Evidence for Prenatal Competition among the Central Arbors of Trigeminal Primary Afferent Neurons

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Previous studies have shown that damage to vibrissa follicles in newborn rats and mice does not alter the brainstem representations of the remaining vibrissa as demonstrated by staining for mitochondrial enzymes such as cytochrome oxidase (CO) succinic dehydrogenase. This study asked whether this lack of effect might be due to the fact that the trigeminal primary afferents in rodents are already quite well developed at birth. We assessed this possibility by using CO staining to evaluate patterns in the brainstems of pre- and postnatal rats. A vibrissa-related pattern began to emerge in trigeminal nucleus principalis and subnucleus interpolaris (Spl) by embryonic day (E-) 19 and appeared fully developed by the day of birth (P-0). We also made partial lesions of the vibrissa pad on E-15–20 and on P-0, killed pups on P-5–7, and measured the size of the CO-stained patches in Spl on both sides of the brainstem. The correspondence between CO patches and clusters of primary afferent terminal arbors was verified in some animals by combining transganglionic horseradish peroxidase tracing and CO staining. Vibrissa pad damage on E-15–18 resulted in significant (20.1–36.9%) increases in the average area of the remaining CO patches in Spl ipsilateral to the lesion. Vibrissa pad damage on E-19, E-20, and P-0 produced small (6.2–8.9%), but insignificant, increases in patch size in Spl ipsilateral to the lesion. We used anatomical and electrophysiological methods to determine whether our lesions altered the trigeminal innervation of surviving vibrissa follicles. We recorded single trigeminal ganglion cells from 12 rats that sustained vibrissa pad lesions on E-17. As in normal rats, all of the 49 vibrissa-sensitive ganglion cells isolated in the lesioned animals were responsive to deflection of one and only one vibrissa. We also dissected 11 deep vibrissal nerves from intact follicles in adult rats that sustained fetal vibrissa pad damage on E-17, and counted numbers of myelinated axons in 1 μ m plastic sections. These data were compared with counts from corresponding follicles on the intact side of the face. The average number of myelinated axons innervating follicles in the damaged vibrissa pads was 196.8 ± 27.9 , and that for the corresponding contralateral nerves was 194.6 ± 25.7 . These data suggest that competitive interactions among the central arbors of trigeminal primary afferents in fetal life may influence the development of central vibrissa repre-

sentations and, further, that lesion-induced central changes need not be correlated with alterations in the peripheral innervation of undamaged follicles.

Development of the mature size and location of the terminal arbors of axons innervating a given target is thought to involve interactions among these fibers in which they compete for some limited resource provided by that target. Such interactions are imputed when ablation or reduction in the activity of one set of afferents results in increased terminal arborizations of fibers that remain or retain normal patterns of activity (Sherman and Spear, 1982). Interaxonal competition has been indicated to occur in the development of the neuromuscular junction (Betz et al., 1980; Bennett et al., 1986), the primary afferent innervation of the skin (e.g., Scott, 1984; Smith and Frank, 1988), and both retinofugal (e.g., Lund and Lund, 1971; Rakic, 1981; Chalupa and Williams, 1984; Sretavan and Shatz, 1986; Coleman and Beazley, 1989) and geniculocortical (Wiesel and Hubel, 1963; Guillery, 1972a; LeVay et al., 1980) projections.

Existing data suggest that such interactions may not play a role in the trigeminal primary afferent innervation of the brainstem. A number of investigators have used cautery of subsets of vibrissa follicles to assay the effects of peripheral lesions upon trigeminal primary afferent development. Both Belford and Killackey (1980) and Durham and Woolsey (1984) employed this paradigm in conjunction with staining for succinic dehydrogenase (SDH) or cytochrome oxidase (CO) to demonstrate the patches corresponding (Bates and Killackey, 1985) to the trigeminal primary afferent innervation of the brainstem. Both of these studies produced the same result: there was no expansion of the terminal arbors of undamaged afferents into territory normally occupied by axons projecting to the damaged follicles. These results led both Belford and Killackey (1980) and Durham and Woolsey (1984) to conclude that competition was *not* likely to play a role in shaping the *postnatal* development of the primary afferent innervation of the trigeminal brainstem complex.

One important limitation of these experiments is that surgical manipulation of the afferent input to the brainstem was carried out only *after* birth. Both CO staining (Erzurumlu and Killackey, 1983) and transganglionic transport of horseradish peroxidase (HRP) (Jacquin and Rhoades, 1985) have shown that the trigeminal innervation of the brainstem has a relatively adultlike organization in newborn rats. It may thus be that the lesions carried out by Belford and Killackey (1980) and Durham and Woolsey (1984) were simply too late to demonstrate competitive interactions among trigeminal primary afferents innervating the whisker pad. Data from a number of systems have demonstrated that there are limited sensitive or critical periods after which

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experimental manipulations have relatively limited effects upon axonal development (compare the results of Chalupa and Williams, 1984, and Sretavan and Shatz, 1986, with those of Guilery, 1972b, with respect to the effects of pre- and postnatal enucleation upon the innervation of the LGNd by axons from the remaining eye).

In an effort to determine whether competitive interactions during prenatal development play a role in shaping the primary afferent innervation of the trigeminal brainstem complex, we made peripheral lesions in rats on embryonic days (E-) 15–20 and on the day of birth (P-0) and used CO staining and transganglionic HRP transport to determine whether or not such lesions altered the remaining trigeminal innervation of the brainstem near the end of the first postnatal week. In order to determine whether changes observed in brainstem were associated with alterations in the peripheral innervation patterns of surviving trigeminal ganglion cells, a small number of adult rats that sustained prenatal peripheral damage were used to assess the numbers of myelinated axons in follicle nerves and the receptive fields of individual trigeminal (V) ganglion cells.

Materials and Methods

Experimental animals. Ninety-eight perinatal and 15 adult Sprague-Dawley rats from 22 litters provided data for this study. Timed pregnancies were obtained by placing two females 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 this day was designated E-0.

Fetal follicle cauterization. Mystacial vibrissa follicles were cauterized in different litters on E-15–20 and on the day of birth (P-0). Fetal whisker follicle cauterizations were accomplished by anesthetizing pregnant females with ether, making a midline abdominal incision, and exteriorizing the uterine horns. The uterine horns were transilluminated with a fiber-optic light and viewed through a dissecting microscope. A parylene-coated tungsten microelectrode attached to the wand of an electrocautery device (Birtcher, model 732 Hyfrecator, 750 kHz spark gap oscillator) was inserted through the uterine wall and amniotic sac to contact the left vibrissa pad. The electrocautery was then activated for 2 sec. Following follicle cauterization of all embryos in a litter, the uterine horns were replaced and the abdominal incision in the dam was sutured closed. Manipulated pups were allowed to come to term. Animals used in the recording experiments and for counts of follicle nerves survived at least 45 d. All other animals were killed on P-5–7.

Cytochrome oxidase staining. All perinatal animals were anesthetized with ether and perfused transcardially with sodium phosphate-buffered saline (pH 7.4) followed by 4% paraformaldehyde in the same buffer. Brains were removed, and frozen 50 μ m coronal sections of the trigeminal brainstem complex were cut and collected in phosphate-buffered saline (PBS; pH 7.4). Free-floating sections were transferred into freshly prepared CO incubation medium that contained 75 mg of diaminobenzidine, 35 mg of cytochrome C (type III, Sigma), and 6 gm of sucrose in 130 ml of 0.1 M phosphate buffer (PB; pH 7.4) (Wong-Riley, 1979). The incubation solution was saturated for 5 min with 5% CO₂/95% O₂. The incubation was carried out at 37°C for 12–24 hr and arrested with several rinses in PBS when there was clear differentiation of "patches" in the brainstem. Stained sections were rinsed in PBS and plated out of gel-alcohol solution onto glass slides. Slides were allowed to air dry, dehydrated in 100% alcohol, cleared in xylene, and coverslipped with Permount.

In order to evaluate the normal development of the vibrissa-related CO pattern in the trigeminal brainstem complex, fetuses (at least four from each age) from rats E-15 through P-1 were harvested, anesthetized with ether, and perfused in the manner described above. The brainstems of these animals were processed for CO as described above.

Staining of damaged vibrissa pads. In order to assess the extent of the damage to the periphery, the facial skin from the lesioned animals was removed, dehydrated in graded alcohols, and embedded in paraffin. Fifteen micrometer sections were cut through the vibrissa pad parallel

to the skin surface. Alternate series of sections were stained with standard hematoxylin and eosin or a reduced silver stain for nerve fibers (Ungewitter, 1951).

Data analysis. Cytochrome oxidase-stained sections were examined with a Nikon Optiphot microscope equipped with a drawing tube. Sixty-six brains in which the vibrissae representations in the trigeminal brainstem complex could be completely and unambiguously reconstructed from serial sections were chosen for analysis. Areal analysis of the vibrissae-related cytochrome oxidase pattern was carried out in subnucleus interpolaris (SpI). Of the three representations of the vibrissae in the trigeminal brainstem complex, the pattern in this subnucleus is the easiest to discern due to its large volume and the high contrast between the patches and "interpatch" regions (Belford and Killackey, 1980).

Patch perimeters on both sides of the brainstem were drawn from six to eight serial sections through SpI. On the intact side, only the patches corresponding to the four most caudal vibrissae (1–4) in rows A–E were drawn. On the lesioned side, as many of these same patches as could be unambiguously identified also were drawn. Measurements of the cross-sectional areas of patches in these reconstructions were obtained with the aid of a graphics tablet. The mean cross-sectional area of CO patches (total area measured/number of patches measured) for each side was calculated. The percent difference between the average cross-sectional areas on the two sides was then determined.

The resultant values were then used in a one-way analysis of covariance, with age at the time of lesion as the independent variable and the number of patches on the side of the brainstem ipsilateral to the damaged whisker pad as the covariate. Post hoc evaluations of the percent changes in rats lesioned at a given embryonic age were made by means of Scheffe tests.

Because previous results from thalamus and cortex (Woolsey and Wann, 1976; Durham and Woolsey, 1984) indicated that patches in rows adjacent to the one that was damaged were more likely to increase in size than those in more distant rows, we carried out one additional analysis. Here, cases in which damage was restricted to one or two rows of vibrissae rather than a portion of the follicles in most or all rows were analyzed on a row-by-row basis. The results were then used to carry out a one-way analysis of variance, with the number of rows between the damaged row and the row analyzed as the independent variable. Post hoc comparisons here were also made by means of Scheffe tests. The level of statistical significance for all tests was set at $p < 0.05$.

To determine whether the changes observed in SpI were also apparent in the principal trigeminal sensory nucleus (PrV), measurements of CO-stained patches were carried out in 13 brains (from the above sample) in which the pattern in this nucleus was clear.

Horseradish peroxidase labeling of V primary afferents. In order to determine the relationship between patches of CO reactivity and trigeminal primary afferents in rats that sustained fetal follicle cauterizations, these axons were directly labeled in five rats aged P-5 that sustained follicle damage on E-16. Six injections (1 μ l each) of an HRP cocktail containing 6% wheat germ agglutinin-conjugated HRP (Sigma), 0.5% cholera toxin HRP (List Biological Lab), and 10% free HRP (Sigma type VI) were made throughout the lesioned whisker pad using a pipette with a 30–50 μ m tip. Following a 48 hr survival time, animals were anesthetized with ether and perfused transcardially with physiological saline, a fixative solution containing 2% paraformaldehyde, and 1.5% glutaraldehyde in sodium phosphate buffer, and a 10% buffered sucrose rinse. Brains were removed and stored overnight at 4°C in 10% buffered sucrose. Coronal sections through the brainstem were cut at 50 μ m on a freezing microtome. Alternate sections were processed for HRP reaction product according to the method of Mesulam (1978) or for CO reactivity using the methods described above.

Recording from V ganglion cells. Twelve adult rats that sustained fetal follicle cauterizations were anesthetized with sodium pentobarbital (60 mg/kg, i.p., with 0.1 ml of 1.5% atropine sulfate), the trachea was cannulated, heart rate recording leads were fastened to the chest, and the rat was then placed in a stereotaxic apparatus. Following a midline incision in the overlying skin, the dorsal calvarium was removed from the side ipsilateral to the damaged whisker pad, the forebrain aspirated and the trigeminal ganglion exposed. Wound edges were infiltrated with a long-lasting local anesthetic (Nupercaine, CIBA). The rat was then paralyzed with 10 mg of gallamine triethiodide, and artificial respiration was initiated. Anesthesia and paralysis were maintained with hourly doses of sodium pentobarbital and gallamine triethiodide (10 mg and 4 mg, respectively). Body temperature was held at $37 \pm 1^\circ\text{C}$ with a feedback-controlled heating blanket.

Figure 1. CO-stained sections through PrV and Spl of rats killed on E-18 through P-0. On E-18 (*A* and *B*), there is dense CO staining in PrV and Spl, but no pattern is apparent. On E-19 (*C* and *D*), segmentation is apparent within the dense staining in PrV and Spl, but the pattern cannot be related to the pattern of the vibrissae. On E-20 (*E* and *F*), the pattern of CO staining is somewhat more distinct, and by P-0 (*G* and *H*), the pattern observed in older perinatal rats is clearly present. Scale bar, 100 μ m for all panels.

The effectiveness of this anesthetic regimen was evaluated in two ways. First, it was used in rats that were not paralyzed. Such animals lie quietly and are behaviorally unresponsive to external stimuli for at least 1 hr after discontinuance of sodium pentobarbital. Second, rats were allowed to recover from paralysis during the recording experiments, and it was determined that anesthesia was still sufficient to prevent responses to mechanical stimulation.

Tungsten electrodes (impedance, 20–25 M Ω) were visually positioned over the ophthalmic/maxillary portion of the ganglion. The electrodes were slowly advanced through the ganglion with a hydraulic microdrive, and extracellular recordings were amplified and displayed using standard methods. Stimulation was delivered to the whisker pad with hand-held probes using previously described methods (Jacquin et al., 1986). The response properties of each well-isolated unit were determined, and the receptive field was carefully mapped. Particular attention was paid to vibrissa-sensitive units. For these, we determined both the total number of whiskers to which the unit responded and whether or not skin or surrounding guard hairs were included in the receptive field.

The extent of the follicle damage in each of these rats was determined by histological processing of the vibrissa pad using the methods described above and CO staining of the cortex contralateral to the damaged side of the face. The vibrissa-related CO pattern is difficult to see in the brainstems of adult rats, but the pattern remains quite apparent in CO-stained sections through cortical lamina IV.

Counts of myelinated axons in nerves supplying spared follicles. Three adult rats that sustained vibrissa pad lesions on E-17 were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and perfused transcardially

with a washout solution (0.1 M cacodylate buffer containing 0.4% xylocaine and 0.4% heparin, pH 7.4) followed by a buffered fixative solution (3% glutaraldehyde and 3% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.4). Brains were removed, and 50- μ m-thick frozen sections of flattened cortex were cut and reacted for the demonstration of CO in the manner described above. Spared individual vibrissa follicles corresponding to enlarged CO cortical patches as well as the contralateral normal follicles were dissected from the mystacial pad, rinsed in cacodylate buffer, postfixed for 2 hr in 1.5% osmium tetroxide and 1.5% potassium ferricyanide in 0.1 M cacodylate buffer (pH 7.4), dehydrated in a graded series of ethanols, cleared in propylene oxide, and embedded in an Epon-Araldite mixture. Blocks were oriented so that the deep vibrissa nerve was cut perpendicular to its longitudinal axis at the level where the nerve entered the follicle capsule. One-micron-thick sections were cut and stained with toluidine blue. Myelinated axons were counted with the light microscope at 400 $\times$. Each nerve bundle was counted three times, and the modal result was taken as the final fiber count.

Results

Normal development of the vibrissa-related CO pattern in the trigeminal brainstem complex

The earliest age at which any pattern could be discerned in the CO staining of the sections through either PrV or Spl was E-19 (Fig. 1*C,D*). While the pattern at this age could not be related to the vibrissa on a one-for-one basis, there was segmentation

Figure 2. *A* and *B* show the damaged and normal vibrissa pads (hematoxylin and eosin stain) of a rat that sustained a lesion on E-15 and was killed on P-7. On the lesioned side, only rows *D* and *E* remain. All of these follicles were innervated. *C* and *D*, and *E* and *F* show CO-stained sections through PrV and Spl, respectively. The insets are low-power photographs showing both sides of the same brainstem section. Note that only two distinct rows of dense patches of CO reactivity remain in PrV and Spl ipsilateral to the lesion. The arrows on the sections through the vibrissa pads (*A* and *B*) point to dorsal (*D*) and anterior (*A*). Scale bar, 200 μ m for all panels.

Figure 3. *A* and *B* show the damaged and normal vibrissa pads (hematoxylin and eosin stain) of a rat that sustained a lesion on E-16 and was killed on P-5. On the lesioned side, rows *A*, *B*, and *E* are intact, as are the two most caudal follicles in row *C* and the D1 follicle. The small rostral follicles in row *D* (arrows) were also innervated. *C* and *D*, and *E* and *F* show CO-stained sections through PrV and SpI, respectively. The insets are low-power photographs showing both sides of the same brainstem section. Note the correspondence between the pattern of innervated follicles on the face and the patches of CO reactivity in the ipsilateral brainstem. The arrows on the sections through the vibrissa pads (*A* and *B*) point to dorsal (*D*) and anterior (*A*). Scale bar, 200 μ m for all panels.

in both PrV and SpI. The segmentation within both of these nuclei became more distinct on E-20 (Fig. 1*E,F*) and appeared fully developed by P-0 (Fig. 1*G,H*).

Effects of fetal vibrissa follicle damage upon CO staining patterns in the V brainstem complex

Electrocautery of a subset of vibrissa follicles on E-15–18 resulted in significant increases in the average size of the remaining patches of high CO density in the brainstem ipsilateral to the damaged vibrissa pad. Such increases were observed in both PrV and SpI, but for the technical reasons discussed above (Materials and Methods) only SpI was evaluated in most cases. The age at which rats were killed (P-5, P-6, or P-7) had no significant effect upon results, so all data from animals that sustained vibrissa pad damage on a given embryonic day were pooled for analysis.

Figure 2 shows results typical of those obtained from rats that sustained follicle cautery between E-15 and E-18. In this case, the lesion produced a loss of all the follicles in rows A–C (Fig. 2*B*) and there was an absence of the corresponding patches of high CO reactivity in both PrV (Fig. 2*D*) and SpI (Fig. 2*F*). Measurements in PrV and SpI showed increases of 35% and 44%, respectively, in the average cross-sectional area of the patches that remained on the lesioned side, relative to the large vibrissa-related patches on the intact side.

In many cases (Fig. 3), the damage to the vibrissa pad was less extensive than that illustrated in Figure 2. In these rats, many more follicles remained in the damaged vibrissa pad (Fig. 3*B*) and the reorganization in the brainstem was much less dramatic (Fig. 3*C–F*). For the case shown, the increases in average patch size in PrV and SpI ipsilateral to the lesion were 21% and 18%, respectively.

Further examples of the lesion-induced alterations in the CO staining pattern in SpI are shown in Figure 4, and the results from all ($N = 66$) of the cases included in the report are summarized in Figures 5 and 6 and in Table 1. The data presented support several conclusions. First (Fig. 5*A*), cautery of vibrissa follicles on E-15–18, but not at later ages, resulted in significant increases in the average cross-sectional area of the patches of high CO activity that remained in SpI ipsilateral to the damaged vibrissa pad (analysis of covariance: $F = 14.8$; $df = 6,58$; $p < 0.0001$; post hoc Scheffé tests all $p < 0.05$). Second, for the E-15–18 animals, there was a significant negative correlation ($r = -0.65$; $p < 0.01$) between the number of patches that remained in SpI ipsilateral to the lesion and the increase in average patch size. Scatter plots illustrating the correlations for each of these ages are presented in Figure 6. The correlation for the animals that sustained lesions on E-15 (Fig. 6*A*) was -0.82 but was not statistically significant because of the small number of cases in this group. The correlation for the rats lesioned on E-16 (Fig. 6*B*) was -0.76 ($p < 0.05$), that for the rats that sustained lesions on E-17 (Fig. 6*C*) was -0.70 ($p < 0.05$), and that for the rats lesioned on E-18 (Fig. 6*D*) was -0.43 (not significant). Importantly, there was no significant correlation between the age at which lesions were sustained and the number of patches that remained in PrV and SpI ipsilateral to the damaged vibrissa

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Figure 4. CO-stained sections through SpI from five different rats (*A–E*) that sustained damage to the vibrissa pad during fetal life. Note the reduced number and increased size of the patches on the deafferented side of the brainstem. Scale bar, 500 μ m for all panels.

Figure 5. *A* shows the increase (mean $\pm$ SEM) in average CO patch size in SpI of rats killed on P-6 or P-7 that sustained vibrissa pad damage on either E-15–20 or P-0. The increases in the animals that sustained lesions on E-15–18 were statistically significant, and those for the rats lesioned on E-19, E-20, and P-0 were not (analysis of covariance; $F = 14.8$; $df = 6,58$; $p < 0.00001$; post hoc Scheffé tests, all $p < 0.05$). The numbers of animals in each group are listed in Table 1. *B* shows the average increases (mean $\pm$ SEM) in CO patch size for 14 cases in which measurements were made in both PrV and SpI. There was no significant difference between the average increases in these two nuclei. *C* shows

pad. The increases in average patch size and average number of patches remaining ipsilateral to the lesion for each age group are summarized in Table 1. Finally, for the smaller number ($N = 14$) of animals in which measurements were made in both PrV and SpI, the average increases in the two nuclei were in very close agreement (Fig. 5*B*). The average increase (mean $\pm$ SD) in PrV was $29.7 \pm 14.4\%$, and that for SpI was $28.0 \pm 15.3\%$.

Experiments that have examined the effects of *neonatal* vibrissa follicle cauterization upon the representation of the vibrissa pad in thalamus and cortex (Durham and Woolsey, 1984) have shown that the central representations of undamaged follicles *adjacent* to, but not those more distant from, damaged follicles increase in size. In many of the cases we analyzed, the damage to the vibrissa pad was sufficiently extensive so that the “identity” of the remaining follicles was not certain. However, in other cases (e.g., Fig. 2), the identity of the follicles ablated was readily apparent. We used these cases to evaluate the effect of distance between remaining follicles and the increase in cross-sectional areas of their central representations in SpI after damage to the vibrissa pad *in utero*.

We analyzed 17 cases from ages E-15 to E-18 in which damage was limited to one or two rows. We collapsed the data across these fetal ages and then related the average percentage increase in patch cross-sectional area with the “distance” between the row analyzed and that removed (Fig. 5*C*). As is evident from the figure, there was a significant negative relationship between distance and the percentage increase observed ($F = 3.6$; $df = 2,35$; $p < 0.05$). Because of this result, we reanalyzed the results from the animals lesioned on E-19, E-20, and P-0 with follicle ablations that were confined to one or two rows. The data from these rats were pooled in the manner described immediately above. In these animals, even the CO-stained patches adjacent to the damaged row or rows did not show significant increases in average cross-sectional area (Fig. 5*C*).

Relationship between CO-stained patches and primary afferent axons

Bates and Killackey (1985) have demonstrated clearly that the patches of high CO reactivity in the trigeminal brainstem complex of both normal perinatal rats and animals that sustained neonatal damage to vibrissa follicles have an exact correspondence with the distribution of primary afferent axon terminals demonstrated by anterograde transport of HRP. We used transganglionic HRP transport to show that this was also the case in rats that sustained follicle cautery *in utero*. Figure 7 shows adjacent sections through both PrV and SpI processed for HRP reaction product (Fig. 7*A,C*) and CO reactivity (Fig. 7*B,D*). Note the exact correspondence between the patches of primary afferent labeling and CO reactivity.

Response properties of vibrissa-sensitive V ganglion cells in adult rats that sustained fetal cautery of vibrissa follicles

We recorded a total of 49 vibrissa-sensitive trigeminal ganglion cells from 12 rats that sustained cautery of vibrissa follicles on

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the results (mean $\pm$ SEM) of the “neighbor” analysis (see Results) for animals lesioned on E-15–18 (solid bars) and animals that sustained vibrissa pad damage on E-19, E-20, or P-0. There was a significant negative relationship between distance and the percentage increase observed for the former group ($F = 3.6$; $df = 2,35$; $p < 0.05$), but no significant increases were observed for the latter animals.

Figure 6. Scatter plots showing the relationships between number of patches remaining in SpI ipsilateral to the damaged vibrissa pad and the mean increase in average patch size for rats lesioned on E-15–18. The correlation for the animals that sustained lesions on E-15 (*A*) was -0.82 but was not statistically significant due to the relatively small sample size. The correlation for the rats lesioned on E-16 (*B*) was -0.76 ($p < 0.05$), that for the rats that sustained lesions on E-17 (*C*) was -0.70 ($p < 0.05$), and that for the rats lesioned on E-18 (*D*) was -0.43 (not significant).

E-17 and survived to adulthood (>45 d of age). All of these neurons were sensitive to deflection of only one mystacial vibrissa (e.g., Fig. 8*A–C*). Furthermore, none were activated by stimulation of either the guard hairs between vibrissa follicles or the skin of the vibrissa pad. All of the animals used in these studies had fetal lesions that were verified in two ways. First, all had clear abnormalities in the number and distribution of follicles on the vibrissa pad (e.g., Fig. 8*D*) and all had altered cortical CO patterns corresponding to the vibrissae (Figs. 8*E*, 9). These data are thus consistent with the proposal that the increased size of the representations in PrV and SpI of the vibrissa follicles that remained after fetal lesions was not dependent upon increases in the peripheral receptive fields of vibrissa-sensitive V ganglion cells.

Effects of fetal vibrissa pad damage upon numbers of fibers in follicle nerves

We counted the fibers in 11 deep vibrissal nerves from follicles that corresponded to enlarged CO cortical patches, and their

Table 1. Numbers of animals, average increases in CO patch size in SpI, and average number of patches remaining on the side ipsilateral to the lesion

Age	N	Average increase in patch size	Number of patches remaining
E-15	5	27.6 ± 11.3	12.6 ± 2.8
E-16	7	20.1 ± 7.2	13.1 ± 2.2
E-17	10	36.9 ± 13.7	8.9 ± 3.6
E-18	16	29.4 ± 10.4	11.2 ± 4.1
E-19	5	8.6 ± 16.1	9.4 ± 3.4
E-20	10	8.9 ± 16.5	12.4 ± 2.7
P-0	13	6.2 ± 7.6	11.2 ± 3.4

Note that maximum possible number of patches (i.e., those measured on the normal side) is 20. Error terms indicate SD.

Figure 7. Results from two different cases in which HRP transport was combined with CO staining. *A* and *B* show HRP reaction product and CO staining, respectively, in PrV of one rat. Note the correspondence between the two patterns. *C* and *D* show data from Spl from another rat. Again, note the correspondence between the patches of HRP labeling (*C*) and CO reactivity (*D*). Scale bar, 200 μ m for all panels.

contralateral normal counterparts. Examples of material used to make the counts are provided in Figure 10, and all counts and the sizes of the corresponding patches of CO reactivity are shown in Figure 11. The average number of myelinated fibers in the nerves of a follicle with an enlarged patch (196.8 ± 27.9) was not significantly different from that of its corresponding

contralateral normal follicle (194.6 ± 25.7) ($p > 0.05$). The average size of the patches of CO reactivity in the cortex contralateral to the lesions in these animals was 0.25 ± 0.07 mm². The average for the cortical CO patches corresponding to the control nerves was 0.16 ± 0.02 mm² ($t = 4.51$; $df = 10$; $p < 0.005$; paired t test).

Figure 8. *A* shows oscillographs from a trigeminal ganglion cell recorded from an adult rat that sustained vibrissa pad damage on E-17. The cell had a low (<1 Hz) spontaneous firing rate documented by the trace shown in *A*, and it responded (*B*) only to stimulation of the vibrissa indicated by the solid arrow in the photograph of the vibrissa pad (*D*). Stimulation of an adjacent vibrissa (*C*, open arrow in *D*) excited another ganglion cell. Only seven large follicles remained on the damaged vibrissa pad of this animal (*D*), and reconstruction of the pattern of CO staining in lamina IV of the contralateral cortex reflected this same pattern (*E*). The calibration for *A–C* is 1 mV and 1 sec. The lines under the traces in *B* and *C* indicate the duration of the stimulation. Scale bar in *E*, 2 mm; the arrows point toward anterior (*A*) and lateral (*L*).

Discussion

The results described in this article support the conclusion that competitive interactions among vibrissa-related V primary afferent axons during prenatal development play a substantial role in shaping the central terminal fields of these axons. Using essentially the same assay employed by Belford and Killackey (1980) and Durham and Woolsey (1984), the present study demonstrated that cauterization of vibrissa follicles prior to E-19 resulted in significant increases in the CO patches corresponding to the remaining follicles.

Lesion-induced primary afferent reorganization

There is considerable evidence that undamaged primary afferent axons in perinatal rodents can expand their terminal fields after peripheral nerve lesions and, further, that there is a limited sensitive or critical period after which such lesions no longer produce reorganization of the central axons of undamaged sensory ganglion cells.

Rhoades et al. (1989) transected the infraorbital nerve (ION, the V branch innervating the vibrissa follicles) in rats on E-16 and used transganglionic tracing with HRP when these animals reached adulthood to demonstrate expanded central territories for undamaged mandibular axons. Similar lesions in newborn rats produced no alterations in the central terminal distributions of undamaged mandibular V branches (Jacquin and Rhoades, 1985). Rhoades et al. (1983) showed that transection of the ION in newborn hamsters did result in expansion of the terminal fields of undamaged mandibular primary afferents. The hamster is born after a 16 d gestation period (that for the rat is 21 d), and the V system may be less mature in newborn animals of this species. Accordingly, Jacquin and Rhoades (1987) transected the ION in 5-d-old hamsters and found that mandibular sensory afferents remained restricted to their normal territories. Fitzgerald (1985) and Fitzgerald and Vrbová (1985) have reported expansion of the central terminal field of the saphenous nerve after neonatal sciatic nerve damage in rat. The fact that postnatal peripheral nerve lesions in the rat result in primary

afferent reorganization in the spinal cord, but not the V brainstem complex, may reflect the generally rostrocaudal gradient of CNS development (see Ayer-LeLievre and Seiger, 1984, for additional discussion).

Experiments in nonmammalian vertebrates have also demonstrated that peripheral damage during early development can alter the central terminal fields of undamaged axons. In general, the manipulation used in these experiments has been ablation of selected dorsal root ganglia rather than transection of peripheral nerves. Frank and his coworkers (Frank and Westfield, 1982; Smith and Frank, 1988; Mendelson and Frank, 1989; Frank and Mendelson, 1990) have shown that deletions of dorsal root ganglia result in altered central terminal fields by undamaged ganglion cells.

Do the larger patches of CO reactivity represent enlarged central terminal fields by vibrissa-related primary afferents?

The present results and the previous findings of Bates and Killackey (1985) provide strong support for the conclusion that metabolic stains such as CO and SDH provide a very accurate representation of the clustered terminal arbors of vibrissa-related trigeminal primary afferent terminations. However, since CO staining is an indirect method of demonstrating the size and location of these terminal arbors, another potential interpretation of the present results must be considered.

The effect observed in the brainstem might reflect a reduction in the overlap of the central arbors of V axons innervating a given follicle. Indeed, Jensen and Killackey (1987) suggested that one of the consequences of neonatal ION damage upon individual thalamocortical axons innervating the rat's somatosensory posteromedial barrel subfield was a reduction in the overlap of fibers innervating a given small portion of the cortex. The results of Shatz and her coworkers (Stretavan and Shatz, 1986; Garraghty et al., 1988; Roe et al., 1989) suggest an analogous result in the LGNd after removal of one eye in fetal cats. There is an expansion of the terminal arbors of Y-cell axons, but normally sized X-axons are sometimes located in inappropriate parts of the LGNd.

Two aspects of the present data suggest that a reduction in the overlap of the central arbors of primary afferents innervating a given vibrissa follicle is not a likely explanation of the changes observed in the brainstem. First, if overlap of fibers innervating a given vibrissa was reduced, it would likely follow that the segmented patterns of both CO reactivity and HRP-labeled primary afferent terminals would be degraded (i.e., blurred). This is, in fact, what occurs in cortex after neonatal ION transection (Jensen and Killackey, 1987).

The patterns observed with both CO staining and HRP labeling appeared as well defined in the experimental animals as in normal rats. We thus believe that CO staining in animals with fetal vibrissa follicle damage, as in normal animals, provides an accurate representation of the clustering of primary afferent axons in the trigeminal brainstem complex.

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Figure 9. Reconstructions of vibrissa-related CO patterns in a control cortex (*D*) and in cortices contralateral to the damaged vibrissa pads of three rats that were used in the electrophysiological experiments (*A–C*). The arrows in the reconstruction in *D* point toward anterior (*A*) and lateral (*L*), and they also apply to all other panels. Scale bar, 2 mm for all panels.

Figure 10. Photomicrographs of the follicle nerves innervating the C2 vibrissa follicle on the intact (*A*) and damaged (*B*) sides of the face. The nerve on the intact side contained 199 myelinated axons, and that on the damaged side contained 206 myelinated fibers. Scale bar, 50 μ m for both panels.

Does the expansion of the central terminations of undamaged primary afferents necessarily reflect interaxonal competition?

Competitive interactions among the axons innervating a given target are usually imputed when removal (Scott, 1984; Mendelson and Frank, 1989) or reduction in the activity (Guillery, 1972a; Sherman and Spear, 1982) of one subset of afferents results in an increase in the terminal arborizations of fibers that remain or retain normal patterns of activity. The underlying assumption in the interpretation of such results as reflecting competition is that subpopulations of afferent axons compete for some limited commodity at the target (e.g., trophic substance, synaptic sites). We believe that the present results reflect such competitive interactions among the central axons of vi-

brissa-related trigeminal primary afferents, but there are other possible interpretations of our findings.

Smith and Frank (1988) have interpreted the central primary afferent reorganization that follows neural crest lesions in bullfrog to reflect a process that they have referred to as "respecification." They have suggested that the central primary afferent reorganization that follows dorsal root ganglion ablation in bullfrog tadpoles may be a secondary result of *peripheral* axonal sprouting by these same ganglion cells. Changes in the location of the central arbors of these axons may simply reflect their new peripheral targets. Most recently, Mendelson and Frank (1989) demonstrated that removal of the brachial dorsal root ganglion in developing frogs resulted in a small number of neurons in the adjacent dorsal root ganglion "sprouting" into the dener-

Figure 11. Counts of myelinated nerve fibers from 11 follicle nerves from damaged vibrissa pads (*right column*) and matched nerves from the intact vibrissa pads. The nerves counted are designated in the *left column*. The closed contours surrounding each nerve count are drawings of the CO-stained patches corresponding to the follicle in question in

vated triceps muscle and corresponding part of the spinal cord. Importantly, their results indicated that these primary afferents did not develop abnormally large central arbors, but rather central terminations that were appropriate with respect to their new peripheral target. Thus, while competition among primary afferent axons may play a role in the development of primary afferent projections to the periphery (Scott, 1984; Mendelson and Frank, 1989), it need not be imputed in either their normal central development or their lesion-induced reorganization.

It is unlikely that the central changes observed in the present experiment reflect peripheral respecification of the type described above. All of the vibrissa-sensitive ganglion cells recorded in the fetally lesioned rats responded to deflection of only one whisker. This is also the case in normal adult animals (Zucker and Welker, 1969; Jacquin et al., 1986). It is also worth noting the sizes of the peripheral receptive fields of V ganglion cells appear mature at, or prior to, the time at which most of the lesions in this study were carried out. Chiaia et al. (1990) recorded from V ganglion cells in rats as young as E-16. Nearly all of the primary afferents that could be excited by stimulation of a vibrissa follicle at this age had receptive fields restricted to that follicle.

The observation that fetal cauterization of vibrissa follicles did not lead to the development of abnormal receptive fields by the vibrissa-sensitive axons that remained may seem somewhat surprising in view of the fact that both fetal (Chiaia et al., 1989) and neonatal transection of the ION (Jacquin et al., 1986; Rhoades et al., 1987; Chiaia et al., 1988) does result in the development of abnormal peripheral projections by undamaged neurons. However, a major difference between those studies and the present report is that damage in this study was restricted to the periphery rather than to the entire ION. The abnormal receptive fields that developed in those studies most likely occurred as a result of misrouting of regenerate axons and invasion of denervated peripheral territory by undamaged axons from other V branches (Rhoades et al., 1987). The fact that the periphery was damaged directly in the present study probably reduced the likelihood of peripheral sprouting by undamaged axons. Furthermore, regenerate axons may have been inhibited from innervating intact vibrissa follicles because those that remained were already supplied by their normal complement of V axons. The suggestion is supported by the result that undamaged follicles on the damaged vibrissa pad were not innervated by an abnormal number of deep vibrissal nerve fibers.

Does the critical period for central reorganization of vibrissa-related primary afferents depend upon overlap of these fibers at the time of our lesions?

The present results suggest a strong temporal correspondence between the development of segregation of the central terminal

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the contralateral cortex. The average number (mean $\pm$ SD) of myelinated fibers from the follicles in the damaged vibrissa pads was 196.8 ± 27.9 , and that for the corresponding contralateral nerves 194.6 ± 25.7 ($p > 0.05$). The average size of the cortical patches of CO reactivity corresponding to the nerves counted in the lesioned vibrissa pads was $0.25 \pm 0.07 \text{ mm}^2$. The average for the cortical CO patches corresponding to the control nerves was $0.16 \pm 0.02 \text{ mm}^2$ ($t = 4.51$; $df = 10$, $p < 0.005$, paired t test).

arbors of primary afferents innervating different vibrissa follicles and time during which damage to some primary afferents results in increased terminal fields for those that remain. While a vibrissa-related pattern was not fully developed until E-20, some segmentation could be seen in animals killed on E-19. Lesions made later than E-18 failed to produce a significant increase in the size of the CO patches that remained in these animals. This result raises the possibility that some overlap of axon arbors may provide the necessary substrate for interaxonal competition in this system.

It is important to note that overlap between populations of afferent axons is *not* a necessary condition for competition of these populations. This was demonstrated quite clearly by LeVay et al. (1980), who showed that monocular deprivation of a monkey beginning at 5½ weeks of age resulted in a substantial expansion of the ocular dominance columns related to the non-deprived eye. At 5½ weeks of age, ocular dominance columns in monkey show a nearly adult degree of segregation.

There is also evidence that overlap of terminal fields is not a necessary condition for alterations in the central arbors of undamaged primary afferents after peripheral lesions. Neonatal ION transections in hamsters result in an increase in the terminal zone of undamaged mandibular primary afferents (Rhoades et al., 1983). Transganglionic tracing in newborn hamsters indicated no central overlap between mandibular and ION fibers (Jacquin and Rhoades, 1985). Similarly, expansion of the central terminal field of the axons that supply the auriculotemporal sinus hair occurs after postnatal ION lesions (Waite, 1990). By birth, the CO patch related to this whisker is clearly segregated from those of the mystacial vibrissae.

All of the results described in the preceding paragraph were obtained in preparations where large portions of the periphery were denervated. Such damage has been shown repeatedly to result in substantial reorganization of the peripheral processes of primary afferent neurons (e.g., Frank and Westerfield, 1982; Jackson and Diamond, 1984; Scott, 1984; Kinnman and Aldskogius, 1986; Rhoades et al., 1987) that may in turn lead to respecification of the central arbors of these cells (Smith and Frank, 1988). It thus seems likely that the results of these experiments may not be directly comparable to those of the present study.

One other caveat should be raised with respect to the relationship between the development of vibrissa-related segregation in the brainstem as reflected by CO staining and the sensitive period for lesion-induced increases in the central arbors of undamaged primary afferents. There may be a substantial delay between the time when vibrissa-related primary afferents develop a segmented pattern and that when a corresponding CO pattern emerges in the brainstem. This is certainly the case with respect to the development of thalamocortical afferents and SDH patterns in the rodent somatosensory cortex (compare the results of Erzurumlu and Jhaveri, 1990, with those of Killackey and Belford, 1979), and preliminary results (Crissman et al., 1989; R. W. Rhoades, unpublished observations) indicate that this is also true in the brainstem.

In summary, the present results indicate a temporal correlation between the normal development of vibrissa-related segmentation in the brainstem and the ability of peripheral lesions to alter the size of the terminal arbors of undamaged afferents. This correlation and its potential implications must be viewed with caution until normal trigeminal primary afferent development is described with direct axonal tracing techniques.

Summary

The present results support the conclusion that interaxonal competition in the brainstem plays an important role in shaping the terminal arbors of these fibers. There is a period during prenatal development when damage to primary afferents leads to reorganization (probably increases in size) of undamaged axons that is independent of any change in the peripheral innervation patterns of these fibers. Finally, the period when such effects can be induced has at least a rough correspondence with that during which the central arbors of axons innervating different vibrissa follicles may overlap in the brainstem.

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