stimulating electrode that was bipolar and coated with Formvar (AM-Systems). Before each experiment, we determined the maximal EPSP amplitude by increasing the stimulation intensity in small increments until the amplitude of the peak of the negative extracellular potential saturated. The opposing effects of the positive population spike recorded in the stratum radiatum at high stimulation intensities were not taken into account. The strength of presynaptic fiber stimulation was then adjusted to evoke EPSPs that were 25% of the maximal amplitude. After recording baseline synaptic responses for 20 to 30 min, we induced LTP using one of four different protocols. Two of these protocols consisted of two trains of 100-Hz stimulation (1.0-s duration) delivered 20 s apart. For weak intensity 100-Hz stimulation, the stimulation intensity was left at that used to evoke baseline synaptic responses. For strong intensity 100-Hz stimulation, the 100-Hz trains were delivered at a stimulation intensity sufficient to evoke EPSPs that were 75% of the maximal EPSP amplitude. LTP was also induced by theta-burst stimulation protocols that consisted of bursts of four stimulation pulses at 100 Hz delivered with 200 ms between each burst (that is, at 5 Hz). Weak theta-burst stimulation consisted of 25 bursts given at baseline stimulation intensity, whereas strong theta-burst stimulation consisted of 10 bursts delivered at an intensity sufficient to evoke EPSPs that were 50% of the maximal obtainable EPSP amplitude. As controls, age-matched littermates with intact NOS alleles, as well as 129/Sv and C57BL/6 (male and female) mice, were used. The animals ranged from approximately 6 to 23 weeks of age. The results from wild-type mice of different genetic backgrounds and sex were similar, and the results were combined. All values reported are mean ± SEM. We performed statistical comparisons by using Student's *t* tests for two independent means. For immunostaining, $nNOS^-$ and wild-type mice were anesthetized with pentobarbital (100 mg per kilogram of body weight) and killed by perfusion with phosphate-buffered saline, which was followed by perfusion with freshly depolymerized 4% paraformaldehyde (PF) in 0.1 M phosphate buffer (PB). The brains were removed and postfixed in 4% PF in PB for 2 to 4 hours. The brains were then cryoprotected by soaking overnight in 20% (v/v) glycerol in PB. Immunostaining for eNOS and nNOS was performed as described (*13*). Free-floating tissue sections (40 μm) were incubated in affinity-purified eNOS antiserum (1:50 dilution) or affinity-purified nNOS antiserum (1:1000 dilution). Staining was visualized with an avidin-biotin-peroxidase system (Vector Laboratories) with diamino benzidine as a chromagen. Controls for specific staining included preadsorption with excess peptide for eNOS and excess fusion protein (amino acids 1 to 181 of cloned nNOS) for nNOS, which completely eliminated staining for the antisera.

21. J. Larson, D. Wong, G. Lynch, *Brain Res.* **368**, 347 (1986).
22. W. Y. Musleh, K. Shahi, M. Baudry, *Synapse* **13**, 370 (1993).
23. In slices from wild-type animals, 60 min after weak theta-burst stimulation EPSPs were 126.2 ± 5.5% of the baseline (n = 11 animals, 19 slices); EPSPs were 184.2 ± 11.8% of the baseline (n = 6 animals, 10 slices) after strong theta-burst stimulation. In $nNOS^-$ slices, EPSPs measured 60 min after weak and strong theta-burst stimulation were 127.4 ± 8.9% (n = 8 animals, 14 slices) and 176.1 ± 5.2% (n = 6 animals, 13 slices) of the baseline, respectively. For both theta-burst stimulation protocols, the amount of LTP in slices from $nNOS^-$ animals was not significantly different from that seen in slices from wild-type animals (weak stimulation: t [17] = 0.11, not significant; strong stimulation: t [10] = 0.631, not significant).
24. We also observed that homosynaptic long-term depression (LTD) was normal in slices from $nNOS^-$ mice. In these experiments, LTD was induced in hippocampal slices obtained from young animals (4 to 6 weeks old) by 900 pulses of 1-Hz stimulation. One hour after beginning 1-Hz stimulation, EPSPs were 84.2 ± 4.5% of the pre-1 Hz baseline (n = 6 animals, 11 slices) in wild-type slices and 85.1 ± 4.6% of the baseline (n = 5 animals, 10 slices) in $nNOS^-$ slices.
25. Y. Izumi, D. B. Clifford, C. F. Zorumski, *Science* **257**, 1273 (1992).
26. E. S. Furtine, M. F. Harmon, J. E. Paith, E. P. Garvey, *Biochemistry* **32**, 8512 (1993); R. G. Knowles and S. Moncada, *Biochem. J.* **298**, 249 (1994).
27. In wild-type hippocampal slices that were continuously bathed in 50 μM oxyhemoglobin (beginning at least 1.5 hours before induction of LTP was attempted), EPSPs 60 min after weak theta-burst stimulation were 98.89 ± 4.2% of the baseline (n = three animals, six slices; not significantly different from the baseline, $t(2)$ = 0.46, not significant). LTP in slices from $nNOS^-$ mice was also blocked by oxyhemoglobin. EPSPs 60 min after weak theta-burst stimulation were 98.43 ± 3.62% of the baseline (n = three animals, six slices) and not significantly different from pre–theta-burst stimulation levels [$t(2)$ = 0.434, not significant]. Importantly, the effects of hemoglobin on LTP may be due to its ability to bind extracellular CO, another candidate retrograde messenger, and thus to prevent it from reaching the presynaptic terminal.
28. A. Arai and G. Lynch, *Eur. J. Neurosci.* **4**, 411 (1992); G. D. Clark, L. T. Happel, C. F. Zorumski, N. G. Bazan, *Neuron* **9**, 1211 (1992); A. Wieraszko, G. Li, E. Kornecki, M. V. Hogan, Y. H. Ehrlich, *ibid.* **10**, 553 (1993); K. Kato, G. D. Clark, N. G. Bazan, C. F. Zorumski, *Nature* **367**, 175 (1994).

29. E.R.K. is a Senior Investigator and T.J.O. is a Research Associate of the Howard Hughes Medical Institute. E.R.K. and T.J.O. were also supported by National Institute of Mental Health grant MH-45923. S.H.S. is supported by U.S. Public Health Service grants DA-00266, Research Scientist Award DA-00074, and a grant from the William M. Keck Jr. Foundation. T.M.D. is supported by U.S. Public Health Service grant CIDA NS-01578. P.L.H. and M.C.F. are supported by NIH and a sponsored research agreement with Bristol-Myers Squibb.

18 February 1994; accepted 2 June 1994

TECHNICAL COMMENTS

Cortical Reorganization and Deafferentation in Adult Macaques

Until 1991, there was a general consensus that the reorganization of the body map in the primary sensory cortex after deafferentation in adult animals only occurs within 1 to 2 mm of neurons with normal receptive fields (*1*). In addition to the immediate effects of deafferentation, such as the unmasking of existing excitatory inputs from adjacent body parts (*2*), changes to the deafferented part of the map continue over weeks or months (*3*). However, most evidence suggests that plasticity is limited to a zone no wider than the extent of the arbors of thalamocortical axons (*1*). This reinforces the notion (the "unmasking hypothesis") that most of the reorganization occurs through an increase in the efficacy of thalamocortical connections that existed before deafferentation (*4*).

The evidence for limited ability of the adult cortex to reorganize was challenged by T. P. Pons and his colleagues (*5*), who made extracellular microelectrode recordings from neurons in the primary sensory area of the postcentral gyrus of anesthetized adult macaque monkeys more than 12 years after unilateral or bilateral sectioning of the dorsal roots from C2-T4. In the zone within which the arm and hand would normally be represented, Pons *et al.* found that all neurons now had receptive fields on the lower face, as if the entire strip of cortex below the hand area, in which the lower jaw is represented in intact monkeys, had been stretched out over a sheet of deafferented cortex that was 10 to 14 mm long (*5*).

We tested a more parsimonious explanation for the results of Pons *et al.* (*5*). If there were a second representation of the face medial to the arm area, then facial inputs could take over the deafferented cortex from two fronts. There is evidence that the lateral parts of the face and the lower jaw are represented a second time in the "upper head area." This region was originally discovered at the dorsal end of the arm representation by Woolsey *et al.* (*6*), who used evoked potentials with monkeys. Later, the existence of neurons with tactile receptive fields on the face within the upper head area was established (*7*, *8*). Our experiment, using an awake (N_2O-sedated) adult female macaque monkey (*Macaca mulatta*), was designed to confirm that some neurons in the upper head area have receptive fields on the lower jaw and to measure the distance between the two facial representations (*9*).

In agreement with earlier work, we found that the medial boundary of the primary face area overlapped that receiving inputs from the thumb (Fig. 1). Facial fields adjacent to those on the thumb were usually found on the lower jaw or lip (Fig. 2), but neurons with receptive fields on the nose and eyebrows were occasionally found next to those on the thumb or fingers (Fig. 2). Similar relationships were reported by

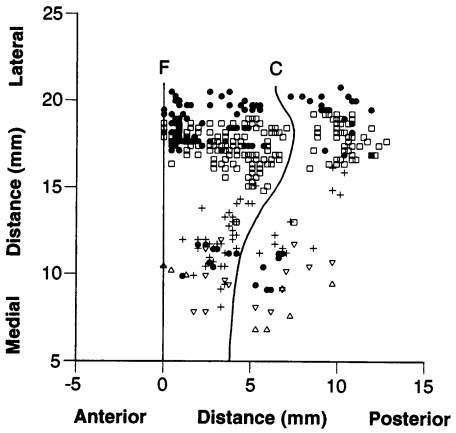

Fig. 1. The recording sites and body representation are shown in a two-dimensional map of area SI, similar to those used by Pons *et al.* (*6*). Histological sections were aligned on the fundus of the central sulcus (0 mm), and recording sites were projected on to the surface of the cortex. Some symbols represent several neurons recorded in close proximity. Receptive fields: orofacial (●), hand (□), arm (+), occiput (▽), trunk (△), fundus of central sulcus (F), crest of the bank of central sulcus (C).

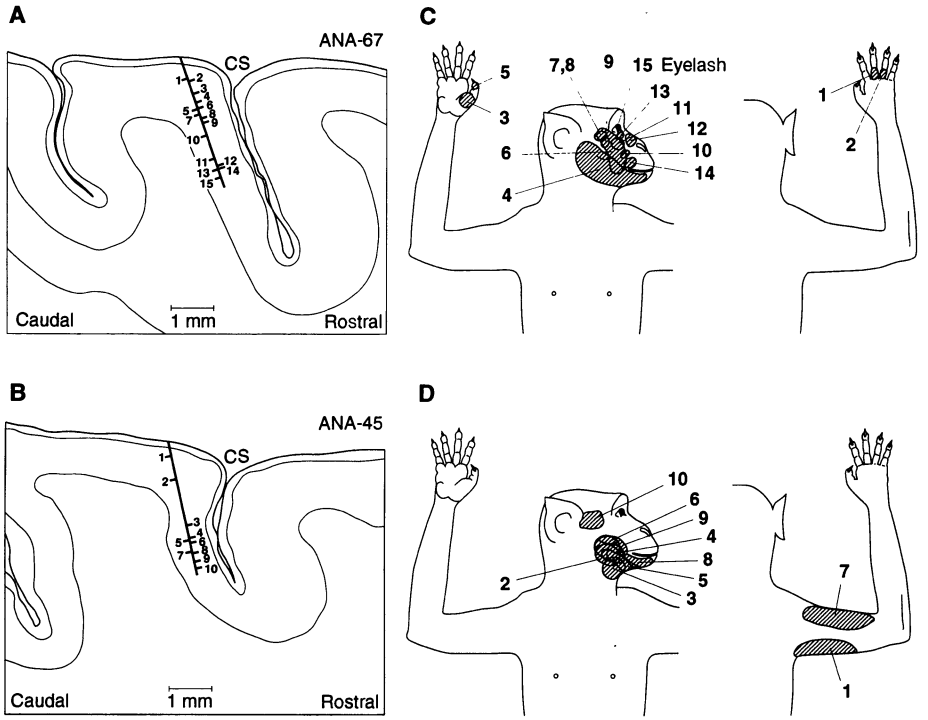

Fig. 2. (**A** and **B**) Parasaggital sections of the cortex about 17.5 mm (A) and 11.4 mm (B), from the midline in Fig. 1, showing electrode tracks and numbered recording sites. (**C** and **D**) The corresponding receptive fields of neurons recorded at each site. (C) Neurons with receptive fields on the thumb (3, 5) are next to those with fields on the lower jaw (4) and cheek (6) at the medial boundary of the primary face area. (D) At the laterlal boundary of the upper head area, receptive fields on the upper arm (1, 7) are next to those on the lower jaw (2, 8) or outer face (6).

others (*7*), although Pons *et al.* imply that only fields on the lower jaw and thumb were adjacent (*5*, p. 1859). We found that the medial and posterior boundaries of the upper head area were situated next to the representation of the trunk (Fig. 1), as suggested by earlier studies (*6*, *7*, *9*). More laterally, trigeminal receptive fields were found next to those on the shoulder or arm (Fig. 2). Our data and those of others (*6*, *7*, *9*) show that the neurons in this area receive inputs from only the eyebrows, posterior parts of the face, lower jaw, and lower lip; the central part of the face from the eyes to the upper lip is represented only in the primary face area (Fig. 2C). However, the fields represented in the upper head area are supplied by the trigeminal nerve, not by branches of area C2 or C3, and they remain after all the cervical roots are cut (*9*). Trigeminal inputs to the upper face area would therefore have remained intact in the monkeys studied by Pons *et al.* The descending tract of cranial nerve V extends as far caudally as C3 (*11*); the fact that only the periphery of the face is represented here may be because this part of the primary sensory cortex is supplied by the upper cervical dorsal horn and not by the more rostral parts of the trigeminal sensory nuclei. This speculation is based on the finding that sectioning the descending tract of cranial nerve V at C1 causes sensory deficits in only those areas that are represented in the upper face area; the central part of the face is spared (*12*).

One question posed by Pons and his colleagues (*5*, p. 1859) was why the zone occupied by the trunk did not expand ventrally. Our results (Fig. 1) show that the capacity for expansion of the area receiving inputs from the parts of the trunk is limited because no more than 2 mm of cortex receiving inputs from the occiput or upper arm divides the neurons in the upper face and trunk area (*13*).

The area of primary sensory cortex that receives inputs from the upper limb and cervical roots measured at least 12 mm in the medio-lateral direction. However, our results show that expansion of the facial representation into this area can take place from two fronts that are separated by approximately 5 mm at the closest point (Fig. 1).

When dorsal roots from C2 to T4 were cut, Bioulac and Lamarre (*9*) found that all of the deafferented area did not immediately become responsive to facial inputs. It may take many years for cortical reorganization to be completed, as Pons *et al.* (*5*) suggest, although there is other evidence that large areas of denervated cortex become responsive to the same inputs as adjacent areas within 2 months (*3*). However, our data (*14*) show that these changes do not require that the spread from adjacent areas be an order of magnitude greater than 1 to 2 mm and thus support the unmasking hypothesis (*1*). Even though it may be necessary to expand the limits to explain the results of Pons *et al.* (*5*), all the changes that they observed could still have taken place within the axonal arbors of thalamocortical cells that received inputs from both the face and the upper limb before deafferentation, because these can be as wide as 3.5 mm in macaques (*15*).

James P. Lund
Département de Stomatologie,
Faculté de Médecine dentaire,
Université de Montréal,
Montréal Québec, H3C 3J7, Canada
Gong-Duo Sun
Department of Physiology,
Nanjing University,
Nanjing, People's Republic of China
Yves Lamarre
Département de Physiologie,
Faculté de Médecine,
Université de Montréal

REFERENCES AND NOTES

1. M. M. Merzenich *et al.*, *Neuroscience* **8**, 33 (1983); M. M. Merzenich and J. H. Kaas, *Trends Neurosci.* **5**, 434 (1982); J. H. Kaas, M. M. Merzenich, H. P. Killackey, *Annu. Rev. Neurosci.* **6**, 325 (1983); M. Calford, *Nature* **325**, 759 (1991).
2. J. Metzler and P. S. Marks, *Brain Res.* **177**, 379 (1979); D. D. Rasmusson and B. G. Turnbull, *ibid.* **288**, 368 (1983); R. W. Dykes, P. Landry, R. Metherate, T. P. Hicks, *J. Neurophysiol.* **52**, 1066 (1984); M. Calford and R. Tweedale, *ibid.* **65**, 178 (1991).
3. M. M. Merzenich *et al.*, *Neuroscience* **10**, 639 (1983); M. Calford and R. Tweedale, *Nature* **332**, 446 (1988); M. M. Merzenich *et al.*, *J. Comp. Neurol.* **224**, 591 (1984).

4. R. W. Dykes and Y. Lamour, *Brain Res.* **449**, 1 (1988); J. T. Wall, *Trends Neurosci.* **11**, 549 (1988); R. W. Dykes, in *Brain Cholinergic Systems*, M. Steriade and B. Biesold, Eds. (Oxford Univ. Press, Oxford, 1990), pp. 294–313.
5. T. P. Pons *et al.*, *Science* **252**, 1857 (1991).
6. C. N. Woolsey, W. H. Marshall, P. Bard, *Bull. Johns Hopkins Hosp.* **70**, 399 (1942); D. P. Ullrich and C. N. Woolsey, *Trans. Am. Neurol. Assoc.* **23** (1954). (In the discussion following this paper, Perfield stated that neither the tongue nor the central part of the face seemed to be represented in the upper face area of man.)
7. D. A. Dreyer, P. R. Loe, C. B. Metz, B. L. Whitsel, *J. Neurophysiol.* **38**, 714 (1975).
8. Pons *et al.* do not refer to an upper face area: in figure 1 of their report, occipital and neck areas are shown, but not an upper face area. In another paper by Pons *et al.* [*J. Comp. Neurol.* **241**, 445 (1985)], cranial occipital and neck fields are described adjacent to the trunk, and some of these extend onto the forehead and to the face just in front of the ear. No neurons had fields on the lower jaw. The only "face" area is the traditional one lateral to the hand (figure 19 of that paper).
9. B. Bioulac and Y. Lamarre, *Brain Res.* **172**, 427 (1979).
10. The surgical and recording methods are similar to those described elsewhere [Y. Lamarre, A. J. Joffroy, M. Fillion, R. Bouchoux, *Rev. Can. Biol.* **29**, 371 (1970); W. Jiang, E. C. Chapman, Y. Lamarre, *Exp. Brain Res.* **84**, 342 (1991)]. Other data had already been gathered from the adult female *Macaca mulatta* used in this experiment before the sensory cortex was mapped. Two or three times per week, recordings were made with glass-insulated tungsten microelectrodes that were inserted through an implanted recording chamber (22 mm in diameter) with a piezoelectric microdrive. The animal was seated in a primate chair during each recording session, which lasted from 1 to 3 hours, and was sedated with a mixture of N_2O (5 liters/min) and O_2 (0.5 liters/min). Receptive fields on the skin, lips, and tongue were stimulated by brushing with cotton swabs. The caudal boundary of the primary motor cortex was established by microstimulation (nine pulses, 300 Hz, 0.1-ms duration). Within the motor cortex, descrete twitches could be evoked at currents of ≤20 μA.
11. T. Humphrey, *J. Comp. Neurol.* **97**, 143 (1952); A. Torvik, *ibid.* **106**, 51 (1956).
12. D. Denny-Brown and N. Yanagisawa, *Brain* **96**, 783 (1973).
13. Measurements made on figure 2C of Pons *et al.* (*6*) suggest that the area receiving inputs from the trunk did expand ventrally after deafferentation because the distance between the dorsal boundary of the leg area and the ventral boundary of the trunk area was about 2 mm greater in the deafferented animal.
14. Only one monkey was used in this experiment. We decided not to kill a second because there was already evidence in the literature that the upper face area existed (*6–8*).
15. P. E. Garraghty and M. Sur, *J. Comp. Neurol.* **294**, 583 (1991).
16. We acknowledge the help of M.-T. Parent and T. Ling with the experiments, which were supported by a Group Grant from the Canadian Medical Research Council. We thank T. Drew, R. Dykes, M. C. Bushnell, and G. H. Duncan for reading the paper.

30 November 1993; accepted 24 March 1994.

Response: Lund *et al.* propose that a lateral expansion of the "upper head" area, combined with a medial expansion of the lower face representation, is a "more parsimonious" explanation of our findings because it does not require (i) a revision of the cortical distance limit for reorganization in adults or (ii) a revision of the currently accepted mechanisms that underlie cortical plasticity. I disagree with these conclusions for the following reasons.

As for the first point, whatever mechanisms may be responsible for reorganization of the cortex, and whether the reorganization spreads from one (medial or lateral) or two representations of the head (*1*), the extent of the reorganization that we found (*2*) was nearly an order of magnitude larger than had previously been thought possible.

As to the second point, the distance between the "upper" and "lower" face representations is a critical issue for Lund *et al.*, but they offer conflicting statements. First, they state that "[t]he area of primary sensory cortex that receives inputs from the upper limb and cervical roots measured at least 12 mm in the mediolateral direction." Likewise, Dreyer *et al.* (*3*, p. 719), referring to the distance between two head representations, have stated, "[o]n the gyral crown these regions are separated by approximately 12 mm of cortical tissue, which receives its input from the contralateral arm and the hand." This distance coincides with other experimental results (*4*), but if the largest mediolateral spread of thalamocortical axons is 3.5 mm, then 7.0 mm (a 3.5-mm lateromedial and a 3.5-mm mediolateral expansion) would be the maximum expansion that the unmasking hypothesis could explain. The "upper head" area extends only 2.0 mm in the mediolateral dimension, and represents chiefly the occiput region instead of the lower face (*3*), but let us here assume that it represents the latter. A distance of 12.0 mm between the two head representations, less 2.0 mm for the upper head representation (*3*), less 7.0 mm for overlap of thalamocortical axons, still leaves 3.0 mm (or 30% of the reorganized cortex) for which the unmasking hypothesis cannot account.

We reported (*2*) that the deafferented and reorganized region ranged from 10 to 14 mm mediolaterally, depending on the animal studied. Reorganization over 14 mm in the mediolateral dimension, less 2.0 mm for the "upper head" area, less 7 mm for the overlap of thalamocortical axons, leaves 5.0 mm (or 45%, of the reorganized cortex) for which there is no accounting (*5*).

Second, Lund *et al.* found that the "upper" and "lower" head regions were separated by only 5.0 mm in one animal (*6*). But in the animals we studied, the distances between the two head representations were a minimum of 8 to 12 mm [a 10- to 14-mm range (*2*) less 2 mm for the "upper head" representation]. Thus, the unmasking of overlapping thalamocortical connections is insufficient to explain our results.

The unmasking hypothesis also does not explain why the overlap of thalamocortical arbors would be restricted only to the representations of the lower face and upper limb, or why there would be no overlap of thalamocortical arbors representing the rest of the face and the upper limb, or of arbors representing the trunk and the upper limb (*5*).

Ullrich and Woolsey (*7*) demonstrated the presence of trigeminal input to the "upper head" region in macaques by recording neural activity from animals with dorsal rhizotomies. They also demonstrated the presence of the dominant cervical inputs to the "upper head" area (*3*, *7*). Lund *et al.* do not add new empirical observations regarding the extent of, or the possible mechanisms responsible for, the reorganization we reported. Our findings (*2*) of an expanded face representation still require a reevaluation of both the upper distance limit for cortical reorganization, as well as the mechanisms responsible for it.

Tim P. Pons
Department of Neurosurgery,
Bowman Gray School of Medicine,
Wake Forest University,
Winston-Salem, NC 27157–1029, USA

REFERENCES AND NOTES

1. In note 8 of their comment, Lund *et al.* state that "Pons *et al.* do not refer to an upper face area." We are aware of this area, and we cited "[reference 9 in (*2*)]" the original paper describing this region [C. N. Woolsey, W. H. Marshall, P. Bard, *Bull. Johns Hopkins Hosp.* **70**, 399 (1942)]. Woolsey and co-workers also did not use the label "upper head," but "Occiput, Ear & Side of Head" in their summary (figure 14, p. 428, of their 1942 paper).
2. T. P. Pons *et al.*, *Science* **252**, 1857 (1991).
3. D. A. Dreyer, P. R. Loe, C. B. Metz, and B. L. Whitsel [*J. Neurophysiol.* **38**, 714 (1975)] also indicate that the "upper head" area represents chiefly the occiput and receives mostly cervical inputs. They state (p. 719) "The medial region of cortex, which receives a less substantial trigeminal input, has been shown by evoked potential studies to represent chiefly the occipital aspect of the head and, in this paper, it will be refrred to as 'the upper head area of SI.' " In figure 9 of their summary, Dreyer *et al.* also use "Occ." to label this region, not "upper head."
4. T. P. Pons *et al.*, *J. Comp. Neurol.* **241**, 445 (1985); T. P. Pons *et al.*, *Brain Res.* **335**, 350 (1985); R. J. Nelson, M. Sur, D. J. Felleman, J. H. Kaas, *J. Comp. Neurol.* **192**, 611 (1980).
5. The hypothesis concerning the overlap of thalamocortical axons contradicts anatomical evidence in adult monkeys which shows that regions of the thalamus projecting to the cortical hand and face representations do not overlap [E. G. Jones and T. P. S. Powell, *Brain* **93**, 37 (1970); E. Rausell and E. G. Jones, *J. Neurosci.* **11**, 210 (1991); *ibid.*, p. 211; E. Rausell *et al.*, *ibid.* **12**, 4088 (1992); T. P. Pons and J. H. Kaas, *J. Comp. Neurol.* **240**, 16 (1985)].
6. Perhaps methodological differences account for the discrepancy in the reported distances between the two head representations. We only considered receptive fields (RFs) encountered in serially consecutive recording site locations to be adjacent (RFs 1 and 2, 2 and 3, and so on, but not 1 and 5). We would not have considered the RFs shown on digits 3 and 4 (for recording locations 1 and 2) to be adjacent to those on the face (recording locations 4, and 6 to 14) as do Lund *et al.* in their figure 2A and 2C. Likewise, it does not appear that RFs 11 to 13 on the nose were adjacent to those on the thumb (recording site 5 in their figure 2A) or that receptive fields on fingers other than the thumb adjoin the face based on the data presented in their figure 2.
7. D. P. Ullrich and C. N. Woolsey, *Trans. Am. Neurol. Assoc.* **79**, 23 (1954).

26 April 1994; revised 16 June 1994; accepted 28 June 1994