that direct tetrodotoxin application to the SI cortex does not prevent vibrissae-related pattern formation in this structure. There are

Table 1. Parameters at P3 for normal and ION-sectioned rats

Parameter	Normal	ION sectioned
No. of axons	24	27
Branch number		
Minimum	6	5
Maximum	22	32
Group mean	10.4	15.5
SE	1.4	5.8
Arbor width, μm		
Minimum	75	75
Maximum	265	410
Group mean*	144	249.2
SE	6.4	33.2
Total arbor length, μm		
Minimum	239	282
Maximum	945	1619
Group mean	505.6	790.1
SE	84.6	248.2

*$U = 0.000$; $P = 0.034$.

currently no studies of neuronal activity levels in the somatosensory system of unanesthetized developing rodents. Studies in anesthetized developing rodents indicate that both spontaneous activity and evoked activity are weak but present during this time period (23, 24). That the situation is a complicated one is indicated by two studies that have examined the role of activity-dependent processes on pattern formation under normal conditions and after peripheral manipulation. Neither study (25, 26) found evidence for activity-dependent processes in normal development, and only one of the two (25) found evidence for such processes to be operating in cortex after the peripheral manipulation.

The best evidence that activity is a signal that influences the formation of thalamocortical axon arbors comes from a study of cat primary visual cortex. Antonini and Stryker (27) have assessed the effects of binocular tetrodotoxin blockade of the optic nerve on the morphology of individual thalamocortical axon arbors during development. Binocular tetrodotoxin blockade is roughly comparable to the present approach, in that it affects the entire receptive surface projecting to one hemisphere. Antonini and Stryker (27) found unusually widespread arbor branches that failed to retract during development and also axon arbors that lacked the patchy organization characteristic of their normal counterparts. Although the experimental manipulations of Antonini and Stryker were begun well after thalamocortical axons had begun to cluster in layer IV, their data are in agreement with the present results in indicating that progressive events play a role in arbor formation and that a peripherally derived signal appears to promote clustering. However, differences between results of the two studies include the apparent absence of regressive events in normal development of the thalamocortical axons in the rodent somatosensory cortex and the likely role of impulse activity as the candidate signal in cat primary visual cortex. These differences may be related to a feature of cat primary visual cortex that has no analog within rat SI. That is the necessity to align two sets of thalamic afferents, each of which originates in one of the eyes and carries information about the same point in visual space. In this case, impulse activity in the form of temporally correlated firing may provide the cue for alignment; regression of axonal branches may be a necessary part of the alignment process. Given the preexisting topographic order in thalamocortical projections to rat SI that is detectable on P0 (20) and the fact that they are related to a single peripheral source, no such alignment process may be necessary, and therefore impulse activity may play a more subtle role such as promoting terminal arbor clustering, which would not be detected in the experimental paradigms em-

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ployed to date. In this vein, it is worth noting that Y-axon arbors projecting to area 18 of cat visual cortex apparently develop by progressive growth (28). These strategy differences in formation of connections both within and across species underscore the necessity of studying a variety of systems to determine the full range of cortical growth strategies.

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