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An investigation of the cortical control of forepaw gripping after cervical hemisection injuries in rats

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Abstract

Previous studies in mice have demonstrated that forepaw gripping ability, as measured by a grip strength meter (GSM), is dependent on the contralateral sensorimotor cortex, but this dependency changes after hemisection injury at cervical level 4 (C4). Initially, the mouse fails to grip with the forepaw ipsilateral to the hemisection but gripping recovers. Additionally, a mouse's gripping by the contralateral paw becomes independent of the sensorimotor cortex, indicating a reorganization of cortical control of gripping function (Blanco et al, 2007). Here we explore whether a similar reorganization occurs after cervical hemisection injuries in rats. We show that as in mice, unilateral lesions of the sensorimotor cortex impair rats' gripping by the contralateral paw. We also confirm from previous studies that cervical hemisections in the impair rats' gripping by the ipsilateral paw. In contrast to mice, however there is minimal recovery of gripping after complete lateral hemisections and secondary lesions of the sensorimotor cortex continue to impair rats' gripping by the contralateral paw. Thus, forelimb gripping ability as measured by the GSM is dependent on the contralateral sensorimotor cortex in rats even after a cervical hemisection.

Keywords

spinal cord injury; hemisection; plasticity; functional reorganization; recovery of function; cortico-spinal tract; sensorimotor cortex; rat; digital flexors; upper extremity; hand function

INTRODUCTION

Most spinal cord injuries (SCI) in people occur at the cervical region, and for the individuals with this level of injury, recovery of upper extremity function is the highest priority (Anderson, 2004). These facts motivate efforts to develop models of cervical SCI in rodents and techniques to assess forelimb function. In this regard, recent studies have revealed that assessment of gripping ability with a grip strength meter (GSM) provides reliable, quantitative measurements of deficits and recovery of forelimb motor function after cervical spinal cord injuries in rats

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and mice (Anderson et al, 2004, 2005, 2007). Given this, it is now of considerable importance to define which pathways mediate gripping function. Although it has not been established definitively which descending pathways mediate gripping ability, the corticospinal tract (CST) is implicated by virtue of the fact that the task requires extension and then flexion of the digits in order to grasp the bar of the GSM. This interpretation is further supported by the fact that at least in mice, gripping ability is abolished following unilateral lesions of the sensorimotor cortex, although recovery does occur over time (Blanco et al, 2007).

Mice show recovery of gripping ability after cervical hemisection injuries (Anderson et al, 2004) and also after cortical motor lesions (Blanco et al, 2007). Surprisingly, after mice recover from a lateral hemisection in the cervical region, lesions of the motor cortex ipsilateral to the hemisection do not affect gripping ability by the contralateral paw. These results suggest that there is reorganization of cortical control of forelimb motor function after cervical spinal cord injury.

The goal of the present study was to determine if forelimb gripping ability in rats is dependent on the contralateral sensorimotor cortex and whether there is reorganization of cortical motor control after cervical hemisection similar to that seen in mice. Complete cervical hemisections in rats leads to permanent impairment of gripping function, as measured by the grip strength meter (Anderson et al, 2005, 2007). It has not been established whether forepaw gripping depends on the sensorimotor cortex in rats. Studies showing that forepaw function as measured by a reaching task is impaired following cortical lesions suggest that voluntary gripping would depend on the sensorimotor cortex (Gharbawie et al, 2007, Castro, 1972, Whishaw and Kolb, 1988, and Anderson et al, 2007).

To address these questions, in the present study we assessed forepaw gripping ability in rats after lesions of the sensorimotor cortex alone and after cervical hemisection injuries. We show that forepaw gripping ability in rats is disrupted by lesions of the sensorimotor cortex, even when the cortical lesion follows a cervical hemisection lesion.

METHODS

Experimental animals were female Sprague-Dawley rats from Harlan, Inc., San Diego, CA that were 200–250g at the beginning of each experiment and between 2–8 months of age. Rats were housed in groups of three to five and maintained on a 12/12 h light/dark cycle in a room with controlled temperature and humidity. All procedures were approved by the Institutional Animal Care and Use Committee of the University of California Irvine.

Three separate experiments were carried out. In the first, rats (n=5) received unilateral lesions of the left sensorimotor cortex and were then trained on the GSM (procedure explained below) beginning 2 days post-lesion. Grip strength data was collected starting 8 days post-lesion, and was assessed until 68 days post-lesion. The rats then received a secondary lesion of the right sensorimotor cortex, and grip strength testing continued for 57 additional days. To confirm the ablation of the cells of origin of the corticospinal tract (CST), these rats received injections of retrograde tracer FluoroGold (FG) into the spinal cord to retrogradely label neurons that project into the spinal cord. As a positive control, a separate group of rats (n=5) received unilateral lesions of the left sensorimotor cortex and 6 days later, received injections of FG into the spinal cord. Rats were kept for 7 days after the FG injections.

In experiment 2, rats (n=10) were tested 8 times over 17 days in the GSM task and then received complete right hemisection injuries at cervical level 4 (C4). Grip strength was tested for 43 days after the spinal cord hemisection injury. Then the rats received secondary lesions of the right sensorimotor cortex. Grip strength testing then continued for 37 days after the cortical lesions. To trace the projections from the surviving cortex, rats received intracortical injections

of tetramethylrhodamine and biotin conjugated dextran amine, 10,000 MW, lysine fixable (mini-ruby-BDA, Molecular Probes, Eugene, OR) 38 days following the cortical lesions and were kept an additional 19 days.

In experiment 3, rats (n=10) were handled, trained and measured on the GSM initially as in experiment 2. The surgical procedures for right lateral hemisection at C4 and right sensorimotor cortex lesion were identical. Grip strength testing was identical until 13 days after the right sensorimotor cortex lesion. At that time, a different individual took over the GSM testing and used a modified testing procedure so that gripping was encouraged by allowing the rat more time to grip the bar (one trial every six seconds vs. one trial every 2 seconds) and by moving the palm of the forepaw back and forth along the bar to provide sensory cues. This modified testing continued until 45 days post-lesion. Rats then received BDA injections into the surviving cortex, and survived for 17 days.

Grip strength testing

Rats were handled for 5 consecutive days and trained for an additional 5 days so that they would become accustomed to being gently held in the way that is required for grip strength testing. Grip strength was assessed as described in Anderson et al, 2005 using a grip strength meter (GSM) designed by TSE-Systems and distributed by SciPro, Inc. For testing, rats were gently held so their back legs were supported with one forelimb lightly restrained. The paw being tested was brought to the bar, the rat was allowed about one second to establish a grip, and the rat was then gently pulled back in one smooth motion until grip was released. The time between trials averaged two seconds. Positive grips were scored when the rat grasped the bar immediately with all fingers, and after release, the paw was relaxed and not clenched. Grips were not scored if the bar was hooked by a claw or by spastic clenched paw (in the case of rats with cervical spinal cord injuries). Gripping force was defined as the maximum force recorded on the GSM before the rat released the bar. Rats were given a maximum 25 trials per session. Testing stopped after 4 legitimate grips were recorded for each paw or until there were 15 consecutive trials with no successful gripping.

Sensorimotor cortex lesions

Rats were anesthetized with an intraperitoneal injection of Ketamine and Xylazine (100mg/kg and 10 mg/kg, respectively; Western Medical Supply, Inc., Arcadia, CA). The scalp was shaved and swabbed with betadine, and the eyes were swabbed with Vaseline. The rats were secured in a stereotaxic apparatus (Kopf), the scalp was incised, and the skull was removed from the midline to 4 mm lateral, extending 4 mm rostral to and 4 mm caudal to bregma. Cortical grey matter was gently aspirated down to the level of the white matter using a Pasteur pipette with the tip pulled to a diameter of approximately 300 μ m. A rectangular piece of Gel Foam was placed in the lesion site and the scalp was sutured and swabbed with betadine. Postoperatively, rats received 10ml of 0.9% saline, 0.5mg/kg Baytril, and 0.01mg/kg Buprenorphine subcutaneously and were maintained on an isothermic pad until alert and mobile.

Retrograde labeling of CST neurons with FluoroGold

For fluorogold (FG) injections, rats were anesthetized as above, the neck and upper back was shaved, and the skin was treated with betadine. A midline incision exposed the spinal column at the level of C2-T1, and layers of muscle overlying the vertebral column were bluntly dissected to visualize the transverse apophyses. A dorsal laminectomy was then performed on the fourth cervical vertebra (C4). FG was injected using a 10 μ l Hamilton syringe with a pulled glass tip at 3 sites in the spinal cord: 1mm to the right of the midline and 0.8mm ventral to the dura mater, at the midline and 0.5mm ventral to the dura mater, and 1mm to the left of the midline and 0.8mm ventral to the dura mater. After the syringe was lowered, 0.25 μ l of 4% FG was injected at each site over one minute, and the pipette was left in place for three minutes.

The skin was closed with stainless-steel wound clips, and post-operative care was as described above. Rats were kept for 7 days post FG injection, after which they were killed humanely with an overdose of Euthasol (195mg/ml pentobarbital sodium and 25mg/ml phenytoin sodium; Delmarva Laboratories, Inc., Richmond, VA). When deeply anesthetized, rats were perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS), pH 7.4.

Spinal cord injury surgery

Rats were anesthetized as above, and a laminectomy was performed at the fourth cervical vertebra (C4). A complete lateral hemisection was performed on the right side of the spinal cord using a Moria micro knife, (FST catalog #7040A, Fine Science Tools, Foster City, CA). The microknife was inserted into the spinal cord at the midline, with the blunt back-side of the knife abutting the dorsal blood vessel and the sharp-side facing laterally. The knife was lowered until it touched the ventral surface of the vertebra column, and was then drawn laterally until it exited the spinal cord. This incision was repeated once. The muscle layers were sutured, and the skin was sutured as described above. Post-operatively, rats received 10ml of 0.9% saline, 0.5 mg/kg Baytril, and 0.01 mg/kg Buprenorphine subcutaneously and were maintained on an isothermic pad until alert and mobile. Saline and Baytril were given for 7 consecutive days, and Buprenorphine was given for 3 consecutive days post injury. Bladders were manually expressed for the first week; however, urine retention was minimal after the first day (i.e., bladders were empty or contained minimal urine when expressed). Rats were inspected for weight loss, dehydration, discomfort, and autophagia, with appropriate veterinary care as needed.

Intracortical injections of Mini-ruby-BDA for tract tracing

Stereotaxic injections of miniruby-BDA were made into the left sensorimotor cortex to trace the CST on the side of the hemisection. The main purpose was to aid in the recognition of spared CST axons. Rats were anesthetized and prepared for surgery as above. The skull overlying the sensorimotor cortex was removed with a dental drill, and BDA (100mg/ml) was injected at a total of six sites with a 10 μ l Hamilton microsyringe with pulled glass tip. Coordinates for the injection sites were 1.5mm and 2.5mm lateral to the midline at 1mm rostral to bregma, at bregma, and 1mm caudal to bregma at a depth of 1mm from the cortical surface. At each site, 0.5 μ l of tracer was injected over approximately 5 minutes. After all injections were complete, the scalp was sutured and rats were left on a water jacketed heating pad until they recovered from anesthesia. Post-operatively, rats received 10ml of 0.9% saline, 0.5 mg/kg Baytril, and 0.01 mg/kg Buprenorphine subcutaneously and were maintained on an isothermic pad until alert and mobile.

Histology

For fluorogold tracing, brains and spinal cords were carefully dissected out and tissue was postfixed in 4% paraformaldehyde in 0.1M PBS, pH 7.4. Brains and spinal cords were placed in 30% or 27% sucrose respectively overnight for cryoprotection. Once the tissue sank in this solution, it was frozen in a block of TissueTek (VWR International, West Chester, PA). Brains were sectioned in the coronal plane, collecting sections every 100 μ m to be analyzed under the fluorescence microscope. A 1.5 cm section of spinal cord was taken containing the injection site extending from about C2 through T1. This block was sectioned at 20 μ m in the horizontal plane maintaining serial order.

For BDA tracing, tissue was collected and processed as above. Brains were sectioned in the coronal plane, and representative sections taken every 500 μ m were stained with H&E. A 1.5cm block was taken containing the spinal cord lesion site extending from about C2 through T1. This block was sectioned at 20 μ m in the horizontal plane maintaining serial order. One section every 40 μ m was stained for BDA as described in Steward et al, 2004. Although mini-ruby

BDA projections can be visualized with fluorescence, histochemical staining of BDA allows better visualization of the fine axonal arbors.

Statistical Analysis

On each testing day, the average force (grams) each rat exerted with each forepaw was calculated from four trials per paw. A repeated measures two-way ANOVA was performed to identify differences between paws and time post-injury. The Bonferroni test was used for post-hoc analysis to correct for multiple comparisons.

RESULTS

Experiment one

Cortical lesions impair gripping ability by the contralateral forepaw—After unilateral lesions of the left sensorimotor cortex, rats gripped with the left forepaw (mean gripping force averaged 170 ± 15 g), but failed to grip with the right forepaw for the first 4 weeks following the lesion. Figure 1 illustrates the average of all rats and figure 2 illustrates the data from individual animals. Three of the 5 rats (#11, 12, and 13) failed to grip with the right paw throughout the testing period whereas two rats (#14 and 15) eventually recovered the ability to grip. Rat #14 suddenly was able to grip with the right forepaw 36 days after the left cortical lesion. Interestingly, once the rat began to grip with the right paw, the mean grip strength was not significantly different from the left paw (133 ± 4.1 g for the right paw and 132 ± 4.0 g for the left). Rat #15 exhibited day-to-day variability in gripping with the right paw starting 74 days after lesion of the left sensorimotor cortex. The rat gripped on some days, then failed to grip and then began to grip again for several days, etc. Again, when the rat did grip with the right paw, the average grip strength was similar to the left paw (Figure 2).

After the secondary lesion of the right sensorimotor cortex on day 68, almost all rats failed to grip with the left paw. Four of the 5 rats (#11, 12, 13 and 15) did not grip with the left paw at any time after the secondary lesion of the right cortex. Rat #14 initially failed to grip but suddenly began to grip with the left paw 9 days after the right sensorimotor cortex lesion. A few days later, the rat again failed to grip with the left paw, but gripping re-appeared a few days later at previous levels and continued for the remainder of the experiment. An important implication of this on-and-off gripping is that the rat is able to grip with the affected paw, but does not do so on particular testing days.

Histological assessment of the cortical lesions—The lesions of the sensorimotor cortex extended from approximately 4mm rostral to bregma to 4.5mm caudal, and from the midline to 4mm lateral, eliminating the gray matter down to the level of the sub-cortical white matter in most of the rats. No FG positive cells were seen in either side of the cortex in rat #11, 12, 13, or 15, although FG labeled cells were abundant in subcortical regions including the hypothalamus and brainstem of all rats. A representative case is shown in Figure 3. The lesions were not complete in rat #14, however. On the left side, the cortical lesion extended 1.5 mm lateral to the midline; on the right side, there was sparing of approximately 1mm of cortex medially so that the lesion extended from 1mm to 3mm laterally to the midline. A few FG positive cells were detected in the area lateral to the lesion margin on the left side of the brain 1mm rostral to bregma and also at 2.5mm caudal to bregma. A few FG positive cells were also detected on the right side at 2.5 and 3.5 mm caudal to bregma. It is noteworthy that animal #14, in which the cortical lesions spared some CST neurons, was the only rat that recovered consistent gripping after the cortical lesions.

To confirm that FG injections effectively labeled the cells of origin of the CST, a separate group of rats received unilateral lesions of the sensorimotor cortex and FG injections. In these

rats, abundant FG positive neurons were seen on the intact side confirming the efficacy of the retrograde labeling of the cells of origin of the CST, and confirming again the ablation of the cells of origin of the CST on the side of the lesion (Fig. 4).

Experiment two

Do rats exhibit the reorganization of cortical control of gripping after cervical hemisection injuries that occurs in mice?

Prior to spinal cord injury, rats in Experiment 2 gripped with both forepaws to the same extent, and average grip strength was similar to what was seen in previous studies (Anderson et al, 2005). Figure 5 illustrates the average of the data for all rats, and Figure 6 illustrates the graphs for the individual rats. Two rats died immediately after the C4 hemisection injury, and another died 54 days after hemisection, thus the final group size was seven. Data from the three rats that died are not included in the graphs because histological assessment of these lesions was not possible.

After the right hemisection lesion, rats failed to grip with the right forepaw for the duration of the 14 week testing period with the exception of one rat. Rat # 5 failed to grip with either forepaw, and this persisted for the duration of the testing period. Rat #3 failed to grip initially with the right forepaw, but recovered the ability to grip with some variability in gripping performance. The rat did not grip with its right forepaw for the first 27 days; gripping suddenly appeared on day 28, was not seen the following testing session on day 30, and then reappeared again on day 32. There were three more testing days where the rat did not grip the bar in a normal fashion, but for the majority of the time, rat #3 was able to successfully grip the bar. The differences in outcome for #3 and #5 are likely due to differences in the extent of the cervical hemisection injury (see below for description).

It should be noted that although all rats but #3 failed to grip normally, they did exhibit some ability to grip in an abnormal way that did not meet the criteria for scoring. Specifically, these rats closed their paw around the bar slowly, and failed to relax their forepaw after being gently pulled off the bar. Some of the rats would make a fist with their paw and use only the tips of their digits to grip the bar. In keeping with the scoring criteria for this experiment, these abnormal grips were scored as zero.

In addition to the impairment in gripping performance, rats also exhibited other signs of forelimb impairment after the hemisection. Specifically, the right paw was held in a flexed position for the first three weeks, and was not used for locomotion. At about 3 weeks, most of the rats (all but rat #5) gradually began to use the paw for walking and other tasks. In keeping with the scoring procedure described by Anderson et al (2005), when paws were held in a fixed flexed position during grip strength testing, gripping was scored as zero even when the stiff paw generated force on the bar as the rat was pulled away.

After the right cortical lesion on day 43, all rats failed to grip with the left forepaw; this impairment persisted for the duration of the experiment. There was, however, a qualitative difference in the overall forelimb motor impairment produced by cortical vs. spinal cord injury. After cortical lesions, the left forepaws were flaccid when held at the GSM rather than flexed as seen after cervical spinal cord injury. Observation of rats in their home cage revealed that there was no obvious impairment in use of the left forelimb during locomotion after right cortical lesion. This was in contrast to an obvious impairment in locomotion, grooming and other tasks in the home cage with the right paw after cervical hemisection.

Histological assessment of the lesions and relationship to gripping ability—Rats received BDA injections into the cortex contralateral to the hemisection lesion to assist in assessing whether lesions completely transected the CST. In 1 rat, labeling was adequate to

determine CST integrity; in 6 rats, BDA labeling was too light to be useful, and the completeness of the lesion was based on histological appearance alone.

To define the extent of lesion, horizontal sections were examined in serial order, and the lesion at each dorsal/ventral position (every 40 μ m) was classified as complete (extending to the midline), incomplete, or over-hemisection (extending past the midline). In two rats (#7, and #8) the hemisections were complete, fully transecting the dorsal corticospinal tract and gray matter to the central canal on the right side (histology from rat #8 is illustrated in Figure 7A, D, and G). These rats did not regain gripping ability at all in the right forepaw. Four rats (#2, #3, #4 and #10) the lesions did not completely transect the right side of the spinal cord (histology from rat #3 is illustrated in Fig. 7B, E, and H). Rat #3 regained gripping ability in the right forepaw 32 days post spinal cord injury; rats #2, #4 and #10 did not grip with the right forepaw at any time after the lesion. In one rat (#5) the lesion was complete on the right and extended across the midline so that much of the dorsal column on the left was also damaged (an over-hemisection, see Fig. 7C, F, and I). This rat failed to grip with either forepaw after the spinal cord injury.

The specific region of spinal cord that was left intact may explain the differences in gripping ability between the rats in which the hemisection was incomplete. The transection in rat #3 did not reach the midline at any point dorsally, medially or ventrally. This left almost the entire dorsal CST intact as well as the grey matter around the central canal. In rat #4, the most dorsal region of white matter in the right dorsal column was partially intact; the grey matter, and the ventral white matter were transected completely to the midline. Rats #2 and #10 also had incomplete hemisections that spared the medial/dorsal part of the dorsal column, and left a small part of the dorsal CST intact. The lesions extended to the midline in the ventral region of the dorsal column and also in the central grey matter, but were incomplete in the ventral spinal cord.

The cortical lesions in the rats of experiment 2 were similar to those in experiment one. We could not directly assess sparing of CST neurons in the cortex, however. These rats did not receive FG injections because they had a cervical hemisection.

Experiment three

Gripping values obtained by GSM testing of spinal cord injured rats depends on the testing procedure—During the course of one experiment to assess reorganization of cortical control of gripping ability, we discovered that the values obtained by GSM testing of spinal cord injured rats depended on the testing procedure. We discovered this when a different individual took over the GSM testing 13 days after the right cortical lesion and used a modified testing procedure. Trials were spaced at longer intervals, the rat's paw was kept in contact for a longer period of time, and the rat's paw was moved along the bar to provide sensory cues to encourage gripping. Using this modified testing procedure, rats that did not grip with the standard testing protocol did eventually grip sufficiently to generate force.

The overall design of this experiment was the same as in Experiment 2. Rats were trained on the GSM, received a right lateral hemisection at C4, and then a secondary lesion of the right sensorimotor cortex 46 days later. Figure 8 illustrates the average of the data for all rats, and Figure 9 illustrates the graphs for the individual rats. One rat died immediately after the C4 hemisection injury. Another rat (#9) was euthanized due to infection 8 days after the cortical lesion and prior to the change in the testing procedure (individual graph for rat #9 not shown). Thus the final group size was eight.

After the spinal cord injury, all rats failed to grip with their right paw. Similarly, following right cortical lesion, seven of eight rats failed to grip with their left paw. On the 13th day

following the cortical lesion (59 days post hemisection), the testing procedure changed as described. Using the modified testing procedure, four rats generated force by an abnormal grip using the right paw and all eight rats were able to use an abnormal grip to generate force with the left paw. Abnormal grips were scored when gripping and/or release of the bar was executed slowly. Abnormal grips differed from tightly clenched paws that hooked the bar because the digits did extend and flex, albeit in an abnormally slow fashion. These were seen only when additional time was provided for gripping and release, and inclusion of this category of gripping increased the variability in whether or not the rat gripped on particular testing days and in the force generated during the grip. Four of the rats (#4, #5, #7, and #10) began to grip with their right forepaw the day that the testing procedure changed (59 days post hemisection). Following right cortical lesion, one rat (#1) did not show any impairment in gripping ability with the left forepaw except for two non-consecutive days (13 and 17 days post cortical lesion). All other rats (#2, #3, #4, #5, #7, #8, and #10) failed to grip with their left paw from the time of right cortical lesion until 13 days post cortical lesion when the modified testing procedure was used.

It should be noted that the discrepancy between testers was not discovered during the course of the experiment because data were collected blind and analyzed after the experiment was completed. This turned out to be an advantage because we could then go back and determine exactly what the differences were in the testing procedure.

Histological assessment of the lesions and relationship to gripping ability—In experiment 3, BDA labeling was adequate to determine CST integrity in 5 rats; in 3 rats, BDA labeling was too light to be useful, and the completeness of the lesion was based on histological appearance alone.

In two rats (#8 and #10) the hemisection was complete, fully transecting the dorsal corticospinal tract and gray matter to the central canal on the right side (histology from rat #10 is illustrated in Figure 10A, D, and G). Neither of these rats exhibited normal gripping with their right forepaw. Rat #10, however, did exhibit abnormal gripping when the testing procedure changed as described above. Both #8 and #10 failed to grip normally with the left paw immediately following right sensorimotor cortex lesion, but showed abnormal gripping ability when the testing procedure was modified. In four rats (#1, #4, #5 and #7), the lesion was incomplete, and did not completely transect the right half of the spinal cord (histology from rat #5 is illustrated in Figure 10B, E, and H). In these 4 cases, the transection disrupted most of the dorsal CST, but not all of it. Despite having an incomplete lesion, none of these rats exhibited normal gripping with the right forepaw after spinal cord injury. Three rats (#4, #5 and #7) exhibited abnormal gripping by the right paw during the modified testing procedure, whereas rat #1 continued to exhibit no gripping ability by the right paw.

In two rats (#2 and #3), lesions were complete on the right and extended across the midline so that much of the dorsal column on the left was also damaged (an over-hemisection; histology from rat #3 is illustrated in Figure 10C, F, and I). Rat #2 failed to grip normally with both forepaws for the duration of the experiment but did show abnormal gripping with the left paw during the modified testing procedure. Rat #3 failed to grip normally with the right paw after hemisection and showed only transient abnormal gripping with the right paw during the modified testing procedure. The rat failed to grip normally with the left forepaw, but showed abnormal gripping ability when the testing procedure was modified.

The cortical lesions were similar to those in experiment one (Fig. 1) except for rat #1, in which the lesion area was smaller, sparing some of the rostral sensorimotor cortex. The lesion did not extend further than 2mm caudal to bregma on the right side of the brain. Grip strength data for this rat is described above. Rats in experiment 3 did not receive FG injections because they

had a cervical hemisection, so we could not directly assess sparing of CST neurons in the cortex.

DISCUSSION

The goal of this study was to assess whether forepaw gripping as measured by the GSM depended on the contralateral sensorimotor cortex in rats and whether this dependency changed after hemisection injuries at C4 as occurs in mice. We found that gripping ability was impaired following lesions of the contralateral sensorimotor cortex in rats, as is also true in mice. However, the extent of recovery from both cervical hemisection and sensorimotor cortex lesions in the two species is different. Our results confirm previous studies (Anderson et al., 2005), showing that rats exhibit minimal recovery of gripping ability after cervical hemisection injuries as measured by the grip strength meter. We further show that lesions of the sensorimotor cortex in rats impair gripping by the contralateral forepaw and that there is minimal recovery of gripping for up to 14 weeks. This is in contrast to mice that exhibit substantial recovery of gripping ability after sensorimotor cortex lesions. Third, we show that after C4 hemisections, secondary lesions of the ipsilateral sensorimotor cortex continue to impair gripping ability by the contralateral forepaw. Finally, we show that gripping function, as measured by the GSM, depends on the testing procedure. In what follows, we discuss each of these points.

In rats, lesions of the sensorimotor cortex and cervical spinal cord cause lasting impairments in gripping by the contralateral forepaw

Our results demonstrate that gripping by the forepaw depends on the integrity of the sensorimotor cortex. This, together with the nature of the gripping response, implicates the CST in forepaw gripping function, although it is also possible that cortical control is mediated through brainstem relays. The same basic dependency exists in mice except that mice recover gripping ability after cortical lesions whereas rats do not at least under the testing conditions used here.

One possible explanation for the differential recovery in mice vs. rats has been previously discussed in Anderson et al, 2005. The GSM measures forepaw gripping in both rats and mice, but there is a difference in the way the test is administered between the two species. Rats are supported by holding their body and are brought toward the bar of the GSM which they grasp; in contrast, mice are held at the base of the tail suspended over the bar. The motivation to grip the bar may be different in these situations because mice are hanging by their tail whereas rats are supported. These different testing conditions may also activate different reflex pathways involved in posture and balance in addition to those responsible for voluntary motor control. For example, mice extend their forelimbs before gripping the bar because they are suspended by their tail. Being suspended is likely to motivate mice to grip for stability. Rats are supported, which would reduce activation of pathways responsible for posture and balance, and motivation to grasp the bar is probably less. In this situation, gripping may be controlled primarily by the CST. This could account for the lack of recovery following cervical hemisections in rats. There is also the possibility of a differential training effect. Mice could have benefited more than rats from the same amount of experience in the GSM testing paradigm. Since the mice were suspended by their tails, repeated trials on the task could have shifted gripping control to another anatomical substrate, allowing recovery.

Another possible explanation relates to the organization of the CST in rats vs. mice. In mice, there are substantial re-crossing connections at cervical levels so that CST projections are partially bilateral. For example, following BDA injections into the sensorimotor cortex, there are extensive terminal arbors ipsilateral to the labeled tract, and a moderate number of CST axons extend across the midline at segmental levels to arborize on the side ipsilateral to the

injection. Such re-crossing CST axons are seen in normal rats, but are not abundant (Maier et al., 2008). Thus, it is conceivable that such re-crossing axons mediate the recovery of gripping that is seen following both cervical hemisections and sensorimotor cortex lesions in mice. In this regard, it is noteworthy that in rats, unilateral lesions of the CST at the level of the medullary pyramid lead to increases in the number of re-crossing CST axons, suggesting trans-midline sprouting. This is accompanied by partial recovery of forelimb function as measured by the horizontal ladder task (Maier et al., 2008). Forelimb function is further improved when the unimpaired forelimb ipsilateral to the pyramid lesion is restrained. In this situation, the number of re-crossing CST axons is greater than seen when the impaired forelimb is restrained during the recovery period (Maier et al., 2008). These findings implicate re-crossing CST axons in forelimb motor recovery, at least in the horizontal ladder task.

It is important to note that in the study by Maier et al, the link between the number of recrossing CST axons and forelimb recovery is not perfect. For example, in animals in which the non-impaired forelimb is restrained, and in which recovery is enhanced, the number of recrossing CST axons is not significantly greater than the number seen in rats in which neither forelimb is restrained. In contrast, in animals in which the impaired forelimb is restrained, the number of re-crossing CST axons is less than seen in rats without any restraint. Thus, restraint of the impaired limb may diminish the trans-midline sprouting of CST axons that would otherwise occur. Further studies will be required to elucidate the relationship between trans-midline sprouting of CST axons and recovery of forelimb motor function.

Reorganization of cortical control of gripping

After mice recover from a lateral hemisection in the cervical region, lesions of the motor cortex ipsilateral to the hemisection do not affect gripping ability by the contralateral paw (Blanco et al, 2007). These results suggest that there is reorganization of cortical control of forelimb motor function, as measured by the GSM task, after cervical spinal cord injury in mice. The reasons for the difference between mice and the rats in this study are not clear. Possible explanations include the difference in testing procedure discussed above, differences in response to training (that is, differential learning based on experience), and anatomical differences including differences in CST organization. In addition, the degree to which gripping depends on the contralateral cortex could also change as a result of cortical reorganization as has been demonstrated in humans (Wrigley et al, 2009, Kaas et al, 2008) and primates (Nishimura et al, 2007). The full explanation of the differences between species will likely require an understanding of the mechanisms responsible for the both the recovery and reorganization seen in the respective species.

Gripping function as measured by the GSM depends on the testing procedure

The inadvertent finding in experiment 3 was that gripping could be promoted by adapting the testing procedure. Our standard testing procedure is designed to test normal gripping function. Adopting strict gripping criteria and only scoring grips that are similar to uninjured rats allows deficits to be detected, thus defining the extent of impairment. On the other hand, the modified procedures facilitate detection of abnormal gripping or recovery based on alternate strategies. This makes the grip strength meter a very valuable tool for assessing forepaw function after cervical spinal cord injury in rodents. The finding that gripping can be detected when abnormal grips were scored implies that different tracts may be responsible for normal gripping versus abnormal gripping. Even when there is a complete lesion of the CST, some rats were able to grip the bar when given more time and more tactile stimulation. This is particularly important to consider because of the clinical implications. Even partial recovery can be very beneficial to a person with a cervical spinal cord injury. This suggests that there are neuron tracts other than the CST that may be important to consider for therapeutic interventions.

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Figure 1. Forepaw gripping ability is dependent on the contralateral sensorimotor cortex
 Average grip strength of the right and left forepaws after an aspiration lesion of the left sensorimotor cortex (day 0) followed by the same lesion of the right sensorimotor cortex (day 68). Each point on the graph represents the average of four gripping scores of the right or the left forepaw from all the rats in this experiment. Eight days after injury to the left sensorimotor cortex, mean gripping force across all animals was 172 ± 15.4 g (mean $\pm$ standard deviation) for the left paw and 0g for the right paw. Error bars represent standard error of the mean (n=5).

Figure 2. Forepaw gripping ability of individual rats after cortical lesion

Grip strength of the right and left forepaws of individual rats after an aspiration lesion of the left sensorimotor cortex (day 0) followed by the same lesion of the right sensorimotor cortex (day 68). Rat #14 is the only one that showed recovery of affected forepaw after both lesions. The recovery time was between 36–38 days after the respective cortical lesion. Rat #15 showed recovery of only the right forepaw 74 days after the left cortical lesion. The group data are plotted in Figure 1. Error bars represent standard error of the mean.

Figure 3. Bilateral cortical lesion

Panels A, C, E, and G represent the left side of the brain at different positions relative to bregma, indicated by the numbers. The arrows indicate the lesion boundaries. Panels B, D, F, and H show the corresponding right side of the brain, which also has a cortical lesion. Arrows here also indicate lesion boundaries. A cross section through the midbrain (I) shows FG labeling in cell bodies of the red nucleus. Scale bar=100microns, cx=cortex, str=striatum, hipp=hippocampus, cc=central canal, m-red nucleus.

Figure 4. Cortical lesions ablate CST neurons

Panels A, C, E, and G represent the left side of the brain with cortical lesion at different positions relative to bregma, indicated by the numbers. The arrows indicate the lesion boundaries. Panels B, D, F and H show the right side of the brain with cortex intact and robust FG labeling in the CST neurons. Panels I and J are sections through the midbrain.

Scale bar=100 microns, cx=cortex, str=striatum, hipp=hippocampus, cc=central canal, rn=red nucleus.

Figure 5. Sensorimotor cortex lesion still impairs gripping ability after spinal cord injury
 Average grip strength (n=8) of the right and left forepaws before and after right lateral hemisection at C4 followed by right sensorimotor cortex lesion. Days -17 through -1 is the pre-injury period of baseline measurement, day 0 is the time of spinal cord injury, and day 43 was the time of cortical lesion. Error bars represent standard error of the mean.

Figure 6. Forepaw gripping ability of individual rats after cervical hemisection followed by cortical lesion

Grip strength of the right and left forepaws of individual rats before and after right lateral hemisection at C4 (day 0) followed by right sensorimotor cortex lesion (day 43). The group data are plotted in Figure 5. A description of the lesion (histological verification) is included in each graph. Error bars represent standard error of the mean.

Figure 7. Representative complete, incomplete and over-hemisection lesions in experiment two
Horizontal sections of injured spinal cord illustrate how lesion extent was classified. Panels A, D, and G are from rat # 10 and show a complete hemisection that fully transects the right side of the spinal cord at the level of the dCST (A), the central canal (D), and the ventral column of the spinal cord (G). Panels B, E, and H are sections from rat #5 and show an incomplete hemisection at the dCST (B), cc (E), and vc (H). Panels C, F, and I are sections from rat #3 and show an over-hemisection where the lesion damages the left side of the spinal cord at the dCST (C), cc (F), and vc (I). Scale bar=500 μ m, dCST=dorsal corticospinal tract, cc= central canal, vc=ventral column.

Figure 8. Gripping function depends on the testing procedure

Rats received a right lateral hemisection at day 0, and then a right cortical lesion on day 46 post hemisection. Each point represents the mean across all rats. The pre-injury values are indicated on the graph as days -11 to -1. Modified testing procedure began 13 days post cortical lesion (59 days post hemisection). Error bars represent standard error of the mean.

Figure 9. Forepaw gripping ability of individual rats from experiment three

Grip strength of the right and left forepaws of individual rats before and after right lateral hemisection at C4 (day 0) followed by right sensorimotor cortex lesion (day 46). The group data are plotted in Figure 7. A description of the lesion (histological verification) is included in each graph. Error bars represent standard error of the mean.

Figure 10. Representative complete, incomplete and over-hemisection lesions in experiment three Horizontal sections of injured spinal cord illustrate how lesion extent was classified. Panels A, D, and G are from rat #8 and show a complete hemisection that fully transects the right side of the spinal cord at the level of the dCST (A), the central canal (D), and the ventral column of the spinal cord (G). Panels B, F, and H are sections from rat #3 and show an incomplete hemisection at the dCST (B), cc (E), and vc (H), Panels C, F, and I are sections from rat #5 and show an over-hemisection where the lesion damages the left side of the spinal cord at the dCST (C), cc (F), and vc (I). Scale bar=500µm, dCST=dorsal corticospinal tract, cc= central canal, vc=ventral column.