Lesion-induced reorganization in the brainstem is not completely expressed in somatosensory cortex

(cuneate nucleus/sciatic nerve/horseradish peroxidase/brachial plexus/single cell recording)

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Communicated by James M. Sprague, University of Pennsylvania Medical Center, Philadelphia, PA, February 2, 1995

ABSTRACT Electrophysiological and neuroanatomical methods were used to determine the extent to which neonatal forelimb removal altered the organization of the cuneate nucleus and representations of the fore- and hindlimbs in the primary somatosensory cortex of adult rats. Neonatal forelimb removal resulted in invasion of the cuneate nucleus by sciatic nerve primary afferents and development of cuneothalamic projection neurons with split receptive fields that included both the hindlimb and forelimb stump. Mapping in the primary somatosensory cortex of the neonatally manipulated adult rats demonstrated abnormalities, but the major change observed in the cuneate nucleus was demonstrable at only a few (5%) cortical recording sites in the remaining stump representation and there were none at all in the hindlimb representation. These results suggest that lesion-induced brainstem reorganization may be functionally suppressed at either the thalamic or cortical level.

Understanding the mechanisms that underlie central nervous system reorganization following peripheral nerve damage remains a major goal of neurobiology (see refs. 1 and 2 for recent reviews). Functional reorganization following peripheral nerve damage in infancy or adulthood, while quite variable, has been well documented in the somatosensory cortex (1, 2). Similarly, evidence for functional reorganization at subcortical somatosensory stations has been reported (3–8). However, relatively few studies have examined the consequences of peripheral nerve damage at more than one level of the neural axis. Such an approach is a necessity if we are to fully define both the range of changes that occur in the central nervous system and the relation between alterations at different levels of the neural axis.

We have examined the consequences of neonatal forelimb removal in both the brainstem and the neocortex of the adult rat in order to compare and contrast the results of this manipulation at the two levels. We chose to examine the brainstem because this is the first synapse in the ascending somatic lemniscal system and the first place where afferents arising from two different peripheral locations (forelimb and hindlimb) terminate in close proximity, in the nuclei cuneatus (Cu) and gracilis (Gr), respectively. We chose primary somatosensory cortex because of the well-studied and readily assessable map of the entire body surface that characterizes this structure.

MATERIALS AND METHODS

Neonatal Forelimb Removals. Pups >12 h old were anesthetized by hypothermia until immobile. The left forelimb was amputated with iridectomy scissors below the shoulder and the

brachial artery was sealed by electrocautery. The stump was infiltrated with local anesthetic (0.7% bupivacaine) and the skin was closed with cyanoacrylate adhesive. The pups were rewarmed and returned to their mothers.

Tracing of Sciatic Nerve Projections. Eight adult rats that sustained neonatal forelimb amputation and six normal animals were anesthetized with a combination of ketamine (100 mg/kg) and xylazine (20 mg/kg) and the sciatic nerve was labeled with a mixture of wheat germ agglutinin- and cholera toxin-conjugated horseradish peroxidase (HRP) as described (9). After a survival period of 3–4 days, rats were killed with an overdose of sodium pentobarbital (80 mg/kg) and perfused transcardially with physiological saline followed by a solution containing 1% paraformaldehyde and 2.5% glutaraldehyde in phosphate buffer (pH 7.4). The brainstem and spinal cord were removed and sectioned, and HRP-labeled axons were demonstrated according to the protocol of Mesulam (10). Alternate sections were processed for cytochrome oxidase histochemistry according to the method of Wong-Riley (11).

Recordings from the Dorsal Column Nuclei. Rats were anesthetized as described above and prepared for recording from the dorsal column nuclei as described (9). Briefly, the trachea was cannulated and the brachial plexus and sciatic nerve were exposed. The rats were placed in a stereotaxic head holder and ventilated mechanically, and bipolar hook electrodes were positioned. The brachial stimulating electrode was placed just proximal to the origin of the median, ulnar, and radial nerves and the sciatic nerve electrode was placed ≈1.5 cm distal to the sciatic notch. The rats were paralyzed with gallamine triethiodide (30 mg, delivered i.p.) and maintained in a state of paralysis and light anesthesia for the duration of the recording session with periodic injections of gallamine triethiodide (20 mg, i.p.) and urethane (200 mg, i.p.). A concentric bipolar stimulating electrode was inserted into the ventral posterolateral thalamus and its position was confirmed by recording multiple unit activity.

The medulla was exposed from a point ≈1 mm rostral to the obex to the first cervical vertebra. The brainstem surface was photographed at ×44 magnification to provide a map for recording microelectrode placements and was then covered with warm silicone fluid. Single units were recorded with micropipettes (20–60 MΩ) filled with 3 M KCl saturated with fast green FCF. Cutaneous receptive fields were defined with tactile stimuli delivered with brushes and blunt probes. In addition to cutaneous responses, responses to stimulation of deeper tissues were also noted. Regions of the skin from which responses were evoked were plotted on body surface drawings.

Conduction velocities of the afferent inputs to the Cu neurons were determined from measurements of the response latency to electrical stimulation (0.1-ms pulses ranging from 4

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Abbreviations: Cu, cuneate nucleus; Gr, gracile nucleus; HRP, horseradish peroxidase; acv, average conduction velocity.

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to 12 V) and the anatomical lengths of the afferent pathways were measured at autopsy. The transsynaptic nature of responses to electrical stimulation was determined by their inability to follow 100-Hz trains of stimuli.

Thalamic projecting Cu neurons were identified by secure responses to high-frequency electrical stimulation and collision between evoked orthodromic and antidromic spikes (12).

To confirm the position of recorded neurons in the Cu, several electrode tracks in each experiment were marked by dye ejection or electrolytic lesions made with metal electrodes at sites of unit recordings made with micropipettes. At the end of each recording session, the rat was given a lethal dose of sodium pentobarbital (80 mg/kg) and perfused in the manner described above. Brainstems were removed, cut into 50- μ m-thick sections, and stained with cresyl violet.

Recordings from the Primary Somatosensory Cortex. Standard multiple-unit recording and receptive field mapping techniques were used to assess the representation of the body surface in the cortices of six normal adult rats and six adult animals that sustained neonatal forelimb removal. Recordings from the latter animals were made from the cortex contralateral to the neonatal lesion. Rats were anesthetized as described above and placed in a stereotaxic head holder. A midline incision was made in the scalp, the skull overlying the dorsal cortex was removed, and the dura was incised and reflected. The surface of the cortex was photographed at $\times 44$ magnification to record the placement of microelectrode penetrations and then covered with warm silicone.

Mapping was accomplished by using the procedures described by Wall and Cusick (13) and Rhoades *et al.* (9). Briefly, unit clusters and occasional single units were recorded with varnish-coated tungsten microelectrodes ($Z = 0.9\text{--}1.3\text{ M}\Omega$) and cutaneous receptive fields were defined as described above. Electrode penetrations spaced $\approx 200\text{ }\mu\text{m}$ apart were made in a roughly rectangular array and multiunit activity was recorded at depths between 500 and 750 μm . Between 100 and 150 penetrations were required to map areas and produce the boundaries of the forelimb stump and hindlimb representations shown in Fig. 3. Within these representations, all penetrations responded to somatic stimulation. Outside the boundary lines, penetrations were either silent or responded to other body regions such as the trunk, lower jaw, or mystacial vibrissae.

Several electrode tracks in each experiment were marked with electrolytic lesions. At the end of each mapping experiment, the animal was given a lethal dose of sodium pentobarbital (80 mg/kg) and perfused as described above. Cortices were flattened, sectioned, and processed for cytochrome oxidase as described above.

RESULTS

Reorganization of Sciatic Nerve Projections to the Dorsal Column Nuclei. In normal adult rats, the terminations of labeled sciatic nerve axons in the dorsal column nuclei are restricted to Gr (Fig. 1*A* and *B*). In all of the rats that sustained

FIG. 1. Polarized dark-field photomicrographs showing labeling in the dorsal column nuclei resulting from injection of HRP into the sciatic nerve of a normal adult rat (*A*) and three adult rats that sustained neonatal amputation of the ipsilateral forelimb (*C*, *E*, and *G*). The borders of Cu are denoted by dotted lines. The same sections are shown at higher magnification in *B*, *D*, *F*, and *H*. Note the extension of labeled sciatic afferents into the medial one-half of Cu in the animals that sustained forelimb amputations (*C*–*H*) and their absence in the Cu of the normal animal (*A* and *B*). (Bar = 150 μm .)

Table 1. Functional properties of Cu neurons in adult rats that sustained ipsilateral neonatal FL amputations

Experimental condition	Total recorded neurons	No. of SF neurons	SF neurons projecting to thalamus	No. of DF neurons	DF neurons projecting to thalamus	acv, m/s
Six normal adult rats	48	47	14	0	0	16.30 ± 4.3 (SF, brachial; <i>n</i> = 47)
Six adult rats, neonatal left FL removed	49	24 (9 stump, 4 trunk, 2 neck, 5 face, 3 HL, 1 tail)	1	20	6	12.8 ± 3.7 (SF, brachial; <i>n</i> = 9) 19.1 ± 10.8 (SF, sciatic; <i>n</i> = 4) 10.6 ± 4.1 (DF, brachial; <i>n</i> = 18) 18.5 ± 7.0 (DF, sciatic; <i>n</i> = 18)

SF, single field; DF, double field; FL, forelimb; HL, hindlimb.

neonatal forelimb removal, fibers labeled by sciatic nerve HRP injections extended into Cu. These axons reached all portions of the nucleus, but were most dense in its medial half (Fig. 1 C-H).

Functional Reorganization in Cu. A total of 48 single cells, 14 of which were antidromically activated from the contralateral thalamus, were recorded from Cu in six normal rats (Table 1). Forty-seven of these neurons had receptive fields restricted to the forelimb. No receptive field could be located for the remaining cell. All of the neurons recorded were activated by stimulation of the brachial plexus [average conduction velocity

(acv) = 16.3 ± 4.3 m/s] and none was activated by electrical stimulation of the sciatic nerve.

The results from rats that sustained neonatal forelimb amputation were substantially different. Forty-nine Cu neurons were recorded from six of these animals. Twenty-four cells had single continuous receptive fields and one of these projected to the thalamus (Table 1). Fifteen cells had receptive fields consistent with the normal representation within this nucleus (nine on the forelimb stump, four on the trunk, and two on the neck). Nine cells had clearly abnormally localized receptive fields (five on the face, three on the hindlimb, and

FIG. 2. Example of a Cu cell with a split receptive field. Blackened areas on the rat (A) show the cell's receptive fields. Responses to cutaneous stimulation (arrows above traces) are shown in B. Trace 1 shows the response to stimulation of the stump. Trace 2 shows the response to stimulation of the hindlimb, and trace 3 depicts the unit's spontaneous activity. The response to electrical stimulation of the brachial plexus is shown in C. A series of 10 pulses at a frequency of 100 Hz produced a single spike with a latency of 4.0 ms. The response to electrical stimulation of the sciatic nerve is shown in D. A single pulse produced multiple spikes with the initial spike having a latency of 6.5 ms. The response to stimulation of the thalamus is shown in E. A series of 10 pulses at a frequency of 100 Hz produced a corresponding series of spike peaks with a fixed latency of 1.8 ms. The results of a collision test used to verify the antidromic activation of this cell are illustrated in F. Trace 1 shows a typical response (latency of 4.0 ms) to a single pulse applied to the brachial plexus. Trace 2 shows a thalamic stimulation artifact (arrow) that has been delayed 5.5 ms after the start of the sweep, followed by the spike ~1.8 ms later. The third sweep was initiated by brachial plexus stimulation. The orthodromic spike was followed by a 1.5-ms delay and then the thalamic stimulation. Note the lack of antidromic action potential (thalamic stimulation artifact at 5.5 ms). (G) Photomicrograph shows location of a lesion (arrow) marking the cell whose responses are shown in A-F. Cu and Gr are identified on the contralateral side. (Bar = 250 μm.)

one on the tail). Nine cells with stump receptive fields also responded to electrical stimulation of the brachial plexus ($avc = 12.8 \pm 3.7$ ms) and four with receptive fields on the hindlimb (three) or trunk (one) responded to stimulation of the sciatic nerve ($avc = 19.1 \pm 10.8$ ms).

Twenty cells recorded from amputees had split receptive fields that typically included a portion of the stump and the hindlimb (Fig. 2A). Six of these neurons were antidromically activated from the contralateral thalamus (Fig. 2E) and 18 were activated by electrical stimulation of the brachial plexus (Fig. 2C and F; $avc = 10.6 \pm 4.1$ m/s) as well as by stimulation of the sciatic nerve (Fig. 2D; $avc = 18.5 \pm 7.0$ m/s). We also recorded one cuneothalamic projection neuron with a split receptive field that included the face and stump. Five cells recorded from the rats that sustained neonatal forelimb amputations did not have receptive fields. Both the percentage of Cu cells with split receptive fields and cells with receptive fields that included the hindlimb were significantly higher in the rats that sustained neonatal lesions ($P < 0.01$, Fisher's exact test).

Functional Reorganization Cortex. Examples of the cortical limb representations of two normal rats and of two animals that sustained neonatal forelimb amputation are shown in Fig. 3. Organization of the maps in the two groups is quite similar. There is an elongated region in which the forelimb or, in the manipulated rats, the stump is mapped. Abutted against this area is a smaller region devoted to representation of the hind-

limb. The sizes of the forelimb (stump) and shoulder representation in the normal rats and in the experimentally manipulated rats ($n = 6$ in each condition; 6.82 ± 0.84 vs. 6.68 ± 1.57 mm²) was not statistically different ($P > 0.05$). Likewise, the size of the hindlimb representation (3.03 ± 0.31 vs. 3.38 ± 0.51 mm²) did not differ significantly between the two groups ($P > 0.05$).

No cortical sites responsive to hindlimb stimulation were found within the forelimb representation of normal rats. In the experimentally manipulated rats, the region normally devoted to the forelimb contained a representation of the stump and surrounding shoulder. In addition, a small number of sites were responsive to stimulation of both the stump and hindlimb, but they were quite rare. Such sites were found in five of the six experimentally manipulated rats (e.g., see Fig. 3C and D) and the average percentage of such sites was $5.3\% \pm 3.0\%$ ($P < 0.05$). No unit clusters responsive to both stump and hindlimb stimulation were recorded within the hindlimb representation of the experimentally manipulated animals.

DISCUSSION

The present results provide evidence that neonatal removal of the forelimb results in anatomical and functional reorganization in the brainstem such that $>40\%$ of Cu neurons have receptive fields that express convergent input from both

FIG. 3. Examples of the forelimb and hindlimb areas within the somatosensory cortices of normal adult rats (A and B) and adult rats that sustained neonatal amputation of the contralateral forelimb (C and D). Note that the stump (ST) and hindlimb (HL) areas of manipulated rats are similar in shape and size to the forelimb (FL) and HL areas of the normal rats. Split receptive fields (♦) were detected rarely in the ST areas of manipulated animals and not at all in the FL areas of normal rats. Traces from two unit clusters with split receptive fields are shown in E. In each case, trace 1 shows the response to stimulation (arrow) of the ST and trace 2 shows the response to stimulation of the HL. (F) Bright-field photomicrograph is a cytochrome oxidase-stained section of the cortex, whose map is shown in D. Placement of electrodes to produce electrolytic lesions is indicated by asterisks numbered 1–4 in D. Sites for these lesions were chosen based on the microelectrode recordings which indicated that each site was adjacent to the ST representation. The actual electrode lesions numbered 1–4 are shown in F. The mystacial vibrissae (V) are located medial and posteriorly to the ST representation. Orientation arrows in F indicate the anterior (a) and lateral directions and apply to A–D. (Bar = 1 mm.)

forelimb- and hindlimb-related primary afferents. We also provide evidence for functional reorganization at the cortical level in the sense that a representation of the forelimb stump and shoulder completely fills the region normally devoted to the forelimb. However, cells with convergent receptive fields were found at only a small (5%) fraction of recording sites within the forelimb representation and not at all in the hindlimb representation. Given that antidromic stimulation provided evidence that Cu neurons with convergent receptive fields project to thalamus, our results provide strong evidence that information expressed at one level of the neural axis can be suppressed at higher levels.

Subcortical Reorganization. The anatomical experiments demonstrate that sciatic afferents extend throughout most of Cu in the rats that sustained neonatal forelimb amputation and the electrophysiologic recordings indicate that at least some of these fibers are likely to be large-caliber, low-threshold primary afferents. This is in line with a previous report from this laboratory demonstrating anatomical sprouting of sciatic primary afferents into Cu and limited functional convergence within this nucleus as measured by unit cluster recording following fetal forelimb removal (9). The present anatomical results are somewhat surprising since a number of previous studies have provided relatively little anatomical evidence for sprouting of low-threshold primary afferents after postnatal peripheral nerve damage (refs. 14–17, but see also refs. 18 and 19 for evidence that such sprouting does occur). The fact that neonatal peripheral damage can alter subcortical functional organization has also been demonstrated in a number of previous experiments (e.g., see refs. 8, 20, and 21).

Cortical Reorganization. The organization of the primary somatosensory cortex in the animals that sustained neonatal forelimb removal was not normal in the sense that it contained a representation of the stump rather than the distal forelimb and a small number of unit clusters within this representation were excited by hindlimb stimulation. However, the changes observed were relatively modest compared to those in the brainstem. The area devoted to representation of either limb was not significantly different than normal. These results appear similar to those of Waite and Taylor (22), who reported that cauterization of vibrissae follicles in newborn rats did not result in responsivity to distant body parts in the portion of the cortex normally representing these long whiskers. Similarly, Waite (8) observed only a very slight expansion of the cortical representation of the digits into the region normally representing the vibrissae after neonatal transection of the infraorbital nerve in rats. In contrast, Donoghue and Sanes (23) did note sites responsive to face stimulation in the region that normally mapped the distal forelimb after forelimb amputation in rats.

Relationship Between Brainstem and Cortical Reorganization. The most striking aspect of the present results is the contrast between the reorganization observed in the brainstem and the small degree to which it is reflected in the cortex. The idea that some level of the normally functioning nervous system may not express all, or indeed, may actively suppress some, inputs is not novel and is supported by several lines of evidence. First, in a number of instances the precision of cortical functional representations is apparently greater than that of their underlying thalamocortical projections (1, 24, 25). Second, experiments that have manipulated either the periphery or central neural structures have demonstrated changes in the part(s) of the body surface represented by a given central neuron or recording site that occur too rapidly to be explained by the growth of new connections and that therefore must reflect connections that are normally present but not expressed (e.g., see refs. 4 and 26–32).

In this context, the suppression of hindpaw inputs within the cortical representation of the altered forelimb may be a

reflection of normally operating dynamic processes. A similar process has been reported to occur within the visual system of one type of Siamese cat, “midwestern” (33). In these cats, a genetic anomaly results in the misrouting of a subset of optic nerve fibers to the lateral geniculate, and in this case the topographically inappropriate anatomical input is functionally expressed in the lateral geniculate but is suppressed in primary visual cortex. We would interpret this and our own experiment as a general tendency on the part of the nervous system to suppress local information that is inappropriate in the sense of it being incongruent with the larger body of information within which it is embedded. While in the case of the Siamese cat the suppression occurs at the cortical level, the locus of the suppression in our own experiment is unclear and needs further investigation. However, the surprising nature of the present results emphasizes the need to investigate central reorganization following peripheral damage at multiple levels of the neural axis.

The excellent assistance of Dr. Theodore H. Bowlus, Dawn M. Allan, and Beth Figley is gratefully acknowledged. This work was supported by National Institutes of Health Grants NS 28888, DE 07734, and DE 08971.

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