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Effects of Swimming on Functional Recovery after Incomplete Spinal Cord Injury in Rats

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Abstract

One of the most promising rehabilitation strategies for spinal cord injury is weight-supported treadmill training. This strategy seeks to re-train the spinal cord below the level of injury to generate a meaningful pattern of movement. However, the number of step cycles that can be accomplished is limited by the poor weight-bearing capability of the neuromuscular system after injury. We have begun to study swimming as a rehabilitation strategy that allows for high numbers of steps and a high step-cycle frequency in a standard rat model of contusive spinal cord injury. The purpose of the present study was to evaluate the effect of swimming as a rehabilitation strategy in rats with contusion injuries at T9. We used a swimming strategy with or without cutaneous feedback based on original work in the chick by Muir and colleagues. Adult female rats ($n = 27$) received moderately-severe contusion injuries at T9. Walking and swimming performance were evaluated using the Open-Field Locomotor Scale (BBB; Basso et al., 1995) and a novel swimming assessment, the Louisville Swimming Scale (LSS). Rats that underwent swim-training with or without cutaneous feedback showed a significant improvement in hindlimb function during swimming compared to untrained animals. Rats that underwent swim-training without cutaneous feedback showed less improvement than those trained with cutaneous feedback. Rats in the non-swimming group demonstrated little improvement over the course of the study. All three groups showed the expected improvement in over-ground walking and had similar terminal BBB scores. These findings suggest that animals re-acquire the ability to swim only if trained and that cutaneous feedback improves the re-training process. Further, these data suggest that the normal course of recovery of over-ground walking following moderately-severe contusion injuries at T9 is the result of a re-training process.

Keywords

contusion injury; rehabilitation; re-training; swimming

Introduction

Hindlimb Stepping during locomotion in mammals is thought to involve descending pathways that originate in the midbrain (mesencephalic locomotor region; MLR), pass through relays in the ventromedial medulla (VMM), to descend in the ventral white matter of the spinal cord to the lumbar enlargement (Jordan, 1998). These descending locomotor command fibers are thought to innervate central pattern generating circuitry (CPG) located in the rostral part of the lumbar enlargement serving to initiate and maintain hindlimb stepping during locomotion (Noga et al., 1995; Magnuson et al., 1999; Hadi et al., 2000; Loy et al., 2002). Functional

evidence of the MLR-VMM-CPG pathway can be observed during fictive locomotion, recorded from hindlimb nerves in pharmacologically paralyzed, decerebrate cats, which can be elicited using a constant stimulus of 15 hz in the MLR (Shik et al., 1966; Noga et al., 1991) or brainstem (Shefchyk et al., 1990). Hindlimb stepping on a treadmill can also be induced by MLR stimulation, even when one hindlimb is completely deafferented, and is often maintained for some time following bilateral hindlimb deafferentation (Grillner and Zangger, 1984). The pattern of hindlimb muscle activation during MLR stimulation in a deafferented cat is very similar to the intact animal, apart from the response amplitudes of key extensor muscles which are reduced compared to normal, leading to a relatively flexed limb position during stance (Grillner and Zangger, 1975, 1984). These and other detailed observations (Hiebert and Pearson, 1999) suggest that afferent feedback plays an important role in regulating limb extension (weight-bearing) during the stance phase of stepping, but that the underlying pattern of the centrally generated rhythmic activity is not dependent on phasic descending or afferent input. The underlying rhythm appears to be a function of the intrinsic characteristics of the neurons and circuitry in the spinal cord.

Thus, functional locomotion requires both supraspinal inputs, which serve to initiate and maintain locomotor movements, and afferent feedback from the limbs that modulates the step cycle (Grillner, 1975). Three different types of afferent feedback are thought to play important roles in the various phases of locomotion (Grillner, 1975; Muir and Steeves, 1995). First of all, proprioceptive input from muscle spindles and Golgi tendon organs signal limb-loading during the stance phase and limb position throughout the step cycle (Pearson et al., 1993). Secondly, afferent input from joint capsules signal the extremes of limb position. Hip joint afferents in particular are effective at triggering of the transition from stance to swing phases (Grillner et al., 1975; Muir and Steeves, 1995). Finally, feedback from cutaneous receptors signal plantar foot contact during stance and dorsal foot contact during swing if an obstacle is encountered or the foot is dragging (Muir and Steeves, 1995; Bouyer and Rossignol, 2003).

Following spinal cord injury, descending input onto interneurons of the CPG is reduced while afferent inputs remain intact. Deficits in hindlimb function during walking are proportional to white matter loss in the ventral half of the spinal cord following contusion or laceration injuries or demyelination of the thoracic spinal cord (Basso et al., 1996; Schuck et al. 2002; Loy et al. 2002). For more than two decades, hindlimb afferents have been used to activate and re-train spinal cord locomotor circuitry in a variety of animal models. Studies in rats (Multon et al., 2003), chicks (Muir and Steeves, 1995), and humans (Harkema et al., 1997; Abel et al., 2002; Hicks et al., 2003) have revealed that locomotor training after incomplete spinal cord injury leads to improved function. Barbeau and Rossignol (1987) demonstrated that, even following complete spinal transection, adult cats could be taught to walk if trained on a treadmill with body weight support and a strong afferent drive provided by pinching the tail or stimulating the perineal region. In this paradigm, several weeks of intensive training is required suggesting that the amount of activity may play a role in re-activating or re-training the spinal cord circuitry required for normal oscillatory function.

In order for walking to be reestablished however, a small amount of cutaneous input must be present. A recent study by Bouyer and Rossignol (2003) used a cat model of weight-supported treadmill rehabilitation to demonstrate that spinalized animals could be re-trained to walk on a treadmill provided that some cutaneous inputs to the hindpaw were left intact. Even with progressive loss of cutaneous afferents, the cats regained function with re-training. Complete absence of cutaneous inputs, however, resulted in permanent loss of plantar placing and weight bearing during locomotion despite several months of re-training (Bouyer and Rossignol, 2003). These observations indicate that cutaneous afferent input from the paw is a critical component of weight-supported treadmill training in spinalized cats.

Clinical rehabilitation after moderate and severe spinal cord injuries, however, can be more problematic due to the fact that most patients with ASIA grade C or D injuries have difficulty achieving weight-bearing limb movements without external support (Dietz et al., 1994, 1995; Harkema et al., 1997; Abel et al., 2002; Maegle et al., 2002; Pepin et al., 2003). In rodents, swimming is a form of locomotion that involves repetitive stepping-like movements of unloaded limbs. Unlike treadmill walking, which employs a harness to support body weight, swimming relies on natural buoyancy as a means of weight support. In addition to the buoyancy, another potential advantage of employing swimming as a rehabilitation strategy in rats after experimental spinal cord injury over treadmill walking is that swimming involves a higher number of step cycles being produced by the central pattern generator circuitry. Thus swimming may allow moderately and severely injured rats to achieve more step cycles during a given training period than they would with traditional treadmill training.

Swimming and walking are naturally occurring behaviors in both rats and chicks that share some important features including the phasic relationship between the right and left alternation of limb flexion and extension. During swimming, however, both cutaneous feedback and loading of the limbs are significantly reduced compared to the levels during walking (Muir and Steeves, 1995). While sensory feedback has been shown to contribute to the recovery of locomotor function after spinal cord injury (Edgerton et al., 1992, Muir and Steeves, 1995; Harkema et al., 1997), only one study (Muir and Steeves, 1995) has looked at the specific role of cutaneous, or exteroceptive, feedback in the recovery of hindlimb function following an incomplete injury. They examined the role of phasic cutaneous feedback during swimming in spinal cord injured chicks. Since swimming normally involves very low levels of both proprioceptive (loading) and cutaneous feedback, the latter was provided by adding buoyant centrifuge tubes suspended from the bottom of the pool that touched the feet of the chicks during swimming. This model allowed Muir and colleagues to isolate the effects of cutaneous feedback on hindlimb locomotion. Results from this study showed an improvement in hindlimb function in chicks that were exposed to phasic cutaneous feedback compared to chicks that swam without this input. Since rats are also natural swimmers and to date no other study has examined the role of cutaneous feedback in locomotor recovery in these animals, we hypothesized that cutaneous feedback would be beneficial to locomotor re-training of rats with incomplete spinal cord injuries.

The current study examines the role of swimming, with and without cutaneous feedback on locomotor recovery after incomplete spinal cord injury in rats. We have chosen to focus on animals with moderately-severe contusion injuries of the thoracic spinal cord because the majority of these animals recover some degree of weight-supported stepping, but have severe and persistent deficits in hindlimb function during over-ground walking (Basso et al., 1995, 1996; Magnuson et al., 1999). It is our hypothesis that animals involved in a swimming-based rehabilitation program will show greater functional recovery than untrained animals as assessed by the Basso, Beattie, and Bresnahan (BBB) Open-Field Locomotor Scale (Basso et al., 1995) and a novel assessment developed in our laboratory, the Louisville Swimming Scale (LSS). Furthermore, we hypothesize that cutaneous feedback during swimming will lead to enhanced locomotor performance in both the open field and in the water.

Methods

Pre-Training and Testing

One week prior to surgery, animals were exposed to the swimming pool for several minutes per day for 5 consecutive days. During this time the research personnel responsible for swimming and testing handled the animals on a daily basis and fed them sweetened cereal as treats. Rats are natural swimmers and were quickly acclimatized to the swimming sessions. The swimming pool is a Plexiglas chamber 60-in long, 7-in wide, and 12-in deep, that provides

a nominal swimming distance of 48 inches. There is an adjustable ramp at one end of the pool covered with a computer mouse pad that provides exceptionally good traction to allow even injured animals to exit the pool. The pool has a plexiglass lid that is easily removed and covers all but 8–10 inches of the pool length. The lid was designed to slide freely along the length of the pool to expose either the exit ramp or the entry point at the far end of the pool. The pool was filled to a depth of 8 inches with warm tap water (27–30°C) for each swimming session and was thoroughly cleaned daily. Each animal was carefully transferred from its home cage to the far end of the pool, opposite the ramp. The lid was slid over the entry point of the pool to prevent their exit until they reached the ramp. Uninjured or minimally injured animals rapidly learn to swim to the ramp to get out or to wait to be transferred back to the far end of the pool. In practice, it takes approximately 3–4 sec of swimming and 4–5 sec of transfer time per lap. In this way, upwards of 30 laps can be completed in each 4-min session. We conducted three 4-min sessions each morning and afternoon for each animal, totaling 24 min of swimming per day. Uninjured or mildly injured animals use 12–15 complete “step” cycles (flexion-extension of each hindlimb) to swim the length of the pool, which means that the spinal cord will produce between 360 and 450 step cycles per 4-min swimming session, or up to 2700 step cycles each day after spending a total of 24 min in the pool.

Cutaneous Feedback

Cutaneous feedback during swimming was provided by inverted centrifuge tubes suspended from a stainless steel grid on fishing line. The tubes used were the standard 1.5-mL size with snap lids. Each row consisted of eight tubes placed 12 mm apart. Alternate rows were staggered and rows were separated by 10 mm. This resulted in the maximum possible density of tubes given the 1.0×1.3 mm cap size (cap lip adds 0.3 mm in one direction). The tubes are suspended approximately 4–5 inches from the plate (to the top of the tube) ensuring that the animals contact the tubes but have great difficulty making contact with the metal plate. The tubes provide cutaneous feedback that feels to our fingers like trying to grasp small ice cubes floating in water, but without the cold. There is definite contact, but little in the way of resistance or support.

Spinal Cord Injury

Twenty-seven female Sprague-Dawley rats weighing 160–200 g were used for these experiments. All procedures involving experimental animals were performed according to the guidelines of the University of Louisville Institutional Animal Care and Use Committee (1996). Each animal was anesthetized with Nembutol sodium solution (0.8 cc/100 g, i.p.) and was given prophylactic antibiotics (gentomycin sulfate, 15 mg/kg, s.c.). Body temperatures were continuously monitored during surgery and were maintained at 36–37°C until the animal recovered from the anesthetic. A dorsal midline incision was made over the mid-thoracic spinal cord. A single level laminectomy was performed at the T9 vertebra. The spine was immobilized using clamps applied to the T8 and T10 spinous processes. The NYU Impactor (W. Young, Rutgers, NJ) was used to produce moderately-severe (25 g-cm) weight-drop contusion injuries centered on the T10 segment of the spinal cord. After injury, the wounds were closed in layers and a topical antibiotic ointment was applied to the incision. The animals were placed in recovery cages on heating pads until they recovered from the anesthesia. Injured animals were housed individually and received daily post-operative care, including manual bladder expression until adequate spontaneous voiding occurred. Five animals served as controls and received laminectomies without contusion injuries.

Training Design

Prior to training the animals were randomly divided into three groups: group 1, trained with cutaneous feedback ($n = 8$); group 2, trained without cutaneous feedback ($n = 7$); and group 3

untrained ($n = 7$). On post-injury days 14–17, the animals were placed in the water for one 4-min training session per day, and were assisted by the handlers when necessary. On post-injury days 21–23, the animals were trained for two (day 21) or three (days 22 and 23) 4-min sessions per day, according to their capabilities as a group. By day 24, few of the animals required assistance but all still relied on their forelimbs for forward motion. On post-injury days 28–67 (the final phase of training), the animals trained for three 4-min sessions each morning and afternoon (total of 24 min of training) for 4 days each week. The group receiving cutaneous feedback swam with buoyant tubes suspended from the bottom of the pool, which touched the skin of their hindpaws and hindlimbs as they swam. The rats in the non-swimming group remained in the swimming room for the duration of the sessions each day. They were handled daily and received sweetened cereal as treats with the trained animals at the completion of each training session.

Behavioral Outcome Measures

The BBB involves placing the animal in an open field (36 inches in diameter) and evaluating hindlimb function for 4 min. This evaluation was performed weekly by the same two scorers, who were presented the animals in random order. The low end of the scale (0–7) is characterized by individual joint movements, whereas the intermediate (8–13) and high (14–21) ends of the scale are characterized by weight support and coordination and paw position, respectively. Animals were assessed preoperatively and at approximately weekly intervals thereafter. Scores were analyzed with repeated measures analysis of variance (ANOVA) and followed by Student Newman-Keuls post hoc t -tests when appropriate.

The Louisville Swimming Scale (LSS) is an 18-point scale (0–17) with three ranges: 0–5, 6–11, and 12–17 (Table 1). This assessment was designed to evaluate swimming performance based on three primary components, forelimb dependency, hindlimb activity and body position. While the categories shown in Table 1 were developed using the basic structure of the BBB scale as a rough guide (Basso et al., 1995), a strong emphasis was placed on the different swimming patterns of both injured and normal rats. When a normal rat swims, it is completely dependent on its hindlimbs for forward locomotion and the forelimbs are used only occasionally to steer. The trunk remains at an angle of 20° or less to the water surface and the hindlimbs kick in an alternating fashion to move the animal through the water. The trunk is rotationally stable while the animal is moving. In contrast, animals with moderately-severe spinal cord injuries are completely dependent on their forelimbs for forward locomotion, display a tail down body angle greater than 45° and/or are rotationally unstable showing 45° or more of body roll (about the long axis). With the LSS scale, animals that score in the 0–5 category are poor swimmers that rely heavily on their forelimbs for forward locomotion and have little or no hindlimb movement. Animals that score in the 6–11 range are intermediate swimmers with occasional to frequent hindlimb movement but with some retained dependency on their forelimbs for forward motion, occasional to consistent trunk instability and a mild (21–45°, tail down from horizontal) body angle during forward locomotion (Fig. 1). Animals that score 12 and higher have consistent hindlimb movement, little to no forelimb dependency, little to no trunk instability and frequent to consistent alternating hindlimb movement.

Swimming performance was evaluated using the LSS scale prior to injury, at 11 days post-injury and at approximately weekly intervals thereafter. For each testing session, the animals swam one 4-min session and were scored by two independent observers and a third person who videotaped 1 min of each swim session with a Sony MiniDV digital camera. The initial LSS evaluation, on post-injury day 11 and prior to any training, was done without centrifuge tubes. Starting on post-injury day 18, rats in the cutaneous feedback group were evaluated with the centrifuge tubes in the pool and the animals in the standard swimming group and the non-swimmers were tested without the tubes. The water depth and temperature were kept constant.

Early in the study, the videos were used to confirm body angles and trunk instability scores, by consensus among the scorers. At the completion of the training, on post-injury day 70, all the animals were assessed using the LSS Scale without cutaneous feedback. For the terminal assessment (day 70), the animals were presented to the scorers in a random order.

Statistical Analysis

LSS scores were analyzed with repeated measures analysis of variance (ANOVA) and followed by Student Newman-Keuls post hoc *t*-tests when appropriate.

To examine the relationship between the BBB and LSS scores, scatterplots were constructed and the lines of best fit were plotted for the total data set and also for the individual groups. Pearson correlation coefficients were calculated for each line of best fit with the level of significance set at 95%.

Histological Outcomes

The rats were euthanized on post-injury day 73 with excess anesthetic (ketamine/xylazine, 80 and 10 mg/kg i.p., respectively) and perfused transcardially with calcium free tyrodes and 4% paraformaldehyde. The spinal cords were removed and post-fixed in the same fixative overnight, followed by cryoprotection in 30% sucrose. Spinal cord tissue was cut at 30 μ m and mounted onto glass slides. One set of slides was stained with Cresyl violet as previously described (Hadi et al., 2000). Every second section was photographed with a digital SPOT camera (Medical Diagnostics) attached to a Macintosh G4 computer. The sections were then traced using a Wacom Intuos (Vancouver, WA) drawing tablet with Appleworks 6.0 and saved as a bit-map file. The bit-map images were opened in NIH image and the areas of white matter, gray matter, and cavity were calculated for each section (Magnuson et al., 2005). The injury epicenter was determined as the section with the least spared white matter. The area measurements were transferred to an Excel spreadsheet and graphed. The spared white matter at the injury epicenter was calculated as a percent of the white matter area taken from uninjured animals (Magnuson et al., 2005; Takami et al., 2002).

Results

At 11 days post-injury, and prior to any swim training, all of the animals were highly dependent on their forelimbs for forward motion and received LSS scores (tested without tubes) in the poor category (0–5). By post-injury day 18, after the first week of swim training (a single 4-min training session per day for 4 days), the animals being trained and tested with cutaneous feedback had an average LSS score of 8.0 ± 3.0 (intermediate) while the swimmers that were trained and tested without cutaneous feedback and the non-swimmers remained in the poor category with scores of 4.5 ± 2.8 and 2.3 ± 1.0 , respectively (Table 1 and Fig. 2). All of the animals were at least somewhat dependent on their forelimbs for forward motion and animals in the cutaneous feedback group had frequent (51–95%) or consistent (96–100%) hindlimb movement. The animals in the standard swimming and non-swimming groups showed occasional (6–50%) hindlimb movement, at most (Fig. 2). Likewise, animals in the non-swimming group had more frequent episodes of moderate to severe trunk instability compared to the animals receiving swim training. Figure 1 illustrates a typical injured and a normal rat swimming without cutaneous feedback and the relationship between the buoyant tubes and hindpaws for an injured rat during swim training.

By post-injury day 40, the mean LSS score for animals in the cutaneous feedback group was significantly higher than for the other groups (time: $F = 43.0$, $df = 4, 72$, $p < 0.001$; group: $F = 5.8$, $df = 1, 18$, $p < 0.01$; Fig. 2). Likewise, the group trained and tested without cutaneous feedback had significantly higher LSS scores than the non-swimmers (group: $F = 9.0$, $df = 1$,

18, $p < 0.05$). The animals receiving cutaneous feedback had frequent or consistent hindlimb movement and showed little dependency on their forelimbs for forward motion (seldom or occasional, $<50\%$). Likewise, they had fewer episodes of trunk instability and an improved body position in the water. The majority of animals in the standard swim group had frequent hindlimb movement, occasional forelimb dependency and frequent episodes of moderate trunk instability. The non-swimmers had seldom or no hindlimb use ($\leq 5\%$), consistent forelimb dependency ($>95\%$) and frequent episodes of moderate to severe trunk instability ($>50\%$). By the final week of the study, the animals receiving cutaneous feedback during training had an average LSS score of 11.4 ± 3.3 , while the standard swimming and non-swimming groups had average scores of 7.3 ± 3.2 and 2.6 ± 1.6 , respectively. On post-injury day 70, the animals were re-tested as they were on day 11, without cutaneous feedback. The animals were presented in random order. Both training groups scored significantly better than the untrained group (Fig. 2), and the group trained with cutaneous feedback scored better than the no-feedback group (8.9 ± 3.3 and 7.9 ± 3.7 , respectively); however, this difference was not statistically significant.

No significant differences in the Open Field BBB scores between the swim groups were found at any time point tested (time: $F = 0.75$, $df = 6$, 126 , $p > 0.05$; group: $F = 1.0$, $df = 1$, 21 , $p > 0.05$; Fig. 3). Animals in the cutaneous feedback group had an average terminal BBB score of 10.9 ± 1.3 while the animals trained without cutaneous feedback and the non-swimming group had average BBB scores of 10.1 ± 1.5 and 9.9 ± 1.5 , respectively. Sham animals had an average terminal BBB score of 20.8 ± 0.5 . Table 2 provides brief explanations of various levels of the BBB scoring system with the scores of 8, 9, and 10 in bold.

In order to better understand the relationship between swimming and walking, the terminal LSS scores were plotted against the BBB scores. Regression lines of best fit were plotted to illustrate the relationships. A significant correlation between the LSS and Open Field BBB scores was present for the group trained with cutaneous feedback (Fig. 4, $p < 0.05$). The correlation for the group trained without cutaneous feedback approached significance ($p = 0.054$) and the relationship for the non-swimming group was not statistically significant.

Evaluation of the amount of spared white matter at the injury epicenter for animals in each of the three groups showed no significant differences. Figure 5 provides an example of a moderately-severe (25 g-cm) contusion injury showing Nissl stained sections taken from the injury epicenter and 2.0 mm rostral to the epicenter. Table 3 represents the average percent of spared white matter for each of the three groups.

Discussion

One of the goals of the present study was to extend to rats the work by Muir and Steeves (1995), who showed that cutaneous feedback during swimming could positively influence the recovery of hindlimb function following an incomplete (hemisection) spinal cord injury in chicks. Therefore, using a rat model of spinal cord injury, we set out to determine (1) if swimming-based rehabilitation would result in improved recovery of hindlimb function during walking and/or swimming and (2) if cutaneous feedback during swimming would enhance any improvement in hindlimb function. The results support our hypotheses, in part, by demonstrating that a swimming-based rehabilitation strategy involving supplemental cutaneous feedback can bring about improvements in the recovery of hindlimb function in the water. However, swimming-based rehabilitation had no effect on hindlimb function during overground walking.

In the present study, the animals that were trained with cutaneous feedback showed significant improvement in their LSS scores from 3.1 at post-injury day 11 (tested without cutaneous feedback) to 11.4 at day 68. Similarly, the group that was trained and tested without cutaneous

feedback showed improvement in swimming, however, their mean terminal LSS at post-injury day 68 was 7.9, which is significantly lower than the group trained and tested with cutaneous feedback. These groups of animals had initial, post-injury BBB scores of 3, indicating that they had movement of two of the three joints, and terminal BBB scores of 10.9 and 10.1, respectively, indicating that they demonstrated occasional weight-supported stepping that lacked coordination (Basso et al., 1995). These observations show some similarities to those reported by Muir and Steeves (1995) who demonstrated in the chick that supplemental cutaneous feedback during swim training appears to enhance the recovery of function following an incomplete spinal cord injury. In their study, cutaneous feedback during swim training substantially enhanced the swimming activity of the leg on the injured side during training, as assessed using kinematics and angle-angle plots for the knee and ankle (Muir and Steeves, 1995).

The animals that did not receive swim training had initial and terminal mean LSS scores of 2.1 and 3.3, respectively, indicating that they were completely dependent on their forelimbs for forward motion during swimming throughout the study. These animals had initial and terminal BBB scores of 2.1 and 9.9, respectively, indicating that they were similar to the groups that received swim training when walking over-ground. This finding replicates in rats that reported by Muir and Steeves (1995) for chicks who showed that trained and untrained animals showed similar levels of hindlimb recovery when walking despite the striking differences when swimming.

Between post-injury days 11 and 18, animals received only 4 min of training, with or without cutaneous feedback, per day for 4 days (on post-injury days 14, 15, 16, and 17). For the animals trained and tested in the presence of cutaneous feedback this resulted in a 4.5-point increase (3 to 7.5) in their mean LSS score. Animals trained and tested without cutaneous feedback showed only a 1.5-point increase (3 to 4.5) over the same period. We suggest that some of the increase seen for animals trained for only 16 min over 4 days with cutaneous feedback may be due to the direct influence of cutaneous feedback on hindlimb movement in the water. Swimming is not normally associated with cutaneous feedback such as that supplied by the centrifuge tubes, and we observed that cutaneous input to the dorsal surface of the foot (during forward motion supplied by the forelimbs with the hindlimbs dragging) appeared to induce hindlimb flexion. During forward motion the flexed limb often then made contact with the tubes on its plantar surface (Fig. 1), which appeared, in turn, to induce an extension of the limb.

On post-injury day 70, a terminal LSS assessment was done without cutaneous feedback for all three experimental groups. During this assessment the animals were presented in random order and no pre-training was provided to animals in group 1 to acclimatize them to swimming without the centrifuge tubes. The LSS scores for groups 2 and 3 remained stable compared to previous assessments, however the average LSS score for group 1 dropped from 11.4 to 8.9. This suggests that the lack of cutaneous feedback had an acute negative effect on their swimming ability, but on average they remained better swimmers than those in group 2 that were trained and tested without cutaneous feedback.

Thus, in both the chick hemisection and rat thoracic contusion models of spinal cord injury, significant functional improvement can result from a swimming-based rehabilitation program involving supplemental cutaneous feedback. However, in the present study we found that significant improvement in hindlimb function during swimming is training dependent and appears to be enhanced by supplemental cutaneous feedback, but is not dependent on it. In contrast, Muir and Steeves (1995) reported that very little improvement in the activity of the leg on the injured side could be achieved in the absence of cutaneous feedback. This may reflect the different injuries used and the pattern of spared tissue. The moderately-severe contusion injuries we used showed approximately 10% white matter sparing at the injury epicenter,

compared to an uninjured spinal cord, and this sparing was largely bilaterally symmetrical. However, the hemisection injury used by Muir and Steeves (1995) spared 50% of the white matter at the injury epicenter, on one side of the spinal cord. One could speculate that the small amount of bilateral sparing following a contusion may be sufficient to reduce the strict dependency on cutaneous feedback of the circuitry below the injury shown in the chick model. There are, however, many other issues to consider, including the relatively immature state of the chick model.

Bouyer and Rossignol (2003) showed recently that cutaneous afferents from the foot are absolutely necessary for successful re-training of hindlimb walking on the treadmill in completely spinalized cats. They found that complete hindpaw deafferentation rendered treadmill retraining impossible, even if non-specific sensory input is provided (tail pinching). In the contusion model of spinal cord injury the lumbar spinal cord is not completely isolated from the higher CNS and sensory input from the hindlimbs and paws is intact. Our results showed that swim training in the absence of any supplementary cutaneous feedback can result in improved hindlimb function during swimming in parallel with the normal progression of recovery of over ground walking. This suggests that while cutaneous feedback may be absolutely necessary for re-training of a stereotypic locomotor activity that is normally associated with substantial cutaneous input from the paws, it is not necessary for re-training of swimming, an activity not normally associated with cutaneous input. However, our results show that supplemental cutaneous input, that may be inappropriate for the behavior of swimming, can be used to enhance, at least acutely, hindlimb movements during swimming thus potentially improving the re-training process.

In the present study, the majority of animals achieved plantar paw placement during stance with or without weight-support (BBB scores of 9 or 8, respectively) by week 5 regardless of which training group they were in (Fig. 3). This observation is of interest for two reasons. First of all, it suggests that moderate swim training, with or without cutaneous feedback, does not appear to alter the time course of functional recovery of hindlimb movements in the open field. Secondly, it suggests that the cutaneous and proprioceptive (limb-loading) sensory feedback associated with plantar paw placement may be a key factor allowing spinal cord injured animals to achieve substantial improvements in hindlimb function during over-ground locomotion. This implies that achieving plantar paw placement allows these animals to re-train themselves resulting in functional improvements up-to and including weight-supported stepping that lacks consistent forelimb-hindlimb coordination (BBB scores of 10–11). Recent work by Van Meeteren et al. (2003) and Lankhorst et al. (2001) suggests that increasing hindlimb activity with plantar paw placement, in the form of voluntary wheel running in an enriched environment, may be reflected as small but significant improvements in hindlimb function during over-ground walking. While the mechanisms behind such plastic changes in spinal cord and neuromuscular function are not known, it has been shown that post-spinal cord injury allodynia is reduced more effectively by treadmill exercise than it is by swimming (Hutchinson et al., 2004), suggesting that lasting changes to sensory input and processing can result from activity-based rehabilitation strategies that include cutaneous and proprioceptive (limb-loading) feedback. It has also been shown that activity-based rehabilitation strategies can induce modest but significant changes in factors and receptors thought to be involved in plasticity in the spinal cord and elsewhere in the central nervous system (Gomez-Pinilla et al., 2002).

The corollary to these observations also appears to hold, in that improvements in swimming performance are dependent on a re-training process, which did not occur in the non-swimmers and was enhanced in the group trained with cutaneous feedback. We can conclude from these observations that moderately-intense swim training (24 min per day) with or without cutaneous feedback, significantly enhances the recovery of functional hindlimb movements during

swimming without significantly influencing either the time course or end point of the recovery of functional hindlimb movements in the open field. Swim training with cutaneous feedback may be even more effective, however our results do not allow us to distinguish a significant long-term effect of cutaneous feedback from a direct or acute influence of the centrifuge tubes on hindlimb movement. We will attempt to tease out these differences in a future study by doing LSS assessments on all experimental animals in the presence and absence of cutaneous feedback at each time point.

It has been reported by a number of authors in both animal models and human spinal cord injury (Harkema et al., 1997; De Leon et al., 1998a,b; Hutchinson et al., 2004) that post-injury training is task-specific. Animals that are trained to stand show improved standing but not walking, and animals that are trained to walk, but not to stand show improvements that are specific to walking. We hypothesize that our results demonstrate this phenomenon in two natural modes of locomotion, walking and swimming. The animals that are exposed to swimming on a regular basis (with or without cutaneous feedback) show recovery of hindlimb function that parallels, qualitatively and quantitatively, the normal course of recovery seen for over-ground walking. Animals not exposed to water show little to no improvement in their swimming capabilities. In support of these hypotheses, we found that a scatterplot of the terminal BBB and LSS scores indicates a significant relationship between these two measures for the group that received swim training with cutaneous feedback (Fig. 4). The standard swimming group shows a correlation that approaches significance while the non-swimming group shows a lack of correlation between these scores. Qualitative similarities include hindlimb alternation during swimming and stepping in the open field. Quantitative similarities include the amount of hindlimb movement during swimming (none, seldom, occasional, frequent, consistent) and the amount of stepping in the open field (occasional, frequent, consistent).

In summary, three critical points can be made from these data. First of all, animals with 25 g-cm contusion injuries at T9 show substantial recovery of hindlimb function during walking regardless of the swim training they undergo. We interpret this to indicate that these animals are re-training themselves to walk and that this re-training process is not influenced by their swim training or lack thereof. The majority of 25 g-cm injured animals achieve plantar paw placement while standing by 3–5 weeks post-injury. This means that the spinal cord will be receiving cutaneous and proprioceptive input appropriate for stance (plantar surface of the paw and limb-loading), suggesting that re-training of the spinal cord can occur spontaneously while the animals are moving about in their cages. Secondly, animals that are not trained to swim following a 25 g-cm contusion injury at T9 do not spontaneously recover significant hindlimb function during swimming. Finally, animals that are trained to swim following injury recover significant hindlimb function during swimming and this recovery can be enhanced if the training includes phasic cutaneous feedback. Overall, our results support the concept of task-specific training effects following spinal cord injury and provide support to the earlier studies pointing to the importance of cutaneous feedback during training. Unfortunately, our findings cannot be used to provide support for the clinical use of a training strategy using un-loaded limbs because of the likelihood that our animals were experiencing substantial training effects during their normal daily activities. However, the present study presents a model of retraining that has the potential to provide substantial insight into the mechanisms of plasticity and learning in the spinal cord circuitry caudal to an incomplete injury.

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FIG. 1.

Shown are pictures illustrating both normal and injured rats swimming without cutaneous feedback. The panel on the right demonstrates an injured animal swimming with cutaneous feedback. Note the differences in the body angle and the position of the forelimbs between the injured and normal rats. The picture on the right illustrates the relationship between the hindlimbs and the buoyant tubes used to provide cutaneous feedback.

FIG. 2.

Shown are the Louisville Swimming Scale (LSS) scores from 25g-cm injured animals over a 68-day period. One group received daily swim training with cutaneous feedback, one group was trained without cutaneous feedback, and one group remained untrained. As assessed by the LSS, the animals trained with cutaneous feedback (1) had significantly higher swim scores compared to the animals trained without feedback at post-injury days 40 and 68, and compared to the untrained animals at days 24, 40, 54, and 68 ($p < 0.05$). The animals in the group that was trained without cutaneous feedback (2) had significantly higher swim scores than the untrained animals (3) at post-injury days 40 and 68. The group of sham animals had significantly higher swim scores than the injured groups at all time points tested.

FIG. 3.

Shown are the Basso, Beattie, and Bresnahan (BBB) Open-Field Locomotor scores for animals in each of the four experimental groups. The sham animals had significantly higher BBB scores compared to animals in the three injured groups at all testing points. There were no significant differences between any of the injured groups.

FIG. 4.

Shown is a scatter plot of the terminal (week 9) Basso, Beattie, and Bresnahan (BBB) and Louisville Swimming Scale (LSS) scores for all animals. Pearson correlations and lines of best fit were calculated for each group. The group trained with cutaneous feedback showed a significant positive correlation between the LSS and the BBB scores ($p < 0.05$).

FIG. 5.

Shown are photomicrographs (4×) of Nissl (Cresyl violet)–stained sections from a 25 g-cm injured spinal cord 2.0 mm rostral to the epicenter (**A**) and at the T9 injury epicenter (**B**). Adobe Photoshop™ was used to adjust the brightness and contrast, remove the background, and transfer the photomicrographs to gray scale.

Table 1
Louisville Swim Scale: Summary

Category	Range		Description
HL movement	0	N	Any hindlimb movements are counted
	4	C	
HL alternation	0	N/S	Strict R/L alternation over two complete cycles
	3	C	
FL dependency	0	C	FL movements are associated with forward motion; steering movements are not counted
	4	N	
Trunk instability	0	F/S	Rotation about the long axis of the body
	4	N or S (Mi)	
Body angle	0	Se	Tail down angle relative to water surface
	2	N/Mi	

Each range goes from none (0%; N), through occasional (1–5%; O), to frequent (51–95%; F) and consistent (96–100%; C). Trunk instability and body angle includes none (0°; N), mild (1–20°; Mi), moderate (21–45°; Mo), and severe (46° and up; Se). Poor swimmers score, 0–5; intermediate swimmers, score 6–11; good swimmers, score 12–17. Normal Sprague-Dawley rats score a perfect 17.

Table 2
Functional Characteristics of the Basso, Beattie, and Bresnahan Scale

Score	Indication
0	Complete plegia of the limb
7	Extensive movement of all three joints without weight support
8	Plantar paw placement without weight support
9	Plantar paw placement with weight support during stance only
10	Occasional weight support during stepping, no coordination
13	Consistent weight support with some coordinated stepping
14	Consistent coordinated stepping with plantar weight support, incorrect foot placement, posture and tail position
21	Normal locomotion

Shown are some of the key features of the BBB Open Field Locomotor Scale that are of particular importance for the current study. The range of scores received by animals in the current study is shown in boldface.

Table 3
Histological Measures: Injury Epicenter

Group	Spared white matter
Cutaneous feedback	8.6 ± 5.3
No feedback	11.0 ± 1.5
Non-swimmer	8.8 ± 6.4

Data presented as percentage of normal (uninjured controls) ± SD. No significant differences were found between any of the swim groups.