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Challenges of animal models in SCI research: Effects of pre-injury task-specific training in adult rats before lesion

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Abstract

A rarely explored subject in animal research is the effect of pre-injury variables on behavioural outcome post-SCI. Low reporting of such variables may underlie some discrepancies in findings between laboratories. Particularly, intensive task-specific training before a SCI might be important, considering that sports injuries are one of the leading causes of SCI. Thus, individuals with SCI often underwent rigorous training before their injuries. In the present study, we asked whether training before SCI on a grasping task or a swimming task would influence motor recovery in rats. Swim pre-training impaired recovery of swimming 2 and 4 weeks post-injury. This result fits with the idea of motor learning interference, which posits that learning something new may disrupt learning of a new task; in this case, learning strategies to compensate for functional loss after SCI. In contrast to swimming, grasp pre-training did not influence grasping ability after SCI at any time point. However, grasp pre-trained rats attempted to grasp more times than untrained rats in the first 4 weeks post-injury. Also, lesion volume of grasp pretrained rats was greater than that of untrained rats, a finding which may be related to stress or activity. The increased participation in rehabilitative training of the pre-trained rats in the early weeks post-injury may have potentiated spontaneous plasticity in the spinal cord and counteracted the deleterious effect of interference and bigger lesions. Thus, our findings suggest that pre-training plays a significant role in recovery after CNS damage and needs to be carefully controlled for.

1. Introduction

Spinal cord injury (SCI) results in deficits of sensory, motor and autonomic function below the level of the injury. Thus, depending on its severity, SCI may lead to significant disability and to a steep drop in the quality of life. Unfortunately, no truly successful treatments exist for SCI including the most common intervention, rehabilitative training (Fouad & Tetzlaff, 2012). One of the reasons for a lack of effective treatments is the challenge in reproducing

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results with animal models, let alone human subjects (Filli & Schwab, 2012). The complexity of animal models is frequently underestimated in part due to missing details in published research, a challenge that could be rectified by applying reporting standards as proposed by Lemmon and colleagues (Lemmon et al., 2014). For example, the presence/absence of outliers, treatment side effects and negative outcomes are rarely revealed. Another factor that is frequently ignored and not described sufficiently is the training of the animals prior to experimental injury. This is important information, given that treadmill exercise in rats was reported to increase levels of brain-derived neurotrophic factor (BDNF; Gomez-Pinilla et al., 2001) and other factors associated with plasticity, such as neurotrophin-3 (NT-3; Ying et al., 2003). Elevation of BDNF levels as a result of training/exercise counteracts a SCI induced decline in BDNF (Gomez-Pinilla et al., 2012). BDNF is a neurotrophin associated with cell survival, neurite outgrowth, remyelination, and neuroprotection (Weishaupt et al., 2012). Hence, animals trained before SCI might display a better recovery following injury than animals left alone in their cages.

In addition, recovery following nervous system injuries frequently involves compensation, or the implementation of a new movement strategy to make up for the loss of function (Hurd et al., 2013). Consequently, one could hypothesize that pre-training a task will make it more challenging to learn a compensatory strategy following injury. This is based on findings that rehabilitative training in one task may interfere with performance in another task in animal models (Girgis et al., 2007; de Leon et al., 1999). In human subjects, a related phenomenon, termed proactive interference, occurs when learning a new motor skill is adversely affected by a previously learned skill. This phenomenon has, for example, been reported in subjects who first learned the forehand tennis stroke and then had difficulty learning the backhand stroke (Eason & Smith, 1989). Thus, the role of pre-injury training of specific tasks could translate easily to the clinical setting. It may be that the highly variable pre-injury activities and types of exercise humans engage in could influence treatment and rehabilitation decisions and ultimately functional outcomes after injury. Therefore, for the present studies, we explored the effect of task-specific training applied pre-SCI on functional recovery using two different rat models of SCI and two different motor tasks; grasping in animals with a cervical dorsal quadrant lesion and swimming in animals with a thoracic contusion lesion. We chose these models to examine a unilateral fine-motor task with the former (Whishaw et al., 1998) and a bilateral locomotor task with the latter (Smith et al., 2006). The two paradigms involve the use of compensatory movement strategies, which might be susceptible to motor learning interference. Specifically, rats often scoop or drag food pellets rather than actually grasp pellets following cervical SCI (Hurd et al., 2013), and after thoracic SCI, rats switch from relying on their hindlimbs to their forelimbs to swim (Smith et al., 2006).

2. Materials and Methods

2.1. Subjects

For the grasping study, adult female Lewis rats (N = 20; Charles River Laboratories, Wilmington, MA, USA) were housed in groups of 5 rats per large (18" × 14") cage. For the swimming study, adult female Sprague-Dawley rats (N = 14; Harlan, Indianapolis, IN, USA)

were housed 2 per cage in smaller (10" × 18") cages under a 12:12h light-dark cycle. All the animals were acclimatized to their respective facility for a minimum of 1 week prior to the start of the experiments. For each study, rats were divided into two groups, the pre-trained group ($n = 10$ & 7, respectively) and the non-pre-trained group ($n = 10$ & 7 respectively). For the grasping study, all the animals were food restricted to 9 g per animal on the day before training sessions and were otherwise fed *ad libitum*. Pre-trained rats received daily grasping (SPG) training for 4 weeks before the injury, while the non-pre-trained rats did not.

For the swimming study, pre-trained animals were swim trained daily, receiving 6 × 4 minute swim sessions 5 days a week for 4 weeks as described previously (Magnuson et al., 2009). During this same period of time, untrained animals were moved to/from the exercise room and were handled daily. In both studies, subject weights ranged between 210–240 g at the time of the lesion. Experimental procedures were approved by the University of Alberta Health Science Animal Care and Use Committee (Grasping) or by the University of Louisville Institutional Animal Care and Use Committee (Swimming).

2.2 Surgery

Grasping Study—Rats received a dorsolateral quadrant (DLQ) spinal cord lesion at the cervical level on the same side of their preferred paw, ablating the ipsilateral corticospinal (CST) and rubrospinal (RST) tracts. Surgical procedures were carried out under isoflurane anaesthesia (5% for induction; 2.7–3% for maintenance). Once anaesthetized, the rats were shaved and mounted into a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). The surgical site was disinfected with chlorhexadine digluconate (Sigma-Aldrich Canada Ltd., Oakville, ON, Canada) and the eyes were protected with the lubricant Tears Naturale (Alcon Canada, Inc., Mississauga, ON, Canada). A skin incision was made over the cervical region of the spinal cord and the muscles were dissected apart. This was followed by a laminectomy of the C4 vertebra. The lesion was performed at the C4 level with a custom-made surgical microblade. Muscles were sutured with vycril 5-0 (Johnson & Johnson Medical Pty Ltd., Sydney, NSW, Australia) and the skin was stapled with 9 mm stainless steel clips (Stoelting Co., Wood Dale, IL, USA). After the surgery, the rats were given 0.03 mg/kg of buprenorphine (Temgesic, Schering-Plough, Kirkland, QC, Canada) and 4.0 ml of saline. This was followed up by a 0.02 mg/kg dose of buprenorphine 8 hrs later and 2.0 ml of saline if the animals showed signs of dehydration.

Swimming Study—Rats received a moderate (12.5 g-cm) thoracic (T10) spinal cord contusion delivered by the New York University Impactor as described previously (Magnuson et al., 2009). Briefly, surgical procedures were carried out under Pentobarbital (IP, 50 mg/Kg) anesthesia and all animals received prophylactic antibiotics (Gentamicin, 15mg/Kg sc). Once anaesthetized, the surgical site was shaved and disinfected with chlorhexadine digluconate (Sigma-Aldrich, St. Louis, MO). The eyes were protected with Lacri-lube (Allergan, Irvine, CA). A midline dorsal skin incision was made exposing the thoracic and upper lumbar spinal column and the muscles were dissected to expose the vertebral laminae from T8 to T11. This was followed by a laminectomy of the T9 vertebra and, following securing the vertebral column with clamps at T8 and T10, delivery of the contusion to the T10 spinal cord leaving the dura intact. Muscles were sutured with vycril

5-0 (Ethicon Endo-Surgery, Blue Ash, OH) and the skin was closed with stainless steel staples. After the surgery, the rats were given 0.05 mg/kg of buprenorphine (Henry Schein, Indianapolis, IN, USA) and 5.0 ml of saline. This was followed up by a 0.03 mg/kg dose of buprenorphine 8 hrs later and additional saline if the animals showed signs of dehydration.

2.3 Pre-training and Rehabilitation

Grasping Study—Pre-training and rehabilitation were undertaken based on Whishaw et al. (1998). The testing apparatus was a clear Plexiglas chamber (38.5 cm l × 12.4 cm w × 45 cm h), with a slit at the front through which the rats could grasp a sucrose pellet (45 mg/each) from a shelf. The pre-trained group received daily 10 min training sessions on the SPG task for 4 weeks 5 days a week before the surgery. During this time, the untrained group was habituated to the task conditions. Untrained animals were placed in the SPG chamber with the reaching slit blocked off for 10 min. In order to determine the paw preference of the untrained animals, the rats in this group were allowed to reach through the slit for 1–3 sessions before the lesion. Untrained rats were allowed to reach for more than one session if they failed to reach during the first session or if they reached with both paws, making their paw preference ambiguous. Rats from both groups received rehabilitative training on the SPG task for 6 weeks/3–5 days/week for a total of 25 rehabilitation days starting 1 week post-spinal cord injury. Grasping success rate was calculated by dividing the number of pellets grasped by the number of reaching attempts. The highest success rate achieved by each rat during rehabilitation was used to calculate the final average success rate. Weekly average success rates post-injury were also calculated. Some rats compensate after injury by dragging pellets across the shelf and shoveling the pellets into their mouths (termed “scooping”), rather than grasping the pellets “properly”. To quantify this type of compensation, the number of pellets scooped was divided by the number of attempts, and the averages/week were compared between groups.

Swimming Study—Swim pre-training and post-injury assessments were undertaken based on Smith et al., (2006) and Magnuson et al., (2009). Swimming was performed in a clear Plexiglass tank (150 cm l × 20 cm w × 30 cm deep) with a black neoprene covered ramp at one end onto which the rats could easily climb. The pre-trained group received 6 × 4 min swimming sessions daily, 5 days each week for 4 weeks, with the final session 3 days before the spinal cord injury surgery. Throughout the pre-training period, animals in the untrained group were handled daily and were transported to and from the training room with the trained animals. Rats from both groups received rehabilitative swim training, 4 days/week for 6 weeks post-spinal cord injury, as described previously (Smith et al., 2006; Magnuson et al., 2009). Swim training started 2 weeks post-injury with 2 × 4 min sessions each day and ramped up to the maximum of 6 × 4 min sessions over the first 2 weeks (8 days) of training. The initiation of swim training was delayed until 2 weeks post-injury in order to avoid stressing the cardiovascular system during the period of vascular permeability that is thought to make the injury epicenter and penumbra susceptible to increased inflammation (Smith et al., 2009). Swimming was assessed weekly (on Fridays) using the Louisville Swim Scale (LSS; Smith et al., 2006b) and hindlimb kinematics (Magnuson et al., 2009). Normal animals swim bipedally, with their hindlimbs only and use their forelimbs only for steering and obstacle avoidance. After a moderate thoracic SCI animals initially rely on their

forelimbs for propulsion, but quickly re-learn to use their hindlimbs. The LSS is a visual scale (0–17) based on forelimb dependence, hindlimb movement and alternation in addition to body position during a 4 min swimming session. Animals that score 0–5 rely heavily on their forelimbs for propulsion and show very little hindlimb movement, poor body angle (tail-down) and rotation (on their side). Animals scoring 6–11 utilize both forelimbs and hindlimbs for propulsion, but retain a poor body position and may not kick consistently with their hindlimbs. Animals scoring 12–17 display consistent hindlimb movement with alternation rely little on their forelimbs for propulsion and have a good body position in the water (parallel to the water surface and correct dorso-ventral orientation).

2.4 Other Behavioural Measures

2.4.1 Grasping Study

Movement Rating Scale: Video recordings were made at the end of the rehabilitation period with a high-speed video camera (IPX-VGA, 210 fields/s). Three reaches for the lesioned limb of each rat were qualitatively analyzed. A reach was broken down into 10 components and each component was rated on a 3-point scale. If the movement was normal, a score of (2) was given. If the movement was performed abnormally, a score of (1) was given. If the movement failed to occur, a score of (0) was given. In cases where there was uncertainty as to the presence of a movement, a score of (0.5) was given. The component movements analyzed were based on the description from Whishaw et al. (2003).

Horizontal Ladder Task: Rats were trained on the horizontal ladder task 5 weeks post-SCI for two sessions on 2 consecutive days with five runs a session. Testing was conducted 2 days after the last training session. The animals had to cross a ladder with unevenly spaced rungs from a neutral cage to reach their home cage. The home cage with cage mates served as the positive reinforcement. During testing, a camera (JVC, 60 fields/s) was mounted on a tripod in front of the ladder. Six runs were recorded for each rat, three in the left to right direction and three in the opposite direction. However, only three runs were assessed for each animal, those with the lesioned forelimb at the front in the recording. Frame by frame analysis of forelimb placements within a 60 cm stretch of the ladder was performed. Placements at the start or end of the ladder were excluded. Placements of the lesioned forelimb were rated on the foot fault 7-point scale (Metz & Whishaw, 2009), with higher scores indicating better paw placement.

2.4.2 Swimming Study—Hindlimb function during overground stepping was assessed pre-injury and every second week post-injury using the Basso, Bresnahan and Beattie (BBB) Locomotor Scale as described previously (Basso et al., 1995; Magnuson et al., 2005). Briefly, animals were observed moving about in an open field (empty children's wading pool, 39" in diameter) for 4 min. Hindlimb movements were scored by two experienced raters that were blind to the experimental groups. In addition to the LSS (described earlier) and the BBB scales, hindlimb function during swimming was objectively assessed using 2D kinematics, as described previously (Magnuson et al., 2009). Black sharpie marks were placed on the iliac crest (IC), the ischial tuberosity of the hip (H), the lateral malleolus of the ankle (A) and the metatarsophalangeal joint of the 4th toe (T). These markers were tracked in high-speed (60Hz) video (Basler 602f, Basler Inc., Exton, PA) taken during 3 pool laps

where the animal swam continuously and smoothly through the cameras field of view. A minimum of 6 complete stroke cycles were digitized using MaxTraQ (Innovision Systems, Columbiaville, MI) and angle-angle plots were created for the IC-H-A and H-A-T angles using a three-segment, two-angle hindlimb model that avoids the inaccuracies of the rodent knee (Magnuson et al., 2009; Kuerzi et al., 2010). The angle-angle plot areas were then calculated, representing the in-phase excursions of the two angles, a measure that is exquisitely sensitive to changes in swimming stroke kinematics (Magnuson et al., 2009).

2.5 Perfusion, Tissue Collection and Histology

Grasping Study—Rats were euthanized with a pentobarbital overdose (Euthanyl; Biomedica-MTC, Cambridge, ON, Canada), and perfused with 4% paraformaldehyde. The brain and spinal cord were then dissected and post-fixed overnight in 4% paraformaldehyde, after which the tissue was cryoprotected in 30% sucrose for 2–4 days. Spinal tissue was cut into blocks, covered in Tissue Tek (Sakura Finetek, Torrance, CA, USA), mounted onto filter paper and frozen at -60°C in 2-methylbutane (Fisher Scientific, Ottawa, ON, Canada). Tissue crosssections containing the lesion were cut at $25\text{ }\mu\text{m}$ in a Cryostar NX70 (Fisher Scientific, Ottawa, ON, Canada) and staggered on four slides (Fischer Scientific, Ottawa, ON, Canada). The slides were stored at -20°C until further processing.

Spinal cord cross-sections were counterstained with cresyl violet. Sections were warmed at 37°C for 1 hr and rehydrated in TBS for $3 \times 10\text{ min}$. Slides were then immersed in fresh 0.5% cresyl violet for 4 min in a fume hood. To remove excess cresyl violet dye, the slides were quickly dipped in distilled water before the tissue was serially dehydrated in increasing ethanol concentrations (50%, 75%, 99%, and 99%) for 2 min each. This was followed by $2 \times 2\text{ min}$ clearing steps in xylene. After clearing, the slides were coverslipped with permount.

Fifteen sections $200\text{ }\mu\text{m}$ apart including the lesion epicenter were analyzed. Images of the tissue were captured under bright-field microscopy and the image processing program ImageJ 1.43 μ (National Institutes of Health, Bethesda, MD, USA) was used to measure the area of necrotic tissue. Tissue was considered necrotic if cresyl violet stained inflammatory cells invading the tissue. The lesion volume (V_l) was obtained with the Cavalieri method (Hains, Saab, Lo, & Waxman, 2004) using the formula $V_l = \Sigma(a \times d)$ with (a) as the lesion area of each section and (d) as the inter-section distance. The total volume of the spinal cord block analyzed was extrapolated from the total area of a representative section rostral to the lesion epicenter. Percent lesion volume was calculated by dividing the lesion volume by the volume of the total spinal cord block. For two-dimensional qualitative analysis, lesions were reconstructed on schematics at the C4 level and the lesioned area was calculated as a percent of the total cross-sectional area. Additionally, spared white matter area was calculated as a percent of the cross-sectional area.

Swimming Study—Rats were euthanized with a pentobarbital overdose (250mg/Kg. i.p.) and perfused with 4% paraformaldehyde. Spinal cords were dissected out and post-fixed overnight, also in 4% paraformaldehyde. Spinal cords were then cryoprotected in 30% sucrose for 2–4 days, blocked to isolate the injury epicenter $\pm 3\text{mm}$, mounted in OCT cutting media and $30\text{ }\mu\text{m}$ sections were prepared on a Zeiss Microm cryostat and mounted on

charged microscope slides (Fisher Scientific, Pittsburgh, PA, USA) in five sets. One set of sections were stained using Eriochrome Cyanin, as described previously. A minimum of 6 sections were photographed, opened in iDraw (Apple Computer, Cupertino, CA) and densely stained “spared” white matter was traced using a Wacom Intuous drawing tablet (Wacom, Vancouver, WA). Percent spared white matter was determined using cross sectional areas in ImageJ as compared with age, gender and weight matched controls. The section with the minimum percent spared white matter was identified as the injury epicenter.

2.6 Statistical Analysis

Data were analyzed with GraphPad Prism 4 for the grasping study and with IBM SPSS v21 for the swimming study (GraphPad Software Inc., La Jolla, CA, USA; IBM SPSS v21, IBM, Armonk, NY, USA). For the grasping study, unpaired two-tailed t-tests were used to make between-group comparisons when the data followed a normal distribution. Normality was tested with the D’Agostino-Pearson omnibus test. Mann-Whitney U tests were employed otherwise. Repeated measures two-way ANOVA with Bonferroni post-test was used to compare weekly scooping and grasping performance during rehabilitation. Pearson’s correlation coefficient was used to determine whether there was a relationship between two variables. The data presented are mean values $\pm$ SEM. The threshold for significance was set at a P-value of < 0.05 . For the swimming study, LSS, BBB and angle-angle plot area data were analyzed with repeated measures ANOVA. Following a significant main effect, comparisons between groups were performed using Tukey HSD post-hoc t-tests. Data are presented as mean $\pm$ SD, and results are considered significant for P-values < 0.05 .

3. Results

3.1 Grasping Study

3.1.1 Single pellet Grasping—Reaching scores post-SCI were compared between rats that were trained to grasp for 4 weeks before the injury and rats that were not. Average weekly grasping scores were compared. Two-way factor analysis of variance revealed a significant main effect of time ($F(5, 90) = 6.31, P < 0.001$). Average weekly success rate improved over time. No significant main effect of treatment ($F(1, 90) = 1.74, P = 0.204$) was found. No differences in grasping performance between pre-trained and non-pre-trained rats were found (Fig. 1B). There was no significant interaction between time and treatment ($F(5, 90) = 0.427, P = 0.829$). Furthermore, the highest scores obtained by each animal during the 6 weeks of rehabilitation were compared between groups. In line with the weekly data, there was a non-significant trend for pre-trained rats ($36.6 \pm 10.4\%$) to successfully retrieve more pellets through the reaching slit than non-pre-trained rats ($24.1 \pm 11.5\%$; Fig. 1C). Use of compensatory movement strategies did not differ between the groups. There was no significant main effect of time ($F(5, 90) = 0.94, P = 0.461$), treatment ($F(1, 90) = 0.70, P = 0.413$) or a significant interaction between time and treatment ($F(5, 90) = 1.13, P = 0.350$). That is, pre-trained and non-pre-trained rats scooped pellets to a similar extent after injury (data not shown). Reaching frequency in the first 4 weeks of rehabilitation was also analyzed to determine if rats differed in their motivation to perform the task shortly after their injury. Rats trained before the injury reached significantly ($37.9 \pm 3.95; P < 0.05$) more times than untrained rats (27.9 ± 2.14 ; Fig. 1D). However, by the last 8 days of rehabilitation

training, there was no longer a statistical difference in the number of attempts ($37.4 \pm 3.79\%$ versus $33.2 \pm 3.95\%$ for the trained and untrained groups respectively; Fig. 1E).

3.1.2 Movement Rating Scale—To provide a qualitative outcome measure, a movement rating analysis was conducted. No significant differences between the untrained and trained group were found on any of the 10 components of the grasping motion, although a trend was found for trained rats to perform better at the elbow in, advance, arpeggio, grasp, supination I, supination II, and release components of the grasping movement, while untrained rats tended to perform better at digit extension (Fig. 1F).

3.1.3 Horizontal Ladder—To assess the effect of pre-training on an untrained task, the rats were tested on the horizontal ladder task. The number of errors per step made by rats from the pre-trained ($17.5 \pm 5.00\%$) and untrained ($15.3 \pm 3.45\%$) groups were not significantly different (Fig. 2B). Foot fault scores were also compared. Again, no group differences were found, with rats from the pre-trained group obtaining mean scores of 4.51 ± 0.305 and rats from the untrained group obtaining scores of 4.69 ± 0.139 (Fig. 2C). There was a trend for pre-trained rats to take more time crossing the ladder (7.47 ± 1.42 s) than untrained rats (5.07 ± 0.523 s), but the trend did not reach statistical significance (Fig. 2D).

3.1.4 Lesion Size—Histological analysis revealed that rats trained before the injury had significantly greater lesion volumes than rats that were not pre-trained ($P < 0.05$; Fig. 3A). The average spinal cord lesion volume of pre-trained rats was $16.2 \pm 1.98\%$ and that of untrained rats was $11.3 \pm 0.573\%$. However, when the cross sectional area of damaged tissue was compared between groups, there was not a significant difference, although a similar trend existed with pre-trained rats having lesion areas of $32.9 \pm 3.91\%$ and untrained rats $26.4 \pm 2.04\%$ (Fig. 3B). Cross-sectional spared white matter was also not significantly different between groups. Pre-trained rats had $41.27 \pm 2.491\%$ and untrained rats had $46.31 \pm 1.134\%$ spared white matter (Fig. 3C). No correlation was found between the lesion volume and grasping success rate of animals from both groups ($r = -0.435$, $P = 0.209$ for pre-trained rats and $r = -0.257$, $P = 0.474$ for non-trained rats; Fig. 3D). There was also no relationship between lesion area and success rate ($r = -0.557$, $P = 0.095$ and $r = -0.274$, $P = 0.444$ for pre-trained and non-pre-trained rats respectively; Fig. 3E). Lastly, no correlation between spared white matter area and success rate was found ($r = 0.488$, $P = 0.153$ for pre-trained rats and $r = 0.288$, $P = 0.420$ for non-trained rats; Fig. 3F). These results indicate that with the lesion size variability in this experiment, lesion size was not predictive of success scores.

3.2 Swimming Study

3.2.1 Swimming and Stepping—Swimming and stepping scores for the two experimental groups, pre-trained and non-trained, were obtained every second week starting at week 2. BBB scores were not different at any time point, and these are shown for week 2 only in Figure 4. These animals achieved frequent weight-supported stepping with their hindlimbs with no forelimb-hindlimb coordination, a level of recovery often reported for animals with 12.5g-cm NYU injuries at T9 or 10 (Basso et al. 1995; Smith et al., 2006, Magnuson et al., 2005, 2009). Despite the very similar BBB scores, the animals that

received swim training pre-injury had significantly lower LSS scores at weeks 2 and 4 post-injury (Fig. 4). These animals scored less than 5 on the LSS at week 2 post-injury, indicating that they had very little hindlimb movement, were almost totally dependent on their forelimbs for forward motion and in addition had severe trunk instability. In contrast, the animals that received no swim training pre-injury scored 7–8 at week two postinjury indicating that they exhibited, at a minimum, occasional hindlimb movement, occasional hindlimb alternation and only moderate trunk instability, while retaining a high degree of dependence on forelimbs for forward motion. In addition to the LSS, we also assessed the hindlimb movements during swimming using a 2D kinematic approach where the limb is assessed as a three segment, two-angle model and the knee is ignored, as can be seen in Figure 5A. When the two angles measured (iliac crest-hip-ankle and hip-ankle-toe) are plotted against each other, the resultant ellipsoid describes the movement and position of the limbs by its size, shape and position on the graph. Fig. 5B shows a single swimming stroke for one representative pre-trained animal at 1 week post-injury indicating very little movement of the extended proximal limb segment (iliac crest-hip-ankle) and somewhat larger excursion of the distal limb segment (hip-ankle-toe), while the mean angle-angle excursion (plot area) is significantly lower than for the untrained animals (Fig. 5C). As we have observed previously (Smith et al., 2006; Magnuson et al., 2009) post-injury swim training resulted in significant improvements in both LSS scores and hindlimb kinematics over the first 4–6 weeks post-injury, however, even after 2 weeks of daily swim training, the animals that received swim training pre-injury had lower LSS scores (4 weeks post-injury). Since angle-angle plot area was not different at 4 weeks, it can be concluded that aspects of the LSS other than hindlimb movement, such as trunk instability or hindlimb alternation, were lagging.

3.2.2 Lesion Size—The percent spared white matter at the injury epicenter was not different for the two groups (Fig. 5D), averaging approximately 8% in cross sectional area of only densely stained, normal appearing white matter using our standard Eriochrome Cyanin technique (Kuerzi et al., 2010). This shows that while the pre-training negatively influenced post-injury swimming, it did not significantly alter white matter sparing.

4. Discussion

Experimental SCI research is faced with numerous challenges, such as the reproducibility of functional outcomes (Filli & Schwab, 2012). Although animal experiments are designed to control all known variables, inter-laboratory and even intra-laboratory variability is high. Such variability could be partly attributed to poor reporting of methods (Lemmon et al., 2014). One example is that the detailed description of handling/training protocols prior to the start of experimentation is often missing. This is significant, because training pre-injury is known to influence outcomes after CNS damage, which is problematic when comparing experimental outcomes between laboratories. For instance, pre-injury treadmill training was reported to reduce infarct size and edema after cerebral ischemia in rats (Wang et al., 2001). Also, voluntary wheel running before SCI in rats reduces weight loss after the injury (Erschbamer et al., 2006). The present study addressed the question of how training in two different tasks before SCI might influence motor recovery.

Contrary to earlier results, training in the SPG task increased lesion severity after cervical SCI (Fig. 3A). This is unexpected, because exercise prior to SCI was reported to prevent an injury induced drop in levels of BDNF (Gomez-Pinilla et al., 2012), a neuroprotective neurotrophin (Kobayashi et al., 1997). In our study, it may be that pre-training did not increase BDNF levels sufficiently to confer neuroprotection. The lesion may have been aggravated in pretrained rats due to stress. It is widely accepted that delaying the onset of rehabilitative training is beneficial in rats with incomplete SCI (Krajacic et al., 2009; Smith et al., 2009). One of the possible explanations for this is that handling/training is stressful for animals. It has long been known that mere movement of rat cages is sufficient to induce a circulatory shock reaction (i.e., increased heart rate) and alter plasma concentration profiles of molecules linked with stress, including glucose, pyruvate and lactate (Gartner et al., 1980). Thus, it may be argued that since untrained and pre-trained rats were exposed to the experimenter and testing apparatus before the injury, both groups should have experienced similar amounts of stress. However, pre-trained rats went through the experience of repeated unrewarded reaching attempts for longer than untrained rats. Average of the best reaching success scores achieved by the pre-trained group before SCI was only 56%; failed attempts were due to knocking pellets away or dropping the pellets after grasping them. Unexpected omission of a reward is known to produce an aversive emotional reaction, accompanied by increased corticosteroid levels and emission of odours and vocalizations in rodents (Papini & Dudley, 1997). Chronic stress can result in systemic inflammation, exacerbated inflammatory processes and increased levels of apoptosis post-SCI (Bouchard & Hooke, 2014). If pre-trained rats indeed experienced higher levels of stress, this could have led to worsening of the lesion.

Although we did not measure the level of pre-training, rats may have also engaged in more activity within their home cages immediately after the injury compared to non-trained rats. Exercise initiated acutely after SCI was reported to be detrimental to recovery (Kyoung-Hee et al., 2013). Physical activity increases blood pressure and flow at a time when the blood-brain barrier is compromised, leading to greater infiltration of inflammatory cells and necrosis (Silva et al., 2014). Post-contusion angiogenesis peaks at 7–10 days (Popovich et al., 1997; Benton et al., 2008) and these new vessels may be responsible, in part, for the blood-brain barrier weakness and exacerbation of damage during the post-injury stressor of exercise (Smith et al., 2009). However, exacerbation of the injury did not influence the rats' ability to adopt a compensatory reaching strategy post-injury. Pre-trained rats achieved similar reaching scores to untrained rats (Fig. 1B, C & F) and use of "scooping" to compensate for functional loss was analogous between the two groups. Pre-training in the reaching task also did not affect walking across a ladder (Fig. 2B, C, & D). This could be partly explained by our earlier finding that a small variability in lesion size does not necessarily translate into differences in successful use of the forelimb (Hurd et al., 2013). Although pre-trained rats had a larger volumetric lesion size than non-trained rats, all rats had lesion volumes lower than 30% of the 3 mm spinal cord block analyzed regardless of which group they belonged to (Fig. 3A). In addition, lesion volume as measured with a cresyl violet stain does not assess the health of the tissue and the number of surviving neurons.

In contrast to the grasping study, no differences were found in lesion severity in the swimming study, which might be attributed to the fact that only spared white matter at the epicenter was assessed. Similarly, when spared white matter was compared between groups in the grasping study, there was no effect of pre-training on this measure. The findings may also be discrepant because the SCI was at the cervical level in the grasping study and at the thoracic level in the swimming study. Grey matter is more susceptible than white matter to secondary damage spread after SCI (Ek et al., 2010), and cervical spinal cord has a greater percentage of grey matter than thoracic levels (Turnbull, 1971).

Previously, we found that the recovery of swimming and stepping in untrained animals, as assessed by the LSS and BBB scales, were correlated, but that the correlation was stronger when animals received daily swim training (Smith et al., 2006). Locomotor training after a spinal cord injury has been assumed for many years to be largely dependent on re-engaging the pattern generating circuitry using an appropriately applied repetition of limb movements. This approach is successful in the fully transected cat model where outcomes are hindlimb kinematics and EMG during treadmill stepping (Barriere et al., 2008). This approach has proven to be much less successful when applied to incompletely injured rodents when overground performance is a primary outcome (Fouad et al., 2000; Kuerzi et al., 2010) or when applied to incompletely injured patients with either treadmill or overground performance as the outcome (Dobkin et al., 2007; Harkema et al., 2012). Why these apparent disparities exist is unknown, but certainly few laboratories consider the possibility that pre-injury training might influence post-injury plasticity or performance of a locomotor task. In addition to demonstrating that post-injury swim training successfully improves swimming performance without altering overground performance (Smith et al., 2006a, b), we also showed that adult female SD rats do not learn to swim when first exposed to the activity. We found that their hindlimb performance when first exposed to the activity is kinematically similar to that following 4 weeks of daily swim training (Magnuson et al., 2009). This observation suggests that there is no overt plasticity with the initiation or practice of the activity of swimming, yet our current results suggest strongly that some change has occurred and that this change is revealed as a lower level of performance in the amount and quality of hindlimb movement during swimming, over the first few weeks post-injury (Fig. 4 & Fig. 5C). Thus, the current results provide a potent example of how pre-injury task-specific training, in the form of daily swimming sessions for a month, can dramatically influence postinjury outcomes. The data also suggests that this only applies when the task assessed is similar to the pre-injury exercise, as swimming pre-training did not impact overground locomotion as assessed by the BBB locomotor rating scale (Fig. 4). Interestingly, while rats that were trained in grasping recovered well post-SCI (Fig. 1B, C& F), rats trained in swimming performed worse after the injury (Fig. 4 & Fig. 5C). It is known that training in a specific task may lead to a decline in the performance in another task, possibly because training “uses up” limited neuronal circuitry (Girgis et al., 2007; de Leon et al., 1999; Eason & Smith, 1989). The results support the postulate that rats trained to swim before an SCI could not re-train after their injuries as easily as non-pre-trained rats. This may not have applied to rats trained in grasping due to the role of motivation in the SPG task. Spinal cord plasticity post-CNS trauma is most pronounced in the first 2 weeks after injury (Sist et al., 2014). Pre-trained rats were more motivated to grasp 1 week after the

injury, as they were familiar with the task. Additionally, rats were only allowed use of their injured forelimb to grasp; something which would have been quite difficult for rats that had never trained to grasp. Indeed, rats trained in grasping before the injury grasped significantly more times than non-trained rats in the first few weeks after the SCI (Fig. 1D). The number of grasping attempts of untrained rats did not match that of trained ones (Fig. 1E) until after the window of opportunity for plasticity had already closed (Sist et al., 2014). In contrast, swimming is a forced task involving spinal pattern generating circuitry. When first introduced to the water, rats swim in place for a second or two, and then rapidly adopt a horizontal swimming position and make hindlimb kicking movements that do not change with additional practice, suggesting that swimming is a pattern that is not learned but is “hardwired” (Magnuson et al., 2009). Postthoracic contusion SCI, the use of hindlimbs was compromised but not use of the forelimbs. So, rats had access to an intact pair of limbs to propel them forward. Thus, acute swimming forced both pre-trained and untrained rats to activate neuronal networks when plasticity was still high. Despite this, pre-trained rats were not able to engage their hindlimbs significantly at 2 weeks post-injury showing very limited joint excursions, in particular of the proximal (hip-knee) limb segment, relying almost entirely on their forelimbs for forward motion (Fig. 5C). Hindlimb joint angular excursions improved with 2 weeks of daily training (4 weeks post-injury); however, the overall quality of swimming remained significantly different from controls that swam only post-SCI. Swimming ability of pre-trained rats did not recover to the same extent as that of untrained rats until 6 weeks post-injury. Hence, these results suggest that using the “wired” pattern of swimming pre-injury reduced the capacity to engage novel circuits shortly after injury and that both the necessity to retrain a well-established/utilized motor pattern and the motivation to train can have an impact on functional recovery post-SCI.

In conclusion, our results stress the importance of detailed reporting of the training and handling regimes before experimental procedures (i.e., injuries) are performed in order to enhance transparency and reproducibility of animal studies. They also suggest a disparity between cortically dominated fine motor tasks, like grasping, and spinally mediated tasks, like swimming, as regards how acclimatization to the task might influence outcomes.

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Highlights

- Two different models of spinal cord injury and task-specific re-training are used.
- Activity/training pre-SCI is found to influence post-injury recovery.
- Pre-training increased grasp attempts, but did not influence success rate.
- Pre-training negatively influenced ability to swim acutely post-injury.

Figure 1.

Pre-training in the single pellet grasping task (SPG) does not influence grasping ability in rats after a C4 dorsolateral quadrant (DLQ) spinal cord lesion. A DLQ lesion (A) abolishes the CST unilaterally and most of the RST, greatly diminishing grasping ability in rats. Weekly grasping data (B) shows that there was a non-significant trend for pre-trained rats to successfully retrieve more pellets through a slot after an SCI than rats that were not trained before the injury (two-way repeated measures ANOVA P-value 0.204). When the best scores attained by the pretrained and non-pre-trained group throughout the 6 weeks of

rehabilitation were contrasted, a similar non-significant trend emerged (unpaired t-test two-tailed P-value 0.429; C). Pre-trained rats grasped significantly more pellets than non-pre-trained rats during the first 4 weeks of rehabilitation (unpaired t-test two-tailed $P < 0.05$; D), but this effect disappeared by the final days of rehabilitation (unpaired t-test two-tailed P-value 0.457; E). The detailed components of the grasping movement were analyzed 6 weeks after the DLQ lesion and rehabilitative training (E). Movements of pre-trained animals tended more towards normalcy (the scores were higher) than those of untrained rats, except for digit extension. However, no significant effects of pretraining were found on the 10 components of the grasping movement. In (F), Digits mid = digits turned toward body midline. Digits flex = digits flexed. Elbow = Elbow moved to midline. Advance = Forelimb advanced through the slot. De = Digits extend. Arpeggio = Paw pronates from digit 5 through to digit 2. Grasp = Paw closes over the pellet. Supination I = As the forelimb is pulled away, the paw is rotated 90° . Supination II = Paw is rotated a further 45° . Release = Pellet is released into the mouth. (* indicates $P < 0.05$).

Figure 2.

Performance on the horizontal ladder task is not affected by pre-training on the SPG task. In (A), a Lewis rat walks across the ladder. The dashed circle shows (top) correct paw placement and (bottom) a paw slip. Rats trained on pellet grasping did not make significantly more errors (rung misses or slips) than untrained rats when crossing the ladder (unpaired t-test unpaired t-test P-value 0.713; B). Performance on the foot fault scale was similar between groups (unpaired t-test P-value 0.606), with rats from both groups scoring close to the max score of 6 (C). A higher score is indicative of superior walking ability on the horizontal ladder task. Pretrained rats took more time in (s) to cross the ladder than untrained rats (D), but this difference was not significant (unpaired t-test P-value 0.129).

Figure 3.

Lesion assessment and correlations of lesion and reaching success. Lesion volume was quantified using the Cavalieri method, and the lesion volumes of rats trained before the injury were found to be greater than those of rats that were not pre-trained (unpaired t-test $P < 0.05$; A). Using lesion reconstructions, lesion area and white matter sparing were also assessed. In this case, no group differences were found with regards to lesion size (unpaired t-test P -value 0.157; B) or white matter sparing (unpaired t-test P -value 0.083; C). Reaching success was not found to be correlated with the extent of injury in the rostral to caudal

direction (D) for trained (Pearson's $r = -0.435$) and untrained rats (Pearson's $r = -0.257$). Reaching success was not correlated with the extent of damage (E; pre-trained group Pearson's $r = -0.557$ and untrained group Pearson's $r = -0.274$) or with white matter sparing (F) in the two dimensional plane (pre-trained group Pearson's $r = 0.488$ and untrained group Pearson's $r = 0.288$). (*indicates $P < 0.05$).

Effect of Pre-Injury Swim Training on the Recovery of Swimming

Figure 4.

Shown are the Basso, Beattie & Bresnahan (BBB) open field locomotor scores and the Louisville Swim Scale (LSS) scores for the two experimental groups. The LSS, which has a maximum (normal baseline) score of 18, is shown for post-injury weeks 2, 4 and 6. The BBB, which has a maximum (normal baseline) score of 21, is shown for post-injury week 2. Data is shown as mean $\pm$ SD. Asterix shows significant difference for swim trained pre-injury animals compared to those that were untrained pre-injury. ANOVA and tukey post-hoc t-test, $P < 0.05$.

Figure 5.

Shown in A are stylized examples of hindlimb stick figures for swimming to illustrate the iliac crest – hip – ankle and hip – ankle – toe angles. In B, these two angles are plotted against each other and the resulting ellipses show the angular excursions for single swimming strokes to illustrate an uninjured animal and an injured animal at 1 week post-injury. The ellipse areas, calculated using an elliptical fourier analysis, are shown in C. Pre-trained animals had a significantly lower angle-angle plot area (*, independent two-tailed t-test). D. Spared white matter, based on the cross sectional area of eriochrome cyanin stained compact white matter at the injury epicenter, is not different for the two experimental groups.