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Peripheral Influences on the Size and Organization of Somatotopic Representations in the Fetal Rat Cortex

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Nerve lesions at different fetal ages and on the day of birth were used to determine the role of the periphery in establishing territories devoted to representations of different portions of the body surface in rat somatosensory cortex. Transection of the infraorbital nerve (ION), the trigeminal branch that supplies the whisker pad, resulted in a significant reduction in the area within the primary somatosensory cortex devoted to the representation of the mystacial vibrissae in fetal, but not newborn, rats. Such lesions in fetal, but not neonatal, rats also resulted in significant increases in the cortical area devoted to the representation of the lower lip and jaw. There was a significant positive correlation between the reduction in the vibrissae representation and the expansion of that of the lower lip and jaw. Damage to the ION in either neonatal or fetal rats failed to increase significantly the amount of cortex devoted to the representation of the forepaw. These results indicate that the primary afferent innervation of the periphery does influence the amounts of cortex devoted to representations of different parts of the body surface and that the representation of one region can expand significantly when that of another body part is reduced.

[Key words: vibrissae, cortex, infraorbital nerve, barrels, Di-I, plasticity]

A fundamental question to those interested in CNS development is the degree to which epigenetic events control aspects of nervous system organization. This question has been addressed extensively in studies concerned with the development of the mammalian cerebral cortex (for reviews, see Rakic, 1988; O'Leary, 1989; Killackey, 1990). Information currently available provides support for the conclusion that many aspects of cortical organization including the development of patterns of output and cytoarchitectonic organization can be markedly influenced by afferent input (e.g., Sur et al., 1988; Metin and Frost, 1989; O'Leary and Stanfield, 1989; Schlaggar and O'Leary, 1991). At the same time, others have suggested that at least in some areas, major features of cortical organization are intrinsically determined (Rakic, 1988).

The patterns in the rodent's primary somatosensory cortex that are related to the mystacial vibrissae have also been used extensively to ask questions about the degree to which cerebral organization is determined by genetic and experiential factors (for reviews, see Killackey et al., 1990; Woolsey, 1990). A number of studies have shown that damage to the periphery during the first few postnatal days in rats and mice can substantially alter patterns of thalamocortical afferents and the organization of the vibrissae-related cytoarchitectonic units generally referred to as barrels (Woolsey and Van der Loos, 1970). However, studies in which the trigeminal nerve that supplies the vibrissae follicles, the infraorbital nerve (ION), has been transected on the day of birth have raised the possibility that information intrinsic to the cortex may be involved in the formation of the vibrissae-related pattern. Transection of the ION at birth results in an absence of the normal vibrissae-related patches of thalamocortical afferents and barrels, but a pattern with five distinct bands, presumably related to the five rows of vibrissae follicles, can still be seen during the first few postnatal weeks (Killackey and Belford, 1979; Rhoades et al., 1990). It may be that thalamocortical fibers achieve this row organization prior to the time when the neonatal lesions are carried out, but that the pattern simply cannot be visualized until slightly later ages (see Schlaggar and O'Leary, 1991, for evidence supporting this possibility). Alternatively, it is possible that information intrinsic to the cortex is sufficient to guide the development of this pattern.

In the present study, we have addressed this question by depriving the developing trigeminal system of afferent input prior to birth and, in some cases, prior to the age at which thalamocortical afferents reach the telencephalon (see Catalano et al., 1991, for information regarding the timing of this event). We have also used these animals to address another question raised by the experiments of Killackey and Dawson (1989). They showed that removal of a forelimb from rats on embryonic day 16 (E-16) produced an expansion of the representation of the hindlimb *without* a corresponding decrease in the cortical forelimb representation. This result is somewhat surprising in view of the findings of other studies that have examined the effects of input reductions upon cortical organization. For example, early enucleation reduces the size of the primary visual cortex in primates (Rakic, 1988; Dehay et al., 1991; Rakic et al., 1991) and rats (Olavarria et al., 1987), as does dark rearing in mice (Gyllensten et al., 1966). In the present study, we determined whether fetal ION damage reduced the size of the cortical region devoted to the representation of the vibrissae and whether such lesions increased the portions of the cortex devoted to representations of other parts of the body surface.

In these experiments, we utilized an indirect method for dem-

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We dedicate this study to the memory of Hendrik Van der Loos.

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onstrating distributions of thalamocortical afferents, immunostaining for 5-HT. The cortical fibers containing 5-HT immunoreactivity arise from cells in the raphe nuclei (Bennett-Clarke et al., 1991), but their distribution in the primary somatosensory cortex of perinatal rats closely matches that of thalamocortical afferents as visualized by anterograde transport of Di-I (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; Molecular Probes) from the thalamus (Blue et al., 1991; Rhoades et al., 1993). This approach to labeling the representation of the body surface in the cortex has several advantages over other techniques. It provides a much higher signal-to-noise ratio than either Nissl or cytochrome oxidase (CO) staining and it does not rely upon accurate and complete tracer deposits, as is the case with either HRP or Di-I.

Materials and Methods

Experimental animals. Thirty-two neonatal Sprague–Dawley rats from timed-pregnant litters provided data for this study. Timed-pregnant female rats were obtained according to methods described previously (Chiaia et al., 1992). Two females were placed with an experienced male at the start of the animal colony's dark cycle (7:00 P.M.). The animals were separated the following morning, and vaginal smears were examined for the presence of sperm. In the case of a sperm-positive smear, conception was considered to have occurred the previous evening and the day was designated E-0. Following this procedure, the majority of rats were born early on E-22.

Transection of the ION. The ION was transected in one of two ways. While both methods produced effective ION transections, the second method (electrocautery) was introduced because it resulted in considerably higher survival rates. In some animals, nerve cuts were made according to the methods described by Rhoades et al. (1989). Briefly, pregnant female rats were anesthetized with ether, a 25 mm incision was made through the skin and underlying abdominal wall along the midline, and the uterine horns were exteriorized. Each uterine sac was stabilized between the blades of a pair of blunt forceps and the uterine wall and amniotic sac were transilluminated and pierced with a microknife. A 0.5 mm vertical incision was made through the face of each fetus at a point halfway between the developing optic cup and the caudal edge of the vibrissa pad. After completion of the lesion, the microknife was carefully withdrawn and pressure was applied to its point of entry to arrest loss of fluid from the amniotic sac. After surgery was completed for all fetuses in a litter, the uterine horns were returned to the abdominal cavity and the abdominal wall and overlying skin were sutured.

In other animals, methods similar to those described by Chiaia et al. (1992) were used to damage the ION. Pregnant females were prepared and fetuses exteriorized as described above and a parylene-coated tungsten microelectrode attached to an electrocautery device (Birtcher, model 732 Hyfrecator, 750 kHz spark gap oscillator) was inserted through the uterine wall and amniotic sac and positioned beneath the skin in the caudal portion of the vibrissa pad. The electrocautery device was activated for 2 sec. After the electrocautery procedure were accomplished in all embryos in a litter, the abdominal incision was closed in the manner described above.

Additional animals sustained transection of the ION on the day of birth. The procedures used in these pups were described most recently by Rhoades et al. (1990). Rat pups were anesthetized by placing them on a bed of crushed ice until they were immobile and did not respond to any mechanical stimulation. A vertical incision was made caudal to the whisker pad. The ION was visualized with a dissecting microscope and cut with a pair of iridectomy scissors. After completion of the lesions, pups were rewarmed and returned to their mothers.

Animals were killed with ether between postnatal day 3 (P-3) and P-7 and brains were processed in the manner described below. A summary of the animals used in these experiments is provided in Table 1.

Tissue processing. All animals were perfused transcardially with 0.9% saline in 0.1 M sodium phosphate buffer (PBS; pH 7.4, 21°C). This was followed by a fixative consisting of 4.0% paraformaldehyde in the same buffer (4°C). Brains were removed and postfixed for 1–5 d. Cortices were removed, flattened, and cut into 50 μm sections using a freezing microtome. Brainstems were cut into 50 μm coronal sections. Brainstems of all animals were reacted for the demonstration of cytochrome oxidase (CO) activity according to the method of Wong-Riley (1979). Immunocytochemistry for the demonstration of 5-HT was carried out using methods that have been described in detail in several previous publications (Rhoades et al., 1990, 1993; Bennett-Clarke et al, 1991). Briefly, sections were incubated in primary antibody (5-HT; Incstar; 1:2500 dilution in phosphate buffer) for 14–20 hr at room temperature, rinsed with phosphate buffer, incubated for 1 hr in goat–anti-rabbit IgG diluted 1:200, rinsed again, and incubated in Avidin–Biotin complex (Vectastain) diluted 1:50. Following several rinses, sections were reacted with 0.05% 3,3′-diaminobenzidine and 0.015% H_2O_2 in 0.1 M phosphate buffer. After several rinses in phosphate buffer, the sections were plated on gelatin-coated slides, air dried, dehydrated in graded ethanols, cleared in xylene, and coverslipped with Permount.

Table 1. Animals used in the experiments comprising this study

N	Age at time of lesion	Age at death
2	E-15	P-6
3	E-16	P-5
3	E-17	P-3
3	E-17	P-4
2	E-17	P-6
4	E-18	P-6
1	E-19	P-3
1	E-19	P-6
1	E-20	P-6
4	P-0	P-6
3	P-0	P-4
1	P-0	P-5
1	P-0	P-3

In addition to the animals listed, one rat sustained ION transection on E-17 and killed on P-6, and thalamocortical afferents were labeled with Di-I.

The distribution of thalamocortical axons was demonstrated directly in one animal via anterograde labeling with Di-I. The methods used here have been described by Rhoades et al. (1993). The brain was postfixed for 2–7 d and crystals of the tracer were picked up with pipettes (20–30 μm tips) and inserted into the thalamus under a dissecting microscope. After the deposits were completed, the brain was placed in fixative and warmed in a 37°C oven for 4–6 d and then stored in the dark for 2 weeks to 2 months. Cortex was flattened and cut parallel to the pial surface (75–150-μm-thick sections) with a Vibratome and collected in sodium phosphate buffer. Sections were cleared in iothalmate meglumine (Conray, Mallinckrodt) and then placed on a slide in small pools of 50% dextran (Sigma; 67.9 kDa molecular weight) and coverslipped.

Data analysis. The efficacy of all lesions was determined by examination of CO-stained brainstem sections (see Fig. 2). A lesion was considered to be successful if there was no patterned CO staining in the region of the vibrissae representation ipsilateral to the nerve transection.

Patterns of 5-HT staining in the sections through the flattened cortex were drawn with a tissue projector. Three portions of the pattern were measured: (1) any dense staining in the region of the normal vibrissae representation, (2) the outline of the representation of the lower lip and jaw, and (3) the outline of the forelimb representation. Examples of the regions measured from the normal and deafferented hemispheres of one rat that sustained a fetal nerve transection are shown in Figure 1. All measurements were made using a graphics tablet. Data for each of the three areas measured were analyzed with Kruskal-Wallis one-way analysis of variance by ranks, with age at the time of damage as the independent variable. The relationships between changes in the areas devoted to the representations of the three portions of the body surface assayed were analyzed by multiple regression. Comparison of data from the normal and deafferented cortices in animals that sustained damage at a given age was accomplished by means of Wilcoxon *t* tests. The accepted level of statistical significance for all tests was $p < 0.05$ (two tailed).

Results

Before describing the results of our experimental manipulations, it is necessary to provide further details of the normal pattern

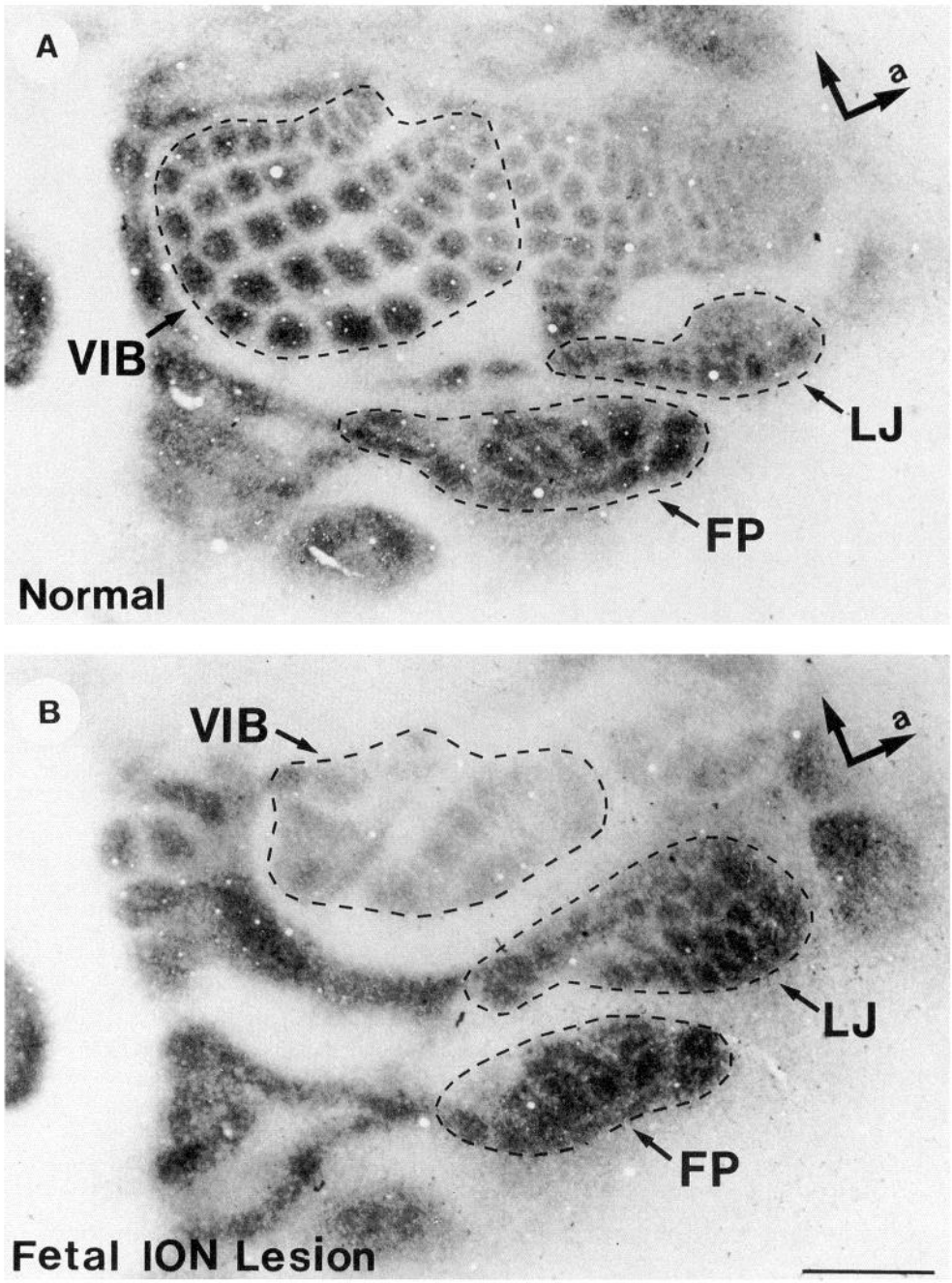

Figure 1. The cortical pattern in a normal rat (*A*) and in a rat that sustained fetal transection of the contralateral ION (*B*). Areas measured are denoted by the *dashed lines*. *VIB*, vibrissae representation; *LJ*, representation of lower lip and jaw; *FP*, forepaw representation. The *arrows* point toward anterior (*a*) and lateral. Scale bar, 500 μm.

in primary somatosensory cortex. Figure 1*A* illustrates this pattern. In this figure, the representation of the mystacial vibrissae and a cluster of sinus hairs on the nares are bounded by the broken line labeled VIB. Immediately anterior is the representation of sinus hairs on the upper lip and furry buccal pad that in the normal animal occupy approximately 25% of the total pattern compared to the approximately 33% of the pattern occupied by the vibrissae representation (see Killackey and Dawson, 1989). Immediately posterior to the vibrissae representation, and excluded from it in our measurements, is a line of clusters running mediolaterally related to the supraorbital and auriculotemporal vibrissae. The peripheral innervation of these vibrissae is carried by the ophthalmic and mandibular branches of the trigeminal nerve and would consequently be spared following ION lesions. This could be confirmed in some cases by the presence of the related clusters in the most lateral portions of the subnucleus interpolaris (Fig. 2 *E–H*).

Figure 1*B* illustrates the cortical pattern in one rat that sustained fetal ION section on E-19. This figure also illustrates the major ambiguity in our results, the inability to distinguish between the representations of the mystacial vibrissae and the furry buccal pad in most of the operated animals. Consequently, in this figure the area outlined and labeled VIB most likely includes some portion of the representation of the buccal pad. Therefore, our measurements most likely underestimate the degree to which the cortical representation of the vibrissae pad was reduced by ION transection. This same problem did not exist for the representations of the supraorbital and auriculotemporal vibrissae. In both normal rats and most of the operated ones, the representation of these vibrissae was separated from

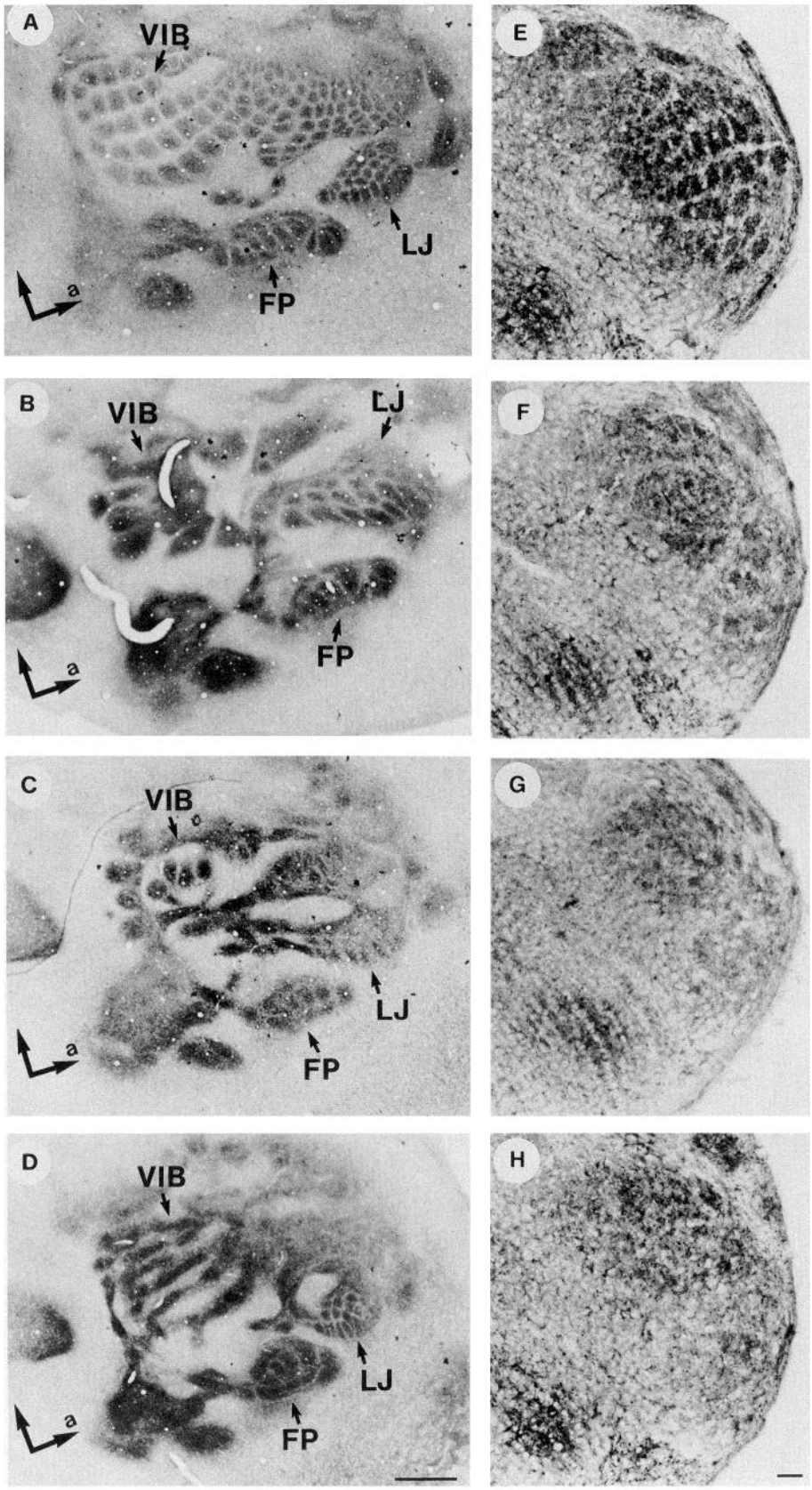

Figure 2. Examples of cortical patterns in rats that sustained ION damage at fetal and postnatal ages. *A*, The rat-tunculus in the normal cortex as revealed by 5-HT immunocytochemistry. *B* and *C*, The organization in two rats that sustained ION damage on E-17. *D* depicts results from a rat that had the ION transected on P-0. There are no vibrissae-related patterns in the fetally nerve-damaged rats, but a row-related pattern is apparent in the rat that received a lesion on P-0. *E–H* are CO-stained sections through the subnucleus interpolaris of each animal. Note the loss of any patterned staining in all of the rats that sustained lesions. Abbreviations are as for Figure 1. Scale bars: *A–D*, 500 μm; *E–H*, 100 μm.

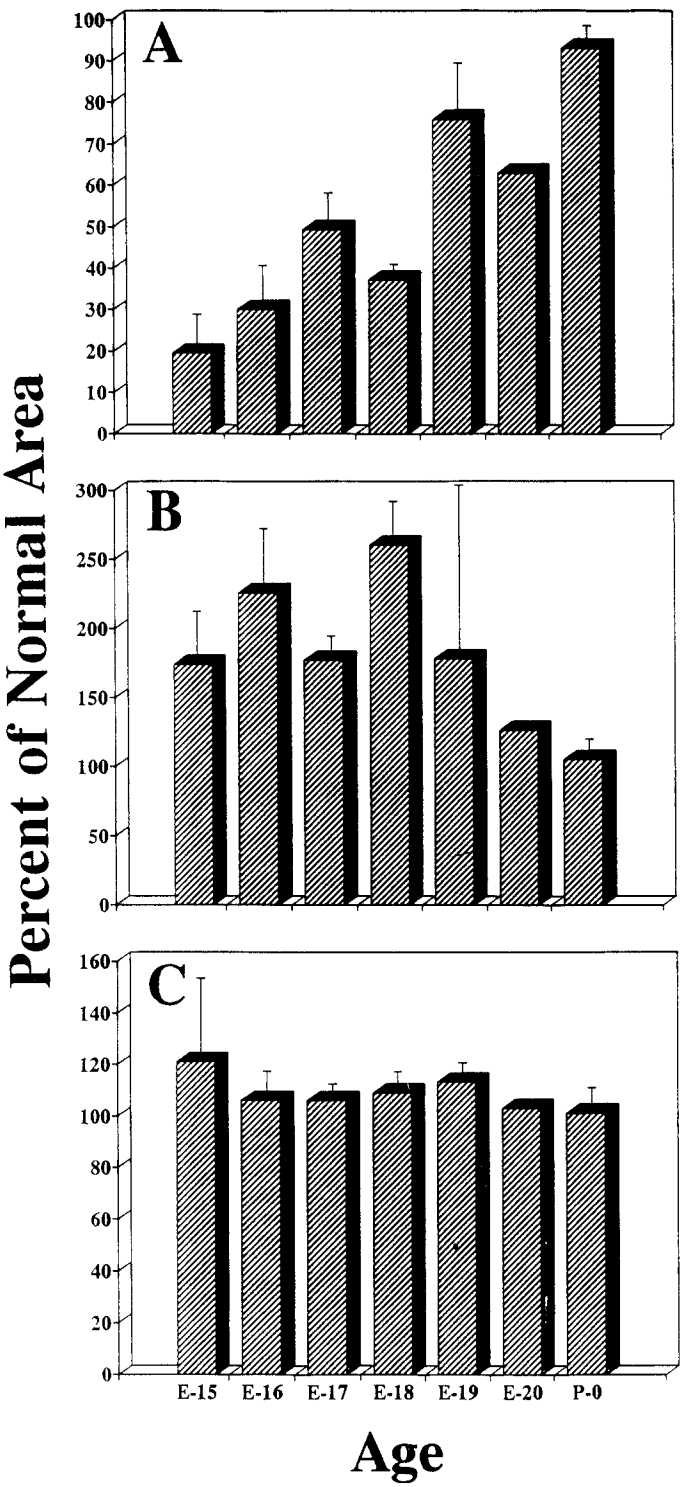

Figure 3. *A*, Cortical area in the deafferented hemisphere devoted to the representation of the vibrissae expressed as a percentage (mean + SD) of that in the normal hemisphere in animals that sustained ION damage at different ages. Note the relationship between age at the time of nerve damage and the reduction in the cortical area devoted to the vibrissae. *B*, Similar presentation of the data for the cortical area devoted to the representation of the lower jaw and lip. *C*, Similar presentation of the data for the cortical area devoted to the representation of the forepaw.

that of the mystacial vibrissae by a band of low staining density. Inspection of Figure 1*B* suggests that the representation of these vibrissae expanded after ION section. However, we did not attempt to quantify this effect. Finally, Figure 1 illustrates the clear and unambiguous representations of the lower jaw and forepaw in both the normal and experimentally manipulated rats.

Anomalous patterning in the brainstem and cortex of rats after fetal and neonatal ION transection

Transection of the ION at or before birth resulted in the absence of the vibrissae-related dense aggregates of CO reactivity that are normally present in trigeminal nucleus principalis, subnucleus interpolaris (Fig. 2*E–H*), and subnucleus caudalis. The regions in which the vibrissae are normally represented are characterized by low levels of uniform CO reactivity. However, clusters related to the supraorbital and auriculotemporal vibrissae can be seen in the most lateral portions of the subnucleus interpolaris.

In cortex, on the other hand, levels of 5-HT immunoreactivity appeared to be roughly normal although the patterns of distribution were clearly anomalous. The effects of ION transection on cortical patterns appeared to be both age dependent and more variable. Transection of the ION on P-0 typically resulted in a cortical pattern chiefly consisting of five bands of 5-HT immunoreactivity (Fig. 2*H*) oriented along the same dimensions as the five rows of clusters related to the mystacial vibrissae in the normal rat (Fig. 2*A*). In contrast, prenatal ION transection resulted in even more anomalous cortical patterns that consisted of dense aggregates of label separated by relatively label-free zones. Both the orientation and size of these dense aggregates varied considerably. These patterns (Fig. 2*B,C*) could not be readily related to the distribution of the vibrissae follicles, although in some cases aspects of the overall pattern could be related to peripheral receptor specializations. For example, in Figure 2*C* portions of the pattern related to the sinus hairs of the nares, and the furry buccal pad can be discerned. Such highly anomalous patterns were seen in all of the rats that sustained prenatal ION transections.

The area of the vibrissae and presumed vibrissae representations

Transection of the ION in fetal, but not neonatal, rats resulted in a significant decrease in the cross-sectional area of the vibrissae representation ($p = 0.01$, Kruskal-Wallis ANOVA by ranks). The changes in the area devoted to the representation of the vibrissae are summarized in Figure 3*A*. The average reductions for the rats that sustained lesions on E-15, E-16, E-17, E-18, E-19, E-20, and P-0 were 80.5%, 70.0%, 51.7%, 63.0%, 12.0%, 37.0%, and 7.2%, respectively. The changes in the animals that sustained ION transections on E-17 were statistically significant ($p < 0.05$, Wilcoxon t test), but those in the animals that received nerve cuts on P-0 were not. While the reductions in the size of the vibrissae representation in rats that sustained ION section on E-16 and E-18 were quite large, the p values (0.10 and 0.06, respectively) were not statistically significant. (Note: because of small sample size, a test of significance could not be accomplished for the rats that sustained nerve damage on E-15, E-19, and E-20.) Our analyses also demonstrated that age at the time of death had no effect upon the lesion-induced change in the representation of vibrissae representation.

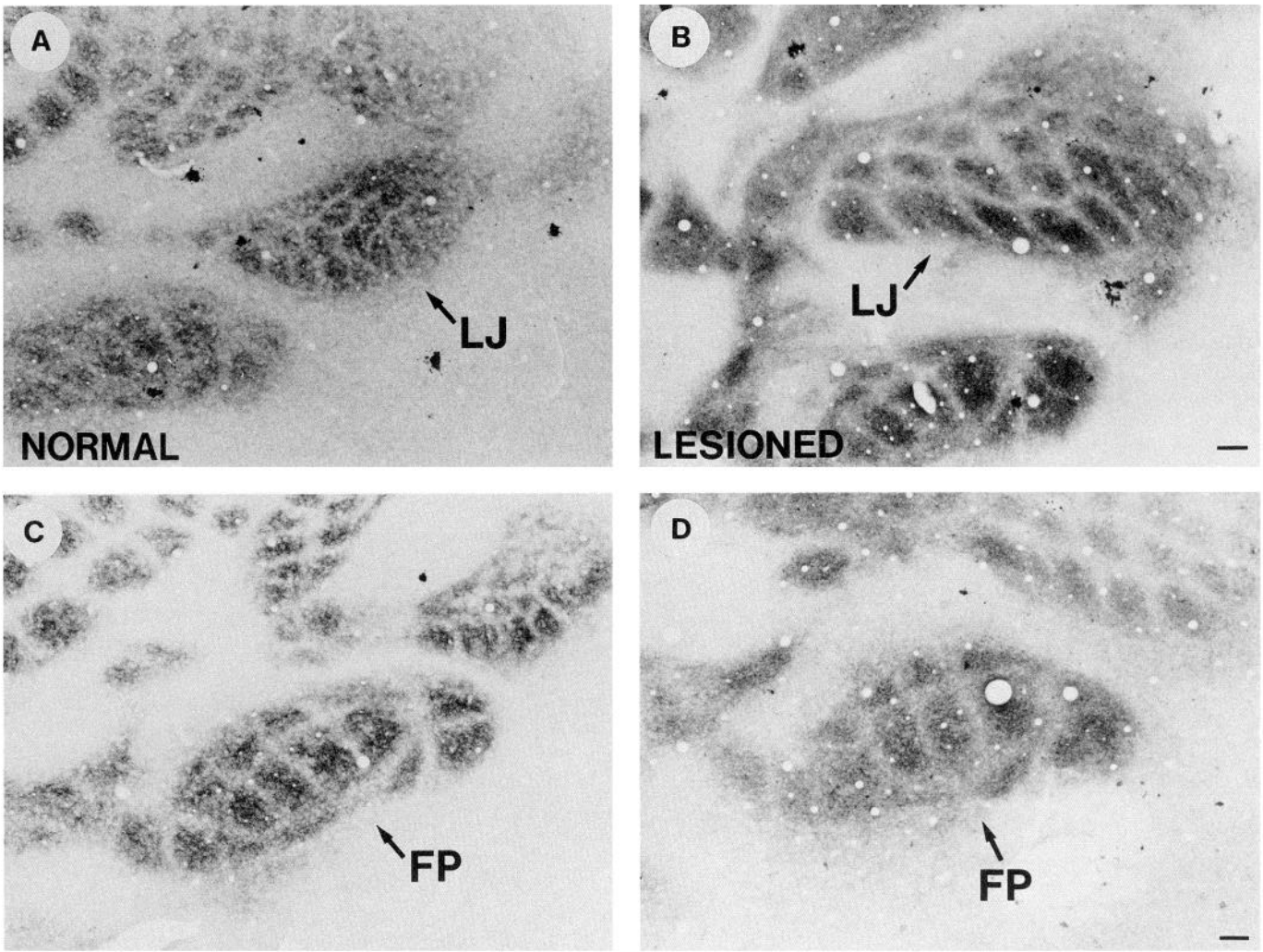

Figure 4. *A* and *B* show the representations of the lower jaw in the normal (*A*) and deafferented (*B*) hemisphere of a rat that sustained ION damage on E-17. Note the marked increase in the representation of the lower jaw on the deafferented side. *C* and *D* show the forepaw representations from another rat that sustained ION damage on E-17. Note the similarity in the size of the representations on the two sides. Abbreviations are as for Figure 1. Scale bars, 100 μm.

The area of the lower jaw and forepaw representations

Fetal ION transection resulted in a statistically significant increase in the cross-sectional area of the cortex devoted to the representation of the lower lip and jaw ($p = 0.001$, Kruskal-Wallis ANOVA by ranks), but no significant change in the cortical area containing the representation of the forepaw ($P > 0.05$).

One example of the increase in the size of the representation of the lower lip and jaw is shown in Figure 4*B*. Significant increases in the cortical area devoted to the representation of the lower jaw were observed in rats that sustained ION lesions on E-17 ($p < 0.05$, Wilcoxon t test; Fig. 3*B*). On the other hand, there was no significant increase in the cortical area devoted to the representation of the lower jaw in animals that sustained ION damage on P-0. When data from all of the animals were pooled, there was a significant negative relationship between the cortical area devoted to the vibrissa pad and that devoted to the representation of the lower jaw ($r = -0.70$, $p < 0.0001$). These results are summarized in Figure 5*A*.

As is apparent from Figures 1, 2, and 4, the representation of the lower lip and jaw is composed of a number of discrete patches. Thus, there are two non-mutually exclusive ways in which the expansion described in the preceding paragraph might have taken place: an increase in the number of patches or an increase in the area of those patches. While somewhat limited, our data favor the latter conclusion. We counted and measured the individual patches in the representation of the lower lip and jaw from five animals (two with E-15 lesions and one each with E-17, E-18, and E-19 lesions) in which the internal organization of the lower jaw representation was most clear. The average number of patches in the normal hemisphere was 19.2 ± 7.04 and that in the hemisphere contralateral to the lesion was 18.0 ± 5.34 ($p > 0.05$, paired t test). In contrast, the average patch size in the normal hemisphere was 0.0149 ± 0.004 mm² and that for the hemisphere contralateral to the damaged nerve was 0.0222 ± 0.003 mm² ($p < 0.01$, paired t test).

Transection of the ION had no significant effect upon the cortical area devoted to the representation of the forepaw (e.g., Fig. 4*D*). The respective changes in area in the rats that sustained ION damage on E-15 through P-0 were 20.5%, 6.0%, 6.0%, 9.5%, 13.0%, 3.2%, and 0.8% (Fig. 3*C*). Further, there was no

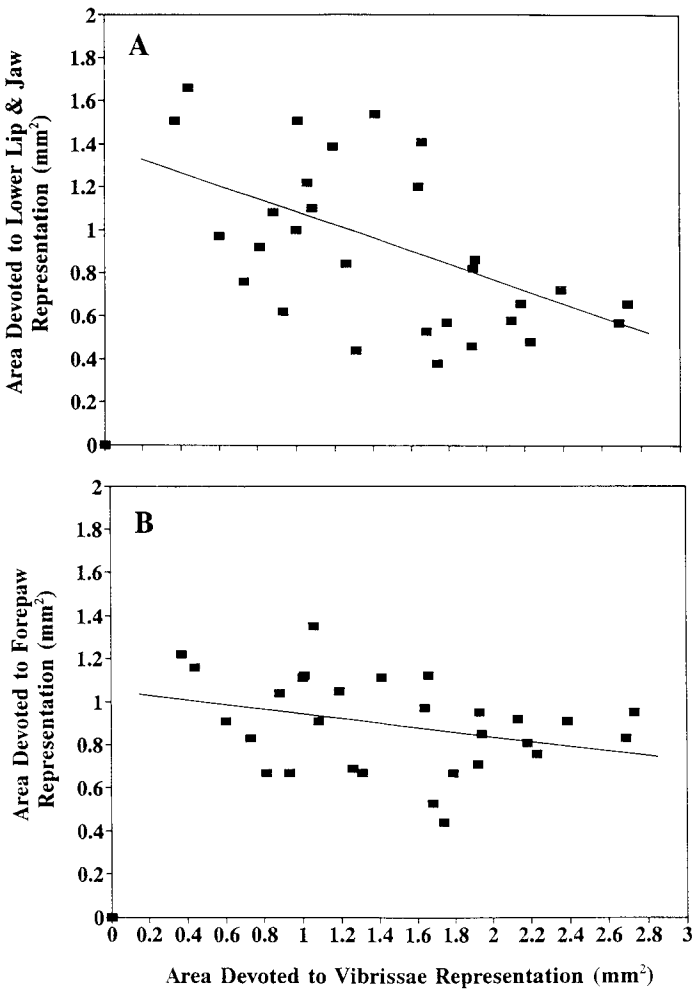

Figure 5. A scatterplot showing the relationship between the area devoted to the vibrissae representation and the increase in that devoted to the representation of the lower lip and jaw of all the animals used in the present study. *B* is a scatterplot showing the lack of a significant relationship between the area devoted to the vibrissae representation and that for the representation of the forepaw in the same animals.

significant relationship between the area devoted to the representation of the vibrissa pad and that devoted to the representation of the forepaw ($r = -0.22$; Fig. 5*B*).

In one rat with a fetal ION transection, thalamocortical axons were directly labeled with Di-I. In this case, the ION lesion spared the innervation of the furry buccal pad and the cortical representation of this peripheral surface is clearly expanded. However, this case also demonstrates both the reduction in size of the vibrissae representation and expansion of the lower jaw seen with 5-HT immunocytochemistry (Fig. 6).

Discussion

The results of this study demonstrate that cutting the ION prior to birth results in a reduction of the cortical representation of the mystacial vibrissae and a concomitant increase in the cortical representation of other peripheral receptor surfaces, most notably the lower jaw. The same lesion failed to produce a significant increase in the portion of the cortex devoted to the representation of the forepaw. This leads us to conclude that establishment of territories in primary somatosensory cortex devoted to particular receptor surfaces is both dependent on an intact periphery and characterized by compartments that exhibit varying degrees of interaction. Our observation of dense aggregates of 5-HT immunoreactivity within portions of cortex that would normally be related to the mystacial vibrissae, and which presumably reflect thalamocortical afferents from the appropriate portions of the ventral posterior nucleus, leads to a second conclusion. Thalamocortical afferents do not terminate diffusely in the afflicted portion of cortex. Instead, they form clusters that, insofar as the methods used in this study allow one to determine, do not correspond to any peripheral receptor organelle.

Technical limitations

Before discussing these results and conclusions in detail, several limitations of the current study should be re-emphasized. First, as noted in Results, it was often difficult to define precisely the portion of the cortex that remained devoted to the representation of the vibrissa pad after neonatal ION lesions. There is little doubt that some errors were made in this aspect of our analysis. Nevertheless, the reductions in the cross-sectional area of the portion of the cortex devoted to the vibrissa pad were so substantial that it is unlikely that errors of the type described would have changed our conclusions.

Second, as noted in the introductory remarks, 5-HT immunoreactivity is an indirect assay of the distribution of thalamocortical afferents. Its use, however, had three very important advantages: high signal-to-noise ratio, ease of specimen preparation, and provision of a permanent record that could be analyzed with light microscopy. The data presented in this study and by Blue et al. (1991) and Rhoades et al. (1993) have demonstrated a close correspondence between Di-I and 5-HT patterns in the developing cortex, and Rhoades et al. (1990) showed a close correspondence between 5-HT and CO patterns. The single E-17 ION-transected rat in which Di-I was used to label thalamocortical afferents exhibited the same changes (i.e., a reduction in the territory devoted to the representation of the vibrissae and an increase in the area of the lower jaw representation) as those observed in the animals where 5-HT immunocytochemistry was used to assay cortical organization. Finally, while 5-HT immunocytochemistry does not label thalamocortical afferents (Bennett-Clarke et al., 1991), it is a presynaptic marker. Thus, statements about cortical areas devoted to the representation of a given part of the body surface cannot be taken as conclusions about distributions of cortical neurons.

Aggregation of axons in the deafferented cortex

The 5-HT immunoreactivity in the cortices of the rats that sustained fetal ION damage was not distributed uniformly. That is, this immunoreactivity tended to form dense clusters of varying shapes and sizes. Direct labeling of thalamocortical afferents with HRP in adult rats that sustained P-0 ION section produces a very similar result (Jensen and Killackey, 1987). If one accepts the argument that the 5-HT immunoreactivity accurately reflects the distribution of thalamocortical axons, then these results suggest that there may be an intrinsically determined tendency for the arbors of thalamocortical axons from ventral posterior nucleus to form patches or clusters regardless of the nature of their afferent input.

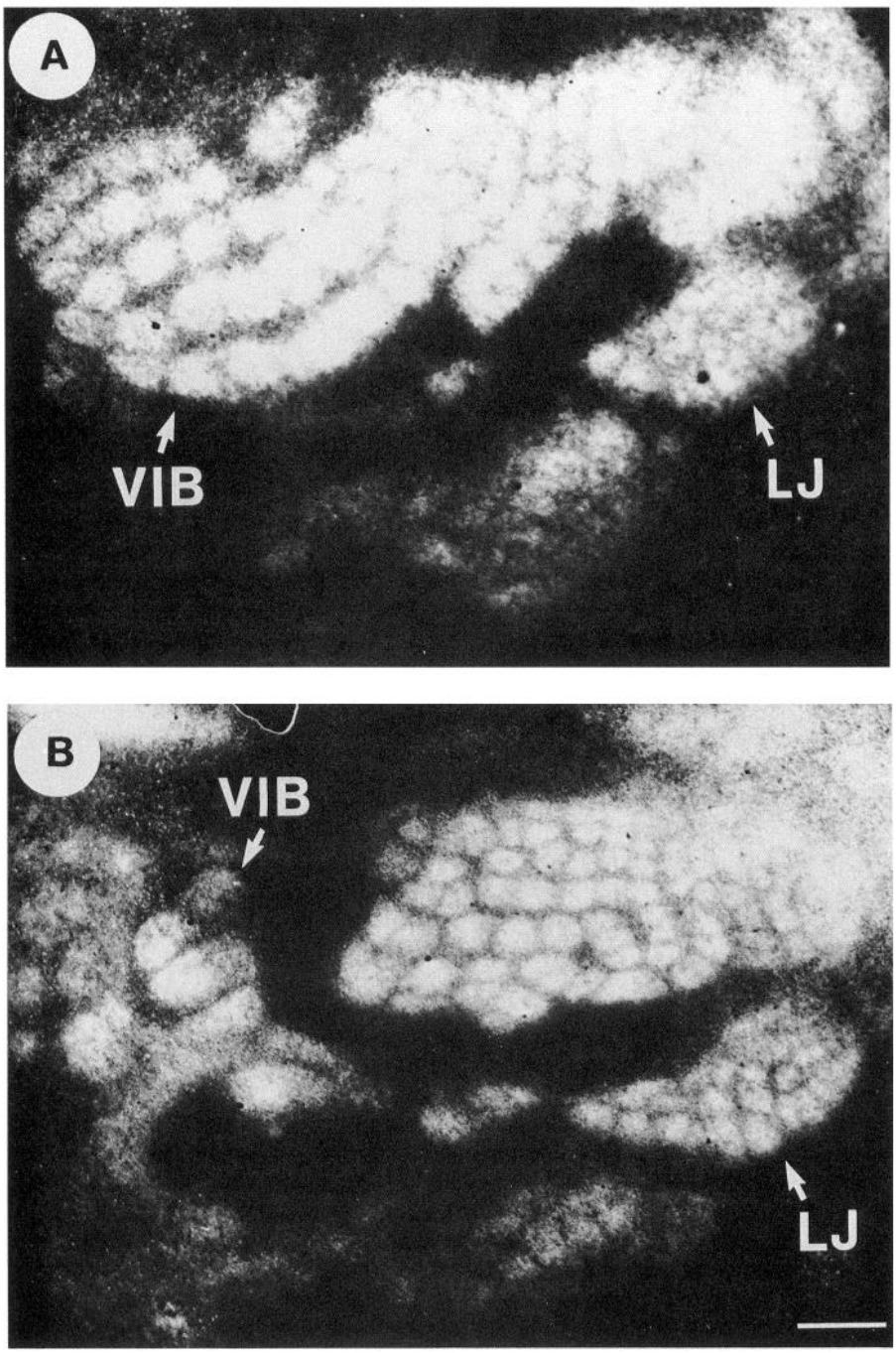

Figure 6. Episcopic fluorescent photomicrographs of a preparation in which Di-I was used to label thalamocortical afferents in a rat that sustained damage to the ION on E-17. Note the absence of a vibrissae representation and the increased size of the representations of the upper and lower jaws in the deafferented hemisphere. Scale bar, 500 μm.

Reduction of the cortical area devoted to the representation of the vibrissae pad after fetal ION damage

There was an age-dependent shrinkage of the representation of the vibrissa pad in the primary somatosensory cortex after ION lesions. Nerve damage in fetal animals resulted in significant shrinkage of this representation while lesions on P-0 did not. Further, ION lesions at early fetal ages seemed to result in both a greater shrinkage and loss of internal pattern organization than did ION lesions at later ages. With regard to these findings, it is important to note that at the time of our earliest lesions (E-15), the ION has made contact with the periphery and the brainstem, has begun to express fasciculation patterns related to peripheral targets (Erzurumlu and Killackey, 1983), and probably contains a greater number of fibers than it will have at any other time during development. The last of these statements is based on the finding of Davies and Lumsden (1984) that peak fiber number in the *mouse* maxillary nerve is achieved on E-13. Thus, E-15 is probably close to the time at which information about the state of the periphery becomes available to the CNS of the rat.

Our interpretation of the present findings is based on two previous hypotheses and focuses on two aspects of the cortical pattern, size and internal organization. First, both Lee and Woolsey (1975) and Welker and Van der Loos (1986) have hypothesized that the size of a barrel, the cortical representation of a vibrissa follicle, is dependent on the amount of afferent innervation supplied to that follicle. Second, Bates and Killackey (1985) hypothesized that during the early postnatal period, the brainstem pattern provides a template for pattern formation

farther centrally and that the degree of peripherally related internal organization in cortical patterns was dependent on the time available for the transfer of pattern information from the periphery to the brainstem. We would extend these two hypotheses both to the prenatal period and to the entire vibrissae pad and its cortical representation. Thus, we would conclude that there is not a fixed cortical region that will represent the vibrissae follicles regardless of their number. Rather, it is the amount of peripheral input in terms of both number of afferent fibers and the time available for them to exert an influence on the brainstem that plays a substantial role in determining the total amount of cortex devoted to the vibrissae representation and the internal organization of that representation. In this context, it should be noted that ION section as we employed it largely changed the time available for the periphery to influence the brainstem.

The shrinkage of the vibrissae representation noted in the present study contrasts with one aspect of a study of Killackey and Dawson (1989). They analyzed succinic dehydrogenase (SDH)–stained sections from the cortices of rats that sustained forelimb removals on E-16 through P-0 and observed no reduction in the area of the SDH patches that they tentatively attributed to the representation of the forepaw. Given the anomalous nature of these "forepaw" patches, the attribution of Killackey and Dawson (1989) may have been faulty. It has recently been demonstrated that the cuneate nucleus of rats sustaining fetal forelimb removal is dominated by input from cutaneous receptors in the remaining stump (Rhoades et al., 1993) and perhaps some of the patches attributed to the forepaw by Killackey and Dawson are related to this input.

Waite and her collaborators (Waite and Taylor, 1978; Waite and Cragg, 1979, 1982; Waite, 1984) have used physiological methods to map the representation of the vibrissae follicles in adult rodents after neonatal peripheral damage and showed reductions in the cortical area devoted to the vibrissa pad. While this contrasts with our own results of a lack in reduction of the anatomically defined vibrissa representation after lesions at P-0, it is important to remember that the relationship between anatomical substrate and physiologically defined maps, particularly after peripheral nerve damage, is an imperfect one (see Killackey, 1989, for a discussion of this issue).

Primary visual cortex is reduced in size in both monkeys (Rakic, 1988; Dehay et al., 1991; Rakic et al., 1991) and rats and mice (Gyllensten et al., 1966; Olavarria et al., 1987) following early peripheral manipulations. Most pertinently, Dehay et al. (1991) report that fetal binocular enucleation results in an age-dependent reduction in the size of monkey primary visual cortex. However, an important distinction between this experiment in the visual system and the present study should be kept in mind. Bilateral enucleation effectively removes the entire visually receptive surface, the retina, and there is an absolute decrease in the size of primary visual cortex. The present experiment removes only a portion of the somatic receptive surface, and while it produces an absolute decrease in the size of the representation of the related portion of the periphery, it is not clear whether the overall size of primary somatosensory cortex is reduced proportionately.

Expansion of the representation of the lower jaw

We provide evidence for an expansion of the representations of the supraorbital and auriculotemporal vibrissae, the furry buccal pad (when incomplete ION section spared its peripheral innervation; see Fig. 6), and the lower jaw following fetal but not neonatal ION damage. Quantitative evidence of expansion was obtained for the lower jaw representation and sample sizes of rats were sufficient to demonstrate a statistically significant increase in the size of this representation following ION damage on E-17. In those animals in which an expansion of the lower lip and jaw representation was noted, this increase reflected an enlargement of the size of individual patches rather than an increase in the number of patches. This result would be expected from the fact that the patches are related to peripheral entities that were not altered as a function of our lesions. This result agrees with the previous findings of Killackey and Dawson (1989), who observed increases in the size of patches comprising the hindpaw representation after fetal forelimb amputations. We would assume that the enlargement of these clusters is a reflection of the fact that there is more cortical territory available for occupation when a portion of the peripheral surface has been damaged. In this case, cluster size is not a direct reflection of peripheral innervation density as it is normally (Lee and Woolsey, 1975; Welker and Van der Loos, 1986).

Overall, there was a statistically significant inverse relationship between the sizes of the lower jaw and the vibrissa representations, suggesting that the sizes of these two representations are interrelated. On the other hand, the same fetal manipulation produced no change in the size of the forepaw representation. These results also parallel those of Killackey and Dawson (1989), who reported an expansion of the hindlimb representation following fetal forelimb removal on E-16 and E-17 and no expansion of the intact representations of either the mystacial vibrissae, furry buccal pad, or lower jaw. Further, the sensitive period for expansion ends at roughly the same time (E-18) in both experiments [note: sample sizes in the present experiment did not allow us to determine if there was a statistically significant expansion on E-18, and while Killackey and Dawson (1989) state that forelimb removal on E-18 did not produce an expansion, their raw data exhibit a trend in this direction, as do the present data]. Thus, in both experiments there appears to be similar spatial and temporal limits to the expansion of a cortical representation. We would suggest that the spatial limits are provided by brainstem adjacencies. The present experiment involved different branches of the same peripheral nerve that innervate the same brainstem compartment, the V complex, while the experiment of Killackey and Dawson (1989) involved primary afferents that innervate two distinct, but closely adjacent, subcortical compartments, the gracile and cuneate nuclei. In neither case did expansion bridge the gap between the trigeminal and dorsal columnar components of the somatosensory system. However, two recent experiments (Bronchti et al., 1992; Rauschecker et al., 1992) have reported cross-modality expansion of the vibrissae representation following neonatal enucleation in mice. These expansions cannot be accounted for in terms of representational adjacencies. Recently, Riddle et al. (1992) have demonstrated that the vibrissae representation undergoes a considerable and differential enlargement during the early postnatal period that they hypothesize is related to high activity levels in this cortical area. Perhaps these cross-modality expansions are related to an even greater dependency on the vibrissae as a source of information about the external world in mice deprived of vision.

How are neocortical areas established?

The present experiment provides clear evidence that epigenetic factors influence the size of discrete portions of the rat primary somatosensory cortex. This finding may also have some rele-

vance to the more general question of cortical specification. Rat primary somatosensory cortex is composed of a group of modules with distinct cytoarchitectonic features, granular cortex, and it is embedded in and surrounded by a matrix of cortex with different less distinct cytoarchitectonic features, dysgranular cortex. The terms "granular" and "dysgranular" reflect the most obvious difference between these two cortical areas, namely, the presence of a relatively dense population of small neurons in layer IV of granular cortex that is much reduced in dysgranular cortex. This is very clear in Nissl-stained sections of adult rat parietal cortex. Moreover, in the adult rat granular cortex and dysgranular cortex differ along a number of other dimensions, further suggesting that they are indeed separate cortical areas. For example, the ventral posterior nucleus projects to the granular cortex and not dysgranular cortex, which in turn receives its major thalamic from the medial portion of the posterior nucleus (Koralek et al., 1988). Dysgranular cortex gives rise to and receives dense callosal connections while these are much sparser in the granular cortex (Koralek et al., 1990). However, the present results suggest some degree of interaction between granular and dysgranular cortex during cortical specification. Current evidence suggests that the areal specification of cortex occurs after neurogenesis within the forming cortex and not at the ventricular zone (Walsh and Cepko, 1992). We would hypothesize that specification processes resulting in the formation of granular and dysgranular cortices commence during the initial ingrowth of thalamic afferents and continue until the major patterns of cortical projections are formed.

For the sake of the argument that follows, we are assuming that dense patches of 5-HT immunoreactivity correlate with granular portions of primary somatosensory cortex and low levels of staining correlate with dysgranular cortex. In this context, it should be noted that in both the normal and experimentally manipulated rats, discrete portions of the representation are separated by gaps of low staining density (e.g., the gap between the representations of receptors in the upper and lower jaws). Given the reduction and expansion of discrete portions of the cortical representations and consequent shifts in the location of the gaps, our results imply that borders between these cortical areas are mutable and not intrinsically fixed.

We would *speculate* that dysgranular cortex with its relatively poor cytoarchitectonic differentiation represents something close to a default condition in terms of the formation of cortical areas. It is the type of cortex that forms in the absence of a strong extrinsic bias. On the other hand, the formation of granular somatosensory cortex with its greater degree of cytoarchitectonic differentiation involves more active processes and is dependent on interactions with peripheral inputs relayed through ascending projections. While there is considerable evidence that thalamic input plays a role in determining aspects of cortical organization (for reviews, see Rakic, 1988; O'Leary, 1989; Killackey, 1990), several recent reports are particularly pertinent to our speculation. First, Windrem and Finlay (1991) have reported that thalamic lesions in P-0 hamsters result in a marked loss of layer IV nonpyramidal neurons and extreme cytoarchitectonic alterations that made "it impossible to locate an area 17 (or O1) boundary in cresyl material." Second, Peinado and Katz (1990) report that the spiny stellate neurons that are the major contributor to the granular appearance of layer IV of rat somatosensory cortex do not undergo their normal remodeling process that restricts their dendrites to layer IV if the ION is sectioned at birth. Third, Schlaggar and O'Leary (1991) have reported that rat visual cortex transplanted to the somatosensory region develops the cytoarchitectonic specializations, barrels, characteristic of primary somatosensory cortex. All of these experiments suggest that thalamic input plays some role in the cytoarchitectonic differentiation that is characteristic of rat primary somatosensory cortex. Finally, early fetal bilateral enucleation in monkey not only reduces the area of striate cortex; it also results in the presence of an adjacent cortical area with cytoarchitectonic features that are considerably less differentiated than those of striate cortex (Dehay et al., 1991; Rakic et al., 1991). Assuming that this anomalous cortical area normally receives lateral geniculate input, one can interpret its less differentiated cytoarchitectonics as further evidence of a default condition in the formation of cortex that occurs in the absence of normal extrinsic factors.

Conclusion

From our point of view, information intrinsic to the neocortex is sufficient to guide the development of a six-layered structure, although further differentiation is clearly at least partially dependent on extrinsic factors. In the case of rat primary somatosensory cortex, the peripheral epithelial surface is clearly one such factor. A second may be thalamic afferents, per se, in addition to their role as a relay. These afferents reach cortex very early and could potentially influence it before peripheral factors (Catalano et al., 1991). In this vein, it is worth noting that the visual cortex and thalamocortical projections of the congenitally anophthalmic mouse are relatively normal although slightly reduced in size (Godement et al., 1977; Olavarria and Van Sluyters, 1984; Rhoades et al., 1984), suggesting that thalamocortical projections alone are sufficient for the formation of relatively normal cortical organization. However, additional research is needed to define more closely both the intrinsic and extrinsic factors which influence neocortical development.

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