Fetal Transplants Rescue Axial Muscle Representations in M1 Cortex of Neonatally Transected Rats That Develop Weight Support

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Giszter, Simon, William Kargo, Michelle Davies, and Motohide Shibayama. Fetal transplants rescue axial muscle representations in M1 cortex of neonatally transected rats that develop weight support. J. Neurophysiol. 80: 3021–3030, 1998. Intraspinal transplants of fetal spinal tissue partly alleviate motor deficits caused by spinal cord injury. How transplants modify body representation and muscle recruitment by motor cortex is currently largely unknown. We compared electromyographic responses from motor cortex stimulation in normal adult rats, adult rats that received complete spinal cord transection at the T2–T4, segmental level as neonates (TX rats), and similarly transected rats receiving transplants of embryonic spinal cord (TP rats). Rats were also compared among treatments for level of weight support and motor performance. Sixty percent of TP rats showed unassisted weight-supported locomotion as adults, whereas ~30% of TX rats with no intervention showed unassisted weight-supported locomotion. In the weight-supporting animals we found that the transplants enabled motor responses to be evoked by microstimulation of areas of motor cortex that normally represent the lumbar axial muscles in rats. These same regions were silent in all TX rats with transection but no transplants, even those exhibiting locomotion with weight support. In weight-supporting TX rats low axial muscles could be recruited from the rostral cortical axial representation, which normally represents the neck and upper trunk. No operated animal, even those with well-integrated transplants and good weight-supported locomotion, had a hindlimb motor representation in cortex. The data demonstrate that spinal transplants allow the development of some functional interactions between areas of motor cortex and spinal cord that are not available to the rat lacking the intervention. The data also suggest that operated rats that achieve weight support may primarily use the axial muscles to steer the pelvis and hindlimbs indirectly rather than use explicit hindlimb control during weight-supported locomotion.

INTRODUCTION

Although effects of spinal cord injury can be partly alleviated by interventions such as transplants or pharmacological therapy (Bregman 1987; Bregman and Reier 1986; Cheng and Olsen 1995; Cheng et al. 1996; Howland et al. 1995; Iwashiti et al. 1994; Miya et al. 1997), some degree of motor function can often develop or recover in the absence of such interventions (Barbeau and Rossignol 1987; Belanger et al. 1996; Edgerton et al. 1992; Hiebert et al. 1994). Therefore it is clearly important to distinguish the functional recovery, measured behaviorally, from the impairment caused by damage to pathways in the CNS, measured anatomically and physiologically (see NIH Pub. 93-3509 1994). Functional recovery is due to several factors; there may be unmasking of otherwise suppressed pathways after spinal shock or alter-
by Miya et al. (1997). At least one segment of spinal cord was completely removed in the transaction by using aspiration under anesthesia by hypothermia. We prepared three groups of animals: 1) T7–T10 spinal cord sections (TX rats), 2) T7–T10 spinal cord transaction plus transplant of E14 spinal cord (TP rats), and 3) control. In our E14 transplants we utilized embryonic segments from midcervical to lumbar spinal cord levels.

**Training and testing**

Rats were trained on several locomotor tasks beginning at weaning, 3 wk postpartum. A full description of training can be found in Miya et al. (1997). Briefly, rats were exercised on a motorized treadmill set at various speeds and trained to cross a narrow (2.5-in.) runway. Test animals were water restricted and rewarded on the treadmill or narrow runway with a dilute sucrose solution. Test animals were videotaped once weekly for evaluation and trained at least three times weekly. For the behavioral testing presented here we classified animals’ locomotion into two categories, 1) a weight supporting (WS) and 2) non-weight supporting (NWS). Animals were videotaped during training as they locomoted completely unassisted by the experimenter. Hindlimb steps of the hindlimb on the camera side of a rat were classified and counted as WS or NWS over a 3-min interval at a treadmill speed of 5 cm/s. WS steps were recognized based on no contact of the trunk, belly, or proximal joints (hip or knee) with the substrate in any limb during the swing and stance phases of the stepping limb. All other types of steps were considered NWS. On the average animals made between 60 and 100 steps in the 3-min interval. In an examination of typical data from our analyses, and neglecting weight support differences among individuals and groups, we found total steps in a 3-min period varied as follows: TX rats made 57 ± 25.2 (n = 8), TP rats 92.7 ± 14.7 (n = 20), and normal 104.7 ± 7.8 (n = 12). By using this method Miya et al. (1997) showed that percent weight-supported steps during locomotion of adult animals, which were operated on as neonates formed a bi-modal distribution with peaks centered on ~20 and 75% weight-supported steps. The classification we used here was based on observations of consistent (>60%) weight support steps in the WS class compared with routine sweeping/scissoring of limbs in the NWS class (<40% weight-supported steps). Our NWS group corresponds approximately to Basso, Beattie, and Bresnahan (BBB) scale ratings of ≤8, and WS corresponds to BBB ratings of 12–14 (see Basso et al. 1995).

**Cortical mapping**

At 2–3 mo of age rats were anesthetized with an injection of ketamine hydrochloride (50 mg/kg), xylazine (5 mg/kg), and acepromazine (0.75 mg/kg) and injected with dexamethasone (5 mg/kg im) to control blood pressure and brain swelling. Subsequent maintenance injections were comprised of ketamine and acepromazine only. The rat was placed in a stereotaxic apparatus, bregma was located and noted, and the skull surface and dura were removed to expose the cortical surface. The animal was electrically isolated from the stereotaxic instrument with rubber caps on ear and mouth bars, and the preparation was checked carefully before and after mapping for short-circuits or capacitative loads, which might compromise current pulse delivery. The cortical surface was kept moist with a shallow saline bath and cotton reservoir. The motor responses that could be elicited from motor areas of both hemispheres of cortex were mapped by microstimulation with fine stainless steel electrodes (FHC ~10 MΩ, initial impedance at 1 kHz, shank diameter 125 μm, and tip <1-μm diam, exposed tip ~5 μm²). Mapping penetrations were arrayed across motor areas in a continuous 0.5-mm grid. Electrode penetrations were vertical with respect to the stereotaxic instrument. Over the area mapped distortions caused by cortical surface curvature or slight movements off precise grid placements caused by blood vessels were small. Stimuli were applied as 0.2-ms total duration constant current bipolar pulses with anodal current leading, at 333 Hz in trains of 300-msec duration. The threshold response and responses at 50 μA were routinely examined. In “silent” areas the maximal current used were 100 μA to confirm absence of any response. The data points in the maps shown were thus all collected at 50 μA, but silent areas were stimulated ±100 μA. To estimate area we considered each penetration at 50-μA current to represent the response in a 250- to 700-μm radius circle (based on Ranck 1975; Tehovnik 1996; Yeomans 1990). Thus areas of recruited tissue were expected to range between just touching square packed circles and overlapping circles with full coverage of tested cortical surface. For analysis, areas were quantified as numbers of such contiguous and adjacent penetrations or in the case of divided or patchy representations (such as the trunk in the normal rat) were assessed by using the total numbers of penetrations. Absolute area was thus not precisely estimated in the square grid. This was based on two possible sources of error. First, there were potentially overlaps of areas activated by individual adjacent penetrations; second, it was possible there were areas within patches between activation circles that were not tested or included in our measure. The potentially missing areas were due to interstices between the adjacent or overlapping circles actually tested by the electrode penetrations. The extent of overlap and size of interstices depended on extent of recruited tissue. The number of such interstices and overlaps also varied with patch shape. However, patch shape variation could not possibly account for the findings reported in the RESULTS. Sites with two response types were counted as contributing area to two representations. Pulse amplitudes and rise times were monitored with a Tektronix oscilloscope to examine voltage changes across a 10-kΩ resistor interposed in series between the preparation ground and the stimulator. Electrodes were replaced if pulse shapes altered radically or desired currents were not achieved. Movements and responding muscles were identified. We used electromyograms to record from chosen muscles with bipolar stainless steel electromyogram pairs (fish-hook, patch, or ball electrodes). Electrodes were chronically implanted in forelimb, hindlimbs, or axial muscles before the mapping experiment or were positioned acutely during the experiment in surgically exposed and identified muscles. Investigators were not blind to animal status during mapping. This was not possible with our laboratory personnel. However, this also allowed more careful search and confirmation of silent areas and careful focus on areas of possible differences. Statistics of standard maps were analyzed and compared by using the MINITAB or S-plus statistical packages.

**Histology**

After mapping, the rats were overdosed with anesthetic cocktail and perfused transcardially with buffered Ringer solution followed by Zamboni’s fixative (4% paraformaldehyde, 0.3% picric acid, and 0.1 M phosphate buffer). The following day the spinal cord was removed. Electrode tracks and electrolytic tracking lesions resulting in local iron deposition were identified in frozen 20-μm sections of cortex after fixation with either Prussian blue staining, counterstained with neutral red, or alternating sections stained with cresyl violet and Prussian blue. The spinal cord was placed in 30% sucrose solution made with 0.1 M sodium phosphate buffer. Histological procedures to examine the spinal cord status followed and are described in detail in Miya et al. (1997). Briefly, consecutive 20-μm sections of the tissue were stained with either nissl myelin stain (Cyanine R followed by cresyl violet) (see Clarke 1981; Shibayama et al. 1998), antibodies to serotonin (5-hydroxytryptamine, 5HT), or calcitonin gene-related peptide (CGRP). The tissue was examined by evaluators blind to surgical procedures or
behavioral status. We used tissue histology to assess cord status, transplant status, and completeness of transection and to detect the possibility of bridging. We were able to detect bridging of some central fiber pathways originating outside the spinal cord across the lesion (5HT) or bridging by small fiber sensory afferents from dorsal roots (CGRP) in the transplant rats.

RESULTS

Our results can be related to three issues: 1) the relationship of cortical organization to lesion, 2) cortical organization alterations attributable to the intervention, and 3) the relationship of cortical organization to motor function. First we will summarize extents of behavioral recovery in injured rats and the histological status of these rats. Second we will address the cortical organization in the context of the issues above.

Behavioral recovery

Before mapping the cortex the level of function of all rats was tested. The levels of recovery were similar to the results of previous studies. In previous work it was found that weight-supported stepping among lesioned rats showed a bimodal distribution (see Miya et al. 1997). The animals were therefore divided into two classes based on consistency of weight support on the locomotor tasks for motivated 3-min bouts of treadmill locomotion (<40% weight-supported steps, NWS class, or >60% weight-supported steps, WS class). Statistical differences in the distributions of motor function between transplant and transect rats were apparent in the data from current and previous work (totals TX n = 14, TP n = 19). Although 68% of transplants had 60% weight-supported steps or better (13/19 WS TP rats), only 28% of transects functioned at that level (4/14 WS TX rats). The distributions of motor performance in the TX and TP groups differed significantly by using a Kruskal-Wallis or one-tailed Mann-Whitney U test (Fig. 3A).

Histological status of surgery and transplants

After sacrifice, the completeness of the spinal cord lesion and the transplants’ status were examined in Nissl-stained parasagittal sections. Axon growth into lumbar cord caused by transplant-mediated regeneration or development was examined with antibodies to 5HT. All 5HT found in lumbar cord derives from descending brain stem pathways. In mid-thoracic segments, there may be sparse 5HT immunoreactive cells associated with autonomic function that are close to the central canal. In normal uninjured rats the descending serotonergic fibers project densely into both the dorsal and ventral horn and also innervate intermediate zones. Histology was evaluated by three independent examiners who were blind to the intervention procedures employed and the level of function. Histology was also used to confirm that cortical stimulation sites were appropriately placed. Examples are shown in Fig. 1. Histology confirmed completeness of transections, integration of transplant tissues, and that some bridging function was provided by transplants for both descending (5HT) and sensory (CGRP) pathways. All WS class TP rats showed some 5HT below the lesion site. However as observed also by Miya et al. 1997 it was not possible to relate the patterns of 5HT below the injury to level of recovery in TP rats. This may not be surprising given that some TX rats also achieved weight support. By our criteria, if transections were complete, the TX rats lumbar cord possessed no detectable 5HT from descending sources and no detectable 5HT in dorsal or ventral horns or anywhere in the gray matter.

Cortical organization of injured rats

At 2–3 mo of age rats were anesthetized, and the motor cortex was mapped with microstimulation. Stimulation length parameters were chosen that were longer than conven-
tionally used (300 ms as opposed to the standard 30-ms long train) and currents $\approx 100 \mu A$ were used in silent areas. In this study we assessed both the recruitment of muscles and the absence of motor responses. We therefore used longer trains to allow the greatest opportunity for temporal facilitation of activity elicited by microstimulation at synapses along the several possible cascades of connections between cortex and motoneurons in lesioned rats. Our train lengths were similar to those used in some of the earliest maps of rat cortex (Settlage et al. 1949). The use of long trains minimized the possibility of false-negatives in our procedures and provided the greatest chance of detecting a functionally relevant physiological connection. Standard maps were generated at 50 $\mu A$ using penetrations in a 500-$\mu m$ grid. Hall and Lindholm (1974) compared and reported little difference between 50- and 250-ms stimulation regimes. However, because our trains differed from the most often used regime, we carefully established that the longer train parameters did not alter the map features of interest or the basic map structure in our control rats.

Our control rats ($n = 8$) had maps of motor cortex (M1 cortex; Fig. 2A), which were fully consistent with published data (Donoghue and Wise 1982; Hall and Lindholm 1974; Neafsey et al. 1986; Wise and Donoghue 1986). The normal motor representation of hindlimb and lumbar axial musculature is contained in an area that is caudal to bregma and $\approx 2.5 \, mm$ of the midline and is believed to overlap primary somatosensory (S1) cortex (Hall and Lindholm 1974; Hummelsheim and Wiesendanger 1986). For the purpose of this study we designate this region the medial-postbregma area (MPB; Fig. 2B). At the current levels we used (10–100 $\mu A$) the hindlimb/lumbar axial muscle representation is separated from the more rostral representation of neck axial muscles by substantial forelimb and shoulder representations. Medially, the region of hindlimb and axial motor cortex terminates about where the vibrissae representations occur in more rostral cortex. Caudal to the MPB area the cortex was motorically silent, i.e., there were no motor responses to our microstimulation across the range of parameters employed.

Typical maps from TX and TP animals in both WS and NWS classes are shown in Figs. 3 and 4. They demonstrate that the normal contralateral hindlimb muscle representation was completely absent in all operates (TX or TP), even at the highest stimulation currents. Despite the possible bridging provided by the transplant, the cortical stimu-

![Fig. 2. Motor cortex maps from normal rats. Maps show cortical areas from which forelimb, vibrissae, hindlimb, and trunk musculature are recruited at 50-$\mu A$ currents. Rostral is at the bottom of each map and caudal is at the top. For comparison purposes all maps in Figs. 2–4 are presented as left cortex in the same orientation, and muscles are referenced as ipsilateral or contralateral to the stimulated cortex. Shading key for regions is at the bottom right of the figure. A: motor map of normal rat. Note that there are 2 axial areas and a hindlimb area. The caudal axial area, posterior to bregma (AP coordinate $<0.0$) overlaps the hindlimb representation and represents more caudal axial muscles. As previously shown, in these data the axial and hindlimb responses sometimes exhibit bilateral effects. Numbers on the map represent sites from which the displayed muscle records were obtained. Muscles shown: (1) contralateral gluteus, (2a) contralateral longissimus, (2b) contralateral biceps femoris, (3a) contralateral iliopectos, (3b) contralateral obiquus externus, (4a) contralateral biventer cervicis, and (4b) ipsilateral biventer cervicis. Muscle 1 is in the hindlimb representation; 2a, 2b, 3a, and 3b are from 2 mixed axial and hindlimb sites; 4a and 4b are rostral axial representation with forelimb overlap. Note biventer muscle activation was bilateral. This was true of several trunk muscles in normal rats, especially in the rostral axial region. Scale bar 1,000 ms. Electromyogram records of muscles at midthoracic segmental levels or below are marked with an asterisk. To allow for the possibility of significant spinal gating of cortical effects in injured animals, muscle activity enhancement or burst prolongation of ongoing activity was also included in the maps of operates as a significant cortically evoked response in trunk musculature. B: summary of normal axial motor regions. The region of cortex caudal to bregma always contained the caudal axial muscle and the hindlimb representation. These representations and the rostral axial representation in the normal rat in A are shown, together with our designation of an area (medial-postbregma or MPB area, from midline to 2.5 mm lateral and from bregma to 2.5 mm caudal) that always contained the caudal axial and hindlimb representations in normal rats.](http://jn.physiology.org/Downloadedfrom)
The average area of the motor sites in the MPB area of (31 sites) recruited (Fig. 5, B), and 100% of the total area of nonfacial motor cortex devoted to trunk in axial muscles (Fig. 5, A). The rat achieved grade 3 (i.e., midthoracic, see text) control of axial muscles in cortex. All axial muscle representation was in the rostral axial representation (i.e., rostral to bregma). Activity of these muscles was either (a) facilitated or (b) recruited de novo during stimulation of cortex. MPB area was mostly silent; a small part of the forelimb representation overlaps the rostral and lateral edge of the MPB area. Muscles shown: (1) contralateral biventer (2) contralateral semispinalis cervicis. Taken together with Fig. 4, these maps show the principal patterns exhibited across the study. Midthoracic or lower axial muscle control was consistently associated with weight support, and hindlimb represents was absent in all operates.

Because there was no evidence for any direct cortical representation or control of hindlimbs, we concentrated further analysis on the two cortical representations of axial muscles. We graded the caudal extent of axial muscles (grades: 1 neck, 2 rostral thoracic, 3 mid to low thoracic, 4 lumbar, 5 tail) activated by the stimulation. We then assessed (1) the caudal extent in the trunk of the axial muscles recruited (Fig. 5, B, and C), (2) the number of rats with representation of axial muscles in the MPB area (Fig. 5D), (3) the average area of the motor sites in the MPB area of cortex (Fig. 5E), and (4) the total area of nonfacial motor responses and percentage of this area of cortex representing axial muscles (Fig. 5F). We found that the MPB axial representation was never present in the TX rats (Fig. 3, A and B). In TX rats the entire MPB area of cortex was motorically silent, except for a few small incursions of the forelimb activation region on the lateral margin of MPB cortex (2–2.5 mm from the midline). In contrast, five of nine TP rats developed motor representation in MPB cortex (e.g., Figs. 4A and 5, D and E). A sixth WT rat had a low trunk representation just rostral to bregma. The remainder of TP rats resembled NWS TX rats (compare Figs. 4B and 3B). Those TP rats that developed MPB representations also always had weight-supported locomotion. The transplants therefore enabled the development of motor function in specific cortical areas in the five of the six transplant recipients, which achieved weight-supported locomotion by our criteria. Transplants rescued motor representation of axial musculature in the caudal areas of MPB cortex in these animals. These areas were, in contrast, motorically silent in TX rats and would, in normal rats, form the second axial muscle representation and the hindlimb representations. These areas give rise to earlier developing fibers of the CST, which could be at or close to the transaction site at the time of the surgery.

We found that the combined trunk and forelimb area in operate rats was greater than the same area in normal rats (31 sites ± 1 SE for operates vs. 25 sites ± 2 SE for normal). This difference was significant (2 sample t-test, P < 0.05). We also estimated the percentage of motorically active nonfacial motor cortex devoted to trunk in our rats. The trunk area in normal rats represents ~30% of the total area of nonfacial motor cortex (including hindlimb areas), both in our data and in other studies (e.g., Hall and Lindholm 1974). Collectively the injured rats showed percentages of nonfacial motor cortex devoted to trunk representations that were significantly greater than those in the normal rats (2 sample t-test P < 0.05; compare Figs. 2A, 3, A and B, and 4, A and B). We also...
observed that the percentages of motor cortex devoted to axial musculature in TX (38 ± 3.4%, mean ± SE) and TP rats (42 ± 3.4%, Fig. 5E) did not differ significantly. However, the presence or absence of hindlimb representations in our rats might significantly bias these fractions. Accordingly we repeated these tests after removal of the sites eliciting exclusively hindlimb responses from the normal rats’ data. After this modification the differences between normal and operate percentages lost statistical significance. Thus it appears that the area of motor cortex representing forelimb and trunk was expanded in operates compared with normals, but the percentage of this cortical area devoted to trunk was similar to the percentage of these same areas devoted to trunk in normal rats. The trunk representation was greater as a fraction of all nonfacial motor areas available, but this was at least partly due to loss of the hindlimb representation in the operate rats.

Examination of axial muscle grades (grades: 1 neck, 2 rostral thoracic, 3 mid to low thoracic, 4 lumbar, 5 tail) showed that the level of locomotor function in the awake behaving animal was strongly related to the caudal extent in the trunk of the axial muscles represented in motor cortex (Fig. 5, B and C). Maximum axial grade achieved correlated closely with weight support ($R^2 = 0.75$ for axial score and weight-support score). All WS class animals without exception (including TX rats in WS class, e.g., Fig. 3A) had axial representations from which midthoracic or more caudal muscles could be recruited. No animal that lacked weight support was able to recruit midthoracic musculature in the axial representations. We also observed that midthoracic muscle activity routinely produced forces and motion at the pelvis either via mechanical coupling or via reflex coupling. These effects included pelvic flexion or pitch, translation, rotation, and yaw. Because more caudal muscles acting directly on pelvis were frequently not directly represented in motor cortex we believe that mechanical and reflex coupling must be important in any cortical control of locomotion in operated rats. The two TX rats with weight support also had midthoracic muscle representations in motor cortex, but these representations were confined to the cortical areas rostral to bregma, which usually represent neck and shoulder. Thus, even in TX rats with weight support, the MPB area was motorically silent. In contrast, in TP rats the caudal most axial representation was in the more caudal motor cortex, and with only one exception it was centered in the MPB region. Sometimes forelimb representations also encroached into this area and overlapped the axial representations in TX rats. Because the segmental level of axial muscles elicited from cortex was consistently correlated with level of weight support, our data suggest that cortical control of midthoracic musculature may be necessary for weight-supported locomotion. Ablation of these representations would directly test this but was not possible in these animals.
Further, the development of mid- to low axial muscle representations in motor cortex associated strongly with the recovery of weight-supported locomotion in lesioned rats. Our results relate to 1) how CNS plasticity may be related to recovery after central lesions, 2) the effects of transplants and other interventions in development and plasticity of the CNS, 3) the development of corticospinal connectivity, and 4) cortical plasticity.

Animals with neural impairments may achieve function in different ways. These include plastic structural reorganization, synaptic strength changes, and the use of redundant pathways. It was demonstrated that transplants increased the likelihood that spinalized rats achieved weight-supported motor function (Miya et al. 1997). However, our data show that the differences between animals with and without this intervention extend beyond a simple difference between the distributions of the transplant and transplant populations’ behavioral recovery. The level of motor function achieved in individual TX and TP rats did not accurately reflect the degree of difference in underlying CNS impairment and reorganization of cortex between TX and TP rats. The transplants unambiguously rescued motor responses of the caudal (MPB) motor areas and recruited more caudal trunk muscles in these representations. This area was silent in injured rats that did not receive a transplant even when functional recovery was similar. Our data thus directly demonstrate that different motor cortex organizations may support similar levels of functional recovery in those TX and TP rats that develop weight-supported locomotion.

The recruitment of low axial muscles from cortex in our lesioned rats could occur in several ways, and these must be considered in any assessment of the possibility of physiologically effective CST regrowth. The CST in rats has various roles in locomotion (Donatelle 1977; Hicks and D’Amato 1977; Soblosky et al. 1996). However other pathways also contribute very strongly to locomotion in the normal uninjured rat, and these systems receive cortical regulation. Various pathways from the cortex might thus mediate the responses detected here, including 1) physiologically effective CST connections across the transection of unknown extent, 2) CST connections to propriospinal systems in cervical or thoracic segments rostral to the lesion, and 3) cortical projections to brain stem systems projecting into spinal cord. Any or all of these could play a role in the recruitment of mid- to low axial musculature from the postbregma areas of S1/M1 overlap cortex. Further, although the transplant enabled the development of this cortical area as a motor structure, CST growth across the transplant may not be the primary reason for this development. For example, it is conceivable that afferent feedback from the caudal trunk may be critical in the development of this motor representation and could in some measure be enhanced by the transplant. It is also important to note that recruitment of low thoracic and axial muscles from rostral M1 cortex was observed in histologically confirmed complete transect rats that were WS as adults. The responses in TX rats were elicited from rostral M1 areas normally representing forelimb, shoulder, or neck muscles. The representations in TX rats suggest yet another set of mechanisms for recruitment of motor responses. The axial muscles recruited had long distributed motor pools that could allow mechanical interaction through the axial

**DISCUSSION**

Taken together, our data suggest that transplants allow the development of axial muscle motor representations in caudal M1/S1 overlap cortex in lesioned rats that were never observed in transect only rats. We also found that no lesioned animals with or without transplants showed any hindlimb representations in the microstimulation maps of M1 cortex.

**FIG. 5.** Significant map features in the populations of TX and TP rats. A: percentage of animals that exhibited >60% weight support in all TP and TX rats examined. TP rats showed significant improvement over transects (P < 0.05, tested with Kruskal Wallis or 1-tailed Mann-Whitney U). B: numbers of operated rats with midthoracic or lower axial representation in M1 motor cortex. These data related very closely to the corresponding levels of weight support in animals in A, i.e., weight-supported rats always achieved midthoracic cortical control. C: average (mean) axial level scores for cortical control in TX and TP rats. A score of three corresponds to recruitment of midthoracic axial musculature. The transplant animals as a group are significantly different in the axial level of cortical control from transects in a 2 sample t-test (P < 0.05). D: numbers of TX and TP rats with medial postbregma (MPB) area of S1/M1 overlap cortex possessing a representation of axial muscles. No transect had recovery of the 2nd MPB axial representation, 5/9 TP rats recovered the representation, and those that did had weight support. E: area of motor activity in MPB area of S1/M1 overlap cortex in TX and TP rats estimated from counts of motorically active sites. This area measure includes both axial or forelimb effects. Forelimb could sometimes be recruited on the lateral margin of the designated area. Transplants as a group had a significantly greater area in the MPB region from which responses could be evoked (P < 0.05, Mann-Whitney U or t-test). F: percentage of motorically active cortex sites in which microstimulation recruited axial muscles with our protocol. This does not differ significantly between TX and TP rats but is greater than the normal percentage (normal percentage 30%) unless the hindlimb representation is excluded. These data are consistent with a larger representation of axial muscles in M1 of operate rats. The increased trunk area of motor activity centered in MPB cortex in TP rats, and the use of part of the MPB area for hindlimb representation in normal rats accounts for most of this difference. TP rats might therefore have slightly larger areas available for other motor representations as a result of the transplant.
skeleton or along the trunks of individual muscles to recruit caudal, physically separated lower thoracic and lumbar motoneurons via stretch reflexes. Stretch reflexes in the separated lumbar cord system may also be abnormally excitable in the injured rats. Dorsal roots that have lost their usual entry segments and targets may grow into either rostral or caudal spinal cord stumps allowing unusual sensory feedback patterns to be established. Finally, it is also conceivable that altered or highly excitable heteronymous reflex patterns may be present as a result of the early transection and/or such reinnervation from dorsal root ganglia orphaned by the transection. These mechanisms could all contribute to recruitment of axial muscles in the transect rat. Conceivably such mechanisms may also play some role in the transplant-mediated functions observed. As will be described, however, current literature strongly supports some CST growth across a transplant.

The extent of CST and other pathway growth enabled by the transplant is likely to impact strongly on the development of cortical organization. At the time of transection, some lumbar CST fibers have reached the lesion site, but the normal M1 map structure is not yet established (Cox and Humphrey 1986) nor are spinal motor pools fully organized. Thus developmental processes occurring within cortex and spinal cord were not yet completed at time of the lesions used in our study (Schreyer and Jones 1982, 1988). Therefore it is likely that reorganization or rescue of CST target interneuronal structures or motor pools and some ascending pathways at the spinal level also contributed to the observed cortical map structures and motor function. In young rats, CST fibers can grow around or through incomplete lesions by using surviving tissues as a bridge to targets (Bates and Stelzner 1993; Bernstein and Stelzner 1983; Schreyer and Jones 1983). Transplants of various types may also serve as bridges (Bregman 1987; Cheng and Olsen 1995; Cheng et al. 1996; Howland et al. 1995; Iwashiti et al. 1994). Nevertheless, CST projections into caudal spinal cord are likely to be reduced and may not extend far. Thus, at best, a reduced CST connectivity to caudal spinal targets underlies the plastic changes that we observed in motor cortex.

All our injured rats are likely to have substantially altered or reorganized their cortical motor functions. Cortical reorganization as a result of injury or experience occurs both during development and in adults, in all mammals examined, including humans (Elbert et al. 1995; Flor et al. 1995; Garraghty and Kaas 1992; Garraghty et al. 1994; Hubel and Weisel 1977; Kaas et al. 1983). The cortical reorganizations in our data have features in common with cortical effects of peripheral nerve damage in adult and neonatal rats (Donoghue and Sanes 1987, 1988; Jones and Schallert 1994; Waite 1984; Wall and Cusick 1986). In neonatal rats, after peripheral lesions, silent M1 areas were only found in the areas of cortex where M1 and S1 overlap (Donoghue and Sanes 1988). These areas of overlap may be particularly plastic or be especially susceptible to loss of targets or feedback in the event of spinal or peripheral damage. The lumbar axial and hindlimb muscle representation in a normal rat is precisely located within the S1/M1 overlap zone (Donoghue and Wise 1982; Hall and Lindholm 1974; Hummelshain and Wiesendanger 1986; Neafsey et al. 1986; Wise and Donoghue 1986), and it was this area of the motor representation that was always silent in transect rats and in which axial motor effects were rescued by transplants.

It is interesting to compare other aspects of the cortical organization of WS transplant and transect rats. Reorganization and plasticity in cortex are often considered to represent reallocation of limited cortical resources. In principle the rescue of the motor MPB axial representation in TP rats increased cortical motor function resources. However, the rescue of the motor responses of the caudal cortical area was not uniquely associated to weight-supported locomotion. About 25% of TX rats achieved weight-supported locomotion without this rescue. The percentage of motorically responsive cortex devoted to axial muscle control did not differ between transect and transplant animals that showed weight support, although their percentages exceeded those of a normal rat. This leads us to speculate that the similar percentages of axial representation in both transect and transplant rats represent an optimal allocation of cortical motor resources in the injured rats. The increase compared with normal may reflect an increased importance and role for cortical control of axial muscles in injured rats. Presumably, in TP rats, the additionally increased total area of cortical territories available for axial muscle representation in motor cortex and the recovery of motor activity in otherwise motorically silent areas of cortex might also allow further improvements in motor control mediated by cortex and the CST compared with transect rats. Such differences might only be observed by more subtle motor tests (Miya et al. 1997).

Both transect and transplant recipient rats that locomoted with good weight support were notably lacking any cortical representation of the hindlimbs. The data suggest that M1 cortex recruitment of midthoracic axial muscles provides sufficient cortical control of the more caudal musculoskeletal complex to allow weight-supported locomotion in adult rats. In principle, the rat could be locomoting after the fashion of a human pulling a cart or rickshaw, with the axial muscles providing balance, additional stabilization, steering control, and some propulsive coupling to the caudal axial and hindlimb system, which otherwise operates largely autonomously (like a legged ‘‘cart’’). The extent to which such axial muscle control could contribute to recovery of weight support after transection injuries generated in adult rats is currently unknown. However it is also worth noting the importance of caution in ascribing any large functional role to the cortical representations and also of caution in ascribing the results described to possible CST regrowth. We are only able to provide an association of this axial cortical control and the recovery. This cortical axial control is embedded in a complex of other mechanisms that may recover in parallel. These include propriospinal and reticulospinal pathways, which may have equal or greater contributions to the recovery of lumbar control and function. Thus we cannot state that the axial control from cortex is critical to the development or recovery of weight support in lesioned animals. Future work will address this question in more detail. However, regardless of the precise role of these cortical motor mechanisms in the weight support of the injured rat, our results show that transplants used in neonates have a substantial effect on development of effective communication and control between cortex and spinal cord. The transplants J) rescued some motor functions in the hindlimb/lumbar areas of cortex
and 2) promoted a gain of several segments in the caudal extent of cortical segmental motor control. A similar gain of one or two neurological levels in a human spinal cord injury would be highly significant.

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