Somatosensory & Motor Research 2002; 19(2): 153–163

Taylor & Francis healthsciences

The effects of long-standing limb loss on anatomical reorganization of the somatosensory afferents in the brainstem and spinal cord

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Abstract

We examined the terminations of sensory afferents in the brainstem and spinal cord of squirrel monkeys and prosimian galagos 4–8 years after a therapeutic forelimb or hindlimb amputation within 2 months of birth. In each animal, the distributions of labeled sensory afferent terminations from remaining body parts proximal to the limb stump were much more extensive than in normal animals. These sprouted afferents extended into the portions of the dorsal horn of the spinal cord as well as the cuneate and external cuneate nuclei of the brainstem (forelimb amputees) or spinal Clarke's column (hindlimb amputee) related to the amputated limb. Such reorganization in sensory afferents along with reorganization of the motor efferents to muscles (Wu and Kaas, *J Neurosci* **19**: 7679–7697, 1999, *Neuron* **28**: 967–978, 2000) may provide a basis for mislocated phantom sensations of missing forelimb movements accompanying actual shoulder movements during cortical stimulation or movement imagery in patients with amputations.

Key words: *amputation, phantom limb, sprouting, primates*

Introduction

Limb amputation severs peripheral nerves including both sensory afferents and motor efferents. Nevertheless, humans with amputation experience sensations of movement in the missing "phantom limb", and tactile stimuli to other parts of the body may be mislocalized to the missing limb (Ramachandran and Hirstein, 1998). Sensations of movement of the missing limb can also occur during motor imagery (Ramachandran, 1993), and when movements of the limb stump are evoked by stimulating motor cortex (Woolsey *et al.*, 1979; Cohen *et al.*, 1991). Understanding the basis of such misperceptions will ultimately depend on determining how amputations alter the sensory and motor systems. We already know that deprived portions of the somatosensory system are reactivated by inputs from remaining more proximal body parts after limb amputation (Rasmusson, 1982; Merzenich *et al.*, 1984; Rhoades *et al.*, 1993; Florence and Kaas, 1995; Florence *et al.*, 2000; Jain *et al.*, 2000), and that such reorganizations are accompanied by the growth of new connections in the brainstem (Florence and Kaas, 1995; Jain *et al.*, 2000) and cortex (Florence *et al.*, 1998). In addition, stimulating the region of primary motor cortex that normally controls movements of the missing limb may evoke movements of the limb stump (Wu and Kaas, 1999; Qi *et al.*, 2000), possibly in part due to the innervation of stump muscles by damaged motor efferents that normally innervate muscles in the missing limb (Wu and Kaas, 2000). Thus, the growth of new connections seems to be a component of sensorimotor adjustment after the loss of a limb.

Here we further investigated the possibility of changes in the brainstem and spinal cord targets of peripheral nerve afferents after the loss of a limb. We were able to study squirrel monkeys and prosimian galagos years after they received therapeutic amputation of an injured limb as infants. We previously reported that primary motor cortex in these primates reorganized so that stimulation of the forelimb portion of motor cortex evoked movements in the stump, and that primary somatosensory cortex had also reorganized so that stimulation of the skin of the stump activated the hand region of the somatosensory cortex (Wu and Kaas, 1999). The projections from the forelimb portion of motor cortex to the cervical spinal cord remained intact, with no obvious change in extent of terminations (Wu and Kaas, 1999), while the columns of motoneurons in the cervical spinal cortex that were previously related to the missing limb had innervated remaining more proximal muscles related to the shoulder or hip (Wu and Kaas, 2000). Here we report how afferents from the skin and muscles of the stump terminate in the spinal cord and brainstem after long-standing amputations. Our results suggest an expansion of terminations of both muscle and skin afferents into the

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ISSN 0899–0220 (print)/ISSN 1369–1651 (on line)/02/020153–11
DOI: 10.1080/08990220220133261

denervated parts of the spinal cord and brainstem. Comparable reorganizations of the somatosensory and motor systems in humans may well account for many of the perceptual abnormalities that occur after the loss of a limb.

Materials and methods

Animals

For this study, we were able to obtain two adult squirrel monkeys (*Saimiri sciureus*) and two adult galagos (*Galago garnetti*) with long-standing therapeutic limb amputations performed by veterinarians after injuries early in life (less than 2 months old). One galago had a hindlimb removed at the hip joint, while the other three primates had a forelimb removed at the shoulder joint. Tracer injections and cortical mapping studies were conducted in these amputated animals when they were between 4 and 8 years of age. Three normal adult squirrel monkeys and five normal adult galagos served as controls. All animal protocols were reviewed and approved by the Vanderbilt Animal Care and Use Committee and followed National Institutes of Health guidelines.

Tracer injections

Animals were anesthetized with isofluorane gas for the muscle injections. After the skin was cleaned with disinfectant, the anatomical tracer cholera-toxin B subunit (CTB, Sigma Chemicals, St. Louis, MO, 1% in distilled water, 20–25 μl/site) was injected with a Hamilton syringe that was used to penetrate skin and enter the muscles in amputated animals and normal controls. In order to cover sufficient muscle groups, injections were placed at 2–4 sites for each body part. Ten minutes following each injection, the syringe was slowly withdrawn from the muscles. Due to spread of the tracer at the injection sites, both muscle spindle and cutaneous afferents were labeled in the regions injected.

For comparisons, patterns of terminations of afferents from the limb of normal control animals were collected by placing tracers in different muscle groups mediating either distal limb or proximal limb movements. For the muscles mediating distal forelimb movements, injections were placed in abductor pollicis brevis, opponens digiti minimi, extensor carpi ulnaris, and flexor pollicis longus. For the muscles mediating proximal forelimb movements, injections were placed in shoulder muscles, including anterior and posterior deltoid, pectoralis major and infraspinatus. For the hindlimb muscle injections, tracers were placed either in foot (abductor hallucis) or hip (gluteus maximus, lliopsoas, and tensor fascia lata) muscles. In addition, direct comparisons of afferent termination patterns from the two body sides were made in the amputated animals by placing bilateral injections symmetrically in muscles mediating remaining stump movements ($n = 3$) or stump tissue overlying the point of amputation ($n = 1$). In two forelimb amputated squirrel monkeys, tracers were placed in the muscles mediating shoulder and remaining stump movement, immediately proximal to the amputation. Patterns of connections were directly compared with those from injections placed in the same muscles of the intact side. Similarly, in a hindlimb amputated galago, tracers were placed bilaterally and symmetrically in the muscle groups mediating hip movements. Because of the long distance and consequently long survival time for the injected tracers to be transported from the hindlimb muscles to the brainstem, the brainstem of the animals receiving hindlimb injections was not processed for histology. Finally, in a forelimb amputated galago, tracer was placed on the stump tissue overlying the point of amputation and patterns of connections were compared with tracer placed on the intact distal forelimb.

Perfusion and histology

Animals were killed following a survival of 3–8 days after tracer injections and a session of stimulating motor cortex with microelectrodes (see Wu and Kaas, 1999; Wu *et al.*, 2000 for detail). The animals were deeply anesthetized with a lethal dose of sodium pentobarbital and perfused through the heart with cold saline followed by fixative (4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4). After the perfusion, blocks of brainstem and spinal cord between C2 to T3 and L3 to S2 segmental levels were removed. Tissue blocks were placed in 30% sucrose buffer at 4°C overnight. Serial sections were cut on a freezing sliding microtome in the coronal plane at 40–50 μm thickness for the brainstem and at 50–60 μm thickness for the spinal cord. Sets of brain sections were immunocytochemically processed for CTB to reveal the distributions of labeled terminals. Other sets were processed for Nissl substance or cytochrome oxidase (CO) to reveal the architectonic boundaries. For details of the histological procedures and methods of identifying the spinal cord segments, see Wu and Kaas (2000).

Results

Here we describe the projections to the dorsal column nuclei and spinal cord of sensory afferents from the muscles and skin of normal limbs and stumps of amputated limbs in two primate species. The major result is that afferents from the stump in amputated animals had expanded their territories of terminations to include portions normally innervated by afferents from the missing limb.

Normal patterns of central terminations of the sensory afferents

The cuneate nucleus and the external cuneate nucleus receive central terminations of the dorsal root ganglion cells innervating the upper limb. Previous studies indicate that each group of primary afferent fibers from a given body part terminates in an elongated column throughout most of the anteroposterior length of these nuclei (Nyberg and Blomqvist, 1982; Bakker *et al.*, 1985; Jasmin *et al.*, 1985; Florence *et al.*, 1988, 1989; Rasmusson, 1988; Culberson and Brushart, 1989; Xu and Wall, 1996, 1999). We placed injections of tracers in the distal limb tissue of three squirrel monkeys and three galagos. No significant differences in central termination patterns were noted between normal control animals and the intact limb in amputated animals. In agreement with previous reports, we found that the pars rotunda of the cuneate nucleus receives inputs from the hand and forearm (Fig. 1). Shoulder injections labeled afferents in a different location at the most lateral margin of the cuneate nucleus immediately surrounding the pars rotunda (Fig. 2 and intact side of Fig. 4). This region corresponds to the pars triangularis of the cuneate nucleus where neurons are responsive to the stimulation of the shoulder as described in the electrophysiological mapping experiments (Xu and Wall, 1996, 1999).

The external cuneate nuclei receive muscle spindle afferents from the upper limb and trunk, and send projections to cerebellum and the superior portion of ventroposterior thalamus relaying to cortical area 3a (Hummelsheim *et al.*, 1985; Jasmin and Courville, 1987; see also Kaas and Pons, 1988 for review). The injections also labeled afferent terminations in the external cuneate nucleus. In agreement with the results of previous anatomical studies (Bakker *et al.*, 1985; Jasmin *et al.*, 1985;

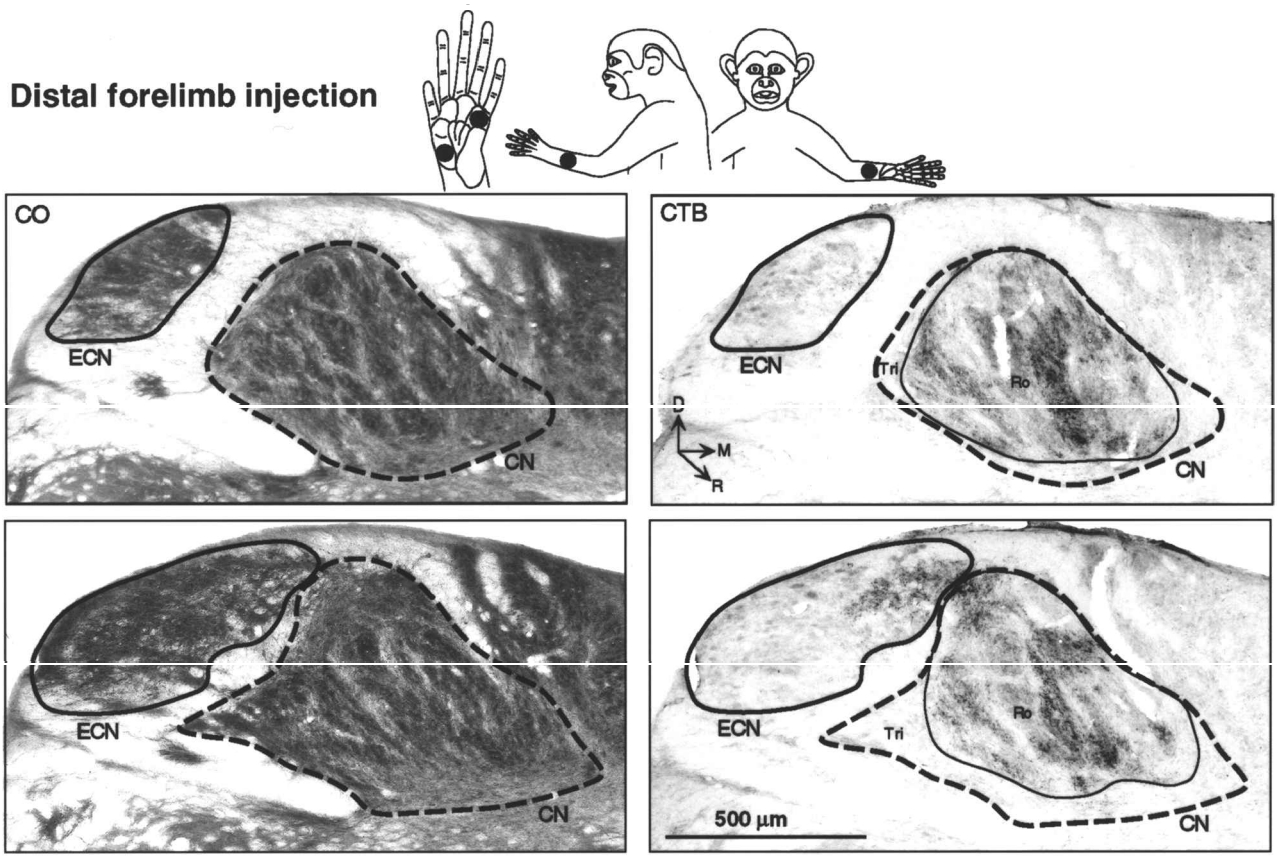

FIGURE 1. Photomicrographs of brainstem cross-sections, showing the anterogradely labeled terminals (right) following tracer injections in the distal forelimb of a normal squirrel monkey. The architectonic boundaries on the right were drawn from the adjacent sections reacted for cytochrome oxidase (CO) activity (left). Pairs of images were digitized and overlaid in Photoshop (Adobe System) based on the surface features and shared blood vessels. Cholera-toxin B subunit (CTB) was injected into the hand and forearm at sites indicated by dots above. The cuneate nucleus (CN, outlined in dashed lines) and external cuneate nucleus (ECN, outlined in solid lines) are marked. The subdivisions of cuneate nucleus, pars rotunda (Ro) and pars triangularis (Tri) are marked and outlined by the thin solid lines. The distal forelimb injections labeled the center portion (i.e., Ro) of the cuneate nucleus as well as the medial portion of the external cuneate nucleus. The sections in the upper panels are 480 μm caudal to those in the lower panels.

Lan *et al.*, 1994), label following distal forelimb injections was found in the middle third of the external cuneate nucleus immediately adjacent to the border of the cuneate nucleus (Fig. 1), whereas label following shoulder injections was distributed lateral to the hand afferents (Fig. 2, and intact side of Fig. 4). In contrast to the cuneate nucleus, sensory afferents from the hand only occupy a very small portion of the external cuneate nucleus, and much of the territory is devoted to inputs from larger muscles. A medial cell column confined to the caudal external cuneate nucleus receives projections from the hand muscles, while the ventral cell column in the posterior half of the external cuneate nucleus receives projections from the forearm and arm muscles. In the caudal external cuneate nucleus, shoulder muscles are represented by a dorsolateral cell column.

Spinal Clarke's column (lamina VII) relays information about position and movement of the leg and lower trunk directly to the cerebellum (Lundberg and Oscarsson, 1956). We placed injections of tracers into the hip and foot muscles of two normal galagos. In Clarke's column, the hip muscle afferents appeared to terminate lateral to those of the foot muscle afferents (not shown). Moreover, in laminae VI and VII of the spinal gray, consistent with earlier results (Culberson and Brown, 1984), the terminations of the entering fibers from the distal hindlimb were distributed more densely in lower segments compared with those from the hip.

We also examined the longitudinal dispersal of sensory afferents within the dorsal horn of the cervicothoracic cord in animals that received forelimb injections or within the lumbosacral cord in animals that received hindlimb injections. In the normal controls, the projections from the normal shoulder formed an extensive labeling in the rostrocaudal domains of the cervical spinal cord, covering C2 to T2 segments, with the densest labeling found between the C2 and C4 levels. In fact, shoulder projections at the level of C2 to C4 segments cover the entire laminae I–V, and have heavy terminations in lamina VI of the dorsal horn. Shoulder projections below the C7 segment were always confined to the most lateral margin of laminae I–V, and have light terminations in lamina VI. By contrast, the terminations from the distal forearm

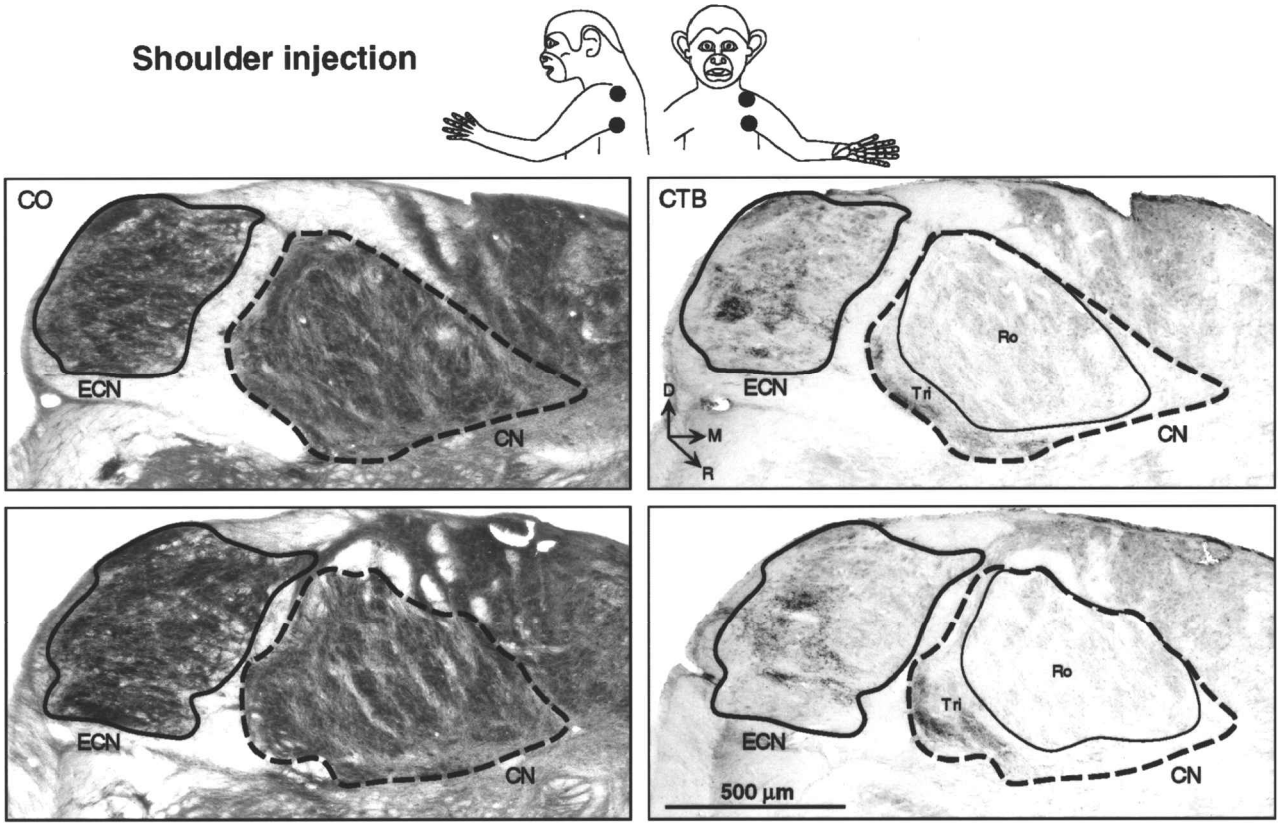

FIGURE 2. Photomicrographs showing the distribution of labeled terminals following injections of CTB into the shoulder of a normal squirrel monkey. Labeled terminals are concentrated in the ventral margin of cuneate nucleus as well as the dorsolateral portion of external cuneate nucleus. Note that the pattern of terminations from shoulder is restricted to pars triangularis (Tri) and therefore is complementary to the pattern of terminations from distal forelimb (cf. Fig. 1). Conventions are as in Figure 1. The sections in the upper panels are 320 µm caudal to those in the lower panels.

have a much more restricted extent. Projections from distal forelimb were found throughout the C4 to T1 segments, with the densest labeling found between CS and C8 levels (see Fig. 6C for summary). Similarly, projections from the normal hip cover laminae I–VII between L3 and S1 segments, with the densest labeling at L3–L5 levels, whereas projections from the foot are restricted between L3 and S1 segments. In the segments where both hip and foot terminations ramify, afferents from the hip are always located more laterally than the afferents from the foot.

Neuronal growth following amputation

Injections in the limb stump labeled larger territories than the matched injections from the intact side or normal controls. In the cuneate nuclei of forelimb amputees, sensory afferents from the stump extended into the pars rotunda, which normally receives distal forelimb inputs exclusively. In each of these cases, the label extended rostrocaudally through nearly all of the cuneate nuclei (Figs. 3 and 4). At higher magnification, the axon terminals could be seen to ramify and have synaptic boutons. While injections of tracer into the stump labeled terminals over most of the pars rotunda, the terminations were not uniformly distributed and instead they formed patchy and irregular terminal ramifications within the CO dense cell clusters.

Similar observations were made in the external cuneate nuclei. Injections placed at the shoulder muscles not only labeled the dorsolateral cell column as in normal limbs, but also clearly labeled the medial column that is normally devoted to hand muscle afferents (Fig. 3). Thus, axons had terminated and sprouted to invade portions of the external cuneate nucleus deprived of their normal inputs as a result of the amputation.

In the single hindlimb amputee, labeled terminations in the lumbar to sacral segments of the spinal cord were directly compared after bilateral and symmetrical injections in the hip. We found that the distribution of labeled terminations on the amputated side was much more extensive than the normal distribution on the intact side (Fig. 5). Moreover, the label from hip injections on the amputated side covered the medial portion of Clarke's column where inputs from the foot usually terminate. The longitudinal extent of the labeled zone covered more spinal cord segments than labeled zones on the intact side of amputee or normal controls.

In the forelimb amputees, we found that projections from the shoulder on the intact side had

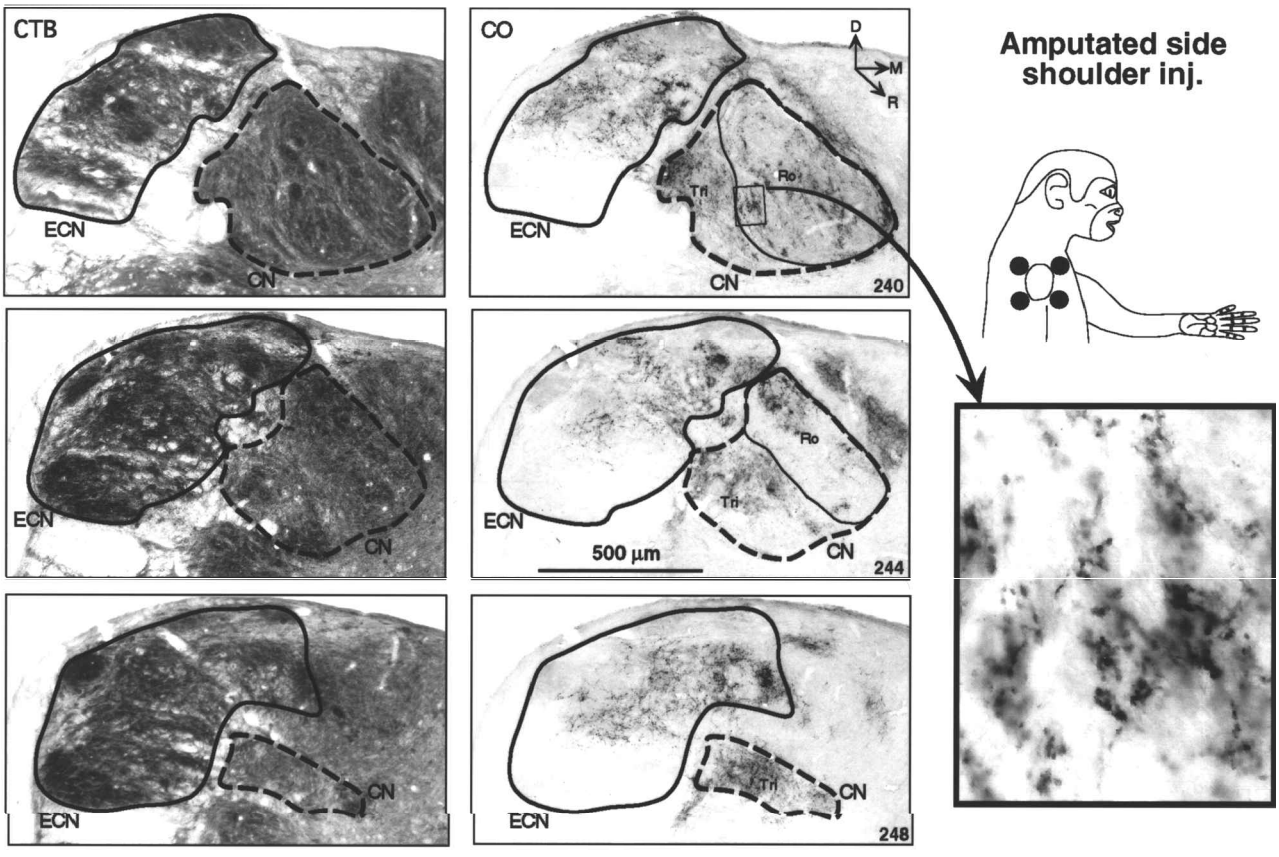

FIGURE 3. Patterns of label following injections placed on the shoulder of a forelimb amputee. The distribution of labeled terminals extends into the central cuneate nucleus and medial portion of external cuneate nucleus that normally only receive inputs from distal forelimb. The box outlines the region that is shown in higher magnification. The enlarged photomicrograph reveals the *en passant* and terminal boutons that are apparent on the labeled axons in the central cuneate nucleus, suggesting functional contact with the deprived region. Also note that the borders of CO-dense cell clusters in amputees are less clear than in normal controls (cf. Figs. 1 and 2). Sections were cut at 160 µm with section numbers listed at the lower right. More rostral sections have progressively higher section numbers. Conventions are as in Figure 1.

termination patterns that matched those in the normal controls. By contrast, projections from the shoulder stump of the amputated side covered larger rostrocaudal domains of the dorsal horn. In these cases, the labeled terminations covered the entire mediolateral extent of the superficial layers in the dorsal horn where the distal forelimb projection is normally located (see Fig. 6C for summary illustration). Similarly, inputs from the hip of the hindlimb amputee had a wider rostrocaudal distribution of label, extending into the S1 segment.

Brainstem neuronal atrophy and 3b reorganization

Consistent with previous results (e.g., Rhoades *et al.*, 1993, Florence and Kaas, 1995; Jones and Pons, 1998), we found that long-standing forelimb de-efferentation results in transneuronal atrophy in the cuneate nucleus and the external cuneate nucleus. In all three cases, the cuneate nucleus and external cuneate nucleus were smaller on the amputated than the intact side. Moreover, the density of CO staining in the pars rotunda on the amputated side was no lighter than on the intact side, suggesting that deafferented neurons still maintain normal levels of metabolic activity. However, in the pars rotunda of the amputated side, the borders between CO dark cell clusters were blurred by the condensation of the clusters within a smaller volume (Fig. 4), and the clusters stained darker for Nissl substance due to an increase in cell packing density within each cluster. Nevertheless, the borders of the shrunken pars rotunda were still distinguishable from surrounding structures. Thus, the larger extents of termination zones of afferents from remaining body parts were not due to any detectable shift of the normal target as a result of shrinkage of the deafferented territory, but instead resulted from the remaining sensory afferents innervating the shrunken pars rotunda (Fig. 4, also see Fig. 6 for summary drawing).

In two of the amputees, we also demonstrated that somatosensory cortex (area 3b) had undergone reorganization so that much of the deprived cortex had become responsive to inputs from the proximal body part (Wu and Kaas, 1999). An example of such reorganization is shown in Fig. 6. Thus, the changes in brainstem innervation patterns likely contribute to the activation of somatosensory cortex.

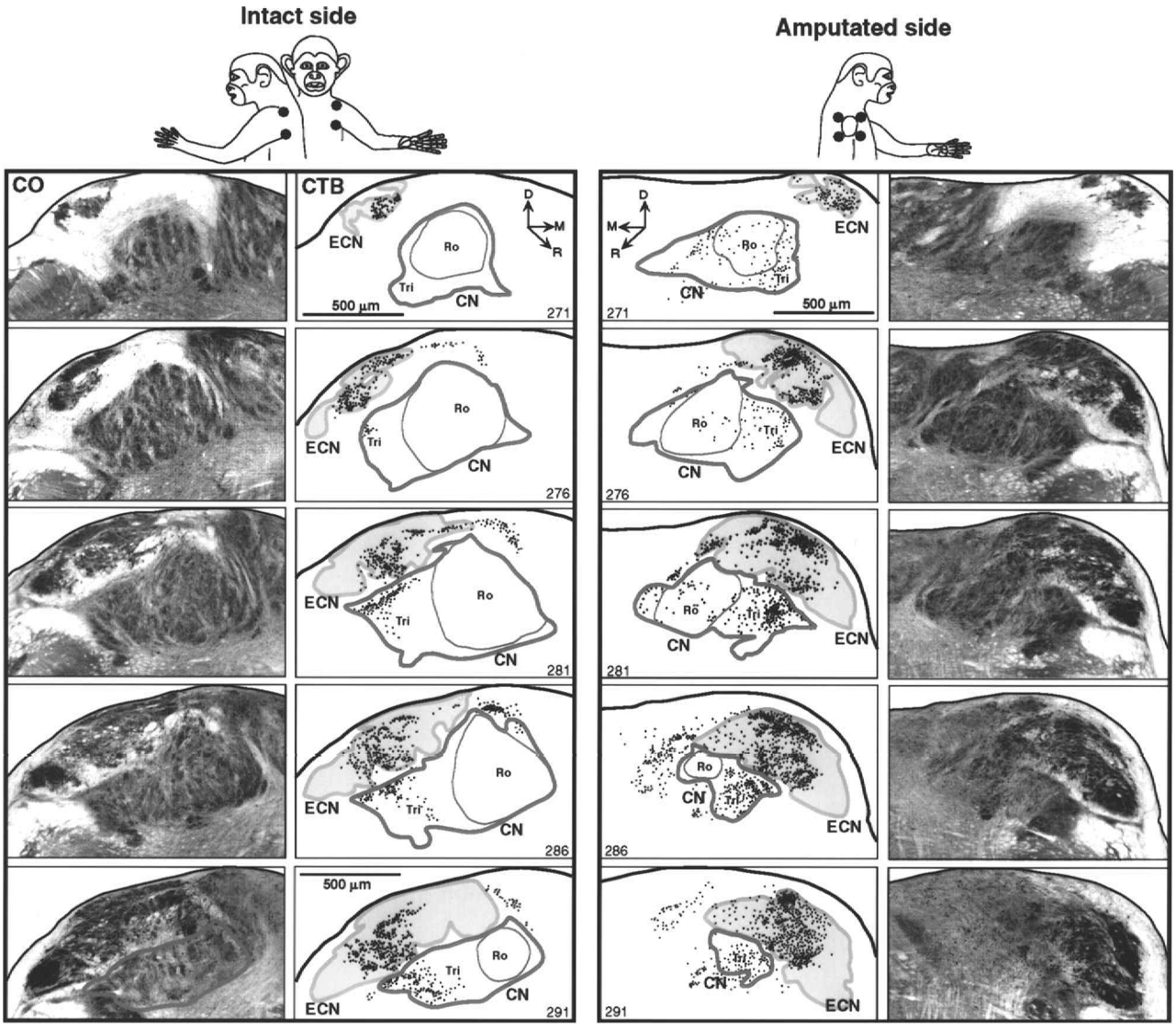

FIGURE 4. Series of brainstem sections showing distributions of labeled terminals after bilaterally matched injections in shoulders of both sides in a forelimb amputee. The distribution of anterograde label (black dots) from the adjacent sections is superimposed on the CO stained sections. The external cuneate nucleus (ECN) is shaded in light gray, and the border of the cuneate nucleus (CN) is marked with a thick gray line. Note that the deprived portion of cuneate nucleus and external cuneate nucleus that normally receive inputs from the distal forelimb is not less intensely stained for CO on the side of amputation (right) than on the intact side (left). However, the deprived portion is now occupied by the labeled terminals resulting from shoulder injections. Also note that the cuneate nucleus and external cuneate nucleus appear apparently smaller on the amputated side in sections cut symmetrically through the two sides of brainstem. Conventions as in Figures 1 and 3.

Discussion

Here we show that long-standing limb loss in primates produced shrinkage of the neuron territories deprived of their normal source of sensory activation. Nevertheless, these territories in the cuneate nucleus and the external cuneate nucleus for the forelimb and Clarke's column for the hindlimb were not completely deprived of sensory inputs since afferents from the muscles and skin of the stump grew into the deprived territories. As a result, these territories expressed levels of cytochrome oxidase that appeared to be normal. These reinnervated neurons undoubtedly relayed to activate deprived populations of thalamic neurons, which in turn activated deprived zones of somatosensory cortex. Thus, such a reinnervation of spinal and brainstem structures is likely to participate in the mislocalization of sensation of movement and touch to a missing limb. Our results and conclusions are related to previous findings below.

The growth of new connections

The loss of a forelimb resulted in the expansion of shoulder afferents from their normal locations to forearm and hand territories in the cuneate nucleus and external cuneate nucleus. Likewise, the hindlimb loss was followed by an expansion of hip inputs into the lower leg and foot territories in Clarke's column. Expansion of afferent terminations in the cuneate nucleus has been observed

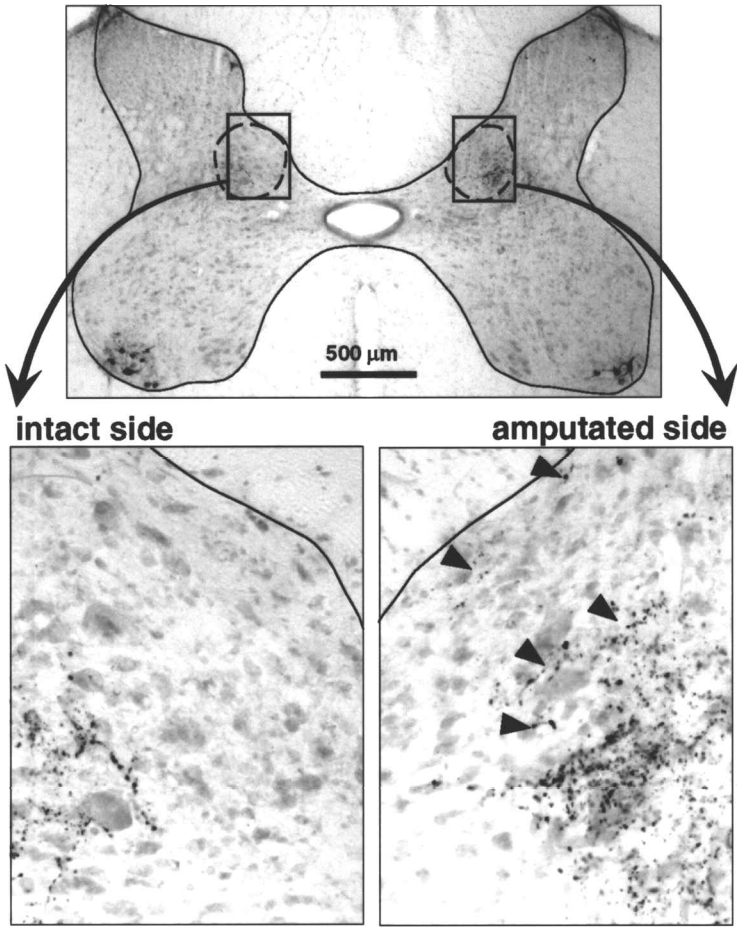

FIGURE 5. Patterns of terminations in Clarke's column (dashed outline) in L3–L4 level of spinal cord of the hindlimb amputee following bilateral and symmetrical injections into the hip muscles. The terminations on the amputated side are denser and extend more medially than the terminations on the intact side, suggesting that the muscle spindle afferents from the hip musculature have taken over the deprived territory that was normally devoted to the feet.

following limb amputation, and dorsal column section (Rhoades *et al.*, 1993; Florence and Kaas, 1995; Jain *et al.*, 2000). The terminations of afferents from the stump of an amputated limb also occupied larger than normal territories in the dorsal horn of the spinal cord (Florence and Kaas, 1995). The sprouting of peripheral nerve afferents into the deafferented dorsal horn of the spinal cord has been an issue of controversy, but such growth has now been repeatedly demonstrated in monkeys and other mammals (see Florence *et al.*, 1993 for review, Florence and Kaas, 1995). Here we show that reinnervations occur not only in the cuneate nucleus but also in the external cuneate nucleus after forelimb loss and in Clarke's column and the dorsal horn after hindlimb loss. Such new growth occurs not only in monkeys, but also in prosimian primates.

Two mechanisms can contribute to anatomical expansion of the central projections observed in the present study. One is that changes in the central terminations result from collateral sprouting of non-deprived axons to grow into the deprived zone. Such sprouting was expected since afferents from the shoulder have been shown to grow into the hand region of the cuneate nucleus after distal forelimb amputation in monkeys (Florence and Kaas, 1995). In addition, some afferents from the face may extend from their normal terminations in the trigeminal nucleus to new locations in the denervated cuneate nucleus after forelimb amputation or sections of forelimb afferents in the dorsal column of the spinal cord (Jain *et al.*, 2000). Forelimb amputation in fetal rats leads to an expansion of remaining dorsal column inputs into the deprived cuneate nucleus (Rhoades *et al.*, 1993), but digit amputation in adult raccoons produced no change in the distribution of inputs from remaining digits (Rasmusson, 1988). The neuronal atrophy consistently observed in this and other studies (Rhoades *et al.*, 1993; Florence and Kaas, 1995; Woods *et al.*, 2000) may have led the adjacent central terminations to sprout and take over the territory, and such alteration can be further amplified by the divergence of the ascending pathway to the cortex (for review, see Kaas *et al.*, 1999; Jones, 2000; Kass and Florence, 2000).

The other possible origin for the anatomical reorganization is peripheral hyperinnervation. Regenerated peripheral branches of severed sensory

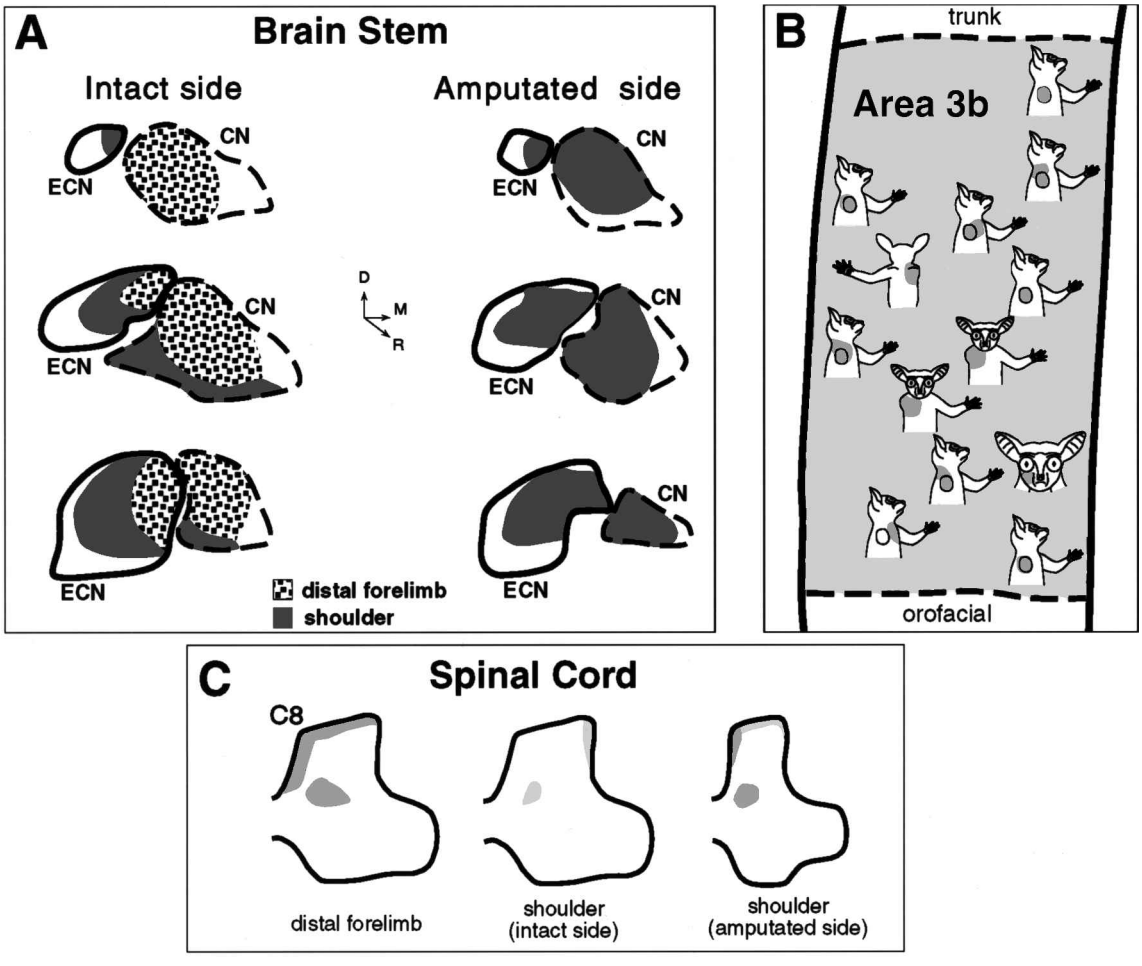

FIGURE 6. (A) A summary of the changes in sensory terminations in the brainstem after long-standing forelimb loss. The deprived region of the brainstem is occupied by inputs from body parts proximal to the amputation. In the animals with a loss of the entire forelimb, the former territory of distal forelimb is occupied by afferents from the shoulder. The cuneate nucleus and external cuneate nucleus are outlined in dash and solid outlines, respectively. Sensory terminations from distal forelimb are marked by a dotted pattern, whereas terminations from the shoulder are marked in dark gray. (B) Microelectrode recording in somatosensory cortex reveals that the majority of the recording sites in the deprived cortex is responsive to the body proximal to the stump. Thick lines delineate the areal borders of 3b, and dashed lines depict the borders between former forelimb (highlighted in light gray), trunk, and orofacial representations. The locations of each recording site are indicated by the body figure of the animal, with receptive field at that site shown in dark gray. (C) Summary drawing of the reorganized terminations in the dorsal column of the spinal cord. At the C8 level, shoulder inputs from the amputated side occupied a larger rostrocaudal extent than those from the intact side, and cover the region that normally receives inputs from the distal forelimb.

nerves may have innervated more extensive skin or muscle spindles surrounding the stump than they had before the amputation. An increased number of terminal ramifications of the severed nerve was found in the dissected skin tissue adjacent to the amputated digit stump (Manger *et al.*, 1996). Thus, severed nerve terminals may make new connections with adjacent tissue, and therefore innervate the skin or muscle spindles more extensively. However, peripheral hyperinnervations do not seem to be responsible for all expansions of labeled regions in the spinal cord and brainstem after peripheral deafferentations. After long-standing forelimb denervation by dorsal column sections, injections of tracers into the chin label part of the deprived cuneate nucleus (Jain *et al.*, 1997). Afferents from the forelimb would not likely hyperinnervate the chin. Dissociating between central sprouting and peripheral hyperinnervation is beyond the scope of the present study, and the two mechanisms can progress in parallel and result in central reorganization.

The atrophy of the cuneate nucleus

Forelimb amputation in monkeys (Florence and Kaas, 1995) and cats (Avendano and Dykes, 1996a, b) causes a shrinkage of the cuneate nucleus, as noted here for monkeys and galagos. The neuronal density increases due to a loss of neuropil and there is some shrinkage of cell bodies, but a significant cell loss probably does not occur (Avendano and Dykes, 1996a, b). The prevention of cell loss may partially, at least, be a consequence of the reinnervation that occurs, although the reinnervation may be sparse. While some reduction in cytochrome oxidase was apparent after forelimb denervation in cats (Avendano and Dykes, 1996a), this was not apparent in the present cases, and a reduction was not noted in a

previous description of the cuneate nucleus in monkeys with forelimb loss (Florence and Kaas, 1995).

The physiological, perceptual, and motor consequences of reinnervation

After forelimb amputation in monkeys, the hand and forelimb portion of primary somatosensory cortex (area 3b) becomes responsive to remaining inputs from the stump, arm, or face (Pons *et al.*, 1991; Florence and Kaas, 1995; Jain *et al.*, 1997, 2000; Wu and Kaas, 1999; Florence *et al.*, 2000). This recovery likely depends in part on cortical mechanisms, including the growth of new cortical connections (Florence *et al.*, 1998), but the growth of new connections in the cuneate nucleus is undoubtedly important as well. After section of nerves to the glabrous hand in monkeys, many deprived neurons in the cuneate nucleus rapidly acquire receptive fields on the innervated dorsal surface of the hand (Xu and Wall, 1999). However, this rapid recovery, which also can be revealed in the deprived portions of cortex (Silva *et al.*, 1996), seems to have the spatial limitation since it only occurs in the representations of the adjacent body parts, such as other portions of the hand. Hence, such a rapid change cannot fully account for the extensive reorganization, and is unlikely to depend on the growth of new connections that occurs after forelimb denervations, as such growth may take as long as 6–8 months to emerge (Jain *et al.*, 1997). After forelimb amputations, reactivations of deprived forelimb representations occur not only at the level of cortex, but also in the ventroposterior nucleus of the thalamus of monkeys (Florence *et al.*, 2000) and humans (see Davis *et al.*, 1998). While the growth of new connections in the thalamus of such monkeys may occur, it has not yet been demonstrated. However, the new growth in the cuneate nucleus likely results in the reactivation of many cuneate nucleus neurons which in turn project to reactivate deprived regions of the thalamus and the cortex, as previously suggested (Florence and Kaas, 1995; Jain *et al.*, 2000). According to present results, this could occur not only for the relay of cutaneous information to the thalamus and cortex, but for the relay of muscle spindle information from the external cuneate nucleus to the ventroposterior superior nucleus of the thalamus to area 3a of the somatosensory cortex (see Kaas and Pons, 1988). We also expect a relay of both types of information from the expected reorganization that has reactivated neurons in nucleus gracilus after hindlimb loss, although this has not yet been demonstrated.

The likely consequence of the reactivation of deprived portions of somatosensory pathways is the mislocalization of peripheral stimuli (see Ramachandran *et al.*, 1992; Ramachandran, 1993; Flor *et al.*, 1995; for review). Direct recordings in the thalamus of a limb amputated patient indicate that neurons that would normally be responsive to stimuli on the missing limb were responsive instead to touch on the stump, and yet electrostimulation of the same neurons evoked sensations of the missing limb (Davis *et al.*, 1998). Electrical stimulation in the leg area of motor cortex of the amputee produced the sensation of movement from the phantom leg (Woolsey *et al.*, 1979). Nevertheless, phantom limb sensations are not limited to tactile sensations, but also include sensations of spontaneous and involuntary phantom limb movement. Such unwanted proprioceptive sensations are likely to be mediated by the same mechanisms as mismatched tactile sensations, as the result of the concurrent reorganizations in both cutaneous and proprioceptive afferents observed in the present study. Spontaneous activity in the reorganized systems may also contribute to the sensation that the missing "phantom" limb is still present (Ramachandran, 1993). In addition, stimulation of neurons throughout the deprived motor cortex can evoke muscle contractions from the body part proximal to the amputated stump (Donoghue and Sanes, 1988; Cohen *et al.*, 1991; Schieber and Deuel, 1997; Wu and Kaas, 1999; Qi *et al.*, 2000). These inappropriate muscle contractions may result in part from the growth of cut motor nerves into remaining shoulder muscles (Wu and Kaas, 2000). Possibly, other new growth occurs in the spinal cord as well.

In humans, the sensation of movement from the missing hand accompanies actual contractions in stump muscles that are evoked by transcranial magnetic stimulation (Cohen *et al.*, 1991) or electrical stimulation of motor cortex (Woolsey *et al.*, 1979). Such stump muscle contractions also occurred when an amputee was asked to move his phantom limb (Ramachandran, 1993). Here, we propose that the concurrently rewired afferent and efferent pathways can generate inappropriate interpretations of afferent signals from the moving stump. The motor commands sent to the phantom limb activate the spinal motoneurons that have been rewired to the muscles proximal to the limb stump (Wu and Kaas, 1999, 2000). Subsequently, the proprioceptive feedback produced from these contracting muscles is sent to activate the deafferented central targets through rewired afferent connections (shown in this study) and thereby evoke the sensation of phantom limb movement.

Acknowledgements

We thank Drs N. Jain and C. Collins for helpful comments on the manuscript. We are also grateful to Judy Ives and Laura Trice for histological assistance. This research was supported by NIH grant NS 16446 to J. H. Kaas.

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