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12. Cells (0.2 g) were collected by filtration and extracted using a 3% SDS-containing buffer. Extraction and immunoblot analysis was performed as described (*4*).
13. Cells were digested overnight in 5% cellulase (RS) and macerase (R10) at 25°C with constant agitation to produce individual cells and cells in small aggregates. The nuclei were stained with 4′6′-diamidino-2-phenylindole, and the fluorescence intensity of individual nuclei was measured using a microphotometer.
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Large-Scale Sprouting of Cortical Connections After Peripheral Injury in Adult Macaque Monkeys

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Distributions of thalamic and cortical connections were investigated in four macaque monkeys with long-standing, accidental trauma to a forelimb, to determine whether the growth of new connections plays a role in the reorganization of somatosensory cortex that occurs after major alterations in peripheral somatosensory inputs. In each monkey, microelectrode recordings of cortical areas 3b and 1 demonstrated massive reorganizations of the cortex related to the affected limb. Injections of tracers in area 1 of these monkeys revealed normal patterns of thalamocortical connections, but markedly expanded lateral connections in areas 3b and 1. Thus, the growth of intracortical but not thalamocortical connections could account for much of the reorganization of the sensory maps in cortex.

The reorganization of somatosensory cortex that has been observed in monkeys with forelimb amputation (*1*) or sensory deafferentation (*2*), and in human amputees (*3, 4*), is presumed to be the basis for the sensation of phantom limbs (*5*) and perhaps phantom pain (*4*). A critical issue is how such large-scale changes are mediated in the adult brain. We previously showed that sprouting of peripheral nerve axons in the brainstem could account for some of the changes in cortical organization after forearm amputation (*1*), but additional mechanisms might be necessary for complete reactivation of deprived cortex. To investigate the possibility that new growth at other levels of the pathway contributes to the cortical reorganization, we studied thalamocortical and corticocortical connections in monkeys that had long-standing injury to the forearm, including arm amputation and wrist fracture. Electrophysiological maps of the cortical forelimb representation in the same monkeys allowed us to relate the patterns of connections to the functional changes produced by the injury.

Small injections of a bidirectional tracer, either wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) or fluoro ruby, were made into somatosensory cortical area 1 of three normal macaque monkeys and four monkeys that had suffered accidental forelimb injuries (*6*) at other primate facilities 1 to 10 years before the terminal experiments were performed (*7*). The injuries resulted in amputation of all the digits on one hand in one monkey, arm amputations in two monkeys and, in a fourth monkey, a wrist fracture that healed with the hand in a ventrally flexed position that rendered it useless (*6*). Although the injuries differed in each of the monkeys, they greatly altered the nature of the effective sensory input to cortex. Thus, these monkeys presented a valuable opportunity to learn about mechanisms of large-scale cortical reorganization that follow major changes in afferent drive.

Injections of similar size and location were placed in the hand representation of the control monkeys and in the reorganized representation of the experimental monkeys (*7*). The locations for the injections were determined from surface landmarks in the normal and injured monkeys and were confirmed by electrophysiological recordings in one of the controls and in all experimental monkeys. Subsequently, extensive electrophysiological recordings were made throughout the deprived zone in cortical areas 3b and 1 of the experimental monkeys. All procedures were performed in accordance with both National Institutes of Health and university guidelines for the care and use of animals in research. This report describes the patterns of label in cortical area 3b (primary somatosensory cortex) and area 1 because we have evidence for reorganization after peripheral injury in these fields. (Labeled neurons and processes also were apparent in other cortical somatosensory areas, including areas 2, 3a, 5, SII, and PV, but it remains uncertain whether or how these connections differ from those in normal animals.)

The distributions of label in areas 3b and 1 in the normal monkeys were similar across animals (Fig. 1). Both in area 1 and in the adjoining somatosensory field, area 3b, clusters of labeled cells and processes were separated from other clusters by zones of little or no labeling (Fig. 1). The label extended across much of the anteroposterior widths of areas 1 and 3b, but this distribution involved limited shifts in representation across the hand, from the palm or the proximal portion of a digit onto the distal digit tips. However, in the mediolateral dimension, where large topographic shifts can occur across the fore-

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limb representation, the distribution of the label was limited (Fig. 1). This results in a pattern of lateral connections that limits the representational spread of activity to near neighbors in normal cortex.

The findings from the normal monkey in which the hand representation was mapped electrophysiologically (*8*) show the normal relationship of the labeled neurons to the representation of the hand. The injection of the representation of digit 2 in area 1 produced label in area 1 that extended only into the representations of the adjacent digit 3 and the palm (Fig. 1). In area 3b, the main focus of label was in the digit 2 representation and extended predominantly into the adjacent representation of digit 3, with sparse labeling extending into the representation of digit 4 (Fig. 1). Thus, neurons in somatosensory cortex are connected predominantly with other neurons that represent that same general region of the body surface; this is consistent with the results of studies that used fluorescent tracers to show cortical connections in macaque monkeys (*9*). Measurements of the extent of retrograde labeling (labeled neurons projecting into the injection sites) in area 1 of the normal monkeys ranged from 2.9 to 4.3 mm with a mean spread of 3.8 mm, and in area 3b the retrograde label ranged from 2.8 to 4.6 mm in mediolateral extent with a mean spread of 3.8 mm. The extents of anterograde labeling (axons projecting from the injection site) were similar to the distribution of retrograde label, but measurements were not made.

In the monkeys with long-standing forelimb injury, the extents of labeled connections in areas 3b and 1 contralateral to the injured forelimb were markedly more widespread than in the normals, even though the sizes of the injections were similar (*7*). The size of the retrogradely labeled zone in area 1 in the experimental monkeys ranged from 5.7 to 8.3 mm (mean, 7.1 mm), and the size of the retrograde projection in area 3b was even more expanded, ranging from 6.9 to 9.2 mm (mean, 7.8 mm). Relative to the labeling in areas 3b and 1 in the normal monkeys, the expansions of the extents of labeling in the monkeys with forelimb injury were highly significant ($P < 0.01$) (*7*). The most numerous and most densely labeled neurons typically were located in the central core of the labeled region. Fewer well-labeled neurons were found beyond the core zone; however, even at the outer extremes of the labeled zones, labeled neurons were readily apparent, and assessment of the labeled extents could be made without ambiguity (Fig. 2). Anterograde labeling usually was located in the same general area as the labeled neurons.

Substantial modifications of the electrophysiological maps in the deprived regions of cortical areas 3b and 1 were also found in the monkeys with forelimb injury (Fig. 2). In the monkeys with the arm amputations, the regions that normally respond to sensory stimulation of the hand had become responsive to the face (laterally) and the remaining upper arm (medially). The monkey that survived 10 years after an arm amputation displayed the largest shift of the face representation into the region where the hand representation had been (Fig. 2). In this case, neurons at many sites responded to stimulation of both the face and the upper arm (Fig. 2C). Neurons with such receptive fields are never found in normal monkeys (*10*). Also, the broad expanse of labeled neurons in areas 3b and 1 of this monkey extended laterally well into the face representation. A comparable expansion of the retrogradely labeled zone was also present in the other monkey with arm amputation (not illustrated). As a result of the expanded distribution of lateral cortical connections, neurons in the normal face representation could activate neurons in the deprived hand representation, and they could cause the neurons to respond to stimulation of the face. The label also extended medially toward the normal location of the arm representation (Fig. 2), so that neurons activated by the arm could also drive deprived neurons in the hand cortex.

In the monkey with wrist fracture, there was a complete representation of the hand, with the digits represented in a lateromedial sequence (Fig. 2E) at least roughly similar to that in normal macaque monkeys (*10*). However, receptive field sizes were strikingly large and often extended across two or more digits, or involved noncontiguous portions of the palm and one or more digits. The labeled neurons in this animal extended throughout most of the hand representation in both areas 3b and 1. In area 3b, and perhaps to a lesser extent in area 1, the label extended beyond the representation of the hand into the forearm representation (Fig. 2E). This broad distribution of cortical connections could cause neurons to respond to stimulation over large regions of the hand, rather than the very precise receptive field distribution that is normally seen in the hand representation of areas 3b and 1 in macaques.

Finally, in the monkey with amputations of digits 1 to 5, the zones in areas 3b and 1 where the digits are normally represented contained expanded representations of the palm (medially) and perhaps an expanded representation of the face (laterally) (Fig. 2D). Many neurons with abnormal receptive fields also were present in this monkey. Neurons typically had very large receptive fields and, at a number of sites in both areas 3b and 1, responded not only to stimulation of the palm but also to stimulation of the face or the back of the hand. The injection produced large foci of labeled neurons in areas 3b and 1 that spanned the medial edge of the face representation and the lateral edge of the

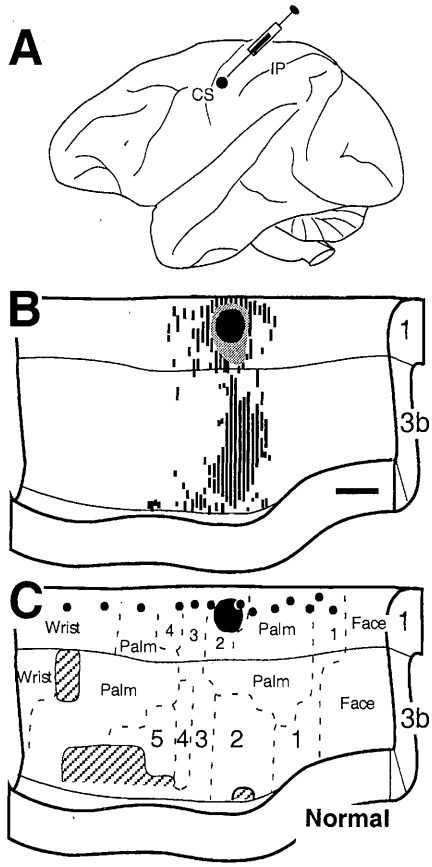

Fig. 1. (**A**) Lateral view of a macaque brain showing the approximate location of the cortical injections into area 1, which occupies the posterior lip of the central sulcus (CS); anterior is to the left. Area 3b is situated ventral to area 1. The hand representations in areas 1 and 3b are located medial to the tip of the intraparietal sulcus (IP). (**B**) Three-dimensional reconstruction of the spatial distribution of retrogradely labeled neurons in cortical areas 3b and 1 of a normal macaque monkey in an en face view of the posterior bank of the central sulcus, following methods described by Pons *et al.* (*10*). Each vertical line indicates the extent of label collapsed across all cortical layers. The distribution of label in every other section has been illustrated. The solid black region indicates the injection site, and the gray fill indicates the halo of dense reaction product around the injection. Lateral is to the right and dorsal is to the top. Scale bar, 1 mm. (**C**) Summary reconstruction of the hand representation in area 3b and a partial representation of the hand in area 1, based on electrophysiological recordings in the same monkey. The reconstructions were generated using methods described in (*10*). The injection site is shown for reference. Numerals indicate digits on the hand. Dashed lines indicate the borders between sensory representations; at every recording location within a sensory representation, neurons had receptive fields that involved similar skin locations. Fine solid lines indicate the borders of cortical area 3b. Stippled regions indicate hairy skin representations.

hand representation (Fig. 2). These may represent normal distributions of label, but there were also additional patches of label at the medial edge of the hand representation (Fig. 2). These connections may reflect new inputs from the palm and wrist to neurons in the deprived hand representation, because widely separated clusters of label such as this have not been seen in areas 3b or 1 of normal monkeys. Connections between topographically distant regions of the hand such as these may lead to the large receptive fields that were observed in this monkey.

The injections also labeled thalamocortical relay neurons in the ventroposterior nucleus (VP) of the thalamus. In normal monkeys, the labeled thalamocortical connections occupy a dorsoventral column in the subnucleus (Fig. 3) where the representation of the hand is located (*11*). The labeled VP neurons in the experimental monkeys had a columnar distribution similar to that seen in the normals. If the forelimb injuries had caused the cortical terminations of VP neurons to grow and activate deprived neurons in hand cortex, we would have expected labeled neurons to be broadly distributed in the hand portion of VP, and even in the more medial face portion of VP (VPM). The measurements suggest that the extents of the labeled zones might be larger in the experimental monkeys than in the normals, but the difference was not significant ($P < 0.05$). The volume of label in the normal monkeys averaged 2.2% of the total volume of the nucleus, with a range of 0.7 to 3.4%. In the experimental monkeys, the average volume of the labeled zone was 3.9%, with a range of 2.0 to 5.1% (*12*). The important feature of these findings is that the thalamocortical projections were at least relatively

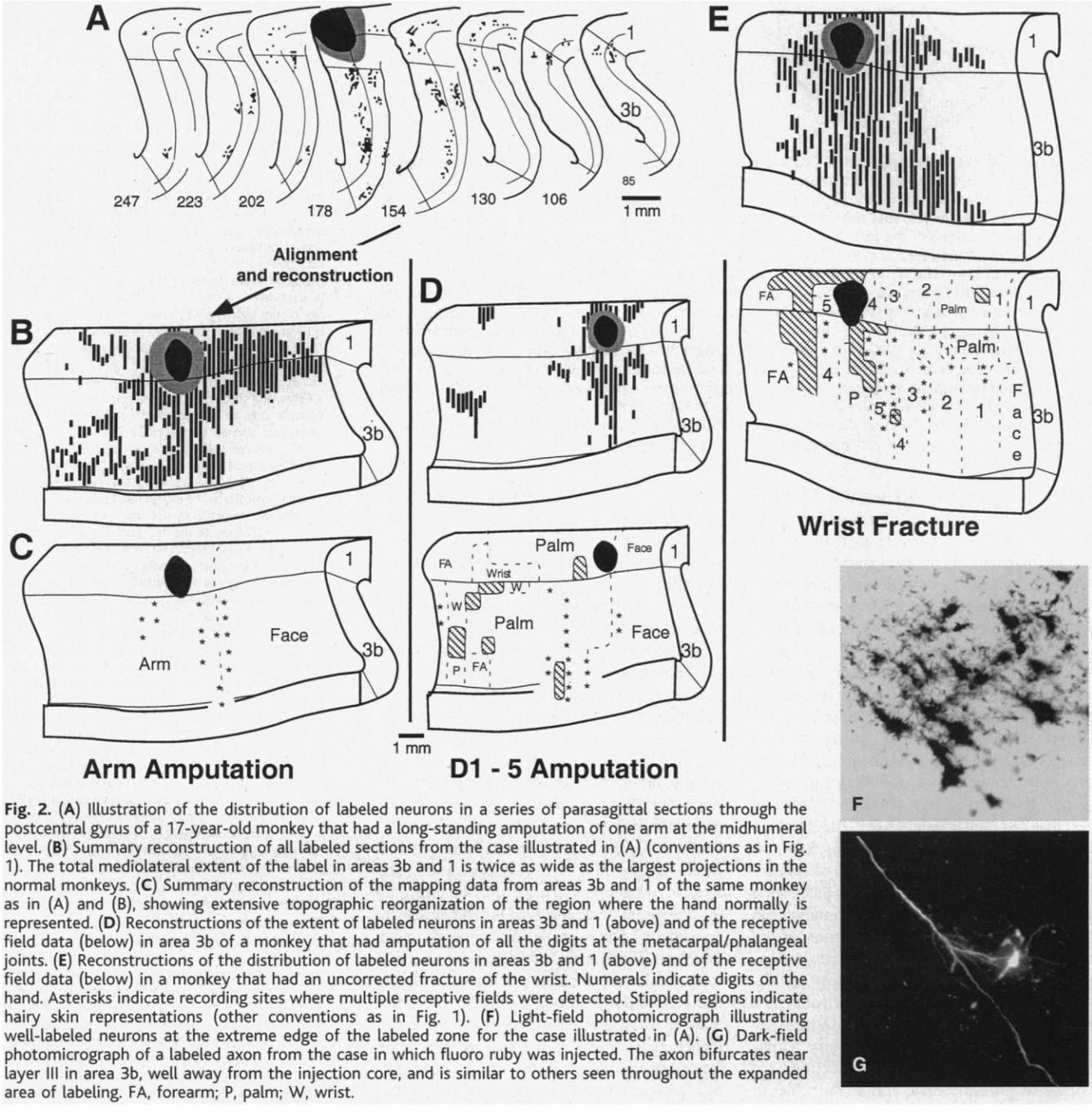

Fig. 2. (**A**) Illustration of the distribution of labeled neurons in a series of parasagittal sections through the postcentral gyrus of a 17-year-old monkey that had a long-standing amputation of one arm at the midhumeral level. (**B**) Summary reconstruction of all labeled sections from the case illustrated in (A) (conventions as in Fig. 1). The total mediolateral extent of the label in areas 3b and 1 is twice as wide as the largest projections in the normal monkeys. (**C**) Summary reconstruction of the mapping data from areas 3b and 1 of the same monkey as in (A) and (B), showing extensive topographic reorganization of the region where the hand normally is represented. (**D**) Reconstructions of the extent of labeled neurons in areas 3b and 1 (above) and of the receptive field data (below) in area 3b of a monkey that had amputation of all the digits at the metacarpal/phalangeal joints. (**E**) Reconstructions of the distribution of labeled neurons in areas 3b and 1 (above) and of the receptive field data (below) in a monkey that had an uncorrected fracture of the wrist. Numerals indicate digits on the hand. Asterisks indicate recording sites where multiple receptive fields were detected. Stippled regions indicate hairy skin representations (other conventions as in Fig. 1). (**F**) Light-field photomicrograph illustrating well-labeled neurons at the extreme edge of the labeled zone for the case illustrated in (A). (**G**) Dark-field photomicrograph of a labeled axon from the case in which fluoro ruby was injected. The axon bifurcates near layer III in area 3b, well away from the injection core, and is similar to others seen throughout the expanded area of labeling. FA, forearm; P, palm; W, wrist.

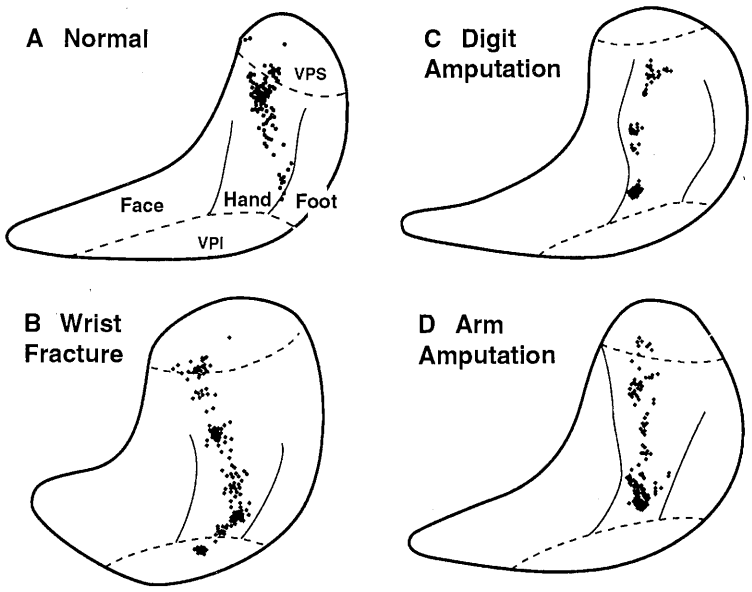

Fig. 3. Frontal sections through the ventroposterior nucleus (VP) of the thalamus, showing the distribution of retrogradely labeled neurons after an injection of area 1 in a normal monkey (**A**) and in monkeys with forelimb injury (**B** to **D**). The sensory representation in VP normally progresses from the face, medially, across the forelimb representation to the hindlimb and tail representation laterally. The locations of the face, hand, and foot subnuclei are indicated in (A). In each panel, black dots represent labeled neurons. In all cases, a column of label is apparent in the lateral subdivision of VP that contains the hand subdivision. Variations in the distribution of label from dorsal to ventral reflect shifts from proximal to distal on the hand representation, which could not account for the physiological changes detected in cortex. In contrast, differences in the mediolateral location of label could involve very large shifts across the body representation, including from hand to face or from hand to foot. Thus, significant changes in the thalamocortical projection would likely involve modifications either in the mediolateral location of the labeled neurons or in their total mediolateral extent. However, few or no differences were detected. Dorsal is to the top and medial is to the left. Heavy solid lines indicate the borders of VP, fine solid lines distinguish the hand subdivision within VP, and dashed lines indicate the borders between VP and ventroposterior superior (VPS) dorsally, and ventroposterior inferior (VPI) ventrally.

normal, whereas the changes at the cortical level were immense. Similarly in the visual system, reorganizations in primary visual cortex of cats and monkeys after focal bilateral retinal lesions were not accompanied by comparably extensive reorganizations in the lateral geniculate nucleus (*13*).

Our study provides evidence of widespread expansions of lateral connections in the region of somatosensory cortex where substantial changes in functional organization have occurred. The expansions of the connections probably reflect intracortical sprouting. The only other demonstration of sprouting in cortex, without direct insult, is in visual cortex of adult cats that had retinal lesions (*14*). The deactivations produced by the retinal lesions were followed by an increase in the density of connections but no growth beyond the preexisting territory. Our results suggest that sprouting can be both within and beyond the framework of the preexisting connections. An alternative explanation for these results is that sparse widespread cortical connections that normally exist in the hand representation of somatosensory cortex were unmasked by the injury. However, if widespread lateral connections are normally present, they should be able to reactivate cortex relatively rapidly after sensory deprivation. But an earlier study has shown that regions of deactivated cortex can remain silent for months after denervation (*15*).

Major cortical reorganizations likely take time to emerge. The present monkeys all had long-standing injuries with the common effect of depriving a broad expanse of cortex of the normal patterns of activation. The implication is that the chronic deprivation of cortex, whether by extensive finger loss, forearm amputation, or wrist fracture, is the key variable in the cortical reorganizations. Apparently, long recovery periods provide an opportunity for new connections in cortex to grow and become effective. Undoubtedly there are additional consequences of the injuries on cortical organization that are specific to the injury. These could include major changes at lower levels of the somatosensory pathway. For example, new inputs expand into the deprived representation of the forelimb in the brainstem after limb amputation (*1*); in this case, the role of new cortical connections may be to synergistically potentiate the other inputs. In contrast, in the case of the wrist fracture where the major impact is disuse, the new cortical connections may serve as an important source of information about the sensory environment, and they may largely account for cortical reactivation. In either case, new growth in the adult brain has important implications for recovery of function after injury, including direct damage to the central nervous system, such as spinal cord injury or stroke. Thus, an important direction for future research will be to identify the mechanisms that lead to such profound changes in intracortical connections.

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7. The injections spanned a mediolateral extent of 0.6 to 1.7 mm, and there were no differences in the ranges of injection sizes for normal monkeys relative to the experimental animals ($P = 0.463$). The location of the injection in area 1 was 1 to 4 mm medial to the tip of the intraparietal sulcus. The hand representations in areas 1 and 3b of macaque monkeys are situated immediately medial to the intraparietal sulcus and extend for ~8 mm medially. In the monkeys with forelimb injury, the location of the injection was based on surface landmarks, and confirmation that they were located in the region that originally represented the hand was obtained by the distribution of label in the thalamus. The procedures used to make the cortical injections and to process the tissue histologically were as described (*16*). Briefly, a small volume (~0.05 μl) of 2% WGA-HRP or 10% fluoro ruby was injected into the posterior bank of the central sulcus. The monkeys that had WGA-HRP injections were either maintained in a lightly anesthetized state for about 12 hours to allow time for the injected tracer to transport (two normal monkeys) or were maintained for microelectrode mapping of cortical area 3b on the same side as the injection (one normal and three experimental monkeys). The monkey that had the fluoro ruby injection was recovered from surgery, and the terminal electrophysiological recording experiment was performed 8 days later. At the end of the survival period, the monkeys were given an overdose of sodium pentobarbital and perfused with paraformaldehyde. In all animals except one, the concentration of paraformaldehyde was 2%; a higher concentration of 3% paraformaldehyde was used in the 17-year-old monkey with the arm amputation, but it appeared to have no discernible effect on the density of the reaction product. The brains were sectioned and processed for tetramethyl benzedine histochemistry (for the monkeys with WGA-HRP injections) or mounted unstained (for the monkey with fluoro ruby injection). To determine whether there were significant differences in the extent of label in the control and experimental monkeys, we performed a Monte Carlo permutation test (on the raw data and on their rank order) for 1000 random replications.

8. The functional organization of cortex was studied using standard multiunit microelectrode mapping procedures, as described (*11*). For reconstruction of the cortical maps for the distribution of label and for the microelectrode recordings, a series of drawings of the labeled cortical sections were digitally scanned into NIH Image, aligned, and rotated to generate a three-dimensional, "en face" view of the distribution of label in area 3b. The receptive field data were superimposed on these 3D reconstructions to produce summary maps of the forelimb representation, using methods described in (*1*).
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Thalamic and Brainstem Contributions to Large-Scale Plasticity of Primate Somatosensory Cortex

Edward G. Jones and Tim P. Pons

After long-term denervation of an upper limb in macaque monkeys, the representation of the face in somatosensory cortex expands over many millimeters into the silenced representation of the hand. Various brainstem and cortical mechanisms have been proposed to explain this phenomenon. Reorganization in the thalamus has been largely ignored. In monkeys with deafferented upper limbs for 12 to 20 years, it was found that the brainstem cuneate and the thalamic ventral posterior nuclei had undergone severe transneuronal atrophy, and physiological mapping in the thalamus revealed that the face and trunk representations were adjoined while the normally small representation of the lower face had expanded comparable to the expansion in cortex. Reorganization of brainstem and thalamic nuclei associated with slow transneuronal atrophy is likely to be a progressive process. When coupled with divergence of ascending connections, it is likely to make a substantial contribution to representational changes in cortex.

Maps of the body surface in the postcentral gyrus of adult monkeys are capable of reorganization under the influence of reduced or enhanced input from peripheral somatosensory receptors (*1*). These reorganizations are likely to be responsible for perturbed sensory experiences that occur after loss of peripheral input from a body part, such as phantom and painful sensations that follow amputation or deafferentation of a limb in humans (*2*). The neural mechanisms that underlie activity-dependent cortical reorganization may be the same as those normally responsible for improvements in perceptual skills that accompany extended sensory experience and, if harnessed, these mechanisms may provide a basis for promoting recovery of function after peripheral or central lesions of the nervous system (*3*). The most extensive reorganization of somatosensory cortex occurred in monkeys in which the dorsal roots of the spinal cord (*4*) or the spinal dorsal columns (*5*) had been cut or a hand had been amputated (*6*). In these cases, after months or years, the representation of the face in the contralateral postcentral gyrus had expanded for a distance of 15 to 20 mm into the adjacent part of the gyrus in which the contralateral upper limb is normally represented.

Mechanisms postulated to explain such extensive reorganization include (i) reorganization in the dorsal column nuclei, which is then projected upward to somatosensory cortex (*5*); (ii) divergence of ascending somatosensory projections whose more divergent synapses have been hitherto silent (*4*); and (iii) reorganization or sprouting of preexisting connections in cortex itself (*1*). Surprisingly, the thalamus, the second synaptic station in the ascending somatosensory pathways and the obligatory relay for all sensory inputs to the cerebral cortex, has been ignored in most explanations.

Earlier data, however, suggest that the somatosensory thalamus is not immune from activity-dependent map plasticity. Expansion of the forelimb representation into the silenced hindlimb representation in the ventral posterior (VP) thalamic nucleus was reported 1 week or more after destruction of the gracile nucleus in rats (*7*). Similar expansions of the VP upper limb representation into that of the lower limb were reported in monkeys after dorsal rhizotomies or section of the gracile fasciculus (*8*). A number of peripheral deafferentation-dependent expansions of receptive fields of individual cells or of the representations of whole body regions have recently been described in the somatosensory thalamus of rats, monkeys, and humans (*9*). We examined the somatosensory thalamus in *Macaca fascicularis* monkeys from the same group in which massive expansions of the cortical face representation after 12 or more years of upper limb deafferentation were first reported (*4*). The thalami and brainstems of the eight monkeys in the group were examined histologically, and in two (one deafferented for nearly 20 years), the thalamus was mapped electrophysiologically and showed extensive reorganization of the body map in a thalamus in which the upper limb representation was affected by severe transneuronal degeneration.

Tactually elicited neuronal activity was recorded in the VP nucleus contralateral to an upper limb that had been denervated by surgical transection of the dorsal roots of the spinal cord from the second cervical (C2) to fourth thoracic (T4) segments (*10*). Microelectrodes spaced at 1-mm intervals entered the VP nucleus in the horizontal plane from behind and in a grid-like pattern. Neuronal responses to light stimulation of the body surface were systematically recorded in 100-μm steps as the electrode was advanced from posterior to anterior through the VP nucleus (*11*). In all eight monkeys, the thalamus, brain stem, and spinal cord were sectioned and stained by the Nissl method, histochemically for the metabolic marker cytochrome oxidase (CO), and immunocytochemically for the neuronal proteins calbindin or parvalbumin (*12*).

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