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Frontal and Frontoparietal Injury Differentially affect the Ipsilateral Corticospinal Projection from the Non-lesioned Hemisphere in Monkey (*Macaca mulatta*)

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Abstract

Upper extremity hemiplegia is a common consequence of unilateral cortical stroke. Understanding the role of the unaffected cerebral hemisphere in the motor recovery process has been encouraged, in part, by the presence of ipsilateral corticospinal projections. We examined the neuroplastic response of the ipsilateral corticospinal projection (iCSP) from the contralesional primary motor cortex (cM1) hand/arm area to spinal levels C5-T1 after spontaneous long-term recovery from isolated frontal lobe injury and isolated frontoparietal injury. High-resolution tract tracing, stereological, and behavioral methodologies were applied. Recovery from frontal motor injury resulted in enhanced numbers of terminal labeled boutons in the iCSP from cM1 compared to controls. Increases occurred in lamina VIII and the adjacent ventral sectors of lamina VII, which are involved in axial/proximal limb sensorimotor processing. Larger frontal lobe lesions were associated with greater numbers of terminal boutons than smaller frontal lobe lesions. In contrast, frontoparietal injury blocked this response, as total bouton number was similar to controls, demonstrating that disruption of somatosensory input to one hemisphere has a suppressive effect on the iCSP from the non-lesioned hemisphere. However, compared to controls, elevated bouton numbers occurred in lamina VIII, at the expense of lamina VII bouton labeling. Lamina IX boutons were also elevated in two frontoparietal lesion cases with extensive cortical injury. Since lamina VIII and IX collectively harbor axial, proximal, and distal motoneurons, therapeutic

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Conflict of interest statement.

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intervention targeting the ipsilateral corticospinal linkage from cM1 may promote proximal, and possibly distal, upper limb motor recovery following frontal and frontoparietal injury.

GRAPHICAL ABSTRACT

Proliferation of the ipsilateral corticospinal projection from contralesional M1 was found in lamina VIII and surrounding subsectors of lamina VII following frontal motor injury (top), potentially supporting axial/proximal upper limb motor recovery. This overall response was blocked following frontoparietal injury, but the projection to lamina VIII remained elevated (bottom).

Keywords

Pyramidal Tract; Frontal Lobe; Parietal Lobe; Corticofugal; Neurosurgical Resection; Plasticity; Spinal Cord; Motor Recovery; Hand Movements

INTRODUCTION

Understanding the role of the non-lesioned, or contralesional primary motor cortex (cM1) in the recovery process of upper limb motor control has gained considerable attention in recent years. Although it is clear that each cerebral hemisphere has a dominant effect on movements performed by the contralateral upper extremity, numerous studies have shown that both hemispheres play a role in mediating unilateral upper extremity movements in healthy adults (Chen et al., 1997; Gerloff et al., 1998; Carey et al., 2006; Davare et al., 2007; Bradnam et al., 2010; Morishita et al., 2011; McCambridge et al., 2011; Uehara, 2011). This has led to the optimistic postulation that the intact hemisphere may play a supportive role in recovery of motor deficits that occur in the more affected limb. Favoring this notion are neuroimaging studies in stroke patients showing that adaptive, functionally relevant cortical reorganization occurs in the contralesional hemisphere following recovery of arm and hand motor function (e.g., Cary et al., 2002; Schaechter, 2002; Jang et al., 2004; Calautti et al., 2007; Riecker et al., 2010; Lotz et al., 2012; Young et al., 2014). Other interest stems from non-invasive brain stimulation (NBS) work showing that excitatory stimulation applied to the cM1 in poorly recovered patients with extensive pyramidal tract injury may facilitate the

motor recovery process (for review see Hoyer and Celnik, 2011; Bradnam et al., 2013; Plow et al., 2014). However, it is equally important to recognize that inhibitory stimulation applied over the cM1, in patients with significant recovery potential and considerable sparing of pyramidal tract output, may also represent a favorable mode of therapeutic intervention (i.e., Schimizu et al., 2002; Murase et al., 2004; for review see Hoyer and Celnik, 2011; Bradnam et al., 2013; Plow et al., 2014).

Another factor that has encouraged study of the non-lesioned hemisphere in motor recovery is the fact that the corticospinal projection (CSP) from the hand/arm region of M1 is bilateral (Kuypers, 1981; for review see Jankowska and Edgley 2006; Baker et al., 2015). Directly related are contemporary studies in non-human primates which have shown that the ipsilateral M1 CSP is weak, and the projection to lamina IX, which contains motoneurons, is extremely weak (Soteropoulos et al., 2011; Morecraft et al., 2013). This is supported by neurophysiological studies showing that M1 is predominately involved in mediating contralateral upper extremity movements, with considerably less influence on ipsilateral and bilateral movements (Tanji et al., 1988; Aizawa et al., 1990; Kermadi et al., 1998). Indeed, it has recently been shown that inducing small, but visibly evoked bilateral proximal limb responses from M1 with intracortical microstimulation (ICMS) require longer stimulus trains that probably activated monosynaptic and polysynaptic pathways (Montgomery et al., 2013). Furthermore, in terms of topography, the ipsilateral M1 CSP preferentially terminates within the dorsomedial (VII_{dm}) and ventromedial (VII_{vm}) subsectors of lamina VII, and to a much lesser extent lamina VIII (Morecraft et al., 2013). Thus, inhibiting this specific projection with NBS in patients with extensive injury to the opposite M1 may block a contributory role of the ipsilateral M1 CSP in the motor recovery process. In theory, this may prevent potential proliferation of the CSP into laminae VIII, which harbors long range propriospinal projection neurons and axial/proximal limb motoneurons. Likewise, this may inhibit migratory sprouting into motoneuron subsectors of lamina IX innervating distal limb muscles. From the perspective of designing effective neurorehabilitative interventions, it is of great importance to explore the compensatory responses of the ipsilateral CSP from the non-injured M1 following both mild injury to M1 in the opposite hemisphere, as well as after extensive injury to M1 and its surrounding cortex.

We previously reported that spontaneous recovery following lateral frontal motor injury (Category F2 lesion), results in an enhanced contralateral corticospinal projection response from spared supplementary motor cortex (M2, SMC or SMA-proper) residing in the lesioned hemisphere (McNeal et al., 2010). This favorable neuroplastic response was topographically specific, occurring only in contralateral lamina VII and the dorsal quadrants of lamina IX at spinal levels C5 to T1 which are critical upper extremity motor-related gray matter targets. In contrast, more recently we found that isolated lateral frontoparietal injury (Category F2P2 lesion) blocks this favorable corticospinal response, and results in deterioration of the M2 CSP in these identical spinal cord subsectors (Morecraft et al., 2015). Considering these opposing responses, it would be of significant interest to study the potential neuroplastic response of the ipsilateral corticospinal projection arising from the contralesional primary motor cortex (cM1) following isolated frontal motor injury, and after combined frontoparietal injury in the rhesus monkey model. Indeed, there are a number of important questions to address in the unaffected hemisphere. First, does isolated frontal

motor injury result in enhanced ipsilateral corticospinal projections originating from the non-lesioned hemisphere as it does for the M2 contralateral corticospinal projection originating from the lesioned hemisphere (McNeal et al., 2010)? Second, does the addition of parietal cortex injury to the frontal motor lesion further enhance the ipsilateral corticospinal projection from cM1 as might be predicted with increased lesion size, or does it have a suppressive, and possible degrading effect on the corticospinal projection system originating from the non-lesioned hemisphere, as we have found for the contralateral CSP originating from spared M2 in the frontoparietal lesioned hemisphere (Morecraft et al., 2015)? Since parietal lobe injury often occurs in the most common form of stroke, middle cerebral artery infarction (Rasmussen et al., 1992; Yoo et al., 1998), this information may have important implications for a large number of stroke patients. That is, are corticospinal projections originating in the non-injured hemisphere vulnerable due to the disruption of somatosensory processing in the opposite (lesioned) hemisphere or do they participate in recovery of hand motor function by enhancing terminal axon connections in the spinal cord.

Since non-invasive brain stimulation applications targeting the unaffected hemisphere are of considerable interest for promoting motor recovery in patients with unilateral cortical injury, and we recently demonstrated that opposing neuroplastic responses occur in the spared M2 CSP following different forms of peri-Rolandic injury, we investigated the effects of frontal versus frontoparietal cortical injury on the ipsilateral corticospinal projection from cM1 in 7 rhesus monkeys following long term (6 and 12 month) spontaneous recovery. The isolated frontal lobe lesion was gradually increased in size in different monkey experiments, as was the severity of the frontoparietal lesion, particularly with respect to the extent of damage to cortex lining the anterior and posterior banks of the central sulcus. Our results demonstrate that combined frontoparietal injury blocks an upregulated neuroplastic response in the cM1 iCSP that accompanies isolated frontal motor injury. However in both lesion scenarios, there are selective increases in terminal labeling in spinal gray matter subsectors harboring motoneurons. Thus, potential therapeutic interventions aimed to enhance the role of the non-lesioned hemisphere in the recovery process may contribute to improved motor function of the more affected upper limb.

MATERIALS AND METHODS

All experimental and behavioral protocols were approved by the University of South Dakota Institutional Animal Care and Use Committee, and conducted in accordance with United States Department of Agriculture (USDA), National Institutes of Health, and Society for Neuroscience guidelines for the ethical treatment of animals. Each monkey (*Macaca mulatta*) enrolled in this study was evaluated by a veterinarian and appraised to be healthy and free of neurological deficit. All monkeys were housed and cared for in a USDA and Association for Assessment and Accreditation of Laboratory Animal Care (AALAC) approved and inspected facility.

Study Design

To accomplish the aims of this investigation, the organization of the ipsilateral corticospinal projection (CSP) arising from M1 in the non-lesioned hemisphere was studied in laminae

VII, VIII and IX at spinal levels C5 to T1 in 3 control monkeys (Morecraft et al., 2013) and in 7 additional monkeys that received cortical lesions produced by coagulation/subpial aspiration (Table 1). The lesion experiments included 3 animals with frontal lobe lesions, each with progressively increased lesion size and corresponding volume (Figs. 1, 2; Table 2), and 4 animals with combined lateral frontal lobe and parietal lobe lesions with differing degrees of damage to cortex lining the depths of the central sulcus (Figs. 3, 4; Table 2). Of the isolated frontal lobe lesion cases, one animal received a lesion of the hand/arm region of the M1 (Category F1 lesion, SDM38), the second received a lesion of hand/arm regions of M1 and adjacent lateral premotor cortex (Category F2 lesion, SDM48) and the third a lesion of the hand/arm regions of M1, the adjacent lateral premotor cortex and the supplementary motor cortex (F3 lesion, SDM50) (Fig. 1). The 4 frontoparietal (Category F2P2) lesion cases involved isolated neurosurgical removal of the hand/arm representation of M1, and adjacent lateral premotor cortex (LPMC), hand/arm region of the somatosensory cortex (S1 or areas 3, 1 and 2) including the rostral most part of area PE (or area 5) of the superior parietal lobule (Fig. 3; see also Morecraft et al., 2015). We intentionally spared the major extent of the posterior parietal lobe (areas PF, PFG, PG and PE: architectonic divisions according to Pandya and Seltzer, 1982) to minimize potential higher-order processing deficits (e.g., visuospatial neglect) from affecting the motor recovery process in our Category F2P2 lesion cases.

Cortical Lesion Method—Detailed descriptions of the surgical preparation, anesthetic procedures, aseptic neurosurgical exposure, monitoring of vital signs, and ICMS method have been previously published (McNeal et al., 2010; Morecraft et al., 2007a; 2013, 2015). Briefly, the preferred hand was identified and the animal was successfully trained on two fine motor behavioral tasks (described below). Then, each animal was anesthetized with isoflurane, placed in a surgical head holder and the cortex was exposed. The animal was transitioned to ketamine anesthetization and the boundaries of the respective hand/arm regions of cortex (opposite of the preferred hand) were identified using ICMS. The animal was transitioned back to isoflurane and the surface vessels supplying or draining the area to be resected were coagulated. Five to 10 minutes after coagulation, the hand/arm part of the cortex was carefully removed by subpial aspiration. This was accomplished using either a pulled glass pipette, or angled Frazier suction tube that was attached to a vacuum aspirator (Schuco-Vac Model 130) by a non-conductive connecting tube. Care was taken to avoid injury to the subcortical white matter. Procedures for closing the dura, bone flap and skin followed those previously described (McNeal et al., 2010; Morecraft et al., 2007a; 2013, 2015). The monkeys survived the lesion for either 6 or 12 months. However, at 33–34 days prior to the fixative perfusion, a second surgery was performed and the injection of tract tracer was made into the hand/arm region of M1 (described below).

Tract Tracing Procedures—With each animal under isofluorane anesthesia, a skin flap was made over the cranium followed by a bone flap over the peri-Rolandic cortical region (McNeal et al., 2010; Morecraft et al., 2013). In both the control and lesion cases the location of the arm representation of M1 was determined by ICMS (Figs. 1, 3) (McNeal et al., 2010; Morecraft et al., 2013). Next, the anterograde neural tract tracer lucifer yellow dextran (LYD) or biotinylated dextran amine (BDA) (Molecular Probes, Eugene, OR) was

injected into the central region of the physiologically localized arm representation of M1 (Figs., 1, 3). Graded pressure injections with a Hamilton microsyringe were made into three separate penetration sites spaced 1–1.5mm apart in a triangular pattern, 3–4 mm below the pial surfaces. In the control cases, 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 were performed in identical fashion with the exception that slightly less tracer volume (total of 0.9 μ l; 0.3 μ l per penetration site) was injected in 6 of the lesion cases and a volume that was equal to the controls (1.2 μ l) was injected in one lesion case (SDM91) (Table 1). The smaller injection volume of LYD was made to insure that any enhanced neuroplastic changes in the lesion animals were not due to larger tracer injections (McNeal et al., 2010). The craniotomy was closed as described previously (Morecraft et al., 2007a,b).

Tissue Processing and Immunohistochemical Procedures—Following the survival period 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 followed by 4% paraformaldehyde and sucrose (McNeal et al., 2010; Morecraft et al., 2013). The cortex and spinal cord were frozen sectioned at 50 μ m in thickness, and the tissue sections were collected and stored as previously described (Morecraft et al., 2013, 2015). One series of cortical and spinal cord tissue sections was stained for Nissl substance for cytoarchitectural analysis using thionin (Morecraft et al., 1992, 2004, 2012, 2013). Additional series of tissue sections were processed using single and double label immunohistochemical procedures for visualization of the injected neural tracers (LYD and BDA) in cortical and spinal cord tissue sections (Figs. 2, 4) as previously described (Morecraft et al., 2007a, 2013, 2014, 2015).

Anatomical Nomenclature and Terminology—For the present report, the distribution of terminal labeling in the spinal cord was evaluated in lamina VII, VIII and IX, where lamina VII was further subdivided into 5 anatomically affiliated subsectors, and lamina IX was subdivided into anatomical quadrants as described previously (Morecraft et al., 2013-see Materials and Methods, Definition of Anatomical Terminology). With respect to the brain injury cases, we utilized the same nomenclature for classifying our frontal motor lesion cases (Category F1 lesion, Category F2 lesion and Category F3 lesion) and frontoparietal lesion site cases (Category F2P2 lesion) as used in our previous publications (Pizzimenti et al., 2007; Nagamoto-Combs et al., 2007, 2010; McNeal et al., 2010; Darling et al., 2009, 2010, 2011, 2013; Morecraft et al., 2015). This terminology is based upon the removal of general anatomical and physiologically defined sectors of frontal and parietal cortex. This classification scheme should not be confused with the nomenclature of the frontal motor areas of Matelli and colleagues (Matelli et al., 1985), which utilize the cytochrome oxidase staining procedure to subdivide the caudal frontal lobe into anatomical subsectors (area F1, area F2, area F3 etc.), nor the anatomical nomenclature of Bonin and Bailey (1947), which subdivide the frontal lobe into anatomical areas based upon the Nissl staining method (area FA, area FB, area FC, etc.).

Stereological Analysis—Terminal boutons of the ipsilateral CSP from M1 were estimated in Rexed's lamina at spinal levels C5 to T1 (Figs., 2, 4; Table 3) using brightfield

microscopy and stereological counting methods (Glaser et al., 2007; West, 2012). Definitions of general anatomical terminology adopted for this study have been described previously (McNeal et al., 2010; Morecraft et al., 2013). The methods used to calculate unbiased estimated bouton numbers at specific spinal levels within spinal cord laminae have been provided in detail in our previous papers (McNeal et al., 2010; Morecraft et al., 2007b, 2013, 2015). Estimates of the total number of terminal boutons within Rexed's laminae (and subdivisions) were determined using the Optical Fractionator Probe and our Neurolucida equipped microscope workstations (Microbrightfield, Colchester, VT, USA) in the identical fashion as previously reported (McNeal et al., 2010; Morecraft et al., 2013, 2015). Every other spinal cord tissue section was used for stereological evaluation. Estimates of the tracer injection site volumes (which included the core volume) and the extirpated lesion site volumes were also calculated (Tables 1 and 2). Specifically, injection site volumes were determined using the Cavalieri probe and same StereoInvestigator software as described in our prior work (Morecraft et al., 2013, 2015). The same stereological probe and method was used to determine the lesion site volume (gray and white matter) (Pizzimenti et al., 2007, Darling et al., 2009).

Statistical Analysis of Neuroanatomical Data—Statistical analyses were performed to determine if significant differences occurred in bouton numbers between the control and lesion animals. Specifically, mixed 2-way repeated measures ANOVAs were used to compare these dependent variables in control versus lesioned animals across the spinal laminae (VII, VIII, IX, for spinal segments C5-T1 – group $\times$ laminae ANOVA) and for total number of boutons distributed among spinal segments C5-T1 (group $\times$ segments ANOVA). We also used a mixed 2-way repeated measures ANOVA to compare bouton numbers within quadrants of lamina IX for segments C5-T1. Mauchly's test was used to determine whether the assumption of sphericity was met when there were three or more levels in a repeated-measures factor (i.e., laminae, segments). 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).

Neuroanatomical Data Reconstruction and Presentation—Publication quality images of injection sites and labeled corticospinal nerve fibers and terminals were captured using a Spotflex 64 Mp 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). Only brightness and contrast were adjusted to maximize discrimination and normalize images for comparative purposes. Cortical surface reconstructions and ICMS maps were developed as previously described using metrically calibrated digital images of the cortical surface (Morecraft and Van Hoesen 1992, Morecraft et al., 2013). Density plots of terminal boutons were made using our Neurolucida microscope workstation to illustrate the topographical distribution of the iCSP from M1 in the controls and lesion cases (Fig. 5). Publication quality line illustrations were created using Adobe Illustrator 10.0 (Adobe Systems Inc., San Jose, CA, USA).

Movement Analysis Procedures

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 (McNeal et al., 2010; Darling et al. 2009). 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).

After reaching stable levels of motor performance on each task, each monkey was lesioned (hemisphere opposite the preferred hand) 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 (Darling et al., 2009, 2011; Morecraft et al., 2015). 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 on the lesioned animals during the brief manual testing sessions.

Behavioral Procedures—Prior to an experimental session the monkey was food restricted for 12–24 hours. Initial training sessions used a “standard” rectangular dexterity board to assess the preferred hand of each monkey as described previously (Nudo et al., 1992; Darling et al. 2009). 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 (Pizzimenti 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.

Pre-lesion data were collected every 1–3 weeks for a total of 18–28 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 predetermined cortical areas. 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 as described previously to identify the first touch of the carrot chip or plate/rod supporting the carrot chip to the end of force application on each trial (Darling et al., 2006). The duration and total applied 3-dimensional absolute impulse were computed for each trial and, along with trial outcome used to compute a performance score. Performance scores were computed and normalized to individual monkey's abilities (i.e., maximum and minimum applied impulse and duration) for each trial at each difficulty level (McNeal et al. 2010).

mDB Data Analysis—Duration of reaching and manipulation, number of manipulation attempts 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). 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) and used to compute the performance scores (McNeal et al., 2010).

Analysis of Hand Motor Skill—We quantitatively assessed motor skill by computing mean divided by the standard deviation of manipulation performance scores over 5 consecutive testing sessions (i.e., 25 trials over an approximately 5 week period) (Pizzimenti et al. 2007; McNeal et al. 2010). 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 (best well - with highest pre-lesion skill for each monkey and a 2nd smaller well with lower pre-lesion skill). Skill was computed for the best 5 consecutive and 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 monkey's strategy changed post-lesion to use additional digits or different digits to perform the task.

Statistical Analysis of Motor Performance—We evaluated the duration (in weeks) from the time of the lesion until the first testing session with an attempt, successful acquisition and 5 successful acquisitions on the mMAP (flat surface task) and mDB (any well) 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 on the best well (well with highest pre-lesion skill) and 2nd smaller well (with pre-lesion skill of about 50% of that on the best well) of the mDB task and on the curved rod task of the mMAP. 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 for the cM1 CSP. Specifically, we considered the number of cM1 CSP boutons in laminae VII, VIII and IX in lesioned monkeys. We previously showed that the increased number of ipsilesional supplementary motor cortex (iM2) CSP boutons were highly correlated with recovery of skill in monkeys with lesions of M1 and lateral premotor cortex (LPMC) (McNeal et al. 2010). More recently, we have shown that the remaining iM2

CSP boutons after M1, LPMC and anterior parietal lobe lesions were also highly correlated with recovery of skill (Morecraft et al. 2015).

RESULTS

In all 7 lesion cases, the LYD injection site in the non-lesioned hemisphere effectively involved M1 lining the anterior bank of the central sulcus (caudal M1 according to Rathelot and Strick 2009), as well as part of M1 residing on the gyral convexity (rostral M1 according to Rathelot and Strick, 2009) (Figs. 1–4). Based on the cytoarchitecture of the region (Preuss and Goldman-Rakic, 1991; Morecraft et al., 2012), this cortex corresponded to area 4. In both regions of M1, dense LYD precipitate was found to thoroughly involve layer V, which harbors the cells of origin for the corticospinal projection (Kuypers, 1981). In all cases the LYD injection site did not involve adjacent primary somatosensory cortex (S1: cytoarchitectonic areas 3, 1, and 2), which lines the posterior bank of the central sulcus (Figs. 2, 4). Axon labeling in the corona radiata, internal capsule, midbrain cerebral peduncle and lateral corticospinal tract were examined in all cases and showed extensive numbers of LYD labeled axons throughout the entire descending course of the M1 corticofugal projection. This supported the conclusion that the corticospinal tract in all cases did not suffer unintended injury at any level of the CNS and that terminal labeling was reflective of a true and effective axon transport environment. The injection site analysis, and ipsilateral corticospinal terminal labeling for our 3 control cases have been previously described in detail (Morecraft et al., 2013). Briefly, the ipsilateral CSP from M1 in our control cases preferentially targeted the dorsomedial (VII_{dm}) and ventromedial (VII_{vm}) sectors of lamina VII (Figs. 5, 6A C, Table 3; for the control cases also see Morecraft et al., 2013, Figs. 9B, 10C). In the frontal motor lesion cases, our initial microscopic analysis revealed that notable ipsilateral terminal bouton labeling occurred in lamina VII and VIII, and in the F2P2 cases lamina VIII and IX (Figs. 5, 6A,B). Dorsal horn terminal labeling was rarely found in all lesion cases. Thus, in all lesion cases stereological evaluation was conducted on the ipsilateral CSP in lamina VII, VIII, and IX and compared to controls (described below).

The histological analysis and total lesion volumes for frontal lobe lesion cases SDM38, SDM48 and SDM50 have been previously reported (Pizzimenti et al., 2007; Darling et al., 2010; McNeal et al., 2010). For the present study, we subdivided the total lesion volume of these 3 cases into caudal (“new”) M1, and rostral (“old”) M1 (according to Rathelot and Strick, 2009) and separately calculated the lesion volume for lateral premotor cortex (LPMCD), ventral lateral premotor cortex (LPMCV) and supplementary motor cortex (M2) when appropriate (Fig. 2; Table 2). Additionally, in the present report the amount of spared cortex for rostral M1 and caudal M1 was determined (Table 2). Finally, the volumetric lesion site information for all F2P2 cases can be found in Table 2, and detailed histological descriptions of each Category F2P2 lesion case can be found in our recent report (Morecraft et al., 2015). It should also be noted that we have previously reported in detail on the specific behavioral/motor recovery effects of all the frontal lobe lesion cases (Darling et al., 2009). The general postoperative observations of motor and somatosensory deficits that occurred in the 4 frontoparietal lesion cases have also been reported, including development of learned nonuse in one frontoparietal lesion case (Morecraft et al., 2015). More detailed

motor recovery analyses of the Category F2P2 lesion cases will be presented in future reports related to effects of lesion volume.

Statistical comparisons of the stereological findings of the cM1 ipsilateral corticospinal projection from the frontal lesion group, frontoparietal lesion group and the control group showed that the frontal lesion induced enhanced bouton numbers in the ipsilateral CSP compared to controls, but there was no difference between the frontoparietal lesion CSP and control CSP. Specifically, comparisons of total labeled boutons in spinal lamina VII, VIII, IX showed a significant difference for group main effect (Fig. 6A, $F_{2,7} = 26.0$, $p < .001$). Post hoc tests showed that monkeys with frontal lobe lesions had more labelled boutons than controls and monkeys with Category F2P2 lesions ($p = 0.002$). However, similar numbers of boutons were observed in both controls and monkeys with F2P2 lesions ($p = 0.99$). We also found differences in distribution of labelled boutons among laminae VII, VIII and IX in the different groups (Fig. 6A, group $\times$ laminae interaction effect - $F_{4,14} = 33.24$, $p < 0.001$). Specifically, lamina VII had the most labelled boutons and lamina IX had the fewest in controls and monkeys with frontal lesions whereas in monkeys with F2P2 lesions, lamina VIII had the most labelled boutons and lamina IX had the fewest. Post-hoc tests showed that the frontal lesion group had more labeled boutons in lamina VII than F2P2 lesion and control groups ($p < .05$). However, there were more labelled boutons in lamina VIII in the frontal lesion and F2P2 lesion groups than controls (respectively: $p < 0.001$, $p < 0.05$, Fig. 6A). For comparison of lamina IX quadrants, there were no significant differences among the groups ($p = 0.477$), no differences among quadrants ($p = 0.29$), and no group $\times$ quadrants interaction ($p = 0.766$). Although not statistically significant, in two F2P2 lesioned animals that had severe lateral cortical damage (SDM87, SDM91) (Table 2), there were elevated numbers of labelled boutons in lamina IX compared to controls (Fig. 6A, B). The distribution of labelled boutons among the 5 subsectors of lamina VII also differed (Fig. 6C, $F_{4,28} = 5.31$, $p = 0.03$) and varied among the 3 groups (Fig. 6C, group $\times$ subsectors interaction effect - $F_{8,28} = 2.89$, $p = 0.08$). Post-hoc tests comparing lamina VII subsectors demonstrated that the ventromedial sector had more labelled boutons than the dorsolateral ($p = 0.005$) and ventrolateral sectors ($p = 0.06$). Post-hoc tests for the group $\times$ subsectors interaction effect revealed that the ventromedial subsectors of the frontal lesion monkeys had more labelled boutons than F2P2 lesion monkeys ($p = .005$) and controls ($p = 0.06$). Monkeys with frontal lesions also had more labelled boutons in the ventral subsector of lamina VII than in controls ($p = 0.03$) and F2P2 lesion monkeys ($p = 0.09$).

We also assessed the segmental distribution of labeled boutons and found that on average there is a progressive decrease of boutons from C5 to T1 but there was considerable inter-subject variation (Fig. 6D). Specifically, we observed that there were differences in numbers of boutons among segments ($F_{2,7} = 6.7$, $p = 0.004$) as C5 had more labelled boutons than C8 and T1 ($p < 0.05$) and C6 had more labelled boutons than T1 ($p < 0.05$). However, there was no statistical evidence of a different segmental distribution among the 3 groups (group $\times$ segment interaction: $F_{8,28} = 1.04$, $p = 0.430$), although the average segmental distribution in F2P2 lesion cases appeared to differ from the distributions in controls and frontal lesion cases (Fig. 6D). Only one control, SDM62, was found to demonstrate labeling in lamina IX (Fig. 6B). With respect to the segmental distribution, labeling occurred in the dorsomedial quadrant at C5 and C7, as well as the ventromedial and ventrolateral quadrants at C7. In

contrast, all but one (i.e., SDM83) of the lesion cases demonstrated terminal labeling in lamina IX (Fig. 6B). In terms of the segmental distribution of lamina IX labeling in frontal lesion cases, labeling occurred in dorsolateral quadrant at T1 in case SDM38. In case SDM50, the ventromedial quadrant contained terminal boutons at C6 and C7, and in case SDM45 the same quadrant had terminal labeling at C7. With respect to the frontoparietal lesion cases, terminal labeling was found in the ventromedial quadrant at C5 in case SDM81, C5 and C8 in cases SDM87 and SDM91, as well as spinal level C7 in case SDM91 (Fig. 5). Finally, terminal bouton labeling was also found in the dorsomedial sector of C5 in case SDM87.

The estimated number of labelled boutons in lamina VIII increased with the number of frontal lobe motor areas lesioned in 3 monkeys (i.e., Fig. 6A – M1 lesion in SDM38; M1 + LPMC lesion in SDM48; M1 + LPMC + M2 lesion in SDM50). Similarly, among the 4 F2P2 lesioned monkeys the volume of the lesion increased progressively from SDM81 through SDM91 (Table 2) as did the estimated number of labelled boutons in lamina VIII (Fig. 6A, 7). Indeed, in both frontal and frontoparietal lesioned monkeys the estimated number of cM1 iCSP boutons in lamina VIII increased with total lesion volume (gray matter and white matter) but the increase with total lesion volume was clearly less in frontoparietal lesioned monkeys than in frontal lobe lesioned monkeys (Fig. 7, note lower slope for F2P2 lesions). Regression analysis confirmed the positive relationships for lamina VIII labelled boutons with lesion volume in monkeys with frontal lobe lesions (Fig. 7, $R^2 = 0.93$, $p < 0.05$) and frontoparietal lesions (Fig. 7, $R^2 = 0.82$, $p < 0.05$). Notably, the number of estimated boutons was also positively correlated with the estimated relative lesion volume to M1c (i.e., as a percentage of the entire M1c arm/hand volume) in Category F2P2 lesioned cases but not in frontal lobe lesion cases, which all had small relative M1c lesion volumes (Fig. 8A). This association was primarily due to effects on lamina VIII (Fig. 8C) as there was only a small increase in number of estimated boutons with relative M1c lesion volume in lamina VII (Fig. 8B).

Recovery of contralesional hand motor function was inversely related to estimated number of boutons of the cM1 ipsilateral CSP in monkeys with Category F2P2 lesions relative to controls. Specifically, there were high positive correlations of post-lesion duration until first attempt in the mDB task with the ratio of number of ipsilateral cM1 CSP boutons in laminae VII and IX to number ipsilateral cM1 CSP boutons in controls, indicating that higher numbers of boutons in F2P2 lesioned monkeys were related to slower onset of recovery (Fig. 9). Similarly, recovery of reaching skill in the mDB task and of manipulation skill in the mMAP curved rod task were inversely correlated with the ratio of ipsilateral cM1 CSP boutons in laminae VII, VIII and/or IX to controls (Fig. 10). We did not examine potential relationships of cM1 ipsilateral CSP boutons in frontal lobe lesioned monkeys because of the large variations in lesion size and lesioned motor areas. However, all those monkeys recovered contralesional hand motor function quite well as reported previously (Darling et al. 2009) and the increasing number of boutons with increasing frontal lesion size (Figs. 6A, 7) is consistent with a positive neuroplastic response in the cM1 ipsilateral CSP. Notably, there were positive correlations of numbers of boutons in laminae VII and VIII with volume of lesions to caudal M1 (M1c) in the F2P2 lesioned monkeys (Fig. 8A,C). This suggests that

larger lesions of the anterior bank of the central sulcus were associated with maintenance of greater numbers of ipsilateral CSP terminals from cM1 in frontoparietal lesioned monkeys.

DISCUSSION

Unilateral injury to the lateral surface of the cerebral hemisphere is one of the most common consequences of stroke. In addition to parts of the affected cortex that escape injury, it is possible that the unaffected hemisphere has great potential to be involved in beneficial aspects of the motor recovery process. In the present study, we have discovered that isolated lesions involving the frontal motor cortex result in an enhanced ipsilateral corticospinal projection (iCSP) from the contralesional primary motor cortex (cM1) at spinal segmental levels C5 to T1 following spontaneous long-term recovery (Figs. 6A, 11). Terminal proliferation of the iCSP increased, as the number of motor areas involved in the frontal lobe lesion increased. Furthermore, versus controls, we found that the terminal distribution shifted significantly, to occupy a more ventral location following frontal motor injury. That is, from laminae VII_{dm} and VII_{vm} in the control cases, to laminae VII_{vm}, VII_v and VIII in the frontal motor lesion cases. This finding is promising, and demonstrates that favorable, upregulated changes in terminal density can occur in parts of the spinal gray matter that have a small number of axon terminals in the non-injured state.

In contrast, this response was blocked following combined lateral frontal motor cortex and lateral parietal somatosensory cortex injury, as total corticospinal bouton number was found to be similar to control levels (Figs. 6A, 12). Specifically, in the frontoparietal experimental cases there was significant deterioration of bouton number in lamina VII that was counter balanced by evidence of elevated numbers of boutons in laminae VIII. In two cases with severe frontoparietal injury, notable increases of terminal boutons were also found in lamina IX (Fig. 6B). These findings show that disruption of somatosensory input to one hemisphere has a suppressive effect on the overall neuroplastic response from the iCSP from M1 in the non-lesioned hemisphere, but the effects on the iCSP to motoneurons (laminae VIII and IX) differs from the iCSP to lamina VII. Since a large number of stroke patients have unilateral frontoparietal injury, our findings indicate that the integrity of intact corticospinal projections originating in the unaffected hemisphere may be at risk, if not considered in the rehabilitation planning, and implementation processes.

Topographical Distribution of the CSP Response and Functional Interpretation

In all of our isolated frontal lobe lesion cases, we found a highly specific iCSP neuroplastic response that was restricted to lamina VIII and the adjacent parts of lamina VII, which we designate as the ventromedial sector of lamina VII (VII_{vm}), and the ventral sector of lamina VII (VII_v) (Morecraft et al., 2013, see Fig. 4B). These two divisions of laminae VII effectively surround lamina VIII, and collectively form a critical part of Kuypers “medial motor system” concept (Kuypers, 1982; for review see Lemon, 2008; see also Morecraft et al., 2013, Fig. 4B). From a functional perspective, the “medial motor system” is related to central nervous system governance of proximal limb (i.e., shoulder) and axial movements whereas in contrast, the “lateral motor system” is related to the steering of distal limb movements. There are numerous anatomical and behavioral building blocks supporting

Kuypers medial and lateral motor system concepts that extend far beyond the current discussion, but for the M1 ipsilateral CSP studied here, highlighting the fundamental spinal cord organization of the medial motor system seems quite appropriate, as it provides important insight as to what parts of the upper limb are likely to be affected by the topographically specific patterns of labeled terminals found in the present study. Central to the medial motor system concept are neural circuits that are organized to preferentially control proximal limb and axial musculature.

From the perspective of the precentral motor cortex, electrophysiological stimulation of the shoulder region of M1 gives rise to shoulder and trunk movements (e.g., Woolsey et al., 1952; Gould et al., 1986; Huntley and Jones, 1991; Godschalk., 1995; Montgomery et al., 2013). The corticospinal projection from this part of M1 preferentially innervates lamina VIII and surrounding parts of lamina VII (Kuypers and Brinkman, 1970) that correspond to spinal sectors VII_{vm} and VII_v. This topographically specific corticospinal projection is also bilateral, underscoring the functional role of this neural system, and the axial/proximal musculature in providing trunk and shoulder girdle stabilization necessary for accurate upper limb movements. At the spinal cord level, lamina VIII contains motoneurons that innervate axial muscles (Sprague, 1948; Holstege 1988). It is therefore reasonable to conclude that cortical input (as well as subcortical or propriospinal input) to lamina VIII specifically affects axial/proximal limb movements, especially at spinal levels C5-C7. Spinal subsectors VII_{vm} and VII_v are also important components of the medial motor system largely due to the intraspinal connectional architecture that characterize the propriospinal neurons occupying these intermediate zone sectors (Sterling and Kuypers, 1968; Molenaar et al., 1974; Molenaar, 1978; Molenaar and Kuypers, 1978; Matsushita et al., 1979; for review see Kuypers, 1982). The majority of propriospinal neurons that reside in sublamina VII_{vm} and VII_v give rise to axons that travel long distances throughout the spinal cord. Along this trajectory these axons give rise to collateral branches and terminals that reenter the spinal gray to end largely in lamina VIII, VII_{vm} and VII_v. In addition to innervating an extensive number of spinal segments, these long projections are bilateral, which fits well with the concept of mediating axial/postural functions. It is important to note that lamina VIII, in addition to harboring proximal limb and axial motoneurons also harbor propriospinal neurons with the same long distance and bilateral connectional properties.

Considering the above, upregulation of the iCSP from cM1 in laminae VIII, VII_{vm} and VII_v that we found in all 3 frontal motor lesion cases most likely represents an effort to assist in the recovery of proximal limb and axial movements in the severely affected upper extremity (Fig. 11). The segmental distribution of the cM1 ipsilateral CSP was larger in terms of number of labeled boutons at C5 and C6 than at C8 and T1 (Fig. 6D), which is also consistent with this deduction, as well as with Kuypers' medial motor system concept as the motoneuron pools controlling the pectoral girdle and proximal limb muscles are primarily located at C5 – C7 (Reed 1940; Jenny and Inukai 1983). Notably, this topographically specific projection was found to increase as the number of frontal motor areas involved in the lesion increased. Our findings of an emphasis on favorable proximal limb reorganization corroborate with the observations of Zaaïmi and colleagues (Zaaïmi et al, 2012), who found in monkeys with long-term unilateral pyramidal tract lesions little to no evidence for enhanced ipsilateral input to hand and forearm motoneurons. From a clinical perspective,

our findings and the interpretation of these results parallel previously reported TMS observations showing that stimulation of the unaffected M1 in stroke patients gives rise to proximal limb movements in the more affected upper limb (Turton et al., 1996; Netz et al., 1997; Alagona et al., 2001; Misawa et al., 2008; Schwerin et al. 2008).

In our frontoparietal lesion cases we found the topographical distribution of ipsilateral corticospinal terminals to be very similar to the frontal lesion cases (i.e., shifting from control locations of VII_{dm} and VII_{vm}, to preferentially targeting laminae VIII, VII_{vm} and VII_v) but total numbers of terminal boutons was similar to controls. Despite this blocked response, in 3 of the 4 monkey cases we found considerably higher numbers of boutons in ipsilateral lamina VIII, providing structural evidence for a neuroplastic response supporting recovery of axial/proximal limb movement in the more affected arm. In addition, as mentioned above, in 2 severely injured F2P2 lesion cases with extensive lateral cortical damage (Table 2: SDM87 and SDM91), enhanced terminal bouton labeling occurred in the ventromedial sector of lamina IX at spinal levels C7 and C8 (Fig. 6A, B; Table 3), suggesting a potential contribution to distal-related motor recovery following frontoparietal injury. Importantly, the ventromedial region of lamina IX contains proximal limb motoneurons (Reed, 1940; Sterling and Kuypers, 1967) and at spinal level C8 harbors triceps motoneurons (Jenny and Inukai 1982) and motoneurons of other distal (i.e., elbow, wrist and hand) extensors (Chicken et al., 2001). Unfortunately, in the latter study it is unclear which additional extensor muscles are represented in the ventromedial region of lamina IX because the injections of retrograde tract tracer were made directly into the cut end of the radial nerve just above the elbow joint level rather than into individual muscles. It is also important to note that Chicken and co-workers (2001) observed dendrites of flexor-related motoneurons (identified by injections into the cut ends of the medial and ulnar nerves just above the elbow) intermingled with the dendrites of extensor motoneurons. Thus, corticospinal terminals ending in this sector of lamina IX may also contact the dendrites of flexor-related motoneurons, in addition to somas and dendrites of extensor-related motoneurons. It is also possible that these increases may be maladaptive following frontoparietal injury and do not assist with recovery of upper limb motor function. For example, such plasticity may contribute to enhancing the extensor synergy (Schwerin et al. 2008) and may partially explain the negative correlations observed between numbers of cM1 iCSP boutons and recovery of reaching and manipulation (Fig. 10). Another potential maladaptive consequence of the Category F2P2 lesion scenario is the significant decrease in terminal boutons in lamina VII that was observed (Fig. 6A, C), since recent non-human primate studies show that C5-T1 spinal interneurons are involved in movement preparation (Fetz et al., 2002), and modulate the control of finger movements and hand grasping (Riddle and Baket et al., 2010; Takei and Seki, 2010, 2013). It will be important to determine in F2P2 lesioned monkeys if forced-use therapy can reverse the deterioration of the CSP to lamina VII, as well as significantly enhance the projection to lamina IX and correlate with positive gains in motor recovery, or require some form of multimodal therapeutic intervention in conjunction with forced-use.

Clinical Implications

Non-invasive Brain Stimulation—Exploring the role of the unaffected hemisphere in the motor recovery process following unilateral cortical injury is of significant interest given the multitude of therapeutic options currently available. Further underscoring this need is the idea that treatment interventions for individual patients that take into account the unaffected hemisphere may eventually be tailored according to the magnitude of injury occurring in the lesioned hemisphere. To elaborate, it appears as if severity of motor dysfunction, and the degree of residual integrity of corticofugal projections from M1 in the lesioned hemisphere, may be critical factors to consider when designing therapeutic interventions with non-invasive brain stimulation (NBS) (for review see Hoyer and Celnik, 2011; Bradnam et al., 2013; Plow et al., 2014; Stinear and Byblow, 2014). In patients with considerable subtotal sparing of pyramidal tract output and promising recovery potential, NBS that *inhibits* the callosal projection from cM1 may represent a favorable mode of therapeutic intervention. Such stimulation may attenuate trans-callosal inhibitory input from the non-lesioned hemisphere to spared pyramidal tract components in the injured hemisphere, thereby promoting favorable contralateral neuroplastic reinnervation of partially deprived subcortical motor-related targets. However, our results of an enhanced iCSP to laminae VIII, VII_{vm} and VII_v suggest that inhibition on the non-lesioned hemisphere in patients that have subtotal injury limited to the frontal motor cortex may also impede a potentially favorable neuroplastic response to support recovery of axial/proximal movement in the more affected limb, and possibly terminal sprouting into lamina IX.

In contrast to this scenario, contralesional hemispheric activity appears to be enhanced in poorly recovered patients that have extensive pyramidal tract damage in the lesioned hemisphere (e.g., Johansen-Berg et al., 2002; Ward et al., 2007; Lotze et al., 2006). This indicates that the contralesional cortex in this patient cohort may be actively involved in the motor recovery effort. Therefore, inhibiting the non-lesioned M1 in patients with extensive damage to ipsilesional M1 may block favorable compensatory neuroplastic responses from descending projections originating from cM1, and possibly descending projections from other contralesional motor areas. It is clear from our frontoparietal injury cases that the overall enhanced neuroplastic response that accompanies isolated frontal lobe injury was prevented. However, in all F2P2 lesion cases, we found evidence of enhanced terminal bouton numbers in lamina VIII, and in 2 of these cases with severe lateral frontoparietal injury (SDM87 and SDM91) we found evidence of increased terminal labeling in lamina IX compared to controls (Fig. 6A,B). In fact, the hand/arm area of M1 in SDM91 was completely removed (Table 2), effectively abolishing all M1 corticospinal tract output to spinal motor areas controlling the contralateral upper limb. Thus, NBS inhibition of the iCSP from cM1 in the unaffected hemisphere in patients with severe motor cortex injury may inhibit favorable axon terminal sprouting in axial/proximal related motoneuron pools (i.e., lamina VIII). Such NBS may also inhibit possible sprouting in distal-related motoneuron pools (i.e., in lamina IX) that may support recovery of elbow, wrist and hand movement.

Bilateral Atrophy of the CSP following Unilateral Parietal Injury—The devastating effects of anterior parietal lobe injury on distal upper extremity movements in non-human

primates has long been known (Kennard and Kessler, 1940; Denny-Brown, 1950; for review see Lassek, 1954), and the potential trophic influence of the parietal lobe on the maintenance of distal extremity integrity, and in particular the hand and forearm, has long been suspected (Guthrie, 1918). In our accompanying study demonstrating the negative effects of an F2P2 injury on the contralateral CSP from spared supplementary motor cortex (M2) in the lesioned hemisphere (Morecraft et al., 2015), we discussed the nature of potentially reduced trophic input as a result of parietal lobe inclusion in a peri-Rolandic injury. Findings from the current study indicate that the loss, or alteration of this potential trophic influence also affects the spared CSP system in the unaffected hemisphere. Possibly due to destroyed interhemispheric projections from the anterior parietal region, the loss of descending S1 projections, or through other complex mechanisms. Thus, in addition to considering the degree of damage and residual components of the ipsilesional M1 for therapeutic planning (Hoyer and Celnik, 2011; Bradnam et al., 2013; Stinear and Byblow, 2014), consideration of the degree of accompanying anterior parietal lobe damage may add prognostic value. For instance, it is possible that gains made in the early recovery period, and corresponding motor recovery plateau, reflect the extent of spared pyramidal tract output in the acute post-injury time frame. However, patients that additionally have anterior parietal lobe injury may benefit from long-term interventions applied after the initial motor recovery plateau is reached. That is, to combat the potential loss of trophic influence on spared pyramidal tract output, and prevent potential long-term deterioration of spared corticospinal projections. Indeed, it has been shown in the clinical literature that deterioration of motor function can occur after patients reach their motor recovery plateau (Sonde et al., 2000; Dhamoon et al., 2012), which would favor the delivery of ongoing therapy in some patients (Thorsén et al., 2005), and possibly those with both lateral frontal motor cortex injury and anterior parietal injury involving the hand/arm representation. It is important to stress that our frontoparietal lesioned animals survived for 6 to 12 months after injury and were allowed to move freely in their cage and use either hand during their activities of daily living, including feeding, climbing and playing with large toys. Despite this high level of motor activity, an upregulated iM1 CSP neuroplastic response did not occur (Fig. 12). A similar lack of a positive neuroplastic response in human patients with anterior parietal damage may be highly detrimental to motor recovery efforts because humans are thought to rely extensively on the corticospinal projection system for producing dexterous hand movements (Cheney et al., 1991; Lemon, 1993, 2008; Porter and Lemon, 1993; Armand et al., 1997; Martin, 2005; Lemon and Griffiths, 2005; Schieber, 2007).

Motor Recovery Observations

We did not predict that within the Category F2P2 lesioned monkey group, those with a better neuroplastic response (i.e., retained higher numbers of cM1 iCSP boutons in lamina VII and increased numbers of such boutons in laminae VIII and IX; Table 3) would have slower and generally poorer recovery of skill in reaching and manipulation (SDM87 and SDM91) (Figs. 9, 10). These findings are consistent with the idea discussed above that without some kind of forced training of the more affected upper limb, reinnervation of spinal neurons by the spared ipsilateral CSP from cM1 may be maladaptive and associated with poorer recovery. Specifically, inappropriate reinnervation patterns may contribute to abnormal synergies that have been observed in human patients (e.g., Schwerin et al. 2008),

although we did not observe such synergies in our frontoparietal lesioned rhesus monkeys. Perhaps such reinnervation patterns in monkeys simply result in altered coordination of proximal and distal upper limb muscles leading to slower, less accurate reaches and poorer grasping of small food targets (i.e., longer grasp durations, increased numbers of lost contacts with the target). The inverse relationship is also consistent with previous studies suggesting expression of ipsilateral pathways after stroke correlates with poor recovery (Ward et al. 2003, Werhahn et al. 2003). Thus, a major question to be considered in future work is whether the contralesional M1 can be recruited appropriately during therapy to contribute to improved recovery of hand motor function following severe frontoparietal injury.

In contrast to the findings in our Category F2P2 lesioned monkeys, the positive cM1 iCSP neuroplastic responses in monkeys with progressively larger frontal lobe motor area lesions were all associated with good recovery of motor function (reported in Darling et al. 2009). Of particular interest is SDM50 as this case received a lesion of the hand/arm areas of M1 + LPMC + M2, thus ipsilesional M2 could not contribute to recovery as in the case of SDM38 and SDM48, yet had comparable skill recovery to these monkeys with smaller lesions (see Table 2 of Darling et al. 2009). It is tempting to speculate that a significant contribution to hand motor function recovery in SDM50 was the increase in the contralesional M1 ipsilateral CSP as reported here, in addition to the contralateral CSP of spared ipsilesional motor areas (i.e., M1c and the cingulate motor cortices), or spinal cord projections from subcortical motor nuclei (e.g., red nucleus (Belhaj-Saif and Cheney, 2000), reticulospinal nuclei (Zaaimi et al., 2012)).

Technical Considerations

In our previous report on the effects of frontoparietal injury (Category F2P2 lesion) on corticospinal projections originating from spared supplementary motor cortex (M2) in the lesioned hemisphere, we discussed in detail, the nature of injecting a lower volume of fluorescein dextran (FD) tract tracer into M2 in our lesion experiments (i.e., 0.9 μ l) compared to our control cases (i.e., 1.2 μ l) (Morecraft et al., 2015, see Discussion: Technical Considerations). The intent of injecting the lower amount of tracer in the lesion cases was to be sure that if we found enhanced numbers of M2 corticospinal terminal boutons in the F2P2 lesion cases versus the controls, as we had discovered following frontal motor (F2 lesion) injury alone (McNeal et al., 2010), this would not be a result of a potentially larger injection site, but reflective of a true, and favorable neuroplastic response. However in our recent report (Morecraft et al., 2015), we found the addition of anterior parietal cortex injury to the frontal motor lesion blocked the upregulated neuroplastic response of the M2 CSP that had occurred following frontal motor cortex injury alone (McNeal et al., 2010) (Fig. 12).

The importance of raising this issue once again, is that the results of the present study further confirm that the dextran tracer volume differences between control cases and lesion cases, had no major effect on the experimental outcomes of our studies. For example, in the current study the control animals received 1.2 μ l of LYD tract tracer in M1, whereas 0.9 μ l of LYD tracer was injected into cM1 in our 3 frontal lobe lesion cases (SDM38, SDM48 and SDM50) (Table 1). Importantly, we found the total number of ipsilateral labeled boutons in

each frontal lesion case was far greater than the control values (Table 3). Thus, demonstrating that the lower volume of tracer (0.9 μ l) is more than enough to label two to three times the total number of ipsilateral boutons compared to controls.

Summary and Conclusions

The polarized findings from the present study, which examined the effects of frontal versus frontoparietal cortical injury on the iCSP from cM1, complement the strikingly different responses that we found for the contralateral corticospinal projection (cCSP) from spared supplementary motor cortex (M2) residing in the lesioned hemisphere (McNeal et al., 2010; Morecraft et al., 2015). Collectively, a positive CSP response occurs after frontal motor injury (Fig. 11). This is characterized by a significant increase in terminal boutons on the more affected, or heavily denervated side of the spinal gray matter from the intact contralateral corticospinal projection arising in the lesioned hemisphere (i.e., M2), and from the intact ipsilateral corticospinal projection arising in the non-lesioned hemisphere (i.e., M1). This favorable response is blocked in both corticospinal projection systems when the unilateral peri-Rolandic frontal lobe injury is accompanied by adjacent parietal lobe damage (Fig. 12). These observations suggest that injury involvement of the primate lateral anterior parietal lobe has a currently undetermined, but detrimental downstream effect on what at first, appears to be intact, healthy corticospinal projection fields. It will be of significant interest to study the effects of parietal cortex injury on other descending corticofugal projection systems, and determine which factors prevent spared corticospinal projection neurons, that normally play a dominant role in control of proximal and distal upper extremity movement, from favorably participating in the motor recovery process. It will be equally important to determine if traditional therapeutic interventions, as well as augmented therapeutic strategies can reverse this unfavorable response.

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COMMON ABBREVIATIONS

cM1	contralesional primary motor cortex
CSP	corticospinal projection
iCSP	ipsilateral corticospinal projection
LPMC	lateral premotor cortex
LYD	lucifer yellow dextran
M1	primary motor cortex
M2	supplementary motor cortex
mDB	modified dexterity board
mMAP	modified movement assessment panel
S1	primary somatosensory cortex

LIST OF ABBREVIATIONS

A	arm
Ab	abdomen
An	ankle
as	spur of the arcuate sulcus
Ax	axial
Cgs	cingulate sulcus
cs	central sulcus
BDA	biotinylated dextran amine
cM1	contralesional primary motor cortex
cc	corpus callosum
cf	calcarine fissure
cs	central sulcus
CSP	corticospinal projection
D	digit
dl (or dLat)	dorsolateral subdivision
dm (or dMed)	dorsomedial subdivision
ecs	ectocalcarine sulcus
El	elbow
F	face
Fr	frontalis
Hp	hip
ICMS	intracortical microstimulation
iCSP	ipsilateral corticospinal tract
ilas	inferior limb of arcuate sulcus
ios	inferior occipital sulcus
ips	intraparietal sulcus
J	jaw (mandible)
Kn	knee
L	leg
LSCT	lateral corticospinal tract
lf	lateral fissure

LL	lower lip
LPMC	lateral premotor cortex
LPMCd	lateral premotor cortex, dorsal part
LPM Cv	lateral premotor cortex, ventral part
ls	lunate sulcus
LYD	lucifer yellow dextran
M1	primary motor cortex
M1c	caudal part of the primary motor cortex
M1r	rostral part of the primary motor cortex
M2	supplementary motor cortex
N	neck
NBS	non-invasive brain stimulation
NR	no response
OO	orbicularis oculi
ots	occipitotemporal sulcus
poms	medial parieto-occipital sulcus
ps	principle sulcus
Roman numerals I-X	Rexed's laminae
ros	rostral sulcus
rs	rhinal sulcus
S1	primary somatosensory cortex
S1c	caudal part of the primary somatosensory cortex
S1r	rostral part of the primary somatosensory cortex
Sh	shoulder
slas	superior limb of the arcuate sulcus
sts	superior temporal sulcus
T	tail
Th	thumb
TMS	transcranial magnetic stimulation
To	tongue
Tr	trunk
UL	upper lip

VF	ventral funiculus
v	ventral subdivision
vl (or vLat)	ventrolateral subdivision
vm (or vMed)	ventromedial subdivision
Wr	wrist

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Figure 1.

Line drawings of the lateral surface of the left and right hemispheres in frontal lobe lesion cases SDM38 and SDM48. In frontal lobe lesion case SDM 50, the line drawings represent the lateral and medial surfaces of the left hemisphere and the lateral surface of the right hemisphere. The location of the frontal lobe lesion site is represented by the blackened area and the location of the lucifer yellow dextran (LYD) injection site is represented in bright yellow. For the LYD injection site, the outer solid line represents the external boundary of the injection site halo and the inner solid line represents the core region. The respective

pullouts on the lesioned hemisphere depict the physiological map obtained using intracortical microstimulation (ICMS) to localize the hand/arm areas prior to gray matter tissue resection. Similarly, the pullout of the LYD injection site depicts the ICMS derived map used to localize the hand/arm area of M1 prior to LYD tracer injection. The central sulcus has been opened in both hemispheres to show the extent of the lesion site as well as the LYD injection site in the depths of the central sulcus. The dashed line near the fundus of the central sulcus represents the cytoarchitectonic border between area 3 (S1) and area 4 (M1). For abbreviations see list.

Figure 2.

Plate of photomicrographs showing representative examples of each M1 cortical injection site (left panels, A, C and E) and corresponding ipsilateral terminal labeling in the spinal cord (right panels, B, D and F) in the frontal lobe lesion cases. **A)** Coronal section showing the LYD injection site (dark blue) in the M1 hand/arm representation in case SDM38. **B)** Terminal labeling of the ipsilateral corticospinal projection in case SDM38 at spinal level C7 showing labeled boutons (arrow heads) in lamina VIII. The inset in the top right is a higher-power (100×) image of terminals (arrow heads) at C7 located close to large cell

bodies (double arrow heads) in the ventral region of lamina VIII. **C)** Coronal section showing the LYD injection site (dark blue) in the M1 hand/arm representation in case SDM48. **D)** Terminal labeling of the ipsilateral corticospinal projection in case SDM48 at spinal level C5 showing labeled boutons (arrow heads) in lamina VIII. The inset in the top right is a higher-power (100×) image of labeled terminals (arrow heads) at C6 located close to a large cell body (double arrow head) in lamina VII_{vm}. The inset in the bottom left is a higher-power (60×) image of labeled terminals at C6 located in the dorsomedial region of lamina VIII. **E)** Coronal section showing the LYD injection site (dark blue) in the M1 hand/arm representation in case SDM50. **F)** Terminal labeling of the ipsilateral corticospinal projection in case SDM50 at spinal level C7 showing labeled boutons (arrow heads) in lamina VII_{vm}. The inset in the top right is a higher-power (100×) image of labeled terminals (arrow heads) at C8 in the ventral region of lamina VIII near the ventral funiculus (VF). In panels A, C and E, the dotted line demarcates the external boundary of the injection site core, the dashed line the external limit of the injection site halo, and each white asterisk denotes the location of a needle track made by the Hamilton microsyringe used to inject the LYD tract tracer. Scale bars = 2 mm in A (applies to C and E); 20 μm for main panel of B (applies to main panels of D and F). Abbreviations: cs, central sulcus; VF, ventral funiculus

Figure 3.

Line drawings of the lateral surface of the left and right hemispheres of frontoparietal lesion cases SDM87, SDM91, SDM81 and SDM83. The location of the lesion site is represented by the blackened area and the location of the lucifer yellow dextran (LYD) injection site in bright yellow. For the LYD injection site, the outer solid line represents the external boundary of the injection site halo and the inner solid line represents the external boundary of the core region. The pullout on the lesioned hemisphere depicts the physiological map obtained with ICMS used to localize the hand/arm areas prior to gray matter resection. The

pullout of the LYD injection site depicts the ICMS map used to localize the hand/arm area of M1 prior to tracer injection. The central sulcus has been opened in both hemispheres to show the extent of the lesion site and LYD injection site in the depths of the central sulcus. The dashed line near the fundus of the central sulcus represents the cytoarchitectonic border between area 3 (S1) and area 4 (M1). For abbreviations see list.

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Figure 4.

Plate of photomicrographs showing representative examples of each M1 cortical injection site (left panels, A, C, E, G) and corresponding ipsilateral terminal labeling in the spinal cord (right panels, B, D, F and H) in the F2P2 lesion cases. **A)** Coronal section showing the LYD injection site (dark blue) in the M1 hand/arm representation in case SDM87. **B)** Terminal labeling of the ipsilateral corticospinal projection in case SDM87 at spinal level C5 showing labeled boutons (arrow heads) in lamina VIII. The inset in the top right is a higher-power (100 $\times$) image of terminals (arrow heads) at C7 that are located close to large

cell body (asterisk) in the ventomedial region of lamina IX. The bottom left inset shows lamina VIIv labeled boutons at C7. **C)** Coronal section showing the LYD injection site (dark blue) in the M1 hand/arm representation in case SDM91. **D)** Terminal labeling of the ipsilateral corticospinal projection in case SDM91 at spinal cord level C6 showing labeled boutons (arrow heads) in lamina VIII close to the ventral funiculus (VF). The inset in the bottom right is a higher-power (60×) image of labeled terminals at C7 that are located close to a large cell body (asterisk) in the ventromedial sector of lamina IX. The inset in the bottom left is a higher-power (100×) image of labeled terminals at C6 located in lamina VIIvm. **E)** Coronal section showing the LYD injection site (dark blue) in the M1 hand/arm representation in case SDM81. **F)** Terminal labeling of the ipsilateral corticospinal projection at C5 in case SDM81 showing labeled boutons (arrow heads) in the ventral region of lamina VIII. The inset in the bottom right shows labeled terminals at C7 that are located in close proximity to a large sell body (asterisk) in the ventral region of lamina VIII. **G)** Coronal section showing the LYD injection site (dark blue) in the M1 hand/arm representation in case SDM83. **H)** Terminal labeling at segmental level C5 in case SDM83 illustrating a dense cluster of labeled boutons (arrow heads). In panels A, C, E, and G the dotted line demarcates the external boundary of the injection site core, the dashed line the external limit of the injection site halo, and each white asterisk denotes the location of a needle track produced by the Hamilton microsyringe used to inject the tract tracer. The brown labeled fibers and terminals in panels B, D, F and H are from a BDA injection site located in different cortical area of interest. Scale bars = 2 mm in A (applies to C, E and F); 50 μ m for main panel of B (applies to main panel of D); 20 μ m for main panel of F (applies to main panel of H). Abbreviations: cs, central sulcus; VF, ventral funiculus.

Figure 5.

Line drawings depicting representative transverse sections through spinal levels C5 to T1 in frontal lobe lesion cases SDM38 and SDM50 (left), control case SDM54 (center), and frontoparietal lesion cases SDM81 and SDM91 (right) illustrating the locations of labeled terminals and terminal clusters (red dots). Only one-half of the spinal cord is shown which represents the distribution of LYD labeled terminals that are ipsilateral to the cortical M1 injection site (i.e., the ipsilateral corticospinal projection from M1). For cases SDM38, SDM50 and SDM 54, the ipsilateral projection was to the right half of the spinal cord as shown, since the M1 injection was made in the right hemisphere. However, for cases SDM81 and SDM91 the ipsilateral corticospinal projection terminated in the left half of the spinal cord (since the M1 injection was placed in the left hemisphere). Thus, for comparative purposes, the spinal cord sections for SDM81 and SDM91 have been flipped in this diagram. Roman numerals in section C5 of SDM54 designate Rexed's laminae and apply to all spinal sections. Laminae VII was subdivided into 5 subsectors (see dashed lines) and lamina IX was subdivided into quadrants (see dashed lines) for stereological analysis. Note the increased labeling in frontal lobe lesion cases SDM38 and SDM50 compared to

control case SDM54, and the sparse terminal labeling in cases SDM91 and SDM81 that is concentrated in lamina VIII. For orientation, dorsal is located on the top of each section and ventral at the bottom. Abbreviations: dm, dorsomedial; dl, dorsal lateral; v, ventral; vl, ventrolateral; vm, ventromedial;.

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Figure 6.

Estimated numbers of boutons of the ipsilateral corticospinal projection (iCSP) from the contralesional M1 by laminae (A), quadrants of lamina IX (B), sectors of lamina VII (C) and spinal segments (D). Data for individual monkeys and mean (M) of the frontal lesion, fronto-parietal (F2P2) lesion and controls are shown. Error bars are 1 SEM. Symbols: * (red) – frontal lesion group number of boutons differs significantly from Category F2P2 lesion and controls, # -frontal lesion group number of boutons differs significantly from controls, + - F2P2 lesion group number of boutons differs significantly from controls, * (blue) - frontal lesion group number of boutons differs significantly from F2P2 group ($p < 0.05$) and there is a trend for difference from controls ($p < 0.09$).

Figure 7.

Estimated number of lamina VIII boutons vs. total (gray matter + white matter) lesion volume for all 10 cases (control, frontal lesion, Category F2P2 lesion). Each plotted point is data from a single monkey. The plotted solid line is the least squares regression line including data from all frontal lesion and control cases. The plotted dashed line is for data from Category F2P2 lesion and control cases. Both regression lines were forced through an intercept equal to the mean estimated number of boutons in control cases.

Figure 8.

Scatterplots of the estimated number of lamina VII+VIII+IX boutons (A), lamina VII boutons (B) and lamina VIII boutons (C) versus caudal M1 lesion volume as a percentage of estimated caudal M1 arm area volume. Each plotted point is data from a single monkey with frontal lobe lesion (circle) or F2P2 lesion (triangle).

Figure 9.

Scatterplots of post-lesion weeks until first attempt on any well of the mDB task versus the ratio of estimated number of boutons in Category F2P2 lesioned monkeys to average of controls in lamina VII (A) and lamina IX (B). Each plotted point is data from a single monkey.

Figure 10.

Scatterplots of recovery of reach skill in the mDB task versus estimated number of boutons in lamina VII (A) and IX (B) and of recovery of manipulation skill in the mMAP task versus estimated number of boutons in lamina VIII (C) and lamina IX (D). Each plotted point is data from a single monkey with a F2P2 lesion. Recovery skill was computed as the ratio of skill over the best 5 consecutive post-lesion test sessions to the last 5 consecutive pre-lesion test sessions (circle) and to the best 5 consecutive pre-lesion test sessions (triangle).

Figure 11.

Summary diagram illustrating the main findings of the current study following isolated frontal motor injury. We found the ipsilateral corticospinal projection (iCSP) from contralesional M1 (cM1) (pathway identified in green) to enhance its terminal projection in lamina VIII and the adjoining ventromedial and ventral sectors of lamina VII (see green arrows in spinal cord section) after isolated frontal motor injury. Also shown are previous findings of the upregulated contralateral corticospinal response from spared supplementary motor cortex (M2) (pathway shown in blue) originating in the lesioned hemisphere

following isolated frontal motor (M1+LPMC) injury (McNeal et al., 2010). Collectively, the findings from both studies demonstrate that frontal motor injury is accompanied by complementary responses of increased corticospinal projections on the heavily denervated side of the spinal cord. The enhanced contralateral corticospinal projection outcome from M2, most likely represents a contribution to motor recovery of proximal, and distal movement in the severely affected upper extremity. Whereas, the enhanced corticospinal projection from M1, most likely represents a contribution to motor recovery of proximal and axial movement in the severely affected upper extremity.

Figure 12.

Summary diagram illustrating the main findings of the current study following lateral frontoparietal injury. We found that the enhanced neuroplastic response of the iCSP from cM1 that occurs following isolated frontal motor injury (shown in Fig. 11), was blocked following lateral frontoparietal injury. Specifically the total number of labeled terminal boutons of the iCSP from cM1 was similar to controls, with a corresponding reduction of lamina VII labeling and an enhanced projection to lamina VIII (green arrows in spinal cord section). Also shown are previous observations of the blocked contralateral M2 corticospinal

neuroplastic response (see blue arrows in spinal cord section) following lateral frontoparietal injury (Morecraft et al, 2015). Collectively, these findings demonstrate that lateral frontoparietal injury prevents the upregulated neuroplastic response that occurs following isolated frontal motor injury in both hemispheres.

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TABLE 1

Description of the Experimental Parameters for Each Case

Case	Lesion Category	Post-Lesion Survival (months)	Sex	Age (y)	Weight (kg)	Area Injected	Tracer/Injections	Total Vol. (µl)	Injection Core vol. (mm ³)	Injection Halo vol. (mm ³)	Post-Injection survival (days)
SDM 54	Control	-,-	M	9.0	9.2	M1 arm	LYD/3	1.2	10.7	64.7	33
SDM 61	Control	-,-	F	4.0	4.3	M1 arm	LYD/3	1.2	13.2	85.5	33
SDM 62	Control	-,-	F	3.3	3.2	M1 arm	BDA/3	1.2	10.4	43.7	33
SDM 38	F1	12	F	21.6	5.6	M1 arm	LYD/3	0.9	9.2	60.4	34
SDM 48	F2	12	F	7.7	5.3	M1 arm	LYD/3	0.9	9.4	65.5	33
SDM 50	F3	12	F	9.6	6.5	M1 arm	LYD/3	0.9	8.6	71.9	33
SDM 81	F2P2	12	F	16.0	6.0	M1 arm	LYD/3	0.9	8.9	64.0	33
SDM 83	F2P2	12	F	4.7	7.0	M1 arm	LYD/3	0.9	8.3	64.3	33
SDM 87	F2P2	6	F	17.5	10.9	M1 arm	LYD/3	0.9	6.7	62.5	33
SDM 91	F2P2	6	F	8.3	5.6	M1 arm	LYD/3	1.2	8.2	74.6	34

TABLE 2

Lesion/Spared Volume Data for F2 and F2P2 Cases (mm³)

Case	Total lesion (white matter)	Total lesion (gray matter)	M2 lesion	LPMCd lesion	LPMCv lesion	M1 lesion	M1 ros spared	M1 ros lesion	M1 caudal lesion	M1 caudal spared	S1 ros lesion	S1 ros spared	S1 caudal lesion	S1 caudal spared	PE/area5 lesion
SDM 38	9.62	102.05	--	--	--	96.73	5.63	5.32	55.23	--	--	--	--	--	--
SDM 48	23.10	221.68	--	89.02	21.69	105.75	0	5.22	47.01	--	--	--	--	--	--
SDM 50	53.66	432.61	121.84	144.30	29.90	133.32	6.61	3.25	43.13	--	--	--	--	--	--
SDM 81	13.55	176.94	--	26.26	8.01	51.65	9.12	22.88	52.61	15.29	63.05	38.35	14.50		
SDM 83	16.11	257.84	--	83.59	--	68.12	--	29.38	29.13	24.11	46.08	41.21	11.43		
SDM 87	56.21	326.32	--	41.61	42.07	107.65	--	32.70	38.24	7.30	22.64	47.31	47.68		
SDM 91	52.94	268.82	--	39.44	--	80.45	--	72.72	--	42.80	18.40	25.61	7.80		

TABLE 3

Ipsilateral Bouton Counts in Each Case by Spinal Lamina¹

		Bouton ²			VII			VIII			IX			IX
Case		#	dLat	dMed	vLat	vMed	v	Total	dLat	dMed	vLat	vMed	Total	
SDM 54	Control	3841	213 (6)	640 (17)	107 (3)	1814 (47)	0	2774 (72)	1067 (28)	0	0	0	0	
SDM 61	Control	3485	0	2230 (64)	279 (8)	697 (20)	0	3206 (92)	279 (8)	0	0	0	0	
SDM 62	Control	4014	335 (8)	1169 (29)	502 (13)	754 (19)	251 (6)	3011 (75)	501 (12)	0	251 (6)	84 (2)	167 (4)	
	Mean	3780.0	183.0	1346.3	296.0	1088.3	83.7	2996.7	615.7	0.0	83.7	28.0	55.7	
	SE	155.7	120.0	572.5	140.0	444.8	102.5	153.6	287.3	0.0	102.5	34.3	68.2	
SDM 38	F1	10067	1088 (11)	3357 (33)	816 (8)	2086 (21)	816 (8)	8163 (81)	1814 (18)	90 (1)	0	0	0	
SDM 48	F2	12600	95 (1)	0	0	4105 (33)	3531 (28)	7731 (61)	4774 (38)	0	0	0	95	
SDM 50	F3	14828	0	166 (1)	332 (2)	4776 (32)	4237 (29)	9511 (64)	5151 (35)	0	0	0	166	
	Mean	12498.3	394.3	1174.3	382.7	3655.7	2861.3	8468.3	3913.0	30.0	0	0	87.0	
	SE	1375.3	426.1	1337.9	290.2	990.1	1277.1	656.5	1292.3	36.7	0	0	58.9	
SDM 81	F2P2	1716	59 (3)	295 (17)	0	236 (14)	118 (7)	708 (41)	949 (55)	0	0	0	59	
SDM 83	F2P2	2248	0	0	0	374 (17)	249 (11)	623 (28)	1625 (72)	0	0	0	0	
SDM 87	F2P2	4525	0	0	0	366 (8)	917 (20)	1283 (28)	2508 (55)	0	551 (12)	0	183	
SDM 91	F2P2	5716	0	289 (5)	0	505 (9)	506 (9)	1300 (23)	3548 (62)	0	0	0	868	
	Mean	3551.3	18.8	150.0	4.0	282.8	222.3	661.8	1534.5	4.0	4.0	4.0	235.8	
	SE	944.3	17.0	97.3	0.0	63.4	203.3	209.7	649.8	0.0	159.1	0.0	231.5	

¹ Percentages of total label in parentheses. Values are rounded to the nearest whole number, except when the value is 0.7 or less.² Total number reflects boutons only from laminae VII, VIII and IX. d, dorsal; v, ventral; Lat, lateral; Med, medial; SE, standard error