Thalamic and Cortical Contributions to Neural Plasticity After Limb Amputation

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Florence, S. L., T. A. Hackett, and F. Strata. Thalamic and cortical contributions to neural plasticity after limb amputation. J. Neurophysiol. 83: 3154–3159, 2000. Little is known about the substrates for the large-scale shifts in the cortical representation produced by limb amputation. Subcortical changes likely contribute to the cortical re-modeling, yet there is little data regarding the extent and pattern of reorganization in thalamus after such a massive deafferentation. Moreover, the relationship between changes in thalamus and in cortex after injuries of this nature is virtually unexplored. Multiunit micro-electrode maps were made in the somatosensory thalamus and cortex of two monkeys that had long-standing, accidental forelimb amputations. In the deprived portion of the ventroposterior nucleus of the thalamus (VP), where stimulation to the hand would normally activate neurons, new receptive fields had emerged. At some recording sites within the deprived zone of VP, neurons responded to stimulation of the remaining stump of the arm and at other sites neurons responded to stimulation of both the stump and the face. This same overall pattern of reorganization was present in the deprived hand representation of cortical area 3b. Thus thalamic changes produced by limb amputation appear to be an important substrate of cortical reorganization. However, a decrease in the frequency of abnormal stump/face fields in area 3b compared with VP and a reduction in the size of the fields suggests that cortical mechanisms of plasticity may refine the information relayed from thalamus.

INTRODUCTION

Peripheral denervations of large extents of the sensory epithelium, as a result of limb amputation, dorsal rhizotomy, and spinal cord injury, can lead to massive functional reorganization in somatosensory cortex (e.g., Elbert et al. 1994; Flor et al. 1995; Florence and Kaas 1995; Florence et al. 1998; Jain et al. 1997; Lane et al. 1995; Pons et al. 1991; Rasmusson et al. 1985; Yang et al. 1994). However, the source of the changes that allows for such large-scale cortical remodeling is unknown. A simple explanation is that changes are induced at multiple levels of the somatosensory pathway, perhaps with amplification as changes are projected from the subcortical relays to cortex. However, few studies have examined the impact of massive deafferentations on subcortical somatosensory structures. After dorsal rhizotomy to denervate the forelimb, extensive reorganization was found in thalamus of monkeys (Jones and Pons 1998; Pollin and Albe-Fessard 1979) and in the dorsal column nuclei of rats (Wall and Egger 1971). In humans that had spinal cord transection, the principle sensory nucleus of the thalamus was reorganized (Lenz et al. 1994). Reorganization in the dorsal column nuclei of rats also was reported after neonatal forelimb amputation (Lane et al. 1995). The only evidence of the subcortical effects of limb amputation in primates is from humans who were undergoing electrophysiological exploration of the somatosensory thalamus during procedures for the treatment of pain. The recordings revealed that in the principal sensory nucleus of the thalamus the large area where the amputated limb would normally be represented had been reactivated by nearby intact representations (Davis et al. 1998; Kiss et al. 1994; Lenz et al. 1998).

What is needed is detailed information about how peripheral perturbations affect each level of the somatosensory pathway so that the relative impact of subcortical changes on cortical organization can be evaluated. Toward this goal we have assessed the impact of long-standing arm amputation on the organization of the ventroposterior nucleus and compared the extent and pattern of change in the ventroposterior nucleus of the thalamus (VP) with that in somatosensory cortex (area 3b) of the same animal. The findings demonstrate that the region of VP deprived of a primary source of sensory activation by arm amputation is completely reactivated by inputs from the remaining stump of the arm and by the face. Moreover, the patterns of reorganization in VP roughly parallel those in area 3b. This suggests that changes induced subcortically after amputation are relayed upstream and may reactivate deprived cortex. However, some of the abnormal receptive field features found in VP were not present in area 3b of the same animal. Thus cortical mechanisms of plasticity may allow for additional modification of the information projected from thalamus.

METHODS

Microelectrode multiunit recordings in the VP of the thalamus and in cortical area 3b were performed in two macaque monkeys (Macaca radiata and M. nemestrina) that each had a forelimb amputation. The amputations were performed years earlier at other facilities for veterinary treatment of accidental injuries. One monkey (Rh8-81) had an amputation at the mid-humeral level at 14 mo of age; it was 7 yr when the experiments were performed. The other monkey (Rh8-110) had an above-the-elbow amputation at 2 yr of age and was 14 yr at the time of the experiments. Before the recording experiment, ketamine hydrochloride (10–20 mg/kg) was given for sedation. A surgical level of anesthesia was achieved using isoflurane gas in O₂. Isoflurane concentrations ranged from 4% during induction to 1% after a surgical level of anesthesia was attained. Multisite microelectrode recordings in thalamus and cortex contralateral to the amputation were made using tungsten microelectrodes (1.0 MOhm resistance at 1 kHz; Micro Probe, Potomac, MD). Vertically directed recording tracks were made in a rectangular gridlike pattern in the region of thalamus where cutaneously elicited neuronal activity was detected. Individual electrode penetrations through thalamus were separated from one another.

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by distances of no more than 0.5 mm; within individual penetrations, recordings were made at intervals of 0.3 mm (case Rh8-81) or 0.5 mm (case Rh8-110). The grid of recordings was extended until the cutaneously responsive zone (presumptive VP) was exceeded mediolaterally and anteroposteriorly to ensure that the entire extent of VP was mapped. Recording procedures for area 3b were the same as described previously (Florence and Kaas 1995; Florence et al. 1998). Briefly, rows of electrode penetrations were directed along the posterior bank of the central sulcus across the presumptive hand representation. We assumed the recordings had exceeded the lateral border of the hand representation in area 3b when a penetration encountered orderly progressions of small receptive fields that only involved the face. Similarly we assumed that the recordings had exceeded the medial border of the forelimb representation when a penetration encountered orderly progressions of receptive fields away from the stump on the trunk or shoulder. The entire forelimb representation of area 3b was mapped in detail in case Rh8-81. In case Rh8-110, although the full extent of the forelimb representation in area 3b was sampled, the total number of electrode recordings made in area 3b was limited because other anterior parietal cortical fields were mapped in addition to area 3b (for a separate study).

The same investigator mapped receptive fields in both VP and area 3b in case Rh8-81 to avoid possible interpretive differences during receptive field mapping. In case Rh8-110, two experimenters mapped area 3b in shifts (one of whom mapped VP); however, the experimenters established common criteria (e.g., Florence and Kaas 1995) for defining receptive fields to minimize experimenter differences. Recordings in area 3b preceded VP because the electrode penetrations directed at thalamus could undercut cortical afferents. Fine hand-held probes and camel hair brushes were used to deliver cutaneous sensory stimulation. Joint manipulation and muscle palpation comprised the high-threshold stimuli. No noxious stimuli were used. Electrolytic lesions were made at selected recording sites for later verification of recording location. After completion of the experiment, the animal was given a lethal dose of sodium pentobarbital and perfused through the heart with buffered saline (pH 7.4) followed by 3% paraformaldehyde. The brain was removed and incubated in 30% sucrose in phosphate buffer (0.1 M, pH 7.4). Cortex was separated from subcortical structures and cut in a plane parallel to the recording tracks. Thalamus was cut in the frontal plane. Series of sections through both thalamus and cortex were reacted for cytochrome oxidase and acetylcholinesterase.

Reconstructions of the recording data were made by drawing series of sections through which the electrodes traversed under low magnification and aligning the receptive field data at the appropriate depths on the tracks. Then architectural information about thalamic and areal borders was superimposed on the drawings. Measurements of receptive field sizes were made by tracing the outlines of each receptive field on a scaled drawing of a macaque monkey in NIH Image software. The measurements for each animal were normalized to the same standard. Two-tailed t-tests (assuming unequal variances) were used to determine whether the mean receptive field sizes in VP and area 3b of each monkey were different ($P < 0.01$). A $\chi^2$ test was used to determine whether the proportions of face fields in the reorganized forelimb representation of VP and area 3b were different ($P < 0.01$).

R E S U L T S

Multunit neuronal activity, elicited with tactile stimulation to the skin surface, was recorded throughout VP contralateral to the amputation. In case Rh8-81, a total of 173 recording sites were judged to be in VP based on the reconstructions of recording tracks. Of those, 82 had receptive fields on the stump of the forelimb and were confirmed to be located in the architecturally defined hand subnucleus. These 82 fields were included in the measurements of receptive field size. In case Rh8-110, a total of 77 recording sites were judged to be in VP; receptive fields on the stump at 40 recording sites were confirmed to be in the hand subnucleus and were measured for size comparisons with cortex. Recordings along tracks that involved medial and lateral locations in VP encountered neurons that responded to stimulation of the face and hindlimb (respectively). Tracks through the central region of VP (where the hand subnucleus is located) consistently encountered neurons that responded briskly to cutaneous stimulation and there was no evidence of silent zones (Figs. 1 and 2). Within the hand subnucleus of VP, neurons at many of the recording sites responded to stimulation on the stump. Most of the receptive fields on the stump were localized to either the medial or lateral surface of the stump, although some receptive fields encompassed the entire stump and occasionally extended onto the trunk and head (Figs. 1 and 2). At many of the recording sites in VP where cutaneous receptive fields were mapped on the stump, responses were also elicited by cutaneous stimulation of the face (stump/face fields). In these instances, the responsiveness of the neurons was robust to stimulation of both the face and stump, although frequently the responses to stimulation of the stump were stronger than the responses to stimulation of the face. At some sites in the hand subnucleus, neurons responded to stimulation of only the face (Fig. 2). These face responses typically were recorded near the medial border of the hand subnucleus adjacent to the normal face representation of VP, suggesting that there had been a latero-ward expansion of the face representation beyond its typical location medial to the hand subnucleus.

In area 3b, receptive fields were determined for neurons at a total of 138 (Rh8-81) and 66 (Rh8-110) recording sites. Of these totals, 92 and 23, respectively, were determined to be in the zone where the hand representation normally is situated. Within this region, the pattern of reorganization was much the same as described previously in monkeys with forelimb amputations (Florence and Kaas 1995; Florence et al. 1998). Most of the neurons in reorganized cortex responded to tactile stimulation of the stump. A few responded to stimulation of the stump and face. The neurons that responded to stimulation of the face typically were located in the lateral portion of the presumptive hand representation and adjacent to the normal face representation. Thus as in VP there appeared to be an expansion of the face representation.

Although the overall patterns of reactivation of the deprived forelimb representations in VP and area 3b were similar, some of the receptive field features in area 3b were not equivalent to those in VP. The most compelling evidence of a difference between the receptive fields in VP and area 3b was the frequency of face inputs in the forelimb representation. The proportion of recording sites that involved the face, including stump/face fields and face only fields, was 53% in VP of case Rh8–81 but only 35% in area 3b of the same monkey. A $\chi^2$ test confirmed that this difference was significant. In case Rh8–110, the proportions of recording sites that involved the face in VP and area 3b were 42% and 25%, respectively. Although the magnitude of the difference between VP and area 3b in this animal was nearly equivalent to the difference in monkey Rh8–81, it was not statistically significant, probably because the number of face fields in area 3b of this monkey was near the lowest allowable number of observations for the $\chi^2$ test.
The difference in the number of receptive fields including the face was not the only discrepancy between VP and area 3b. Even when the fields that involved only the stump were considered, there were differences in receptive field sizes in 3b compared with VP (Fig. 3). Mean size of receptive fields that involved the stump in area 3b for monkey Rh8-81 was $6.5 \pm 2.8$ (SD) cm$^2$ and in VP of this same monkey the mean size of stump fields was $8.9 \pm 5.7$ cm$^2$. In monkey Rh8-110,
the mean sizes of stump fields in area 3b and VP were 10.2 ± 4.8 cm² and 15.5 ± 7.1 cm², respectively. For both monkeys, these differences between VP and area 3b were significant. Differences between the two monkeys in receptive field sizes probably reflect variability in the level or severity of the injury. The differences between VP and area 3b in sizes of stump fields may result from the reduction in area 3b of the number of very large fields found in VP (Fig. 3). This is most apparent for monkey Rh8-81. A composite drawing that shows the total extent of skin surface encompassed by all receptive fields involving the stump in this monkey shows that some of the fields in VP extend beyond the stump and onto the shoulder, neck, and face (Fig. 1D). In area 3b of this same monkey, receptive fields involving the stump are concentrated primarily at the end of the stump (Fig. 1D). The few large fields in area 3b wrap around the medial and lateral surfaces of the stump and adjacent arm (Fig. 1B) but never extend to the shoulder or beyond. In summary, the apparent elimination of some of the face inputs and lack of very large stump receptive fields in the zone of area 3b normally devoted to the forelimb representation suggest that there may be a cortically-mediated suppression of some of the inputs relayed from VP.

**DISCUSSION**

These findings demonstrate that long-standing forelimb amputation produces extensive reactivation of VP as well as area 3b. A number of laboratories have described the patterns of change produced by limb amputation in somatosensory cortex (Elbert et al. 1994; Flor et al. 1995; Florence and Kaas 1995; Florence et al. 1998; Lane et al. 1995; Rasmusson et al. 1985; Yang et al. 1994); however, there has been little information about the effects of amputation on thalamic organization. These results indicate that the forelimb representation in the ventroposterior nucleus becomes completely reactivated after forelimb amputation by intact inputs from the stump of the arm and from the face. These findings are consistent with recent evidence for thalamic reactivation in humans with limb amputation (Davis et al. 1998; Kiss et al. 1994; Lenz et al. 1998). Reorganization in thalamus also has been described after other types of large scale sensory denervations including spinal cord transection in humans (Lenz et al. 1994) and dorsal rhizotomy in monkeys (Jones and Pons 1998; Pollin and Albe-Fessard 1979). Because these types of denervations eliminate sensory inputs to an enormous extent of the sensory representation, it is unlikely that the deafferented zones could be reactivated by existing connections. There has been considerable speculation recently about how such reactivation is mediated.

Earlier evidence from macaque monkeys with long-standing forelimb amputations showed that sensory afferents from the intact stump of the arm sprout new connections into the deprived portions of the cuneate nucleus in the brain stem (Florence and Kaas 1995). New growth from the trigeminal nucleus to the deprived representation in the cuneate nucleus also has been reported in monkeys with limb amputation (Jain et al. 2000). Presumably these new inputs could provide a source of

**FIG. 2.** Receptive fields in reorganized hand representations of VP and area 3b in monkey Rh8-110. Conventions as for Fig. 1.

**FIG. 3.** Bar graphs of distributions of receptive field sizes in VP (black fill) and in area 3b (gray fill) for both amputee monkeys.
activation to deprived neurons in the cuneate nucleus and they in turn could activate deprived neurons in the higher-order somatosensory relays (Kaas et al. 1999). These findings of widespread reactivation of the hand subnucleus in VP by stump and face inputs are compatible with this interpretation. Other factors may contribute to the functional properties of neurons in VP, but the activation specifically from the stump and the face likely reflects the relay of new patterns of activation in the brain stem that were produced by sprouting of intact sensory afferents.

A related issue concerns the extent to which the reorganization in VP is projected to cortex. The similarities that we observed in the patterns of reorganization after limb amputation in VP and area 3b of the same animal indicate that subcortical changes provide at least a substrate for cortical reactivation. Evidence from other studies is consistent with this proposal; peripheral denervations including subcutaneous lidocaine injection (Faggin et al. 1997), transections of nerves to the glabrous hand (Garraghty and Kaas 1991a,b), and cervical dorsal rhizotomy (Jones and Pons 1998; Pons et al. 1991) produce changes in the functional organization of VP and in somatosensory cortex that are strikingly similar, at least in pattern and extent.

However, this is not to say that all aspects of the response properties that emerge in VP after peripheral injury are expressed in cortex. Instead, there were fewer split fields involving both the face and the stump and smaller stump-only fields in area 3b compared with VP. Similarly, in monkeys that had transection of the median and ulnar nerves, receptive fields in the reactivated region of area 3b were significantly smaller than those in the deprived portion of the cuneate nucleus (Xu and Wall 1997, 1999). Also in rats that had forelimb amputation early in life, only 5% of neurons in somatosensory cortex had the unusual characteristic of responsive zones on both the forelimb stump and the hindlimb, whereas 41% of the receptive fields in the cuneate nucleus demonstrated this unusual response property (Lane et al. 1995). When GABA antagonists were administered to cortex while recording in the forelimb cortical representation of the rats that had early forelimb amputation, many more cortical receptive fields that included the hindlimb appeared (Lane et al. 1997). Thus the hindlimb inputs to forelimb cortex apparently were suppressed through GABA-mediated inhibition in cortex.

The present evidence and the related work discussed above give emphasis to mechanisms of reorganization after peripheral denervation that involve the ascending relay of information to the first target in cortex. However, cortical feedback to VP also can have a powerful influence on the functional responses of neurons in the subcortical relays (Ergenzinger et al. 1998; Krupa et al. 1999). Thus the neuronal activity at any one of the relay stations in the circuit may reflect a balance of influences that incorporate both bottom-up and top-down processes. Additionally, some types of plasticity may be mediated only in cortex, without concomitant changes in the subcortical relays. Wang and coworkers (1995) found that coincident stimulation of multiple adjacent fingers produced markedly expanded digit receptive fields in area 3b yet in the same monkeys there was no apparent receptive field change in VP. Thus different types of plasticity mechanisms may be initiated by different types of sensory manipulations, and any given mechanism of plasticity may not affect every level in the circuit equivalently.

We are indebted to L. Trice and M. Varghese for histological expertise. We thank Drs. Olivier Coq and Missy Niblock for helpful advice on the manuscript.

This research was funded by National Institute of Neurological Disorders and Stroke Grant NS-36469 to S. L. Florence with support from the J. F. Kennedy Center for Research on Human Development. Partial support also was provided by Human Frontier Science Plan Organization Grant LTF4398 to F. Strata.

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