Title

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Robotic Rehabilitator of the Rodent Upper Extremity: A System and Method for Assessing and Training Forelimb Force Production after Neurological Injury

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Abstract

Rodent models of spinal cord injury are critical for the development of treatments for upper limb motor impairment in humans, but there are few methods for measuring forelimb strength of rodents, an important outcome measure. We developed a novel robotic device—the Robotic Rehabilitator of the Rodent Upper Extremity (RUE)—that requires rats to voluntarily reach for and pull a bar to retrieve a food reward; the resistance of the bar can be programmed. We used RUE to train forelimb strength of 16 rats three times per week for 23 weeks before and 38 weeks after a mild (100 kdyne) unilateral contusion at the cervical level 5 (C5). We measured maximum force produced when RUE movement was unexpectedly blocked. We compared this blocked pulling force (BPF) to weekly measures of forelimb strength obtained with a previous, well-established method: the grip strength meter (GSM). Before injury, BPF was 2.6 times higher (BPF, 444.6 ± 19.1 g; GSM, 168.4 ± 3.1 g) and 4.9 times more variable ($p < 0.001$) than pulling force measured with the GSM; the two measurement methods were uncorrelated ($R^2 = 0.03$; $p = 0.84$). After injury, there was a significant decrease in BPF of 134.35 g ± 14.71 g ($p < 0.001$). Together, our findings document BPF as a repeatable measure of forelimb force production, sensitive to a mild spinal cord injury, which comes closer to measuring maximum force than the GSM and thus may provide a useful measure for quantifying the effects of treatment in rodent models of SCI.

Key words: grip strength; motor learning; motor system; rat; recovery of function; rehabilitation; robotics; spinal cord injury

Introduction

THERE ARE AN ESTIMATED 1,275,000 PEOPLE in the United States who have sustained an injury to the spinal cord. More than 55% of these injuries occur at the cervical level and thus lead to loss of voluntary control of the upper extremity.[1] Regaining function of the upper extremities is a top priority to increase overall quality of life.[2]

Upper extremity strength, which can be defined as the peak force production capability of an upper extremity muscle group, joint, or the whole limb, is an important aspect of motor function. It is one of the first measurements a clinician performs to assess upper extremity impairment after spinal cord injury (SCI), and indeed the widely used American Spinal Injury Association impairment scale depends on an estimate of strength made using manual muscle testing. Upper extremity strength is clinically important because it does not solely reflect muscle mass, but rather is strongly dependent on the ability of the central nervous system to recruit motor neuronal pools and therefore reflects integrity of descending motor tracts. Upper extremity strength also is important because it is critical for many activities of daily living. Loss of upper extremity

strength (and specifically, grip strength) is one of the best predictors of functional deficit after neurologic injury.[3–6]

Measuring upper extremity strength is relatively straightforward in humans because the assessor can instruct the subject to exert maximal effort against a transducer or, in the case of manual muscle testing, to resist an applied force. Such instruction is not possible with rodents. Thus, many of the most common assessments of rodent upper extremity impairment after cervical SCI, such as the food pellet reaching task[7] and the sticker removal task,[8] do not explicitly test forelimb strength. (For a thorough list of forelimb assessments for rat models, see Kleim and colleagues.[9]) There is, therefore, a mismatch between what is thought to be important clinically in cervical SCI and what is typically measured in studies of rodent models of cervical SCI. Hays and colleagues[10] recently began to address this mismatch in the context of a rat model of stroke. They trained rats to reach for a handle attached to a force transducer and pull it isometrically to reach a predetermined force level in order to receive a food reward. By gradually increasing the target force after the rats achieved consistency in reaching the current target force, they obtained an estimate of rat forelimb force generation capability.

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Here, we developed a quantitative measure of the strength of the rodent forelimb using an adaptive, reward-based approach that makes use of a robotic device. In this device, the Robotic Rehabilitator of the Rodent Upper Extremity (RUE), rats are placed inside a box where they must volitionally reach for and pull on a bar to bring a food reward within reach for eating. This bar connects to a robotic interface through which the force required to complete the pulling task can be modified. To estimate strength, rats were trained to retrieve food pellets against resistance by pulling on RUE and then in some trials, movement of RUE was blocked unexpectedly. We will refer to the peak force the animal generates in this condition as the "blocked pulling force."

This paper describes the use of RUE as a tool for the assessment of upper extremity force production in a rat model of SCI; a future paper will describe its use for rehabilitation (additional details also are available elsewhere[11–13]). We focused on a model of unilateral contusion at cervical level 5 (C5). In this paper, we compare the assessment of forelimb force production via RUE using a new method for measuring force production, the blocked pulling force (BPF) method, with the widely used Grip Strength Meter (GSM).[14–19] We hypothesized that the reward-based BPF method used with RUE would lead to increased motivation to engage in the pulling task and therefore higher forces measured, compared with the GSM. We further hypothesized that we could robustly measure the effect of a mild cervical SCI injury despite paw tone by focusing on the functional abilities of the rat to perform the task of reaching and pulling.

Methods

Animals

Sixteen rats were used (female Sprague-Dawley; Harlan, Inc., San Diego, CA) that were 210–240 g (222.68 ± 2.16 g) at the beginning of the experiment and between 3 and 4 months of age. All the rats were right-paw dominant as determined by their preference to pull with the right hand during the GSM assessments and interaction with RUE. The Institutional Animal Care and Use Committee at the University of California, Irvine, approved all experimental protocols utilized in this experiment.

Rats were handled for three weeks prior to device acclimation. They were maintained on a food-restricted diet in which they received 85% of a normal daily intake as a means to incentivize them to participate during training sessions. Rats were weighed weekly to monitor overall health.

Robotic rehabilitator of the rodent upper extremity (RUE)

We designed a robotic system, RUE, to assess and train the forelimb strength of rats in a self-initiated task that consisted in reaching for and pulling on a bar to retrieve a food reward. The system includes a one degree-of-freedom resistance-based trainer, an automated food reward mechanism, and an acrylic box (15.25 × 30.50 × 30.50 cm) to house the rats during training (Fig. 1A). The width of the box is divided into two halves by means of an opaque acrylic divider to force the rat to pull the bar with a specific forelimb. All assessments in this study were carried out using the right paw.

Rats interacted with the trainer by means of a custom-made metal bar coupled to a voice coil actuator (VCS10-023-BS-01-MH; H2W Technologies Inc., Santa Clarita, CA) that generated the prescribed forces during the assessments. In its resting position, the bar was placed 3 cm above the floor of the acrylic box and in its resting position was within reach for the animals inside the acrylic box (Fig. 1). A linear potentiometer (LCP12A-25-10K; ETI Systems, Carlsbad, CA) was coupled to the voice coil actuator to measure its position. The resistance-based trainer was programmed to behave as a linear spring where resistance force was controlled by varying the spring's stiffness coefficient.

Successful trials were defined as those where the animal pulled past a distance of 2.3 cm and were rewarded with 20 mg chocolate-flavored food pellets (Bio Serv, Frenchtown, NJ). The pellets were delivered to a custom-made food tray placed on the opposite end of the metal bar (Fig. 1B) using an automatic food pellet dispenser (ENV-2030-20; Med Associates Inc., St. Albans, VT) or by placing them in the tray by hand. In its resting state, the bar was retracted so that the food tray remained out of reach behind the front acrylic panel. The reinforced behavior was to bring the food tray within reach by pulling on the bar (Fig. 1C) far enough forward to retrieve the food pellet with the mouth (Fig. 1D). We programmed the robot to hold the bar in the feeding position for two seconds once the rat pulled the food into the chamber and past the 2.3 cm distance. After this period, the robot retracted to its resting position with a large force. There was a possibility that if the animal refused to let go the paw would have been dragged into the window and injured. However, we did not see evidence of this, and the rats never stopped engaging with the device.

We used a USB Data Acquisition board (NI-6009; National Instruments, Austin, TX) interfaced with MATLAB (The MathWorks Inc, Natick, MA) to control the robot and record data at a sampling rate of 1000 Hz. A custom-made user interface was created in MATLAB for the animal trainers to set up and run the assessments.

Measuring forelimb force production

We measured the forelimb force production of each rat once per week using both the GSM and RUE. Concurrent assessments were conducted for 87 days pre-lesion and 200 days post-lesion. On 2 other days each week, all rats performed 3 min strength training sessions with RUE. Briefly, rats were trained with either a constant force—set at a value much lower than their maximum strength—or with an adaptive algorithm that progressively required increasing force production so as to assess maximum strength.[11–13]

GSM. We followed the procedure previously developed for use with the GSM in SCI models.[14] Briefly, the rats were held around the midsection with one forearm restrained by the experimenter and the unrestrained forepaw was brought in contact with the GSM (TSE Systems; Sci Pro, Inc., Sanborn, NY). The rat was held in place until it grasped the bar and then was gently pulled away from the bar by the experimenter. To discount the possible influence of spasticity, the experimenter performed a visual inspection of the hand after the rat released the bar, as specified previously for this assessment.[15] If the fingers remained in a clawed position, then the experimenter registered the measurement as a zero.[15] If the fingers did not remain in a clawed position, the grip strength was defined as the maximal force recorded by the GSM before the rats released the bar. This maximal force was recorded for four attempts and its average computed in each assessment.

RUE. The procedure for the robot-based assessments on the assessment day was as follows. First, the rat was allowed to pull for four to five times without resistance on the bar to reinforce the task-reward pairing. The bar was then blocked by increasing the stiffness coefficient of the robot to a value above the rat's pulling capability. Finally, the rat was allowed to pull for three to five repetitions while the bar was in the blocked state. We then recorded the maximum pulling force exerted during the blocked trials as the rat's maximum forelimb strength for each assessment session. Because the rat voluntarily reached for and gripped the bar and therefore had to voluntarily extend its digits and wrap them around the bar before pulling, it would have been unable to properly complete the task if spasms were present. We therefore did not exclude trials due to the rats' paw configuration after pulling.
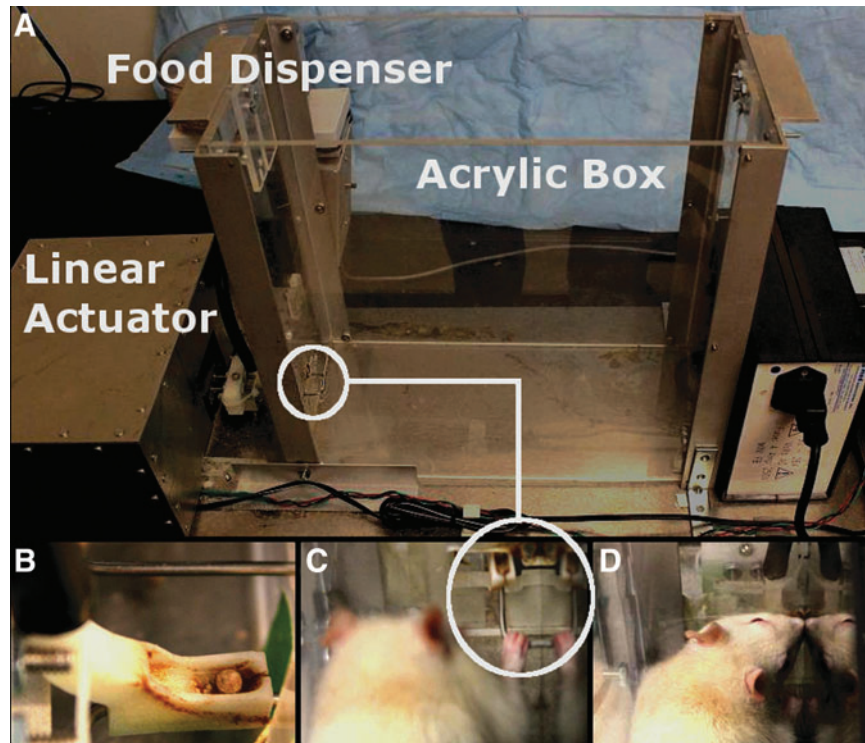
FIG. 1. Robotic Rehabilitator of the Rodent Upper Extremity (RUE). **(A)** The device is composed of a low-friction, backdriveable linear actuator, automated food dispenser, and an acrylic box to house the animal during training. Animals were trained to retrieve a food reward, **(B)** placed outside of the acrylic box and out of their reach, by pulling on a bar **(C)** that was coupled to the linear actuator. Once the animal pulled the food through the window, the animal could retrieve it with the mouth **(D)**. Color image is available online at www.liebertpub.com/neu

Lesion of the spinal cord

For surgery, rats were anesthetized with 1.5–3.0% isoflurane (Western Medical Supply, Inc., Arcadia, CA). Hair overlaying the cervical vertebra was removed by shaving with clippers, the skin was treated with betadine and incised, and the multiple muscle layers overlaying the cervical vertebral column were bluntly dissected. A dorsal laminectomy at C5 was performed with rongeurs. The rat was placed in the stabilizing platform with Addison forceps providing stability rostral and caudal to the laminectomy. The impactor probe (2.5 mm probe) was centered over the exposed spinal cord to the right of the dorsal vein. Unilateral mild lesions with 100 kD of force were created using the Infinite Horizons (IH) Impactor (Precision Systems & Instrumentation, Lexington, KY). After generating the lesion, the muscle was sutured in layers with 5-0 chromic gut (Henry Schein, Melville, NY) and the skin was closed with 9 mm staples (Fisher Scientific, Pittsburg, PA).

Following surgery, rats were immediately placed on a water-circulating heating pad until they recovered from the anesthetic. Post-surgical care included delivery of lactated Ringer's (5 mL/100 g, subcutaneously) for hydration for 3 days and Baytril (enro-flaxacin 2.5 mL/kg, subcutaneously; Western Medical Supplies, Arcadia, CA) for 7 days for prophylaxis against urinary tract infections. The analgesic Buprnex (buprenorphine, 0.01 mg/kg; Western Medical Supplies) was given for 3 days for pain management. Staples were removed at 14 days post-injury. Rats were housed three to four per cage and were monitored twice daily for general health, coat quality (indicative of normal grooming activity), and mobility within the cage. Rats with cervical contusion injuries typically resume these activities the day following injury. In addition, signs of paralysis were monitored, including lack of hind limb movement, tail flaccidity, and unstable/uncoordinated movement. Rats also were monitored for skin lesions on the paralyzed limbs or autophagia of the toes.

Histology

At the end of the testing period (approximately 321 days post-injury), rats were killed with an overdose of Euthasol® (Delmarva Laboratories, Inc., Richmond, VA) and perfused with 4% para-formaldehyde. Dissected spinal cords were post-fixed in 4% para-formaldehyde in 0.1 M buffered phosphate at 4°C. Whole spinal cords were cryo-protected in 27% sucrose prior to embedding in Tissue-Tek® (VWR, Radnor, PA) and flash freezing. A 7 mm segment of the spinal cord centered around the lesion epicenter was collected. Cross-sections measuring 20 μm were taken and thaw mounted onto microscope slides. Sets of three slides were made such that sections on each slide were 200 μm apart and the full 7 mm length of the spinal cord was represented on each slide.

Staining

One set of slides was stained with Ehrlich's Hematoxylin and Eosin. Sections were washed in phosphate-buffered saline (Fisher, Pittsburg, PA), dehydrated through graded ethanols, and de-fatted in Xylenes (Fisher, Pittsburg, PA). Slides were hydrated through graded ethanols to water, stained in Ehrlich's Hematoxylin, washed in water, differentiated in 1% hydrochloric acid in 70% ethanol, washed again in water, blued in 10% ammonium hydroxide, washed again in water, then equilibrated in 95% ethanol before staining in eosin. Excess eosin was removed in 95% ethanol, the slides completely dehydrated in 100% ethanol, cleared in Xylenes, and cover-slipped with DPX.

Data analysis

We used ImageJ software (National Institutes of Health, Bethesda, MD) to measure the areas of healthy and injured gray and white matter. We focused on those sections at or near the lesion

epicenter. Values were expressed as a percentage of the ratio between injured to non-injured halves. The reduction in size was calculated as the ratio between the injured to the uninjured side. We calculated this for the overall hemicord, gray matter alone, and white matter alone.

We measured the reduction in force capability for each rat using a linear regression model (Equation 1; $BPF = \beta_0 + \beta_1 * DPI + \beta_2 * InjuryState + \beta_3 * DPI * InjuryState$), where the BPF was regressed on two factors: 1) the days post-injury (DPI), β_1; and 2) the injury state (set as 0 pre-injury and 1 post-injury), β_2, and their interaction, β_3. The reduction in force capability was thus defined as the absolute change in force (in grams) from pre- to post-injury defined by β_2. β_0 is the y-intercept of the overall model.

Essentially, this means we fit a line to the pre-injury measurements over time, then a second line to the post-injury measurement over time, then measured the difference in the values of the lines at the injury time-point. All statistical analyses were done using MATLAB (The MathWorks Inc, Natick, MA). All measurements reported as significant fall below a significance level of $\alpha = 0.05$.

Results

Exclusion of animals from the analysis

Five of the 16 rats were excluded from portions or all the analysis. One rat did not learn to pull with RUE and was excluded from both the pre- and post-lesion analysis of BPF. Three rats were excluded from the post-injury data analysis following histological review of their lesions; two had not sustained an injury and the other one had a lesion that spanned both sides of the spinal cord. The fifth rat was removed from the post-lesion analysis because it did not engage in the pulling task following the injury. These last four rats were included in the pre-lesion analyses as the exclusion criteria described was inapplicable before the lesion.

Measurements of forelimb force production

During their twice-per-week training sessions, rats pulled on the bar with either low-force or adaptively increasing forces to retrieve the food. During their once-a-week assessment session, we blocked the bar. In this case, the rats increased their pulling force to a value at least 1.9 times greater than the peak force during the training trials (Fig. 2).

Pre-injury, both the RUE and the GSM successfully measured forelimb pulling force for all rats. BPF measurements with the RUE were, on average, 2.64 times higher than the forces measured with the GSM (Fig. 3C; BPF, 444.6 ± 19.1 g; GSM, 168.4 ± 3.1 g), a significant difference (t-test, t[29] = −19.1; $p < 0.001$), and the two methods were uncorrelated (Fig. 3D; $R^2 = 0.03$; $p = 0.84$). Additionally, measurement variability within rats was, on average, 4.86 times greater with the RUE than with the GSM, a significant difference (Fig. 3B; F-test, F[15,14] = 0.03; $p < 0.001$). The BPF was consistent across weeks, increasing slightly. The test–retest reliability (ICC2,1) of the GSM and BPF were 0.24 and 0.61 respectively.

Following injury, we recorded non-zero BPF with RUE for all available rats, but recorded GSM force measurements for only one rat (Fig, 3E) because the other rats failed to meet the paw-placing criterion of placing all fingers on the bar. BPF decreased after the cervical SCI by an average of 134.35 g ± 14.71 g (or $28.3 \pm 2.7\%$ relative to pre-injury BPF), a significant decrease (Fig. 3B; t-test, t[10] = −9.13; $p < 0.001$).

Histology analysis

There was a $16.9 \pm 6.0\%$ decrease in the surface area of the white matter of the injured side relative to the control side (Fig. 4; t-test; $p = 0.01$). There was also a decrease in the surface area for the gray matter of $56.8 \pm 4.9\%$ for the injured side relative to the uninjured side (Fig. 4, one-sample t-test; $p < 0.001$), compared with the control side. The injured hemicord (right hemicord) decreased in size by $30.8 \pm 4.8\%$ relative to the control hemicord (left hemicord; Fig. 4, t-test; $p < 0.001$).

We quantified the effect of the reduction in size of the gray and white matter on the performance with RUE by regressing the change in BPF from pre- to post-injury with the reduction in lesion volume of the injured side. There was a tendency for larger force loss with larger reductions in both white and gray matter, but the regression was not statistically significant (Fig. 5; white matter, $R^2 = 0.28$, $p = 0.10$; gray matter, $R^2 = 0.24$, $p = 0.13$). There was a similar trend in total hemicord area, but the regression was not statistically significant (Fig. 5; $R^2 = 0.32$; $p = 0.07$).
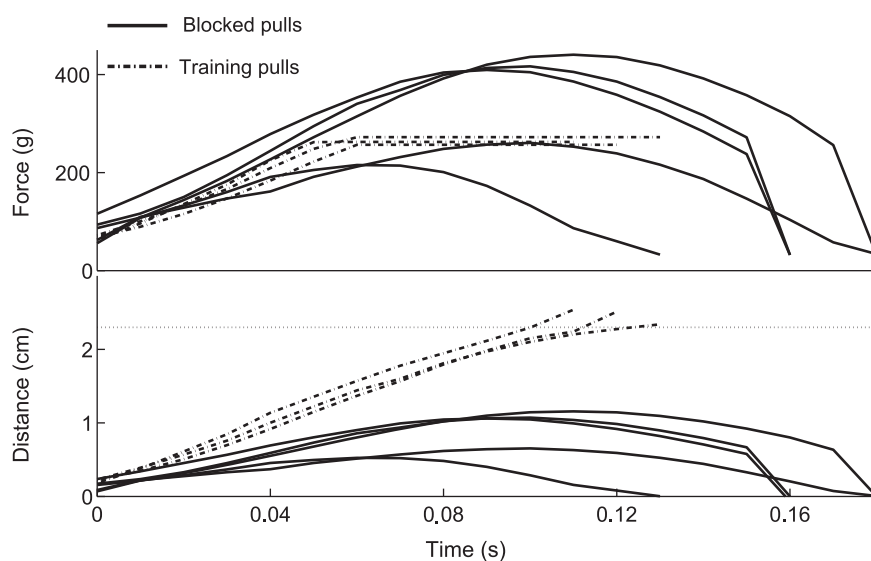

FIG. 2. Sample pulling forces from one rat during a training session (dashed) and a blocked pulling assessment session (solid) immediately prior to the injury. When the bar was blocked, the rat exerted more force, but never succeeded in pulling the bar the 2.3 cm needed to bring the food pellet into the chamber. For the last two blocked pulls, the rat exerted less force.
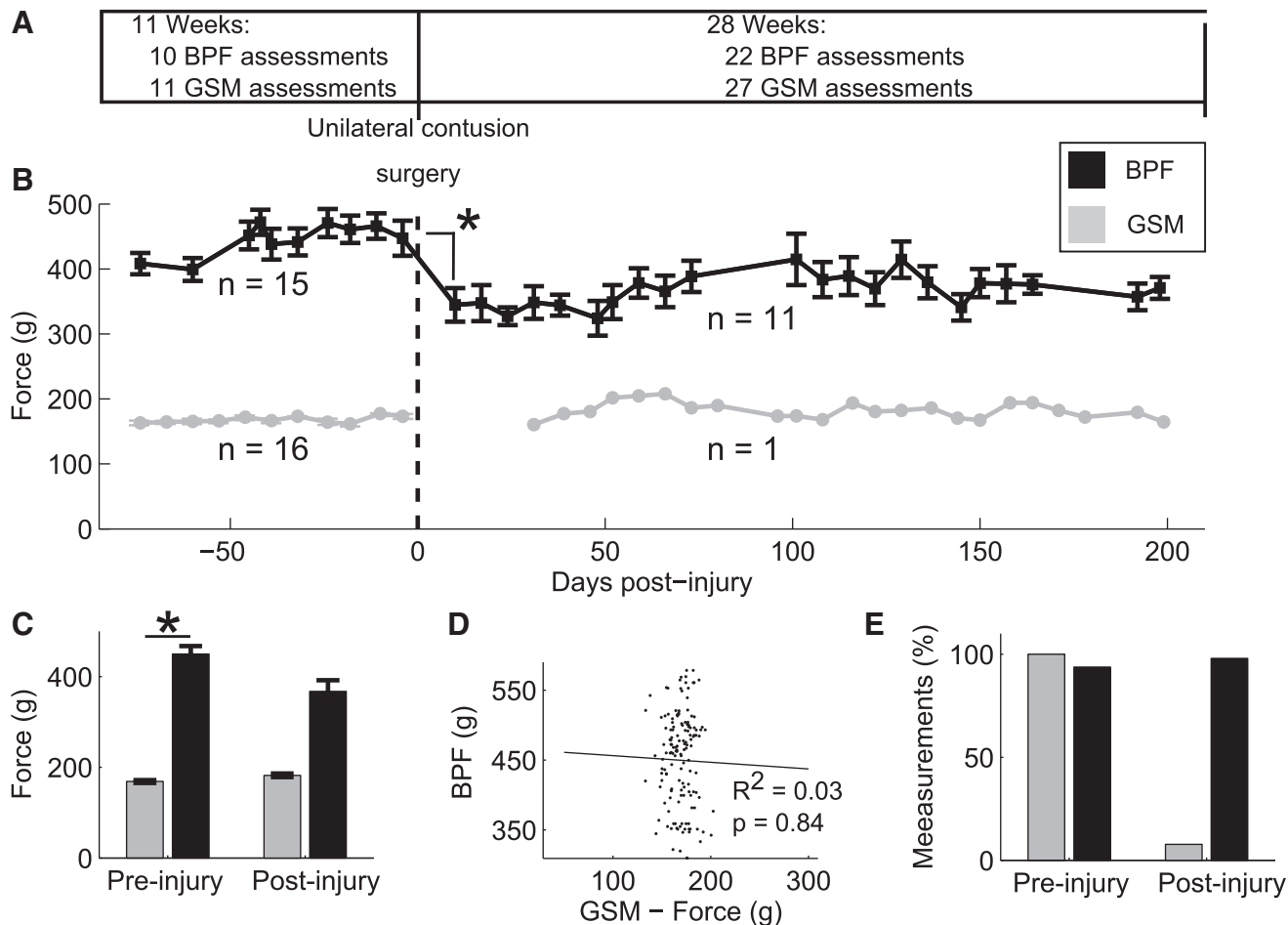
FIG. 3. Assessment of forelimb pulling forces. **(A)** Timeline of the study. Rats trained for 11 weeks pre-lesion and were assessed 10 times with the Robotic Rehabilitator of the Rodent Upper Extremity (RUE) and 11 times with the grip strength meter (GSM). They then trained for 28 weeks post-lesion and were assessed 22 times for blocked pulling force (BPF) with the RUE and 27 times with the GSM. **(B)** Mean pulling force over time for both methods. The RUE measured a significant decrease in force after the injury of $134.35 g \pm 14.71 g$ (*t*-test, t[10]$= -9.13$, $p < 0.001$). The sample size, *n*, dropped post-injury for BPF because of lack of lesion ($n = 2$), bilateral lesion ($n = 1$), and failure to pull ($n = 1$). The sample size dropped to $n = 1$ for GSM measurements because the other rats failed to meet the paw-placing criterion of placing all fingers on the bar **(C)** Prior to the injury, BPF measured with the RUE was on average 2.6 times higher than pulling force measured with the GSM (*t*-test, t[29]$= -19.17$, $p < 0.001$); **(D)** BPF and GSM measurements were uncorrelated. **(E)** Percent of rats from which measurements were successfully obtained.
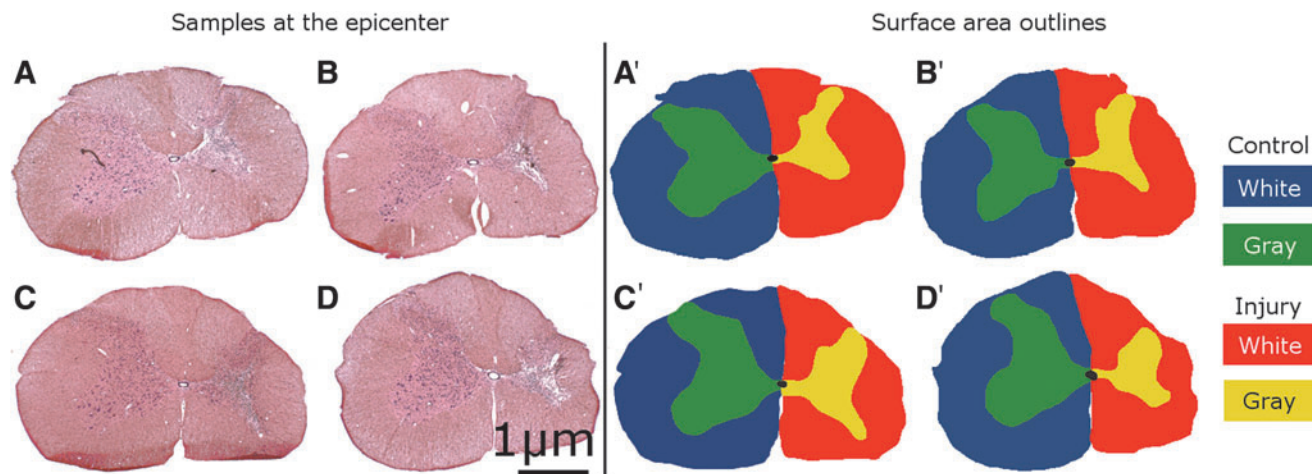

FIG. 4. Samples of the epicenter of the lesion. **(A-D)** Representative samples of cross sections at the epicenter of the unilateral contusion injury. **(A′-D′)** Samples of the selections of white and gray matter for each hemisphere used to quantify the reduction in area. There was a significant decrease in the surface area of both the white ($16.9 \pm 6.0\%$; *t*-test, $p < 0.001$) and gray matter ($56.8 \pm 4.9\%$; *t*-test, $p < 0.001$), compared with the uninjured side. Color image is available online at www.liebertpub.com/neu
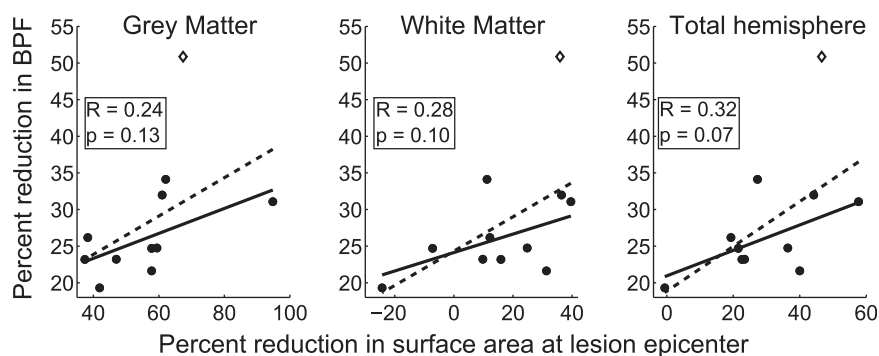
FIG. 5. Reduction in white and gray matter and its relationship to reduction in blocked pulling force (BPF). There was one rat (marked with the diamond) whose BPF was reduced much more than the other rats; this rat was not removed from the analysis since its histology did not give us a reason to do so. Here, we show both fits (i.e., including the diamond [dotted line], and not including the diamond [solid line]) and in each plot report the regression parameters that include the diamond. There was a trend for relationship between BPF and white matter area at the lesion epicenter, but the regression was not statistically significant (dotted line, $p = 0.096$; solid line, $p = 0.13$). The same was true for the relationship between BPF and gray matter area (dotted line, $p = 0.13$; solid line, $p = 0.07$), and the total hemisphere area (dotted line, $p = 0.07$; solid line, $p = 0.08$).

Discussion

Here, we report the development of the RUE, and its use to assess the forelimb strength of rats before and after a unilateral cervical SCI using the BPF paradigm. RUE has several potential advantages for measuring forelimb force production over current standard methods, such as the Grip Strength Meter, and it could provide a useful new tool for rodent models of cervical SCI.

Comparison with other assessments of forelimb force production

To address the need to study forelimb force production in rodent models, researchers have developed techniques for assessing upper extremity strength in rodents. In the pasta matrix reaching task used in rodent models of stroke,[16] rats are presented with varying diameters of pasta and the experimenter measures the maximum pasta diameter the animal can break.[16] A limitation of this approach is that the measurement resolution is determined by the number of pasta diameters presented.

In another approach to assess the forelimb strength of rats after a stroke, Hays and colleagues recently developed the "isometric pull task."[10] They trained rats to pull on an isometric bar attached to a force transducer; if the rat exceeded a target force, it received a pellet food reward. An experimenter progressed the rats to higher target force levels in six predefined stages, such as, for example, being able to obtain 30 pellets in a 30 min training session. Before inducing a stroke, the final target force was 120 g, which the rats had to achieve with 85% success for ten consecutive sessions. Forelimb strength was then defined as the mean peak force the rats achieved in these sessions. After the induced stroke, the rats were again presented with the 120 g target force for four sessions. They achieved this force only on 35% of the pulls; forelimb strength was again measured as the average peak force on these pulls and declined significantly.

For the uninjured animals in both studies, the forelimb strength measured with the isometric pull task was much lower than the BPF measured with RUE (isometric pull task, 154 ± 3 g vs. RUE, 446.6 ± 19.1 g) even though both sets of rats were similar (female Sprague-Dawley, about 4 months old, starting weights of around 250 g, and food deprivation of 85% body weight). These differences in force measurements likely relate to several differences in the

protocols. Most importantly, the use of the BPF paradigm seems to have motivated a greater effort from the rats, presumably because they were conditioned to expect a reward at a certain force, and then reacted by pulling more strongly when the bar became "stuck" (Fig. 2). Second, rats in RUE trained closer to their maximum pulling strength because the adaptive algorithm changed—for each individual rat—the force required to complete the task based on the success of the previous trial (instead of the previous session). Third, the food reward in RUE was placed directly on the bar that the rats pulled, which also may have led the rats to pull closer to their maximum strength. And finally, the orientation of the bar (horizontal in RUE and vertical in the isometric pull task) required the activation of different sets of muscles. Whatever the reason, the BPF paradigm with RUE appears to obtain a closer estimate of maximum pulling force. Note that the BPF paradigm could be implemented with the isometric pull task simply by disconnecting the force from the reward dispenser on randomly selected trials. However, an advantage of the isometric pull task paradigm is that slowly grading the required force level in stages appears to have resulted in less variability in the peak force measurements.

The final approach that we will discuss is the GSM,[14,15,17–20] which measures the force at which an animal releases its grip from a bar as an experimenter pulls its body away from the bar. While it is clear that animals prefer to grasp a bar presented to them, it is uncertain if the GSM measures strength in the usual sense of "peak voluntary force." Despite this uncertainty, the test has good reliability and has been used successfully to assess the effects of cervical SCI on forelimb function cervical SCI.[14,15,18–20]

A further caveat of using the GSM in cervical SCI is that cervical SCI can create abnormal muscle tone that leaves the paw in a clawed position. Measurements with the GSM in this case may not reflect voluntary control of the animal's grip. That is, the experimenter could hook the paw onto the GSM, passively extend the forelimb to the end of its range of motion and then measure resistance to paw release caused solely by the paw/forelimb tone. To account for this, a standardized procedure has been developed in which experimenters perform a visual inspection of the paw after the animal releases the bar; if the digits remain in a clawed position then the measurement is registered as a zero.[15] This strict criterion for an appropriate grip may cause subtle motor improvements to be unaccounted. Thus, some studies, including the present one, have

been unable to record satisfactory GSM measurements, especially immediately after cervical SCI.[14,18]

RUE measured forelimb force production based on the forces exerted during a food-motivated, self-initiated, blocked pull. When rats were trained to retrieve food mounted on a bar by pulling the bar to their mouths, then that bar was unexpectedly blocked from moving and rats exerted an increased force. Using this paradigm, the pulling forces were on average 2.6 times higher than those measured with the GSM. This difference reveals that rats are capable of exerting more force than is measured by the GSM and the BPF method is closer to measuring peak pulling force than the GSM. Nevertheless, it is still not possible to conclude that RUE measures maximal force that rats are capable of exerting. That is, RUE measurements are not necessarily an accurate measure of strength. For now, then, we prefer to refer to the force measured as the BPF, rather than strength, although it is clear that BPF measures volitional, high effort force production. An important point to consider when using the BPF is that it measures both the grip and pull strength. That is, if a rat cannot properly grip the bar, then it cannot pull the bar to get a reward, and if it can grip the bar, but it cannot pull the bar, then similarly it cannot get the reward. This is also the case when using the GSM since the rat also has to grip the bar and then resist when it is being pulled away.

RUE measured a significant decrease in BPF following even the mild SCI used in this study. Thus, BPF is sensitive measure of the effects of SCI with direct clinically relevance. Although the increased variability of BPF, compared with GSM measurements, may confound evaluations of therapeutic outcomes, the increased variability likely reflects the variability in voluntary drive that one would expect when engaging in this pulling task, and may thus be a necessary consequence of measuring voluntary strength. There may be ways to tune the protocol to maximize rat motivation to decrease variability.

Histological analysis revealed a trend for a relationship between gray matter sparing and forelimb strength although the correlation was not statistically significant. Previous work has suggested that weakness after spinal cord injury and stroke can result from either motoneuron loss, or a decrease in the quantity of descending fibers transmitting motor signals to the motorneuronal pools of the spinal cord, resulting in decreased recruitment of those pools.[21] Thus, BPF may be sensitive to regeneration treatments targeting either motoneuron loss or regeneration of white matter tracts following SCI.

Unlike the GSM, RUE involves volitional pulling (rats reach out and pull on the bar), so the measures cannot be produced as a result of paw spasticity coupled with the force exerted by the investigator. GSM testing protocols following cervical SCI require careful observation of paw placement and omission of measurements that could be produced by a spastic grip. This can be the majority of measurements, which reduced the data base in the present study. With RUE, the rat may grip the limb with a spastic paw, but the total limb pulling force must be actively generated by the rat.

RUE measurements were more variable than the GSM measurements. This is consistent with the idea that the RUE measures volitional pulling force that depends on the motivation level of the animal, which might be expected to vary depending on hunger, time of day, pain, etc. RUE measurements also may be more variable because the animal was allowed to grasp or hook the bar in any way it chose. Variations in grasping posture or even whole body posture may affect the force with which a rat can pull.

On the other hand, compared with the GSM, RUE required a more complex and time consuming training and testing protocol. To motivate participation in the task, rats were food-restricted to 85% their normal food intake. Rats were then habituated and shaped to RUE over two weeks prior to any training.

RUE as a rehabilitation tool

RUE may be useful not only as an assessment tool, but also as a means to rehabilitate forelimb strength, thereby simulating the neurorehabilitation process. Although there was no increase in BPF with repetitive training after the cervical SCI on a group level, the percentage of strength recovery, relative to the pre-lesion abilities of each rat, significantly increased in the group that experienced adaptive strength training that challenged them each training day.[11–13] If training with RUE can increase forelimb force production, the device may be useful for enhancing neuroplasticity and neuroregeneration in rehabilitation treatments[22,23] The training paradigm presented can readily be used in the treatment of other neurological injuries, such as more severe SCI, and brain injury, such as stroke; RUE is currently being used by other research groups to study the motor effects and rehabilitation of these types of injuries.

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