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Selective Long-term Reorganization of the Corticospinal Projection from the Supplementary Motor Cortex following Recovery from Lateral Motor Cortex Injury

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Abstract

Brain injury affecting the frontal motor cortex or its descending axons often causes contralateral upper extremity paresis. Although recovery is variable, the underlying mechanisms supporting favorable motor recovery remain unclear. Since the medial wall of the cerebral hemisphere is often spared following brain injury and recent functional neuroimaging studies in patients indicate a potential role for this brain region in the recovery process, we investigated the long-term effects of isolated lateral frontal motor cortical injury on the corticospinal projection (CSP) from intact, ipsilesional supplementary motor cortex (M2). Following injury to the arm region of the primary motor (M1) and lateral premotor (LPMC) cortices, upper extremity recovery is accompanied by terminal axon plasticity in the contralateral CSP but not the ipsilateral CSP from M2. Furthermore, significant contralateral plasticity occurs only in lamina VII and dorsally within lamina IX. Thus, selective intraspinal sprouting transpires in regions containing interneurons, flexor-related motor neurons and motor neurons supplying intrinsic hand muscles which all play important roles in mediating reaching and digit movements. Following recovery, subsequent injury of M2 leads to reemergence of hand motor deficits. Considering the importance of the CSP in humans and the common occurrence of lateral frontal cortex injury, these findings suggest that spared supplementary motor cortex may serve as an important therapeutic target that should be considered when designing acute and long-term post-injury patient intervention strategies aimed to enhance the motor recovery process following lateral cortical trauma.

Keywords

Pyramidal Tract; Frontal Lobe; Corticofugal; Neurosurgical Resection; Plasticity; Spinal Cord

INTRODUCTION

Several neurological conditions affecting supraspinal nervous system centers lead to paresis with the most common condition being stroke. This devastating disorder is the primary cause of functional disability in modern society (Rosamound et al., 2007) and is typically characterized by pronounced impairment of motor function in the arm and hand (Adams et al., 1997). In most cases, substantial motor impairment occurs contralateral to an injury affecting the precentral region of the frontal lobe or its descending corticofugal projections. The precentral, or lateral frontal region of the hemisphere is critical in this respect because the primary motor cortex (M1) resides in this key region of the cortical mantle and M1 is known to give rise to the most prominent corticospinal projection (CSP) (Biber et al., 1978; Kuypers, 1981; Murray and Coulter, 1981; Dum and Strick, 1991; Galea and Darian-Smith 1994; Rouiller et al., 1996; Maier et al., 2002; Lemon and Griffiths, 2005). Other neurological conditions compromising the lateral frontal cortex such as penetrating brain injury (Sweeney and Smutok, 1983) and closed head injury (Lotze et al. 2006) may also result in upper extremity impairment. Similarly, isolated neurosurgical resection targeting pericentral cortex for removing sensorimotor tumors (Fandino et al., 1999, Korvenoja et al., 2006; Desmurget et al., 2007) and arteriovenous malformations (Inou et al., 1998; Andrade-Souza et al., 2006); or for managing treatment-refractive frontal lobe epilepsy (Pondal-Sordo et al., 2006; Chamoun et al., 2007) and movement disorders (Bucy, 1949a) consequently result in post-operative upper extremity dysfunction. Although the majority of patients in all these clinical scenarios experience paresis on one side of the body, the extent of motor restitution is highly variable with some patients attaining remarkable levels of recovery while others are left with severe disability. In spite of our comprehensive capacity to characterize structural and functional post-injury patient profiles, the underlying neural substrates supporting recovery in patients achieving favorable levels of motor restitution remain unclear. Pinpointing these mechanisms would greatly assist in designing therapeutic strategies aimed to enhance and accelerate the recovery process, particularly in patients with poor or inadequate levels of motor recovery (Nudo, 1999, 2003; Hallett, 2001; Chen et al., 2002; Baron et al., 2004; Cramer 2004; Rossini and Dal Forno 2004; Ward 2004; Schaechter 2004; Ward and Cohen 2004; Johansen-Berg, 2007).

Correlations drawn from human studies (Chollet et al., 1991; Fries et al., 1993; Weiller et al., 1992, 1993, Seitz et al., 1995; Nelles et al., 1999a; Carey et al., 2002; Feydy et al., 2002; Johansen-Berg et al., 2002; Loubinoux et al., 2003; Rossini et al., 2003; Fridman et al., 2004; Luft et al., 2004; Gerloff et al., 2006; Ward et al., 2006; Schaechter and Perdue, 2008) and experimental observations in non-human primates (Kennard 1942; Glees and Cole 1950; Aizawa et al., 1991; Nudo and Milliken, 1996; Rouiller et al., 1998; Liu and Rouiller, 1999; Eisner-Janowicz et al., 2008) suggest that cortical areas residing within the lesioned hemisphere that survive the insult, may act in a substitutional manner to promote recovery by taking over some functions that were once mediated by the damaged cortical areas. For example, following brain injury that involves the primary motor cortex (M1) or its descending projections, it has been shown in recovered hemiplegic stroke patients that the supplementary motor cortex (M2) is significantly activated during tasks requiring arm or fractionated hand movements (Weiller et al., 1993; Seitz et al., 1995; Cramer et al., 1997; Loubinoux et al., 2003; Ward et al., 2003; Luft et al., 2004; Ward and Cohen, 2004; Gerloff et al., 2006). In controls performing the same task, significant activation primarily occurs in the hand part of M1. The shift of enhanced metabolic activity to M2 indicates recruitment of corticofugal projection systems from the medial wall of the hemisphere when performing hand movements once predominantly mediated by the lateral part of M1. Since the lateral wall of the cerebral hemisphere is commonly damaged following brain injury and the medial wall is frequently spared (Sweeney and Smutok, 1983; Bogousslavsky and Regli 1990; Carrera et al., 2007), we tested the hypothesis that recovery of upper extremity motor

function following combined unilateral removal of the arm representation of M1 and lateral premotor cortex (LPMC) will be accompanied by corticospinal terminal fiber and bouton proliferation from the ipsilesional, intact supplementary motor cortex at the chronic (6 and 12 month) post-injury recovery period. We also tested the hypothesis that following motor recovery, subsequent injury of M2 would result in the reemergence of functional deficits in dexterous movement. Our results demonstrate that recovery of dexterous movements from isolated lateral frontal injury is accompanied by selective contralateral terminal axon sprouting and bouton proliferation that is restricted to spinal laminae VII and IX which contain interneurons and lower motor neurons respectively that play a central role in motor function. Following recovery, subsequent injury to M2 leads to rapid reemergence of fine hand motor deficits but not paresis. Accordingly, if M2 and its corticofugal pathway are diagnostically determined intact following subtotal motor cortex damage, M2 should be considered as a therapeutic target for enhancing the motor recovery process after supratentorial brain injury.

METHODS AND MATERIALS

All monkeys were housed, cared for, and maintained in a United States Department of Agriculture (USDA) approved and inspected facility. All behavioral and surgical protocols were approved by the University of South Dakota Institutional Animal Care and Use Committee, and conducted in accordance with USDA, National Institutes of Health, and Society for Neuroscience guidelines for the ethical treatment of experimental animals. Prior to beginning the study, each monkey was evaluated by a primate veterinarian and judged to be healthy and free of any neurological deficit. Proximal and distal movements and range of motion at the joints in both upper extremities of all animals were normal with the exception of SDM55. In this case the interphalangeal joints of digit 3 were permanently extended. However, this animal was able to perform precision opposition with digits 1 and 2 to successfully acquire the food rewards in both motor tests.

Study Design

To accomplish the aims of this study, the organization of the CSP arising from M2 was studied at spinal levels C5 to T1 in 8 rhesus monkeys (*Macaca mulatta*) (Table 1). Four monkeys served as controls (Figs. 1–3) and four monkeys received a controlled neurosurgical lesion using aspiration that involved removal of the arm representation of M1 and LPMC (Figs. 4–6). Neurosurgical exposure was performed as reported previously (Morecraft et al., 2001; 2002, 2007a, 2007b) to induce the lesion and inject the tract tracer and each procedure is described in detail below. In the control group (Figs. 1, 3; Table 1), each monkey was injected with 1.2 μ l of the anterograde tract tracer fluorescein dextran (FD) into the central region of the arm representation of M2 and survived for 33 days prior to sacrifice. Each lesioned monkey (Figs. 4 and 6; Table 1) was injected with 0.9 μ l of the same tracer (FD) into the central region of the arm representation of M2 in the ipsilesional hemisphere 33–34 days prior to sacrifice after 6 or 12 months of recovery from a controlled aspiration lesion targeting the arm area of M1 and LPMC. Following immunohistochemical tissue processing for microscopic visualization of the FD tract tracer in all cases (control and lesion cases), terminal boutons were counted at spinal levels C5 to T1 (Fig. 7; Tables 2–7) and fiber lengths were calculated at levels C5 and C8 using stereological counting methods, which is widely acknowledged as the most accurate method to quantify these neuronal structures (Glaser et al., 2007). Terminal boutons were defined as immunohistochemically labeled terminal-like particles (0.5 – 2.0 μ m in diameter) or swellings on the terminal axons within the spinal gray matter and within the anatomically defined reticulated marginal border (RMB) (Figs. 2, 5, 7). Terminal fibers were defined as all immunohistochemically labeled fibers within the confines of the spinal gray matter and within the anatomically

defined RMB. Finally, in one additional monkey (*Macaca fascicularis*) the arm area of M2 was lesioned using ibotenic acid 7.5 months after recovery from the M1/LPMC lesion and studied for subsequent recovery following the sequential loss of M2.

Before the lesion, all monkeys in the lesion group were trained on 2 simple, fine motor behavioral tasks involving reaching for small food targets using a modified movement assessment panel (mMAP) (Darling et al., 2006) and modified dexterity board (mDB) (Pizzimenti et al., 2007) and were then tested for recovery on these tasks after injury (Fig. 8). Specifically, after reaching stable levels of motor performance on each task (approximately 10 testing sessions), each monkey was lesioned and then tested once every week (on both tasks) for the first 2 months post-injury and once every other week (on both tasks) thereafter. Motor performances on individual trials were quantified from 3-dimensional video recordings of movements to acquire small food pellets in the mDB task (Pizzimenti et al., 2007) and from recordings of 3-dimensional forces applied to small carrot chips for the mMAP task (Darling et al., 2006). The animals in the control group were provided with daily distal upper extremity motor enrichment activities (such as a foraging board) to compensate for potential learning/training induced effects of the lesioned animals during the brief manual testing sessions (maximum of 40 trials with each hand to acquire the food targets).

Neurosurgical and Tracer Injection Procedures

Neurosurgical exposure of the frontal lobe contralateral to the preferred hand (identified using standard methods and a handedness index as described by Nudo and colleagues (Nudo et al., 1992; Pizzimenti et al., 2007) was performed following procedures previously described under isofluorane anesthesia (Morecraft et al., 2002, Morecraft et al., 2001, Morecraft et al., 2007). In the control cases the location of the arm representation of M2 was determined by intracortical microstimulation (Fig. 3). The only exception was case SDM 41 (Fig. 3A). During this surgery, a single arm stimulation site was identified on dorsal convexity at the coronal level of the genu of arcuate sulcus prior to proceeding to surgically expose the medial frontal surface within the interhemispheric fissure. However, after the large bridging veins arching over the interhemispheric sulcus (which empty into the superior sagittal sinus) were ligated to fully expose the medial wall, the cortex was electrophysiologically unresponsive with low physiological currents (i.e., in the microamp range used in all the experiments). Therefore, to minimize damage with high current, the central region of the arm representation in this case was further determined anatomically, and in reference to the single stimulation point, as being located at the coronal levels including the genu of the arcuate sulcus to a level approximately 0.5 mm posterior to this landmark as reported in numerous monkey electrophysiological M2 mapping studies (i.e., Aizawa et al., 1991, Tanji and Kurata, 1985, Mitz and Wise, 1987, Mushiaki et al., 1991, Wang et al., 2001). For the control cases the anterograde neural tracer FD (10% solution in saline comprised of an equal quantity of both 3,000 MW and 10,000 MW volumes) (Molecular probes, Eugene, OR) was injected into the central region of the arm representation of M2. Graded pressure injections with a Hamilton microsyringe were made into three separate penetration sites spaced 1–1.5 mm apart in a triangular pattern, 2.5 – 3.5 mm below the cortical surface at approximately a 10–20° angle from the vertical axis. A total of 1.2 μ L of neural tracer was injected (i.e., 0.4 μ L per penetration site) (Table 1). The tract tracer injections in the lesion cases was performed in identical fashion with the exception that less tracer volume (total of 0.9 μ L; 0.3 μ L per penetration site) was injected (Fig. 6A–D; Table 1). After completing the injections, the cortical field was irrigated with saline and the cottonoid padding was removed from the interhemispheric fissure. The craniotomy was closed as described previously (Morecraft et al., 2001, Morecraft et al., 2002; Morecraft et al., 2007) and the control cases survived 33 days. The same basic

neurosurgical approach was performed to expose cortex to induce the lesion which is described below.

Intracortical Microstimulation and Lesion Methods—After the cortex was surgically exposed under isoflurane anesthetization, the animals were transferred to intravenous ketamine anesthetization for electrophysiological mapping. Specifically, intracortical microstimulation (ICMS) mapping was employed as described in our previous reports (Morecraft et al., 2001; Morecraft et al., 2002; Morecraft et al., 2007a) to define the hand/arm region within M1 and the LPMC prior to neurosurgical resection, and to define the arm/hand region of M2 prior to injection with tract tracer (Figs. 3, 6). Physiological thresholds in our experiments ranged from 0.1 μ A to 90 μ A. Movement threshold was defined as the lowest current that elicited movement observed and agreed upon, by at least two individuals. Following electrophysiological mapping, the animal was returned to isoflurane anesthesia for the remainder of the procedure. The M1/LPMC lesion in all cases was created using the subpial aspiration method as applied in human neurosurgery for the purpose of tissue resection (Figs. 6, 9, 10 – see blackened area on lateral surface; see also Fig. 4B (SDM 48) of Pizzimenti et al., 2007). Specifically, tissue resection was performed using an angled glass pipette or angled Frazier surgical suction tube that was attached to a vacuum source. Arteries and veins directly within the cortical field to be resected were microcauterized to control bleeding. The tip of the micropipette or suction tube was then carefully placed on the cortical surface using microscopic guidance and the gray matter gently removed until the underlying subcortical white matter field was identified. Following the lesion the monkeys were then allowed to survive for 168 days (6 month interval or 24 weeks) or 336 days (12 month interval or 48 weeks) prior to sacrifice (Table 1). Note that 33–34 days prior to sacrifice each lesioned monkey was injected with tract tracer as described above in the Study Design.

As indicated in the Study Design, in one monkey an ibotenic acid lesion (IAL) of M2 was made using a previously described procedure in monkeys (Liu and Rouiller 1998). In our experimental case, the IAL was made 7.5 months after recovery from a M1/LPMC lesion (with the M1/LPMC lesion created using aspiration as described above). Specifically, the IAL was accomplished by defining the arm representation of M2 using ICMS then injecting 1.5 μ L of ibotenic acid 2.5 – 3.5 mm below the cortical surface in each injection site. Injections were made every 1.5 mm across the entire M2 arm representation in 3 rows in the rostral to caudal dimension, with each row having 5 injection sites (totaling 15 penetration sites overall). The first row was located superiorly in M2, the third row inferiorly and the second row in between.

Tissue Processing

Following a survival period of 33–34 days after tract tracer injection, each monkey was deeply anesthetized with an overdose of pentobarbital (50 mg/kg or more) and perfused transcardially with 0.9% saline. Saline infusion was followed by 2 liters of 4% paraformaldehyde in 0.1M phosphate buffer at pH 7.4 (PB), then one liter each of 10% and 30% sucrose in 0.1M PB for cryoprotection. The brain was removed, placed in 30% sucrose in 0.1M PB and stored for 2 to 5 days at 4°C. Post-mortem digital electronic images were taken of the cerebral cortex and spinal cord for data reconstruction.

In all cases the cerebral cortex was frozen sectioned in the coronal plane on a sliding microtome (American Optical 860, Buffalo, NY, USA) at a thickness of 50 μ m in cycles of 10, forming 10 complete series of evenly spaced tissue sections respectively (Figs. 1, 4). Each spinal cord was blocked from the central nervous system, frozen with dry ice and cut horizontally on the sliding microtome at a thickness of 50 μ m in cycles of 6 or 8 forming 6

or 8 complete series of evenly spaced tissue sections respectively (Figs. 2, 5, 7). For both the cortical and spinal cord sections, one series of tissue sections was mounted on subbed slides, dried and eventually stained for Nissl substance using thionin (Morecraft and Van Hoesen, 1992; Morecraft et al., 1992; Morecraft et al., 2004).

Subsequent series of tissue sections through the cortex and spinal cord were then processed using single and double label immunohistochemical procedures for visualization of the neural tracers (Morecraft et al., 2007a). In all monkeys except SDM 70, BDA was injected into a spared cortical region of interest and FD was injected into M2 as described above. In these cases, one series of tissue sections from the cortex and spinal cord was used to process BDA alone (single labeling procedure) using the Vectastain Elite avidin-biotin complex (ABC) labeling procedure (PK-6100 Kit, Vector laboratories, Burlingame, CA, USA). Briefly, the tissue sections were rinsed in 0.05M tris buffered saline (pH 7.4) (TBS) then incubated overnight in TBS with 5% normal goat serum (NGS) and 1.25% Triton X-100. Next, the sections were rinsed in TBS and then incubated in the ABC solution for 4 hours at room temperature. The sections were then rinsed with TBS and incubated in a 0.05% solution of 3, 3' diaminobenzidine tetrahydrochloride (DAB) without nickel enhancement for approximately 10 minutes. Subsequently, 30% hydrogen peroxide (H₂O₂) was added to the DAB solution achieving a final H₂O₂ concentration of 0.012%. The tissue was incubated in the DAB/H₂O₂ solution for another 8–10 minutes yielding an insoluble brown reaction product and immediately placed in TBS to stop the reaction. Following immunohistochemical processing, the BDA stained tissue sections were then rinsed in TBS, mounted on subbed slides, dried, then dehydrated in graded alcohol solutions and coverslipped using Permount. Next, an additional separate series of tissue sections were used for double labeling immunohistochemistry in which both BDA and FD were visualized employing a simple multiple colorimetric detection method. To accomplish this, BDA was reacted first in a full series of tissue sections according to the above protocol staining BDA brown. The same tissue sections were then rinsed in TBS then incubated in TBS with 5% NGS and 1.25% Triton X-100 overnight. The tissue sections were then transferred and incubated in 5% goat serum in TBS with biotinylated anti-fluorescein directed against FD at a dilution of 1:500 (Affinity Purified, Catalog Number BA-0601, Vector Laboratories, Burlingame, CA, USA) for approximately 40 hours. The tissue was then rinsed in TBS then incubated in a solution of ABC for 4 hours at room temperature, rinsed again in TBS and incubated with the Vector SG peroxidase substrate kit (Vector SK-4700, Vector laboratories, Burlingame, CA, USA) for approximately 5–10 minutes yielding a blue reaction product for the second tracer (FD). The sections were rinsed, mounted on glass slides, dried, then dehydrated and coverslipped using Permount. Thus, BDA was stained brown and FD was stained blue in the same tissue sections. In SDM 70, BDA was not injected into the cortex as mentioned above. Therefore, only a single colorimetric detection method was employed using the antibody labeling method described above for visualization of FD. Like all other cases, FD was stained blue using the Vector SG peroxidase substrate kit (Vector SK-4700, Vector laboratories, Burlingame, CA, USA).

To verify that the FD antibody and subsequent tissue labeling process resulted in staining only the injected and transported tract tracer, sections from the rostral prefrontal cortex and caudal occipital lobe were also immunohistochemically processed because these cortical regions are not connected to M2 (Luppino et al., 1993). In all 8 monkey cases, neuronal immunohistochemical labeling was not found in these control tissue sections demonstrating that false labeling was not present in the final tissue specimens used for data analysis.

Definition of Anatomical Terminology

With respect to the main cortical motor areas of interest in this study, the supplementary motor cortex (M2) resides on the medial wall of the frontal lobe and corresponds to

cytoarchitectonic area 6m (Morecraft and Van Hoesen 1992; Wise 1996). This identical area correlates with the SMA, SMA-proper and MII nomenclature and architectonic area F3 (and 6m) as defined by others (Woolsey et al., 1952; Luppino et al., 1985, 1993; Tanji 1994; Zilles et al., 1996). The primary motor cortex (M1) corresponds to cytoarchitectonic area 4 and is characterized by its prominent layers III, V and VI with the inclusion of large pyramidal cells in layer V (Bonin 1949; Barbas and Pandya 1987). The lateral premotor cortex (LPMC) resides on the lateral surface of the frontal lobe, anterior to M1, and corresponds to area 6 (Brodmann 1905). The LPMC was divided into dorsal (LPMCD) and ventral components (LPMCV) as defined by others (Luppino et al., 1985; Barbas and Pandya 1987; Preuss et al., 1996; Dum and Strick 2005; Hoshi and Tanji 2006). These premotor regions were further subdivided based upon cytoarchitecture, connections and movement representation as defined in previous studies (Luppino et al., 1985; Barbas and Pandya 1987; Preuss et al., 1996; Wise et al., 1997; Dum and Strick 2005). Briefly, the caudal part of LPMCD was recognized as area 6DC and the rostral part as area 6DR. The region of the LPMCV was subdivided into a dorsal (area 6Va) and ventral (area 6Vb) subregion (see Fig. 1 of Morecraft et al., 2004).

Three major anatomical subdivisions of the spinal gray are recognized including the dorsal horn, intermediate zone and ventral horn. For the present report, the dorsal horn refers to laminae I–IV, the intermediate zone is comprised of laminae V–VII and the ventral horn contains laminae VIII and IX (Fig. 7A–C, see segmental level C5). Lamina X surrounds the central canal and is considered its own area. In the intermediate zone, a distinct area lateral to laminae V, VI and VII was delineated and termed the reticulated marginal border (RMB) (Fig. 7A–C, see segmental C5). This area was identified by Rexed (Rexed, 1954) and Kuypers (Kuypers, 1981) as possessing extensive dendritic arbors of neurons located in the lateral part of laminae V–VII which protrude into the dorsolateral funiculus. We further subdivided laminae I–VII into medial and lateral halves and lamina IX into quadrants based upon the general musculotopic organization of non-human primate motor neurons (Fig. 7A–C, see segmental C5) (Jenny and Inukai, 1983).

Neuroanatomical Analysis and Data Reconstruction

Localization of the cortical injection site (within the arm area of M2) and the terminal fibers/boutons within the spinal gray matter (C5–T1) was accomplished using brightfield illumination on a BX-51 Olympus microscope (Leeds Precision Instruments, Minneapolis, MN). Attached to the microscope was a high resolution MAC 5000 motorized stage (Ludl Electronic Products, Hawthorne, NY, USA) which was joined to the NeuroLucida and StereoInvestigator neuroanatomical data collection software (MicroBrightfield, Colchester, VT, USA) in a Dell Optiplex GX 280. NeuroLucida system was used to plot the major anatomical structures and their boundaries in Nissl and immunohistochemical stained tissue sections and record the locations of the injection site, labeled terminal axon fibers and terminal-like profiles (boutons) in the immunohistochemically processed tissue sections.

Specifically, in the immunohistochemically processed tissue sections the injection sites were localized by plotting the external boundary of the core region and external boundary of the halo region (Figs. 1, 3, 4, 6). The core region of the injection site was defined as the location of dense immunohistochemically reacted product microscopically characterized by a dense blue-black appearance obscuring cellular detail of the gray matter (Mesulam, 1982). The zone characterized as the halo was defined within the limits of the gray matter (i.e., from layer I to the bottom of layer VI) where the dense precipitate that characterized the core zone diminished (Mesulam, 1982). The external limit of the halo was defined where small grains of reaction product were lightly interspersed among anterogradely labeled axons and terminals boutons, and well-defined retrogradely labeled cell bodies. Matching Nissl stained

tissue sections were used to assist in the cytoarchitectonic and laminar analysis of the cortex containing the injection site.

In immunohistochemically processed spinal cord sections, terminal boutons were plotted in every other tissue section to obtain a general characterization of the topography and relative density of the projection in Rexed's laminae (Fig. 7A–C). As will be detailed below, the same sections were used to estimate bouton number and terminal fiber length in Rexed's laminae using stereology. Immunoreactive terminal-like varicosities (i.e., putative boutons or terminal-like profiles) were defined as small swellings along the terminal fibers that were 0.5 – 2.0 μm in diameter (Wouterlood and Groenewegen, 1985; Freese and Amaral, 2006; Morecraft et al., 2007). For this study, terminal fibers were defined as all labeled axons located within the confines of the spinal gray matter domain and within the anatomically defined reticulated marginal border (RMB).

Publication quality images of injection sites and labeled fibers were captured using a Spotflex 64 mega pixel camera, (Diagnostic Instruments Inc., Sterling Heights, MI, USA, version 4.6), mounted on an Olympus BX51 microscope. Photographic montages of the injection sites and labeled fibers were created using Adobe PhotoShop 7.0 (Adobe Systems Inc., San Jose, CA, USA) (Figs 1,2,4,5). Brightness and contrast were adjusted in the images. Cortical reconstructions (Figs. 3,6,9,10) were developed as previously described using metrically calibrated digital images of the cortical surface (Morecraft and Van Hoesen 1992, 1993; Morecraft et al., 2002). Publication quality line illustrations were created using Adobe Illustrator 10.0 (Adobe Systems Inc., San Jose, CA, USA) (Figs. 3,6–10,13).

Stereological Quantification Methods

After plotting the injection sites and terminal boutons to obtain an overview of the location of each injection site (Figs 3, 6), as well as the general topography and density of the M2 corticospinal projection (Fig. 7A–C), stereological methods were employed to estimate the cortical injection site volume as well as the number of terminal boutons (C5 –T1) and terminal fiber length (C5 and C8) within each subsector of Rexed's laminae. Detailed explanations of our stereological methods have been previously described (Morecraft et al., 2007b).

Specifically, the cortical regions of interest (ROI) to which stereology was applied included the core of the injection site and the halo of the injection site (which included the core) (Table 1). Unbiased estimates of the total injection site volume and core injection site volume were determined using the Cavalieri probe in StereoInvestigator software. To accomplish this, every cortical tissue section spaced 500 μm apart through the injection site in the FD immunostained series of tissue sections was used for analysis. The same probe and method was used to determine the lesion site volume (gray and white matter) as reported in our recent papers (Pizzimenti et al., 2007, Darling et al., submitted) (Table 1). The only modification in the lesion analysis was that tissue sections through the lesion site were spaced 500 μm apart, instead of 1000 μm apart, to increase the sample size.

The spinal cord ROI included Rexed's laminae (and subdivisions) (Fig. 7A–C, see C5; Tables 2–7). Unbiased estimates of the total number of terminal boutons and terminal fiber length within Rexed's laminae were determined using the Optical Fractionator and the Isotropic Virtual Planes probe respectively. To obtain an accurate estimate of the total number of labeled particles of interest (i.e., terminal boutons and axon fiber lengths) in each individual monkey spinal cord, every other tissue section in a complete series of processed sections was used for microscopic stereological analysis and the total number of boutons and total axonal length within each ROI were expressed as total number counted per ROI per animal as previously described (Courtine et al., 2008) (Tables 2–7).

Analysis of Injection Site and Lesion Site Volume—With respect to the injection site, the ROI's included the entire injection (which contained the core) as defined by the limit of the external contour as drawn in StereoInvestigator and the injection site core alone. To use the probe, a rectangular grid array was placed over the ROI. The grid array consists of points of known distances apart. Each tissue section containing the injection site was then analyzed. An accurate estimation of overall injection site volume and injection core volume was then calculated by the StereoInvestigator software. The volume of each lesion site was calculated in the same manner as mentioned above with the exception that gray matter and white matter were examined separately by superimposing the external boundaries of the lesion, as determined in the Nissl sections, onto the corresponding Nissl stained tissue sections in the undamaged hemisphere (Figs. 9Ac, d, 10Ac, d, 10Ba, b; Table 1) (see Fig. 4 of Pizzimenti et al., 2007, right column).

Analysis of Bouton Number and Fiber Length—The methods to calculate the estimated bouton number have been provided in detail in our previous paper (Morecraft et al., 2007b). Briefly, we determined the average section thickness, overall fraction of tissue thickness that would be analyzed, the overall fraction of sectional area, and the overall number of tissue sections under analysis that contain the ROI. Using the sectional area and the average tissue thickness we then constructed counting bricks and counted axon terminal boutons and fiber intersection points using unbiased counting rules (Larsen et al., 1998, West et al., 1991). The stereological parameters included the counting brick dimensions, tissue thickness, counting brick placement, guard zone, dissector height and isotropic plane separation (Morecraft et al., 2007b). The same X/Y counting frame (109.2/71.4 μm) and X/Y grid placement (125.3/241.9 μm) was applied to all case material when performing stereology. The methods to calculate axon fiber length are identical to calculating the bouton number with the exception that virtual planes are added to the counting frame and intersections between the virtual planes and terminal fibers are marked. Specifically, to estimate the total terminal fiber length for both control and lesioned cases, we employed the stereological probe Isotropic Virtual Planes. This probe projects random virtual bisecting planes (spaced 25 μm apart) over a given ROI. Intersections between a terminal fiber and a virtual plane are marked. Upon completion of the probe, the stereological software calculates the estimated length of a given object based upon the spacing of the virtual planes, the number of intersecting fibers with the virtual planes, as well as the ssf, asf, dissector height and tissue thickness similar to the Optical fractionators (Larsen et al., 1998).

Statistical Analysis of Neuroanatomical Data

Statistical analyses were performed to determine if significant differences occurred in bouton numbers and terminal fiber lengths between the control and lesion animals. Specifically, separate mixed 2-way repeated measures ANOVAs were used to compare these dependent variables in control versus lesioned animals across the spinal laminae (I–IV, V, VI, VII, VIII, IX, X and RMB for spinal segments C5–T1). We also used a mixed 3-way repeated measures ANOVA to compare bouton numbers within quadrants of lamina IX for segments C5–T1. In this case the repeated measures factors were: dorsal/ventral regions each subdivided into medial and lateral quadrants. Huynh-Feldt epsilon values were examined to determine whether the assumption of sphericity was met when there were three or more levels in a repeated-measures factor (i.e., laminae). Adjustments in degrees of freedom for *F*-tests were made on the basis of the Huynh-Feldt epsilon values resulting in adjusted *P*-values, which are reported in the Results section. Statistical tests were accomplished using the GraphPad InStat 3 statistical software package (GraphPad Software Inc., San Diego, CA) or Statistica software (Tulsa, OK).

Movement Analysis

Two apparatuses were used to test fine hand/digit motor function: the modified movement assessment panel (mMAP) (Darling et al., 2006) and the modified dexterity board (mDB) (Pizzimenti et al., 2007) as previously described (Fig. 8). Forces applied during manipulation of the carrot chip in the mMAP task were recorded at 200 samples/s using Datapac 2k2 (Run Technologies). Movements of the hand during the mMAP task were recorded using a single digital video camera (Sony, model DCR-DVD301) to provide qualitative assessments of movement strategy and success/failure on each trial. Quantitative video recordings of hand movements during the mDB task from 4 cameras were used to assess spatial and temporal variables (e.g., accuracy and duration of the initial reach, grip aperture at touchdown of the hand, etc.). Video resolution from each camera was 656×492 pixels. A custom-built ($16 \text{ cm} \times 16 \text{ cm} \times 23 \text{ cm}$) rigid frame constructed of $\frac{1}{2}$ " welded rectangular tube steel with 41 marked points (with known locations specified using an Optotrak 3020 system – Northern Digital Inc.) was used to calibrate the volume of the space in which the hand moved during the mDB task using direct linear transformation algorithms (SIMI). These algorithms were also used to compute 3D coordinates of the fingertip and thumbtip at touchdown to assess reach accuracy (i.e., distance of fingertip to food pellet target) and grip aperture. Video data collection began when the portal door was opened to allow the monkey to reach toward the food pellet and continued until the pellet was retrieved into the cage, the pellet was knocked off of the platform, or a 60 s time limit had expired. Video collection was manually triggered and single trial video clips of each trial were manually created and verified.

Behavioral Procedures—Prior to an experimental session the monkey was food restricted for 18–24 hours. The initial training sessions used a “standard” rectangular dexterity board to assess the preferred hand of each monkey as described previously (Nudo et al., 1992). Briefly, the device consists of a Plexiglass platform with four tapered wells of different diameter (well A - 10 mm, well B - 13 mm, well C - 19 mm and well D - 25 mm) routed (1 cm deep) into the surface for the purpose of target food-pellet placement which induced the monkey to reach and grasp. The wells were positioned in a square pattern. A centrally located square window was located directly over the standard Kluver board so the monkey could choose to use either hand to grasp the pellet. A single banana flavored pellet was randomly placed into one of 4 wells and the animal was permitted to retrieve the pellet. The animal was given 1 minute to retrieve the pellet and if it was unsuccessful the pellet was removed and the next trial was initiated. Hand preference was measured over 150 trials over a 3 day period (with 50 trials conducted each day). We recorded: 1) the hand used on the initial reach for that trial; 2) the hand used on subsequent reaches and; 3) the hand used to retrieve the pellet. A reach was defined when the animal's hand passed through the plane of the square portal window located directly above the Kluver board. A subsequent reach was defined if the hand was withdrawn into the cage then extended back through the portal plane and over the Kluver board. The handedness index (HI) was computed as: $(P-50)*2$ where P is the average percentage of initial reaches and retrievals with the preferred hand (hand with the higher percentage of initial reaches and retrievals). HI ranges from 0 (50% of initial reaches and retrievals with each hand) to 100 (all initial reaches and retrievals with the preferred hand).

Training with the mMAP (Darling et al., 2006) and mDB (Pizziminti et al., 2007) devices commenced after hand preference was determined. Full testing sessions with the mDB included 5 retrieval attempts for each of the wells (A–E) for both limbs proceeding from the easiest well (E) to the most difficult (A), for a total of 25 trials with each hand. During post-lesion tests, the more impaired hand (contralateral to the surgically induced lesion) was always tested first to ensure high motivation. Full testing sessions with the mMAP included

blocks of 5 trials at each difficulty level with each hand, thereby giving the monkey 30 opportunities to retrieve carrot chips, 15 with each hand. During the first few post-lesion tests, the more impaired hand was tested first on the flat surface task (easiest task) to ensure high motivation. Thus, when considering both tests, a single testing session consisted of only 40 reaches for each hand.

Pre-lesion data were collected every 1–3 weeks for a total of 6–15 sessions according to each monkey's ability to learn the task and perform consistently. The final five pre-lesion experiments demonstrated relatively stable levels of performance before lesions were made to cortical motor areas (Fig. 11A–C). Post-lesion data were collected from both limbs during weekly experimental sessions for the first two months after the surgery (i.e., one testing session per week). Thereafter, tests were conducted biweekly (i.e., one testing session every 2 weeks). After initial training, it was only during the pre- and post lesion experimental sessions that the monkeys had exposure to the mMAP and mDB devices.

mMAP Data Analysis—Force data from the mMAP task were analyzed by visually identifying the first touch of the carrot chip or plate/rod supporting the carrot chip to the end of force application (i.e., when the carrot chip was removed from the plate supported by the load cell or the rod) on each trial using recordings of applied forces displayed in Datapac 2k2. The accompanying video data was also analyzed to verify these times and to identify trial outcome (see equation 1). The duration and total applied 3-dimensional absolute impulse (see equation 1) were computed for each trial. The total absolute impulse represents the total force applied during target acquisition and is larger if greater forces are applied over longer durations (Darling et al., 2006). Low impulse represents greater skill because the subject uses less force and removes the carrot chip faster.

After pre-lesion data collection was completed, performance scores were normalized to individual monkey's abilities (i.e., maximum and minimum applied impulse and duration) for each trial at each difficulty level (see equation 2). Also note in the performance score calculations that if the monkey failed to successfully acquire the carrot chip that higher scores are given if the monkey exerts larger impulses for longer time periods while attempting to acquire it. This occurs primarily in post-lesion trials when the monkey may abandon the attempt quickly and have very low applied impulse and duration (compared to during pre-lesion testing), which would result in high performance scores even though it was in fact a very poor attempt. With this scoring mechanism, low scores were awarded for weak attempts in which applied forces were low and duration of the attempt was short and higher scores were awarded for strong attempts in which large forces/impulses were exerted over a long duration (but limited to a maximum score of 200, which is also the minimum score for a successful trial).

$$\begin{aligned} \text{TAImp}(n) &= \int F_x dt + \int F_y dt + \int F_z dt \\ \text{TAImp}(n) &= \text{Total Absolute Impulse of trial } n \\ \int &= \text{integral over duration of trial } t \text{ with respect to time (dt)} \\ F_x &= \text{Absolute value of Force applied in left/right direction} \\ F_y &= \text{Absolute value of Force applied in anterior-posterior direction} \\ F_z &= \text{Absolute value of Force applied in vertical direction} \end{aligned} \quad (1)$$

$$\begin{aligned}
 &\text{If outcome} \geq 2 \text{ (i.e., successful grasp and lift/manipulation of the carrot chip) then} \\
 &PS_{mMAP}(n) = \{100 * ((TAImp(n) - \text{Min } TAImp) / TAImp \text{ Range}) + 100 * ((Dur(n) - \text{MinDur}) / Dur \text{ Range})\} * Outcome(n) \\
 &\quad \text{if } PS_{mMAP}(n) < 200 \text{ then } PS_{mMAP}(n) = 200 \\
 &\quad \text{Else} \\
 &PS_{mMAP}(n) = \{100 * ((MaxTAImp - TAImp(n)) / TAImp \text{ Range}) + 100 * ((MaxDur - Dur(n)) / DurRange)\} * Outcome(n) \\
 &\quad \text{If } PS_{mMAP}(n) > 200 \text{ then } PS_{mMAP}(n) = 200
 \end{aligned} \tag{2}$$

Where:

$PS_{mMAP}(n)$ – performance score on mMAP trial n

Outcome(n) – success on trial n (0 for no attempt with the correct hand, 1 for unsuccessful attempt with the correct hand, 2 if the carrot chip is successfully grasped and lifted over the rod but then dropped and not removed from the food chamber, 3 if the carrot chip is successfully grasped and lifted over the rod but then dropped and removed from the food chamber, 4 for successful acquisition without dropping the carrot chip)

MinTAImp – minimum single trial pre-lesion total absolute impulse within a difficulty level for either hand

MaxTAImp - maximum single trial pre-lesion total absolute impulse with a difficulty level for either hand

TAImp Range – maximum single trial pre-lesion total absolute impulse – MinTAImp

Dur(n) – duration of trial n

MaxDur - maximum single trial duration during pre-lesion tests

MinDur – minimum single trial duration during pre-lesion tests

DurRange – MaxDur – MinDur

mDB Data Analysis—Temporal characteristics of reaching, manipulation, and 3D locations of the tips of the index finger and thumb were determined from the digital video files as described previously and used to compute performance scores for reaching, manipulation and an overall score on each pre- and post-lesion trial (Pizzimenti et al., 2007). Measurements taken from video were used to compute reach duration, accuracy, grip aperture, manipulation duration and manipulation attempts (# of times contact between the pellet and a digit was lost and then re-established) on each trial. These measurements were each normalized to the performance ranges for each monkey prior to the lesion (i.e., to maximum/minimum reach and manipulation duration, least/most accurate reach, largest/smallest grip aperture, maximum/minimum number of manipulation attempts). Equations for the reach, manipulation and overall performance scores are given below (equations 3, 4, 5). The reach score was computed from reach duration, accuracy and grip aperture at touchdown for trials in which a reach attempt was made (reach score = 0 if no attempt). The manipulation score was computed from manipulation duration and attempts and a multiplier (0 for no attempt, 1 for failed attempt, 2 for successful acquisition).

$$\begin{aligned}
 &PS_{mDB}(n) = (100 * (Rdur(n) + Gapp(n) + Mdur(n) + Acc(jn) + C(n))) * Outcome \\
 &\quad \text{If outcome} \geq 1 \text{ then } PS_{mDB}(n) \geq 50
 \end{aligned} \tag{3}$$

Where:

$PS_{mDB}(n)$ - performance score on mDB trial n

Outcome(n) - multiplier (0 = no attempt, 1 = failure, 2 = successful retrieval of pellet) on trial n

Rdur(n) - (maximum pre-lesion reach duration – reach duration on trial n)/(maximum – minimum pre-lesion reach duration)

Mdur(n) - (maximum pre-lesion manipulation duration – manipulation duration on trial n)/(maximum – minimum pre-lesion manipulation duration)

Acc(n) - (maximum pre-lesion pellet-index distance at touchdown – pellet-index distance at touchdown on trial n)/(maximum – minimum pre-lesion pellet-index distance at touchdown)

Gapp(n) - (maximum thumb-finger distance – thumb-finger distance on trial n)/(maximum – minimum pre-lesion thumb-finger distance)

C(n) - 1/(1 + number of times contact is lost between a digit and pellet on trial n)

$$RS(n) = mr(n) * (100 * (Rdur(n) + Gapp(n) + Acc(n))) \quad (4)$$

Where:

RS = reach score, mr(n) = multiplier (0 = no attempt, 1 = dexterity board contacted) on trial n; Rdur(n), Gapp(n), Acc(n) as defined for equation 1

$$MS(n) = Outcome(n) * (100 * (Mdur(n) + C(n))) \quad (5)$$

Where:

MS(n) = manipulation score; Outcome(n), Mdur(n), C(n) defined as for equation 1

Analysis of Hand Motor Skill—We quantitatively assessed overall motor skill by computing mean divided by s.d. of manipulation performance scores over 5 consecutive testing sessions (i.e., 25 trials over an approximately 5 week period) (Pizzimenti et al., 2007). These were computed for the performance scores on the mMAP curved rod task (which are computed from manipulation duration and forces exerted during manipulation) and for the manipulation scores on the mDB task (well with highest pre-lesion skill for each monkey). Reach and manipulation performance scores were computed as described previously (Pizzimenti et al., 2007). Note that higher mean performance scores (lower duration, impulse on mMAP; lower duration and fewer lost contacts with the food pellet in the mDB tasks) and lower variability of performances will result in higher skill values. Skill was computed for the last 5 pre-lesion test sessions and for the 5 consecutive test sessions with the highest skill during post-lesion recovery.

We also qualitatively analyzed each lesioned monkey's contralesional hand/digit motions in the mDB and mMAP tasks to determine whether the wrist/hand/digit postures and motions were similar in pre and post-lesion successful trials. Specifically, we compared individual pre-lesion trials and post-lesion trials that received similar performance scores (about equal to the average performance scores in the last 5 pre-lesion test sessions) when possible (i.e., in monkeys that showed recovery close to pre-lesion performance scores). In monkeys that showed poor recovery, we compared the post-lesion trials with the highest performances scores to pre-lesion trials with average performance scores. We were particularly interested to determine whether the monkey's strategy changed post-lesion to use additional digits or different digits to perform the task.

Statistical Analysis of Motor Performance

We evaluated lesion effects on hand motor function from the duration (in weeks) from the time of the lesion until the first testing session with 5 successful acquisitions on the mMAP (curved rod task) and mDB (well with highest pre-lesion skill) tasks in each monkey. Recovery of fine motor skill was defined as the ratio of post-lesion skill (highest skill for 5 consecutive test sessions) to pre-lesion skill measured over the last 5 testing sessions before the lesion. These measures of recovery were entered into single linear regression analyses to test whether recovery was correlated with the ratio of bouton numbers in individual lesioned monkeys to average number of boutons in control monkeys. Specifically, we considered the number of boutons in laminae VII and IX in lesioned monkeys, which were significantly higher than in controls (see Results). Statistical tests were accomplished using the GraphPad InStat 3 statistical software package (GraphPad Software Inc., San Diego, CA) or Statistica software (Tulsa, OK).

RESULTS

Histological analysis in both control and lesion cases revealed that all FD injections were confined to the physiologically defined arm representation of M2 and did not extend into the fundus and lower bank of the cingulate sulcus. In all injection site cases the cortical region containing the core region corresponded to cytoarchitectonic area 6m on the medial wall. The halo was also confined to area 6m on the medial surface and spread to involve the dorsal convexity. Cytoarchitectonically, this region corresponded to the medial most part of area 6DR and 6DC which is also considered by some to be the dorsolateral part of area 6m (i.e., directly on the convexity). Indeed, this dorsally located region of cortex has also been shown physiologically to contain part of the M2 hand representation in the monkey (Woolsey et al., 1952; Tanji and Kurata 1982). All injections involved cortical layer V which gives rise to the CSP and was successfully transported to the spinal cord (Figs. 1–7).

Organization of Terminal Bouton Labeling in Control Cases

The corticospinal projection from the arm representation of M2 was examined in 4 control animals and the estimated numbers of labeled boutons for each Case within each laminae are provided in Table 2 (contralateral projection) and Table 3 (ipsilateral projection). Below we will present the data representing the mean of all four cases since the general distributions were similar across all cases. Collectively, the heaviest amount of terminal bouton labeling was found in the contralateral spinal gray matter with fewer boutons occurring ipsilaterally (Fig. 12A). For example, approximately 81% of the total number of terminal boutons was found contralaterally while 19% were located ipsilaterally.

Contralateral Projection—With respect to the contralateral projection, approximately 80% of the total number of terminal boutons occupied the intermediate zone (lamina V, VI, VII and the RMB); (Figs. 2, 12B; Table 2). A moderate amount of labeling (19%) was found in the ventral horn of the spinal gray matter (laminae VIII and IX). In contrast, less than 1% of total boutons were found in the dorsal horn (laminae I–IV) and central spinal region (lamina X).

Within the intermediate zone, the largest number of terminal boutons was found in lamina VII (77%); (Fig. 12B; Table 2). Within laminae VII, most boutons were located laterally (42%) with comparatively fewer boutons residing in the medial region (35%). Lower percentages of boutons were found in lamina VI (slightly greater than 11% total: 4% medially versus 7% laterally) and the reticulated marginal border (RMB) (10%). Finally, very few boutons were found in lamina V (slightly greater than 1%) and these were primarily located laterally. Thus, there appeared to be a ventral to dorsal trend in the number

of labeled boutons in the intermediate zone. The largest percentage occurred ventrally (i.e., lamina VII) with a progressively diminishing number occurring dorsally (i.e., laminae VI to lamina V). There also appeared to be a lateral to medial trend in the intermediate zone with the highest number of labeled boutons occurring laterally.

In the ventral horn of the spinal gray matter, lamina IX had the highest percentage of terminal boutons (78%) followed by lamina VIII (22%) (Fig. 12B). Focusing only on the total number of estimated boutons in lamina IX (Fig. 12C; Table 4), the heaviest labeling occurred in the dorso-medial and dorso-lateral quadrants. Specifically, 55% were located dorso-medially and 31% were located dorso-laterally. In contrast only 11% were located ventro-medially and 3% ventro-laterally.

Ipsilateral Projection—In terms of the total number of labeled boutons found in the ipsilateral spinal gray matter, the M2 projection terminated mainly within lamina VII (slightly less than 59%) and lamina VIII (41%) (Fig. 12D; Table 3). Within lamina VII most boutons occupied the medial region (90%) with less found laterally (10%). Therefore, the topographical distribution of terminal labeling in ipsilateral lamina VII was in direct contrast to the preferential lateral distribution found in contralateral lamina VII. A few labeled boutons were also noted in the dorsal part of lamina IX in cases SDM 41 and SDM 54 which was not detected in the stereological analysis emphasizing the infrequent occurrence of labeled boutons in this region. Finally, few labeled boutons were also noted in the ipsilateral region of lamina X in Case SDM 77.

Experimental Results of the Lesion Cases

Following the M1/LPMC injury, dexterous finger movements recovered. All lesioned monkeys were consistently successful on both tasks by 6 weeks after injury and continued to gradually improve over the next few months to a relative plateau (Fig. 11A–C). Histological analysis showed that the lesion was confined to the intended lateral motor cortices in all tract tracing experiments. In general, gray matter was completely removed in the ablation cases with the exception of a small portion of M1 residing in the lower region of the anterior bank of the central sulcus which was spared to avoid involvement of the adjacent primary somatosensory cortex (Figs. 6, 9, 10; see also Fig 4B of Pizzimenti et al., 2007, Case SDM 48). In all cases, there was minimal subcortical white matter involvement and no subcortical gray matter involvement (Table 1: gray and white matter lesion volumes). A detailed description of the lesion site in each case is provided below.

Lesion Site Analysis of Case SDM 45 (6 month recovery)—The gray matter arm region of the primary motor cortex in SDM 45 was fully removed with the exception of a few millimeters in the very depth of the central sulcus (Figs. 6A, 9A). The face and hind limb regions of M1 were successfully avoided (Fig. 9Ab, c) as the lesion site continued rostrally from the mid-region of M1 to involve the periarculate region of LPMCd (Fig. 9Aa, d). In the region of LPMCd, the lesion extended over the superior limb of the arcuate sulcus into the caudal inferior region of area 6DR. Cortex lining the depths of the arcuate spur was spared (Fig. 9Aa, d). There was minimal involvement of the subcortical white matter that was confined to the region immediately below the gray matter resection (Fig. 9Aa–d). According to Schmammann and Pandya (2006) this region resides above the long corticocortical projection fiber bundles (i.e., the SLF II and FOF) and is for local fibers entering and leaving the affiliated portion of the frontal motor cortex (i.e., see p. 541, section 81 of Schmammann and Pandya 2006). Interestingly, below the midregion of the lesion, over a distance of approximately 3 mm in the anterior posterior dimension, a small portion of the lesion dipped inferiorly forming a distinctive “V” shape within the white matter field (Fig. 9Aa–d). In the coronal dimension, this location corresponded to a plane from the arcuate

spur through the inferior part of the central sulcus. The tapered apex of the “V” appeared to slightly part the upper most portions of the FOF and SFII of which the latter 2 bundles consist of long cortico-cortical association fibers running perpendicular to the descending trajectory of the white matter lesion (Fig. 9Aa–d). However, the apex of the lesion ended, and did not reach the main body of the FOF or SLFII (see p. 451, section 81 of Schmähmann and Pandya 2006). White matter associated with the medial frontal cortex (M2 and the leg region of M1) and adjacent cingulate cortex was spared as were fiber bundles located below intact area 6V (Fig. 9a, d).

Lesion Site Analysis of Case SDM 70 (6 month recovery)—The lesion site in SDM 70 was restricted to the arm part of M1 and the dorsal part of LPMCv which were both electrophysiologically responsive and gave rise to arm movements prior to removal (Fig. 9B). Unlike SDM 45, SDM 48 and SDM 55, that part of LPMCd directly above the spur of the arcuate sulcus was unresponsive when current in the microamp range was applied and therefore, not removed. The face and hindlimb regions of M1 were spared including a small portion of area 4 (of the arm area) in the anterior bank of the depths of the central sulcus (Fig. 9Ba–d). There was no involvement of area 6DR of LPMCd, and slight involvement of the caudal ventral region of area 6DC below the superior precentral dimple (Fig. 9Bb–d). The upper portion of area 6Va was also involved in the lesion, below the arcuate spur as mentioned above (Fig. 9Ba). In the most rostral region of area 6Va, the lesion involved only layers I–III (Fig. 9Ba) but at middle and caudal levels of area 6Va, layers I–VI were completely removed. Of all the category F2 cases, this case also sustained the least amount of white matter damage (Fig. 9Ba–d; Table 1).

Lesion Site Analysis of Case SDM 48 (12 month recovery)—The lesion site in SDM 48 was similar to that described in SDM 45 (compare Fig. 9A to Fig 10A.; Table 1). For example, the arm region of the primary motor cortex was fully removed with the exception of a few millimeters in the very depth of the central sulcus (Fig. 10B; also see Fig. 4B of Pizzimenti et al., 2007). The face and hind limb areas of M1 were also spared (Fig. 10Ab, c). The lesion extended rostrally involving LPMCd (Fig. 10Aa, d). This included cortex over the superior limb of the arcuate sulcus corresponding to the caudal inferior part of cytoarchitectonic area 6DR and the rostral part of area 6DC (Fig. 10Aa, d) which both contain corticospinal projection neurons. The lesion extended ventrally to involve the dorsal most part of area 6Va while cortex in the depths of the arcuate spur was spared (Fig. 10Aa, d). Damage in the white matter was limited and confined to the immediate subcortex, ranging approximately 2 to 3 mm below the region of putative layer VI, but above the FOF and SFL II as found in case SDM 45. Interestingly, and nearly identical to the location found in SDM 45 (i.e., below the mid-region of the lesion, over an isolated distance of 2–3 mm in the anterior posterior dimension), a small portion of the lesion dipped inferiorly forming a distinctive “V” shape, appearing to slightly part the upper most portions of the FOF and SFII but sparing the main bodies of these bundles (Fig. 10Ab–c). Subcortical regions containing corticofugal projections from motor areas located adjacent to the lesion were spared. As with case SDM 45, white matter below the medial motor areas of SDM48, including the cingulate motor areas were also spared (Fig. 10Aa–d) as were fiber bundles below intact area 6V on the lateral surface (Fig. 10Aa, d).

Lesion Site Analysis of Case SDM 55 (12 month recovery)—The lesion site in SDM 55 was restricted to M1 and LPMCd as with Cases SDM 45 and SDM 48 (Fig. 10B; Table 1). The face and hind limb areas of M1 were spared ventrally and dorsally respectively (Fig. 10Bb, c). Cortex lining the anterior bank of the central sulcus in the region of the arm area was also spared (Fig. 10Bb, c). Comparatively, SDM 45 and SDM 48 had more of M1 removed in this location (i.e., removal of the upper half of the anterior bank

while sparing the lower half). In the lateral premotor region, cortex was removed in the caudal part of area 6DR, rostral part of area 6DC and the dorsal most part of area 6Va. In area 6Va, a small region in the arm area was affected but this involved only layers I–III. The cortex in the fundus and anterior banks of the arcuate sulcus was spared. At the rostral extent of the lesion the cortex lining the depths of the arcuate spur was spared (Fig. 10Ba, d). Very little white matter was affected by this lesion (Table 1) and the shallow, vacated parts of the superficial white matter suggested white matter loss to be fibers entering and exiting the removed, gray matter region located above. Thus, all regions of the SLF were spared as were the corticofugal projection pathways of intact adjacent cortex. Fiber bundles below intact area 6V were also spared as determined in the Nissl stained sections.

Lesion Site Analysis of Case SDM 58 (7.5 month recovery)—In the ibotenic acid experiment the first lesion (aspiration lesion) successfully removed the intended lateral motor areas (the arm area of M1 and the dorsal part of LPMC). The face and leg areas of M1 were spared. With respect to LPMC, the lesion site involved the arm region of architectonic areas 6DC of LPMCd and extended to involve the caudal part of area 6DC. As with all the other cases, a small portion of M1 buried in the depth of the central sulcus and the cortex lining the caudal bank of the arcuate sulcus was spared. The second lesion (ibotenic acid lesion) destroyed all gray matter forming the arm area of M2, sparing cortex lining the fundus and lower bank of the cingulate sulcus that corresponded to the rostral (M3) and caudal (M4) cingulate motor areas. The cortex forming the cingulate gyrus proper (area 24a–b, area 23a–b) was also unaffected by the ibotenic acid lesion. There was minimal subcortical white matter involvement that avoided injury to the FOF and SFII. In addition there was no evidence of subcortical gray matter involvement.

Neuroanatomical Observations of Control versus Lesion Cases

Compared to controls, all lesion cases had elevated numbers of boutons in the contralateral spinal gray matter of C5–T1 as shown in a Group (lesion vs. control) X Laminae (I–IV, V, VI, VII, RMB, VIII, IX, X) repeated measures ANOVA (group main effect: $F_{1,6} = 11.0$, $p = 0.016$). Lesioned cases had 1.5–3X more contralateral labeled boutons than the average of controls and all had more than any of the control monkeys, despite the smaller volume of tracer injected in the lesioned monkeys (Fig. 12B; Tables 2,5). Additional labeled boutons in lesioned monkeys were distributed primarily in lamina VII as shown by a significant group x laminae interaction effect ($F_{7,42} = 7.1$, $p = 0.009$ – note that Huynh-Feldt adjustments were made to account for greater than 2 levels in the repeated measures factors) (Fig. 12B; Tables 2,5). Post-hoc tests (Tukey's HSD) showed group differences in lamina VII ($p < 0.016$), but not in other laminae ($p = 0.5$ for lamina IX, $p > 0.9$ for other laminae). However, numbers of lamina IX boutons also appeared to be higher in lesion monkeys, particularly in the dorsal quadrants. (Fig. 12C; Tables 4, 7). Further testing with a 3-way (group, dorsal/ventral quadrants, medial/lateral quadrants) repeated measures ANOVA showed that lesioned monkeys had higher numbers of labeled boutons in lamina IX than controls ($F_{1,6} = 6.1$, $p = 0.049$), especially in dorsal quadrants (group x dorsal/ventral quadrants interaction effect - $F_{1,6} = 6.6$, $p = 0.042$) (Fig. 12C). There were also main effects showing that the M2 projection had more labeled boutons in dorsal than in ventral quadrants ($F_{1,6} = 48.8$, $p < 0.001$) and in medial than in lateral quadrants ($F_{1,6} = 6.3$, $p = 0.046$) in both groups. Notably, there were no group differences in number of labeled boutons in the ipsilateral CSP ($F_{1,6} = 0.01$, $p = 0.92$) (Fig. 12D; Tables 3, 6), demonstrating that the neuroplastic response was limited to the contralateral pathway. In fact, the highest number of ipsilateral boutons was found in a control case (Table 3, see SDM 54).

Lesion cases also had greater M2 terminal fiber lengths than controls in contralateral, but not ipsilateral, spinal gray matter at C5 and C8 levels (Fig. 12E and F). This was verified using

3-way (group, segmental level - C5/C8, laminae) mixed repeated measures ANOVAs for both contralateral and ipsilateral projections. For the contralateral projection the group effect showed a strong trend for longer fiber lengths in the lesioned monkeys ($F_{1,6} = 4.61$, $p = 0.075$) and a group x laminae interaction ($F_{7,42} = 4.42$, $p = 0.061$ after Huynh-Felt adjustment) demonstrating that lesioned monkeys had higher terminal fiber lengths than controls only in lamina VII (Tukey's post-hoc test - $p < 0.001$) (Fig. 12E). Fiber lengths in lamina IX of lesion monkeys averaged 3 fold higher than controls, but there was high variability among individual monkeys in both groups. The ipsilateral projections showed no group differences, nor were there any significant interactions involving groups ($p > 0.448$) (Fig. 12F). Overall, these data strongly suggest greater axon sprouting in the contralateral M2 projection primarily in lamina VII following injury.

Correlation of Neuroanatomical and Behavioral Observations

Recovery of fine motor function to acquire food targets was closely related to the increased number of boutons in individual lesion cases, especially in the mMAP task. The ratio of number of labeled lamina VII boutons in lesion monkeys to the average number of labeled lamina VII boutons in controls was negatively correlated with post-lesion duration until consistent success in the mMAP curved rod task ($R^2 = 0.99$) and in the mDB task ($R^2 = 0.89$) (Fig. 11D). In contrast, the ratio of labeled lamina IX boutons in lesion monkeys to controls was not strongly correlated with recovery to consistent performance in these tasks (Fig. 11E). Recovery of fine motor skill (to levels that exceeded pre-lesion skill in some monkeys) on the mMAP curved rod task, but not in the mDB task, was positively correlated ($R^2 = 0.99$) with the ratio of number of labeled boutons in lamina IX in individual lesioned monkeys to the average number of labeled lamina IX boutons in controls, but not for lamina VII (Fig. 11G, H). Qualitative assessments showed that all 4 lesioned monkeys recovered to use the same movement strategy as during pre-lesion trials in the mMAP curved rod task and that 3 of the 4 monkeys used the same pre- and post-lesion strategies in the mDB task. Only SDM 55 showed a small change in strategy after the lesion in the mDB (best well) task by contacting the food pellet first with the thumb-tip rather than the index finger-tip.

The animal that recovered after 7.5 months from the M1/LPMC lesion and was subsequently subjected to an ibotenic acid lesion (IAL) of M2 showed an immediate reemergence of motor deficits after the second lesion (Fig. 11C). This was not accompanied by paresis or arm transport deficits but significant impairments in dexterous movements of the hand and digits in both mMAP and mDB tasks (Fig. 11C, F). After the IAL, this monkey showed a similar pattern of motor recovery over the first month after injury as the other 4 monkeys did following the M1/LPMC lesion (Fig. 11A–C). For example, gradual improvement occurred over the first month post-IAL (Fig. 11C, F), but manipulation durations in the mDB task remained well above pre-lesion levels (Fig. 11F).

DISCUSSION

In clinical and rehabilitative settings there is tremendous variability of motor recovery in patients who survive brain injury, ranging from severe hemiplegia to recovery levels so extraordinary they are characterized by functional independence. This wide range of observations suggests that central nervous system mechanisms remaining intact following brain injury contribute to, and account for favorable levels of motor recovery. Our observations show for the first time in the non-human primate that motor recovery of precise, well-coordinated movements of the upper extremity is accompanied by selective corticospinal reorganization from the perspective of both laterality and laminar specificity (Fig. 13). In particular, significant differences in numbers of boutons between the controls and brain injured cases occurred only in laminae VII and IX of the contralateral projection (Fig. 13B) which are both important motor-related spinal cord centers (Kuypers, 1981,

Lemon et al., 2004; Schieber, 2007). Furthermore, within lamina IX significant bouton increases in lesion cases were found only in the dorsal quadrants suggesting preferential plasticity in subregions innervating the proximal and distal flexors as well as the intrinsic hand muscles (Jenny and Inukai, 1983). We also found strong correlations between these increases and recovery of motor function (Fig. 11D and H). Following recovery, the reemergence of distal fine motor deficits as a result of the subsequent lesion to M2 strongly supports a functional role for the increased M2 CSP to laminae VII and IX. Indeed, although structural and functional influence on lower motor neurons is normally weaker from M2 when compared to M1 (Lemon et al., 2002; Maier et al., 2002; Boudrias et al., 2006), the increased presence of boutons in lamina IX in the absence of significant M1 and LPMC CSP terminals would support this supposition. Importantly, no neuroanatomical differences were found in the ipsilateral CSP between the controls and lesion cases or in the contralateral CSP to the dorsally located sensory-related laminae (I–IV) or adjacent laminae of the intermediate and anterior regions (V, VI, RMB, and VIII) (Fig. 12B, D). Thus, isolated lateral motor cortex injury results in specific neuroplastic adaptations from the M2 CSP within a localized, but critical part of the spinal motor control system.

Organization of the Corticospinal Projection from the Supplementary Motor Cortex

Neuronal degeneration observations (Lassek 1954; Kuypers 1981) and studies utilizing the injection of retrogradely transported compounds into the spinal cord (Catsman-Beverrots et al., 1976; Biber et al., 1978; Murray and Coulter 1981; Toyoshima and Saki 1982; Hutchins et al., 1988; Nudo and Masterton 1990; Dum and Strick 1991; Galea and Darian-Smith 1994; Luppino et al., 1994; He et al., 1995) have revealed that cortex corresponding to M2 harbors pyramidal neurons in layer V that project to the spinal cord and the relative number of corticospinal projection cells in M2 appear to be quite prominent when compared to other non-primary motor areas (Dum and Strick 1991; Galea and Darian-Smith 1994). More recent examination of the terminal distribution of the corticospinal projection from the hand/arm representation of M2 projection following injections of wheat germ agglutinin-horseradish peroxidase (HRP) (Dum and Strick 1996; Maier et al., 2002) or biotinylated dextran amine (Rouiller et al., 1996) directly into M2 have shown a robust and mainly contralateral projection to lower cervical levels of the spinal cord. The findings from our control experiments are in close agreement with these observations. For example, Dum and Strick (1991) reported that the M2 projection was primarily contralateral (77% of total terminations) with a more moderate, but still impressive distribution of terminals ending ipsilaterally (23%). Similarly, we found on average, 81% of the terminal projection (i.e., by means of bouton estimation) ending contralaterally with 19% of the terminal projection ending ipsilaterally (Fig. 12A; Tables 2 and 3).

With respect to the contralateral projection, we found significant numbers of terminal boutons in lamina VII of the intermediate zone (Fig. 12B) which is in firm agreement with previous observations (Dum and Strick 1996; Rouiller et al., 1996; Maier et al., 2002). Topographically, we found the heaviest distribution of labeled boutons located laterally in lamina VII in all but one case (Table 2, see SDM 77) which also parallels previous observations (Dum and Strick 1996; Maier et al., 2002). The lateral projection within lamina VII extended into the region of the reticulated marginal border whereas the medial projection extended into adjacent lamina VIII which were both characterized by moderate terminal labeling. We also found moderate terminal bouton labeling in the lateral part of lamina VI and comparatively lighter labeling in the medial part of lamina VI which would be similar to previous accounts of relative labeling found in this region (Dum and Strick 1996). Our findings are also in agreement with previous reports (Dum and Strick 1996; Rouiller et al., 1996; Maier et al., 2002) which show a consistent contralateral projection from M2 to lamina IX of the ventral horn indicating direct influence on lower motor neurons

innervating the upper extremity. For instance, we found the lamina IX projection to represent on average approximately 15% of the total contralateral projection which is similar, but slightly higher than the 11% estimated by Dum and Strick (Dum and Strick 1996). This larger percentage is likely due to our ability to positively distinguish light and sparse labeling in the ventral quadrants which would have likely competed with background levels of staining in the HRP method. Considering our findings of estimated bouton numbers in lamina IX quadrants, and the descriptive observations reported previously (Dum and Strick 1996; Rouiller et al., 1996; Maier et al, 2002), there appears to be a consensus on the general topography of the lamina IX projection suggesting that it involves primarily the dorsal part of lamina IX. From our material, 55% of the total number of estimated boutons occurred dorsomedially with 31% located dorsolaterally. Our findings also indicate that the ventromedial quadrant has slightly more CSP terminations than the ventrolateral quadrant but both regions collectively receive sparse innervation compared to the dorsal quadrants. Finally, we found very few terminals in laminae I – IV of the dorsal horn and most were located laterally once again paralleling the findings of Dum and Strick (Dum and Strick 1996).

Corticospinal Plasticity following Motor Cortex Injury and Clinical Relevance

Studies in rodents have shown terminal axon sprouting in the ipsilateral CSP arising from the non-lesioned hemisphere which have clearly advanced our understanding of the corticospinal response following cortical injury (Rouiller et al., 1991; Kawamata et al., 1997; Chen et al., 2002; Lee et al., 2004; Brus-Ramer et al., 2007; Papadopoulos et al., 2007; Liu et al., 2007; Lapash Daniels et al., 2009). However, these studies have not demonstrated terminal axon plasticity in the contralateral CSP from surviving areas located in the lesioned hemisphere as demonstrated in the present study. Furthermore, our study is the first to identify increased numbers of terminal boutons in addition to terminal fiber length, of which the former is a strong indicator of putative synaptic terminations (Wouterlood and Groenewegen, 1985; Freese and Amaral, 2006). We also report for the first time that corticospinal plasticity occurs selectively within specific gray matter subsectors that are known to play a critical role in movement production. Finally, our study is also the first to apply stereology to quantify these morphological features and demonstrate a correlation between corticospinal terminal sprouting and the return of dexterous movements of the hand following motor cortical injury.

The importance of the findings in our non-human primate experiments (Fig. 13) would appear to be particularly relevant to humans with subtotal brain injury for a number of reasons. Indeed, multiple, homologous cortical motor areas are recognized in each hemisphere of the human and non-human primate (Penfield and Welch, 1951; Woolsey et al., 1952; Woolsey, 1958; Zilles et al., 1995; Picard and Strick, 1996; Roland and Zilles, 1996; Geyer et al., 2000), including M2, and higher order primates rely extensively on opposite hemispheric control of digit/arm function (Kuypers, 1981; Heffner and Masterton, 1983; Lemon and Griffiths, 2005). The structural underpinning of this hemispheric control is in part, rooted in the fact that all these cortical motor areas project primarily to the contralateral spinal cord (Figs. 12A, 13) (Kuypers and Brinkman 1970; Kuypers 1981; Toyohima and Sakai 1982; Galea and Darian-Smith 1994; Dum and Strick, 1996; Morecraft et al., 1997; Darian-Smith et al., 1999) and the direct CSP to the motor neurons in primates plays an essential role in the production of finely coordinated dextrous hand movement (Heffner and Masterton, 1975, 1983; Lawrence and Kuypers, 1968; Davidoff, 1990; Bortoff and Strick, 1993; Lemon 1993; Porter and Lemon 1993; Nakajima et al., 2000; Lemon and Griffiths, 2005; Schieber, 2007; Schieber et al., 2009). Taken together, it seems only logical that favorable recovery would correlate with partial sparing of the contralateral CSP in the injured hemisphere, particularly in humans whose preeminent control over voluntary

dexterous movement is directly related to a massive and highly developed CSP system (Heffner and Masterton, 1975, 1983; Kuypers 1981; Nudo and Masterton, 1990; Lemon and Griffiths, 2005; Schieber, 2007). This is underscored by seminal observations showing impairment of dexterous movement is progressively more profound and longer lasting following precentral cortex removal in the monkey, chimpanzee and human respectively (Bucy, 1949b). Likewise, under this condition recovery of dexterous movements in humans is much slower than the chimpanzee, which in turn is slower and less complete than observed in the monkey.

The significantly greater sprouting of M2 terminals into dorsal than ventral regions of lamina IX may be particularly relevant to recovery of reaching and independent digit movements for precision grasping following lateral cortical brain injury. For example, dorsal regions of lamina IX contain the cell bodies of lower motor neurons innervating the proximal flexors (dorsomedial sector) and distal flexors including the intrinsic hand muscles (dorsolateral sector) in macaque monkeys (Jenny and Inukai 1983) (Fig. 12C). Following brain injury, re-establishing cortical control over extrinsic digit flexors and intrinsic hand muscles may be more important than cortical control of extrinsic extensors for grasping and manipulating small objects held between the digits. This would be particularly critical in the mDB task applied in our study which requires independent control of digit motions (Fig. 11H). In this context, it is important to note that extension of the interphalangeal joints is critical for positioning the fingertips for grasping and this action requires activation of the intrinsic hand muscles (Landsmeer and Long, 1965, Mulder and Landsmeer, 1968; Neumann, 2002). Indeed, activation of only extrinsic digit muscles will not extend these joints due to the musculoskeletal mechanics of the digits. In human stroke and brain injury, the classic signs of hemiparesis are a clenched hand (flexed digits) with a flexed wrist and elbow (and medial rotation at the shoulder) which is typically attributed to increased tone and spasticity of the anterior (flexor) musculature. Restoring cortical control to the flexor muscles permits them to be relaxed to relieve tone/spasticity and allow control over digit grasping movements and reaching movements (see Krakauer, 2005 for review). This also permits the extensors to exert greater net extensor torques as they contract against relaxed flexors, thereby allowing elbow extension for reaching and digit extension to release objects. Thus, our findings that dorsal regions of lamina IX received more M2 CST boutons in lesioned monkeys than the ventral regions are consistent with re-establishing control of flexors and intrinsic hand muscles following lateral cortical injury, which likely contributes to recovery of reaching and precision grasping. This would be supported by our behavioral findings which show that following recovery from lateral cortical injury, subsequent injury to M2 leads to reemergence of fine hand motor deficits (Fig. 11F, I). It should be noted, however, that we also found small increases in bouton number in the extensor regions (ventromedial and ventrolateral quadrants) of lamina IX (Fig. 12C). Thus, cortical control of extensors may also play some role in motor recovery.

Ipsilesional Compensatory Mechanisms and Motor Recovery

Accumulating evidence from human studies also points to a contributing role in recovery for spared, ipsilesional non-primary cortical motor areas particularly on the medial hemispheric wall. Foremost are functional imaging studies in stroke patients which have shown ipsilesional activation in M2 when recovered patients perform movements with their affected hand following damage to the precentral motor cortex (M1) or its descending axons (Seitz et al., 1995; Weiller et al., 1993; Cramer et al., 1997; Loubinoux et al., 2003; Luft et al., 2004; Ward, 2004). Neurophysiological studies in non-human primates also support an important role for ipsilesional M2 in the motor recovery process following isolated lateral motor cortex injury. After extensive training of a motor task, physiological unit activity in M2 is known to decrease, but when M1 is selectively lesioned there is an increase in M2

unit activity when performing the same task, indicating an ipsilesional M2 compensatory response following lateral frontal injury (Aizawa et al., 1991). Similarly, there is an expansion of distal forelimb movement representation in M2 following recovery from injury affecting M1 and LPMC (Eisner-Janowicz et al., 2008). On the other hand, very small lesions of M1 in immature monkeys do not appear to have a physiological effect on M2 following recovery (Rouiller et al., 1998). Although the age at which the lesion was induced in this study differs significantly from our study, and those studies mentioned above, it is possible that recruitment of spared CSP from M2 depends on the degree of damage to the overall lateral CSP system as suggested in human studies (Seitz et al., 1998; Ward and Cohen, 2004; Ward et al., 2007; Schaechter and Perdue, 2008). Indeed, following a lesion restricted to M1, spared lateral premotor cortex may play a primary role in mediating the recovery process as indicated by several neurophysiological observations (Kennard, 1942; Glees and Cole, 1950; Rouiller et al., 1998, Liu and Rouiller 1999; Frost et al., 2003; Dancause et al., 2006) and recent neuroanatomical observations (Dancause et al., 2005). Following recovery from an M1 ablation, subsequent ablation to LPMC results in reappearance of paralysis but again, eventual recovery of upper extremity movement (Black et al., 1974; Liu and Rouiller 1999) providing support for this hypothesis.

In humans, considerable motor recovery accompanied by cortical reorganization can occur in the acute as well as chronic time periods following brain injury (Traversa et al., 1997; Silvestrini et al., 1998; Nelles et al., 1999b; Marshall et al., 2000; Byrnes et al., 2001; Small et al., 2002; Ward et al., 2003; Ward and Cohen, 2004). Our findings support the idea of sustained, chronic phase compensatory mechanisms supporting recovery. However, it will also be of great interest to examine neuroplastic adaptations of the M2 CSP that focus on the acute, post-injury time frame. In conjunction with the present findings, this will provide valuable insight on the temporal profile, or evolution of CSP reorganization following lateral cortical injury, particularly since a rapid and considerable degree of recovery occurs during this time frame (Fig. 11A–C).

Although our findings point to the involvement of the surviving ipsilesional CSP in the motor recovery process, it is important to stress that there are likely to be many other contributing projection systems from M2 including the corticocortical, corticorubral, corticopontine and corticoreticular projection systems. Projections from other spared ipsilesional brain regions, which may have included a small portion of M1 hand/digit areas in the very depth of the central sulcus in our cases, are also likely to contribute to recovery. This would be supported by our IAL experiment, in which deficits in dexterous movements immediately reemerged after injury to M2, but some recovery began to occur in the first month after the M2 lesion (Fig. 11C, F, I).

Technical Considerations

In tract tracing practice, the interpretation of the precise location of the effective injection site has been a fundamental challenge (Mesulam, 1982). Following injection of the tract tracing compound, extracellular dissipation occurs forming upon microscopic examination an indiscriminate *halo* around a dark *core* of reaction product (Figs. 1, 4). The *core* of the injection site is characterized by the presence of very dense histochemical precipitate, where individual axons and perikarya are heavily labeled. This region of the injection site is traditionally considered part of the “effective injection site” implying that tract tracer uptake and axon transport of the tracer actively occurs at this location (Edwards and Hendrickson 1981; Warr et al., 1981; Mesulam, 1982; Conde 1987). The uncertainty of the effective uptake site lies in the interpretation of the injection site *halo*, where there is a gradual decline in the density of labeled perikarya, particularly toward the periphery of the injection site halo (Figs. 1, 4). That portion of the injection site halo that closely surrounds the core is also conventionally considered part of the effective uptake zone (Edwards and Hendrickson

1981; Warr et al., 1981; Mesulam, 1982; Conde 1987). However, beyond this part of the halo uptake characteristics are uncertain. For this reason, and as suggested for interpreting the HRP injection site halo (Mesulam, 1982), we chose to err on the side of caution by slightly overestimating the inject site halo when computing the injection site volumes for both the lesion and non-lesioned cases in the event that neurons located at the very peripheral region of the halo were in fact involved in perikaryal uptake and axon transport of tract tracer to the spinal cord. The peripheral edge of the halo was also defined when labeled cells and axons displayed an orderly pattern of labeling indicating a neuroanatomical projection. This was characterized by the presence of discretely labeled columns or patches of anterograde labeling, or when distinctly labeled somas or axon terminals appeared across layers I, III and/or V. Damaged axons in subcortical white matter pathways by the syringe tip can also be an additional source of tract tracer uptake which should be considered when interpreting the effective uptake zone of an injection site (Mesulam, 1982; Schofield, 1990; Reiner and Honig, 2006). However, our surgical approach and injection procedure was designed to completely avoid needle tract penetration into the subcortical white matter underlying M2 which was verified upon histological analysis. Indeed, below cortex forming M2 are numerous descending pathways gaining entrance to the internal and extreme capsules and corpus callosum for example (Morecraft et al., 2002 - see Fig. 5), as well as numerous corticocortical white matter pathways such as the superior longitudinal fasciculus (Schmahmann and Pandya, 2006). Since we did not induce subcortical white matter damage in our experimental cases as verified in our histological analysis, we did not include this territory when computing the respective injection site halo volumes.

In neuroanatomical tract tracing, choice of tracer, injection site volume, region of effective uptake, axon transport mechanisms, post-injection survival time and tissue fixation are all central variables that could affect the experimental outcome of a tract tracing experiment in the form of labeled reaction product (Warr et al., 1981; Mesulam, 1982; Reiner and Honig, 2006; Morecraft et al., 2009). Therefore, to minimize inter-experimental variability between the non-lesioned experiments and the lesion experiments we used the same tract tracer (FD) and applied the same injection procedure, post injection survival interval and tissue processing methods across all experiments. Furthermore, we made one major adjustment in our experimental design to further minimize these variables and their potential impact on neuroplasticity observations. All of our control animals received a larger volume of tract tracer (1.2uL) in the injection process than all of the lesion animals (0.9 uL) so that injection site size of our lesion cases would be at least similar in size, or even smaller in size than all of the control cases, which was achieved upon microscopic analysis (Table 1). Thus, we intentionally handicapped the lesion experiments so that increases in bouton number and fiber length would be more reflective of a true neuroplastic response and not a consequence of a potentially larger injection site size. It is noteworthy that increased numbers of labeled varicosities occurred only within contralateral laminae VII and IX in the lesion cases but not in other lamina or the ipsilateral projection, which served as an internal controls for tracer volume and transport issues. Finally, with respect to injected tracer volume, it is also possible that our findings from the lesion cases may have underestimated the neuroplastic response in terms of bouton numbers because of the smaller volume of FD injected compared to the controls.

It is important to note that an additional problem with dextran tracers is that they are typically transported both anterogradely (very efficiently) and retrogradely (less efficiently) (Reiner and Honig, 2006). Thus, it is possible that following the FD injection into M2, retrogradely transported FD label to spared corticospinal neurons in M1, LPMC or the cingulate motor areas could then be anterogradely transported to the spinal cord as well. However, with respect to our lesion cases this scenario should have minimal effect on the experimental outcome since an extensive portion of frontal cortex containing corticospinal

projection neurons that terminate at C5 to T1 were removed (Figs. 9, 10). In other words, the control cases would have a distinct experimental advantage over the lesion cases in addition to the advantage of the controls having more volume injected into M2 than the lesion cases. To further address this issue, we examined the spared parts of frontal cortex in the lesion cases and found retrogradely labeled neurons in the spared region of LPMCd and LPMCv. With respect to the origin of the corticospinal projection, that part of LPMCd which contained retrogradely labeled cells projects primarily to the lumbosacral cord as determined by previous work (He et al., 1993) whereas that part of LPMCv containing retrogradely labeled neurons in the lesion cases projects only to spinal levels C1 through C4 (Martino and Strick 1987; Nudo and Masterton 1990; Wise 2006). In the lesion cases we also found some retrogradely labeled cells in the spared part of M1 residing in the anterior bank of the central sulcus (Figs. 9,10) that potentially could have labeled corticospinal terminals. However, most of these FD labeled cells were medium sized pyramidal cells and not of the large pyramidal cell type which project to the spinal cord. It is important to stress that we also found in both the control and lesion cases numerous retrogradely labeled neurons with FD in the rostral (M3) and caudal (M4) cingulate motor areas. It is noteworthy in this respect that both M3 and M4 are known to send prominent corticospinal projections to spinal lamina I–IV of the dorsal horn (Dum and Strick 1996; Morecraft et al., 1997). However, in our cases (both control and lesion) dorsal horn labeling throughout spinal levels C5 to T1 was sparse to non-existent (Fig. 7A–C; Tables 2, 5). Thus, this finding would strongly argue against the possibility that retrogradely transported label through cortical collaterals of corticospinal projection neurons was effectively transported to spinal levels C5 to T1.

Another technical matter relates to the designation of labeled varicosities (boutons) as putative synaptic contacts. It is important to note that as determined by electron microscopic analysis, varicosities of this size on the terminal region of axon fibers, that are immunohistochemically identified with the use of contemporary high resolution anterograde tracers, primarily represent synaptic contacts with local neuron profiles (Wouterlood and Groenewegen, 1985; Freese and Amaral, 2005). Indeed, the goal of this study was not to investigate the microsynaptic organization of the corticospinal projection but to derive an estimate of axon innervation in a large tissue sample based upon terminal – like profiles obtained from the exquisite structural detail offered by the tract tracing procedures applied in the study.

Summary and Conclusions

Clinically, our findings strongly suggest that spared ipsilesional M2 serves as an important internal resource supporting the recovery of arm and hand movements following lateral precentral injury by selectively enhancing its corticospinal linkage to the opposite side of the spinal cord (Fig. 13B). This observation provides support for the notion that recovery of motor function following lateral motor cortex injury occurs in part, by a contribution from corticospinal fibers originating outside of the precentral region in an attempt to assume the functions mediated by the destroyed corticospinal fibers (Denny-Brown 1950; Bucy 1957;). Thus, when possible, M2 should be protected in the acute phase following brain injury and evaluated for structural and functional integrity, then recruited in the early and comprehensive stages of brain injury rehabilitation with additional supportive therapeutic implementation. Limiting inflammation to protect uninjured cortical motor areas (Muir et al., 2007), designing pharmacological applications to enhance corticospinal neuroplasticity (Zaleska et al., 2009) and developing non-invasive therapeutic applications (Curra et al., 2002; Wolf et al., 2002; Harvey and Nudo, 2007) that are aimed to enhance the recruitment of undamaged M2 hold great promise toward promoting the recovery process in patients with subtotal brain injury.

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COMMON ABBREVIATIONS

CSP	corticospinal projection
FD	Fluorescein dextran
IAL	ibotenic acid lesion
LPMC	lateral premotor cortex
M1	primary motor cortex
M2	supplementary motor cortex
mDB	modified dexterity board
mMAP	modified movement assessment panel

LIST OF ABBREVIATIONS

A	arm
AC	anterior commissure
Amts	anterior medial temporal sulcus
Amy	amygdala
As	arcuate spur of the arcuate sulcus
Ca	caudate nucleus
cb	cingulum bundle
cc	corpus callosum
cf	calcarine sulcus
cgs	cingulate sulcus
Cl	claustrum
cs	central sulcus
ecs	ectocalcarine sulcus
D	digit
Ea	ear
El	elbow
FD	Fluorescein dextran
GP	globus pallidus
H	hippocampus
Hp	hip
Hy	hypothalamus
IAL	ibotenic acid lesion

IC	internal capsule
ipcd	inferior precentral dimple
ilas	inferior limb of the arcuate sulcus
J	jaw (mandible)
ios	inferior occipital sulcus
ips	intraparietal sulcus
L	leg
LCST	lateral corticospinal tract
lf	lateral fissure
LL	lower lip
LPMC	lateral premotor cortex
LPMCd	dorsal lateral premotor cortex
LPMCv	ventral lateral premotor cortex
ls	lunate sulcus
M1	primary motor cortex
M2	supplementary motor cortex
MB	mamillary body
mDB	modified dexterity board
mMAP	modified movement assessment panel
N	neck
NR	no response
OC	optic chiasma
OT	optic tract
ots	occipito-temporal sulcus
poms	medial parieto-occipital sulcus
ps	principle sulcus
Pu	putamen
RMB	reticulated marginal border
ros	rostral sulcus
rs	rhinal sulcus
Sh	shoulder
slas	superior limb of the arcuate sulcus
spcd	superior precentral dimple
sts	superior temporal sulcus
Ta	tail
Th	thumb

Tha	thalamus
To	tongue
Tr	trunk
UL	upper lip
v	ventricle
W	wrist
3	4,6,23, Cortical architectonic divisions according to Brodmann(1905)
I-X	Laminae of the spinal cord according to rexed (1954)

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Figure 1.

Plate of low-power digital photomicrographs of immunohistochemically processed coronal tissue sections through the cerebral cortex illustrating the fluorescein dextran (FD) injection site in control cases SDM 41 (**A**), SDM 54 (**B**), SDM 62 (**C**) and SDM 77 (**D**). On the injection site, the dashed line represents the external boundary of the injection site core and the dotted line the external boundary of the injection site halo. The white arrows identify a coalesced labeled fiber bundle emerging from the injection site. The scale bar in A applies to all panels. Abbreviations: as, spur of arcuate sulcus; cb, cingulum bundle; cgs, cingulate sulcus.

Figure 2.

Plate of high-power digital photomicrographs of immunohistochemically processed tissue sections through the spinal cord illustrating fluorescein dextran (FD) labeled terminal axon fibers and boutons (arrowheads) in the gray matter of control cases SDM 41 (**A**, spinal level T1), SDM 54 (**B**, spinal level C6), SDM 62 (**C**, spinal level C8) and SDM 77 (**D**, spinal level C7). In panels A and B the inset is a higher power view of the field marked by the asterisk. In panel A the double arrowhead shows a bouton cluster in close opposition to an adjacent neuron soma in lamina IX. The brown labeled fibers in panel C are a result of labeling from the tract tracer biotinylated dextran amine (BDA) that was injected into another spared cortical region of interest. Roman numerals represent Rexed's laminae. Abbreviations: d, dorsal; l, lateral; m, medial; RMB, reticulated marginal border; v, ventral.

Figure 3.

Line drawings of the medial surface of the cerebral cortex in control cases SDM 41 (A), SDM 54 (B), SDM 62 (C) and SDM 77 (D) depicting the fluorescein dextran (FD) injection sites. The irregular green shape represents the location of the injection site core and the surrounding dashed line the external boundary of the injection site halo. The pullout depicts the physiological map of movement representation obtained with intracortical microstimulation used to localize the arm representation of M2 prior to injection of the tract tracer FD. As mentioned in the Supplementary Materials section, a complete physiological map could not be obtained from SDM 41 because after the large bridging veins over the interhemispheric sulcus were ligated to fully expose the medial wall, the cortex was electrophysiologically unresponsive. Therefore, the central region of the arm representation in this case was determined anatomically (along with the single stimulation point that initially assisted in defining the region prior to ligating bridging vessels) as being located at coronal levels including the genu of the arcuate sulcus to a level approximately 0.5 mm posterior to this landmark as reported in previous monkey electrophysiological M2 mapping studies (e.g., Macpherson et al., 1982; Tanji and Kurata 1985; Mitz and Wise 1987; Aizawa et al., 1991; Mushiake et al., 1991; Wang et al., 2001). Micron bar = 5 mm. For abbreviations see List of Abbreviations.

Figure 4.

Plate of low-power digital photomicrographs of immunohistochemically processed coronal tissue sections through the cerebral cortex illustrating the fluorescein dextran (FD) injection site in lesion case SDM 45 (A), SDM 70 (B), SDM 48 (C) and SDM 55 (D). On the injection site, the dashed line represents the external boundary of the injection site core and the dotted line the external boundary of the injection site halo. The white arrows identify a coalesced labeled bundle emerging from the injection site. The asterisk in panels A and D denote the location of a portion of the lesion site. The scale bar in A applies to all panels. For abbreviations see List of Abbreviations.

Figure 5.

Plate of high-power digital photomicrographs of immunohistochemically processed tissue sections through the spinal cord illustrating fluorescein dextran (FD) labeled terminal axon fibers and boutons (arrowheads) in the gray matter of lesioned cases SDM 45 (**A**), spinal level C7), SDM 70 (**B**), spinal level C8), SDM 48 (**C**, spinal level C7) and SDM 55 (**D**, spinal level C8). In panel C the inset is a higher power view of the field marked by the asterisk. The arrowheads in panel A and C show the location of some bouton clusters in close opposition to an adjacent neuron soma in lamina IX. Roman numerals represent Rexed's laminae. For abbreviations see List of Abbreviations.

Figure 6.

Line drawings of the lateral (top) and medial (bottom) surfaces of the cerebral cortex in lesion cases SDM 45 (A), SDM 70 (B), SDM 48 (C) and SDM 55 (D) depicting the locations of the lesion site (blackened area on lateral surface) and core of the fluorescein dextran (FD) injection site (green irregular shape on the medial surface). On the medial wall the dotted line around the injection site core represents the external boundary of the injection site halo. On the lateral surface, the pullout depicts the physiological map of movement representation obtained with intracortical microstimulation used to localize the arm representations of the primary motor cortex (M1) and lateral premotor cortex (LPMC) prior to neurosurgical resection of the gray matter forming these motor cortices. The pullout on the medial surface is the physiological map of movement representation obtained with intracortical microstimulation to localize the arm representation of M2 prior to injection of the tract tracer FD. Micron bar = 5 mm. For abbreviations see List of Abbreviations.

Figure 7.

Line drawings depicting a representative transverse section through spinal levels C5 to T1 in case SDM 54 (control) (**A**), SDM 45 (6 month recovery) (**B**) and SDM 48 (12 month recovery) (**C**) showing regions in the lateral corticospinal tract containing labeled axons (green dots) and regions of Rexed's laminae containing labeled boutons and bouton clusters (blue dots). Roman numerals in section C5 designate Rexed's laminae and apply to all spinal sections. Laminae I–VII were subdivided into medial and lateral halves (see dashed line) and lamina IX into quadrants (see dashed lines) for stereological analysis. For orientation dorsal is located on the top of each section and ventral at the bottom. Micron bar = 2 mm. Abbreviations: dm, dorsomedial; dl, dorsal lateral; LCST, lateral corticospinal tract; RMB, reticulated marginal border; vm, ventromedial; vl, ventrolateral).

Figure 8.

Diagrams of the modified movement assessment panel (A) and modified dexterity board (B) used in this study to track motor recovery following brain injury. Each device attaches to the animal's cage front and the right and left portals can be closed or opened accordingly allowing for controlled testing of each hand without the need for restraints. The reach path to the respective food targets (carrot chip in the movement assessment panel and food pellet in the modified dexterity board) is identified by the curved arrow. In (A), the straight rod in the modified movement assessment panel (mMAP) test can be replaced with a curved rod for a more difficult task, or removed completely creating an easy task allowing the animal to remove the carrot chip (orange sphere) from the flat surface. In the modified dexterity board (mDB) test, task difficulty depends on the size of the well positioned in the target location which is controlled by rotating the Plexiglas disk to the position in front of the portial opening. For more detailed descriptions of these devices see Darling et al., 2006 and Pizzimenti et al., 2007.

Figure 9.

Line drawings of the lateral surface of each hemisphere of SDM 45 (**A**) and SDM 70 (**B**). Both animals recovered for 6 months after brain injury. Representative coronal sections for each case are located immediately below the lateral views (a–d). For both SDM 45 and 70 the left hemisphere illustrates the location of the cortical lesion (blackened area) and the right hemisphere the location of the superimposed lesion (outlined area) that was used to calculate the gray and white matter lesion volumes. In case SDM 45 (**A**), coronal panels a and b are through the lesioned hemisphere and panels c and d show the location of the superimposed lesion site in the non-lesioned hemisphere (dashed line). In case SDM 70 (**B**), panels a–d are through the lesion site. In each coronal section, the motor region of extirpated cortex is identified by the bold italicized conventions and the spared face (Fa) and leg representations (L) are labeled in the intact gray matter of the primary motor cortex (M1, including the small portion of the arm representation (A) that was spared in the depths of the central sulcus. Pertinent Brodmann's areas are indicated on the coronal sections immediately below the gray matter and the respective boundaries are identified by the arrow heads. For abbreviations see List of Abbreviations

Figure 10.

Line drawings of the lateral surface of each hemisphere of SDM 48 (**A**) and SDM 55 (**B**). Both animals recovered 12 months after brain injury. Representative coronal sections for each case are located immediately below the lateral views (a–d). For SDM 48 (**A**) the left hemisphere illustrates the location of the cortical lesion (blackened area) and the right hemisphere the location of the superimposed lesion (outlined area) that was used to calculate the respective gray and white matter lesion volumes. For SDM 55 (**B**) the right hemisphere illustrates the location of the lesion (blackened area) and the left hemisphere the location of the superimposed lesion (outlined area) that was used to calculate the respective gray and white matter lesion volumes. Coronal panels a and b for SDM 48, and c and d for SDM 55, are through the lesion site. Panels c and d for SDM 48, and a and b for SDM 55, show the location of the superimposed lesion site (dashed line) in the non-lesioned hemisphere. In each coronal section, the motor region of extirpated cortex is identified by the bold italicized conventions and the spared face (Fa) and leg representations (L) are labeled in the intact gray matter of the primary motor cortex (M1), including the small portion of the arm representation (A) that was spared in the depths of the central sulcus. Pertinent Brodmann's areas are indicated on the coronal sections immediately below the gray matter and the respective boundaries are identified by the arrow heads. For abbreviations see List of Abbreviations.

Figure 11.

Panels A, B and C show mean pre- and post-lesion performance scores (+ 1 S.D) on the mMAP curved rod task for two lesion group monkeys (A, B) and a monkey that received a similar lesion, then recovered for 7.5 months, and had a 2nd lesion of M2 arm area using ibotenic acid (C). Panels D and E show relationships between time post-lesion until consistent successful acquisition of the food target in the mDB (well on which the monkey performed best) and mMAP (curved rod) tasks and ratio of laminae VII (D) and IX (E) boutons of each lesion monkey to mean of controls. Panel F shows mean (+1 S.D.) pre- and post- M1+LPMC lesion and post-ibotenic acid lesion reach (blue) and manipulation (red) durations for case SDM58 (well C of mDB task). Panels G and H show relationships between recovery of skill (ratio of post-lesion skill to pre-lesion skill) in the mDB (best well) and mMAP (curved rod) tasks and ratio of laminae VII (left graph) and IX boutons (right graph) for each lesion monkey to mean of controls. Panel I shows forces exerted versus time during the curved rod mMAP task for the worst successful trial in the test preceding ibotenic acid lesion in SDM58 (top) and the best performance one week following ibotenic acid lesion (bottom). Note that similar magnitude forces were exerted at one week after the ibotenic acid lesion, but the carrot chip was not removed from the curved rod. Note also that Fx forces are not displayed because of a problem with the recording. Performance

score at one week post M2 lesion was estimated from F_y (anterior-posterior direction) and F_z (vertical direction) force recordings.

Figure 12.

A) Percentages of all boutons in the contralateral and ipsilateral projections from M2 to C5-T1 for controls (average) and each lesioned monkey. **B)** Estimated numbers of labeled boutons in the contralateral CSP from M2 to C5-T1 laminae in controls (average - Con) and individual lesion monkeys (abscissa). Key also applies to panels D-F. **C)** Estimated numbers of lamina IX boutons in each quadrant in controls (average) and individual lesion monkeys. **D)** Estimated numbers of labeled boutons in the ipsilateral CSP from M2 to C5-T1 laminae in control and individual lesion monkeys. **E)** Estimated total fiber lengths in the contralateral CSP from M2 to C5 and C8 in controls (Con) and each lesioned monkey. **F)** Estimated total fiber lengths in the ipsilateral CSP from M2 to C5 and C8 in controls (Con) and each lesioned monkey. Error bars on the control data represent SEM.

Figure 13.

Summary diagram illustrating the main findings of the study. The left diagram (A) illustrates the corticospinal projection from the supplementary motor cortex (M2) in the control experiments. This projection originates from the medial wall of the hemisphere (top, hinged to left from dorsal view of cerebral cortex on right) and most descending fibers cross the midline at inferior brainstem levels (middle) ending in the spinal cord (bottom). The relative intensity of the projection to spinal cord laminae is indicated by line thickness and arrow size. Denser terminal projections are represented by increased line thickness and arrow head size. Progressively lighter terminal projections are indicated by progressively thinner lines and arrowheads. The right diagram (B) illustrates the M2 corticospinal projection in the brain injury experiments after motor recovery of dexterous upper extremity movements. The lesion is located on the dorsal view of the hemisphere (blackened area) and involved the arm representation of the primary motor cortex (M1) and adjacent part of the lateral premotor cortex (LPMC). Extensive enhancement of the contralateral projection to lamina VII and IX

occurred following the lateral motor cortical injury but not in other contralateral or ipsilateral laminae.

Table 1

Description of the Experimental Parameters in each Case

Case	Sex	Age (yrs.)	Weight (kg)	Area injected	Tracer/injections	Total vol. (μl)	Injection Core vol. (mm ³)	Injection Halo vol. (mm ³)	Post-injection survival (days)	Lesion vol. gray matter (mm ³)	Lesion vol. white matter (mm ³)
Control											
SDM 41	male	4.6	4.8	M2 arm	FD/3	1.2	38.6	84.6	33	N/A	N/A
SDM 54	male	9.0	9.2	M2 arm	FD/3	1.2	33	67.7	33	N/A	N/A
SDM 62	female	3.25	3.2	M2 arm	FD/3	1.2	31.5	60.4	33	N/A	N/A
SDM 77	male	8.3	8.6	M2 arm	FD/3	1.2	14.6	51.6	33	N/A	N/A
6 Mo. Survival											
SDM 45	male	5.4	7.2	M2 arm	FD/3	0.9	25.2	57.9	33	212.6	23.0
SDM 70	male	7.7	8.1	M2 arm	FD/3	0.9	15.2	73.7	34	143.3	7.8
12 Mo. Survival											
SDM 48	female	7.7	5.3	M2 arm	FD/3	0.9	19.9	48.7	33	220.3	23.1
SDM 55	male	12.8	11.9	M2 arm	FD/3	0.9	17.8	37.2	34	207.6	20.5

Table 2

Contralateral Bouton Counts in each Control Case by Spinal Lamina (percentages of total label in parentheses). Values are rounded to the nearest whole number except if the value is 0.7 or less. Abbreviations: Lat, lateral; Med, medial, RMB = reticulated marginal border.

Case	Bouton #	I-III		IV		V		VI		RMB		VII		VIII	IX	X
		Med	Lat	Med	Lat	Med	Lat	Med	Lat	Med	Lat	Med	Lat			
SDM 41	64,075	0	21 (0.003)	0	0	0	627 (1)	2,006 (3)	5,766 (9)	5,515 (8)	18,050 (28)	21,811 (34)	2,507 (4)	7,521 (12)	251 (0.4)	
SDM 62	73,706	0	0	0	0	0	612 (1)	122 (0.2)	2,326 (3)	5,265 (7)	12,856 (17)	31,833 (43)	490 (0.6)	20,202 (27)	0	
SDM 54	85,130	0	12 (0.001)	0	294 (0.3)	0	784 (1)	588 (0.7)	5,485 (6)	5,779 (7)	21,255 (25)	26,936 (32)	9,011 (11)	14,986 (18)	0	
SDM 77	80,504	0	0	0	0	375 (0.5)	900 (1)	7,428 (9)	3,826 (5)	8,703 (11)	32,862 (41)	22,884 (28)	975 (1)	2,401 (3)	150 (0.2)	
Mean	75,853.8	0	8.3	0	73.5	94	731	2,536.0	4,350.8	6,316	21,255.8	25,866.0	3,246	11,277.5	100.3	

Table 3

Ipsilateral Bouton Counts in each Control Case by Spinal Lamina (percentages of total label in parentheses). Values are rounded to the nearest whole number except if the value is 0.7 or less.

Case	Bouton #	I-III		IV		V		VI		RMB		VII		VIII		IX		X	
		Med	Lat	Med	Lat	Med	Lat	Med	Lat	Med	Lat	Med	Lat	Med	Lat	Med	Lat	Med	Lat
SDM 41	4,012	0	0	0	0	0	0	0	0	0	0	2,257 (56)	501 (12)	1,254 (31)	0	0	0	0	0
SDM 62	7,836	0	0	0	0	0	0	0	0	0	0	5,265 (67)	734 (9)	1,837 (23)	0	0	0	0	0
SDM 54	53,187	0	0	0	0	0	0	0	0	0	0	27,524 (52)	2,547 (4)	23,116 (43)	0	0	0	0	0
SDM 77	5,552	0	0	0	0	0	0	0	0	0	0	2,101 (38)	225 (4)	3,076 (55)	0	150 (3)	0	0	0
Mean	17,646.8	0	0	0	0	0	0	0	0	0	0	9,286.8	1,001.8	7,320.8	0	37.5	0	0	0

Table 4

Contralateral Bouton Counts in each Control Case Within each Quadrant of Lamina IX (percentages of total label in parentheses). Values are rounded to the nearest whole number except if the value is 0.7 or less.

Case	Bouton #	DMED	DLAT	VMED	VLAT
SDM 41	7,521	4,763 (63)	2,758 (37)	0	0
SDM 62	20,202	10,285 (51)	6,367 (32)	2,693 (13)	857 (4)
SDM 54	14,986	7,640 (51)	4,604 (31)	2,351 (16)	391 (3)
SDM 77	2,401	2,101 (88)	300 (12)	0	0
Mean	11,277.5	6,197	3,507	1,261	312.0

Table 5

Contralateral Bouton Counts in each Lesion Case by Spinal Lamina (percentages of total label in parentheses). Values are rounded to the nearest whole number except if the value is 0.7 or less.

<u>Case</u>	<u>Bouton #</u>	<u>I-III</u>		<u>IV</u>		<u>V</u>		<u>VI</u>		<u>RMB</u>	<u>VII</u>		<u>VIII</u>	<u>IX</u>	<u>X</u>
		<u>Med</u>	<u>Lat</u>	<u>Med</u>	<u>Lat</u>	<u>Med</u>	<u>Lat</u>	<u>Med</u>	<u>Lat</u>		<u>Med</u>	<u>Lat</u>			
SDM 45	228,150	0	0	0	0	0	471 (0.2)	5,177 (2)	4,236 (2)	1,059 (0.5)	72,950 (32)	86,010 (38)	7,530 (3)	50,717 (22)	0
SDM 48	153,507	0	422 (0.3)	0	0	1,126 (0.7)	1,970 (1)	5,628 (4)	9,990 (6)	6,894 (4)	26,312 (17)	62,613 (41)	5,206 (3)	33,346 (22)	0
SDM 55	100,070	0	0	0	0	6 (0.0005)	1,001 (1)	3,695 (4)	5,696 (6)	3,233 (3)	24,631 (25)	39,410 (39)	2,078 (2)	20,320 (20)	0
SDM 70	204,694	7 (0.00004)	3,228 (2)	166 (0.008)	1,573 (0.8)	662 (0.3)	3,311 (2)	3,807 (2)	7,946 (4)	23,423 (11)	58,931 (29)	68,201 (33)	11,505 (6)	20,941 (10)	993 (0.5)

Table 6

Ipsilateral Bouton Counts in each Lesion Case by Spinal Lamina (percentages of total label in parentheses). Values are rounded to the nearest whole number except if the value is 0.7 or less.

Case	Bouton #	I-III		IV		V		VI		RMB		VII		VIII	IX	X
		Med	Lat	Med	Lat	Med	Lat	Med	Lat	Med	Lat	Med	Lat			
SDM 45	14,752	0	0	0	0	0	0	22 (0.1)	7 (0.004)	15 (0.009)		7,648 (52)	2,942 (20)	4,118 (28)	0	0
SDM 48	7,245	0	0	0	0	0	0	0	0	0		4,328 (60)	1,035 (14)	1,882 (26)	0	0
SDM 55	1,771	0	0	0	0	0	0	0	0	0		1,078 (61)	77 (4)	616 (35)	0	0
SDM 70	34,928	0	0	0	0	0	0	0	0	0		16,305 (47)	1,904 (5)	15,312 (44)	0	1,407 (4)

Table 7

Contralateral Bouton Counts in each Lesion Case Within each Quadrant of Lamina IX (percentages of total label in parentheses). Values are rounded to the nearest whole number except if the value is 0.7 or less.

<u>Case</u>	<u>Bouton #</u>	<u>DMED</u>	<u>DLAT</u>	<u>VMED</u>	<u>VLAT</u>
SDM 45	50,717	25,179 (50)	10,354 (20)	12,948 (26)	2,236 (4)
SDM 48	33,346	13,226 (40)	15,618 (47)	3,236 (10)	1,266 (4)
SDM 55	20,320	9,621 (47)	6,158 (30)	3,156 (16)	1,385 (7)
SDM 70	20,941	13,243 (63)	4,470 (21)	3,228 (15)	0
					0