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ORIGINAL RESEARCH

Mono-directional step-training in spinal rats produces unique stepping patterns in non-trained directions

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Locomotor recovery after spinal cord injury has been studied extensively in the laboratory of Dr. Reggie Edgerton. The current study combines the treatments of epidural stimulation, quipazine, and manual step-training to further examine the locomotor recovery of spinal cord transected rats. Rats were trained to step in one direction (forward, sideward, or backward) and tested for their ability to step in different directions. Results show that rats trained to step in one direction are able to step consistently in different directions. Ultimately, these results have implications for the treatment of spinal cord injury in humans.

Introduction

The physiological mechanisms that underlie mammalian spinal cord injury (SCI) recovery are still unclear; however, research from the past half-century has guided the development of different theories that attempt to physiologically explain motor function improvement[1-5].

One such theory is known as task specificity, which proposes that there are specific circuits in the spinal cord responsible for generating specific motor tasks[3]. Evidence for this theory was observed when spinal cats (full transection of spinal cord) that were only trained to walk, walked significantly better than spinal cats that were only trained to stand3. Despite the loss of supraspinal control after SCI, the spinal cord (below the site of injury) of spinal cats was still capable of commanding the limbs to perform novel motor tasks. Furthermore, the success at different motor tasks was dependent upon the specific training received.

The role of sensory input on motor recovery after spinal cord injury has also been studied extensively. Studies have emphasized the critical role of sensory influence in modulating motor output. Studies show that a combination of sensory input from epidural stimulation, a serotonin receptor (5-HT) agonist, and step training, brings about stepping in spinal rats. Step training is administered by eliciting locomotion of the paralyzed limbs using a treadmill[6].

To what extent does sensory input influence task specificity in spinal rats? In this experiment, spinal rats are trained to forward step, sideward step, or backward step and are tested for their ability to step in trained and other untrained tasks. The current hypothesis is that spinal rats will recover stepping ability best in the specific direction at which they are trained (Figure 1), because they receive sensory afferent input from that specific direction during training. Additionally, the hypothesis states that during testing the further a rat is from its trained direction, the worse it will perform.

The results indeed show task specificity, as predicted by the hy-

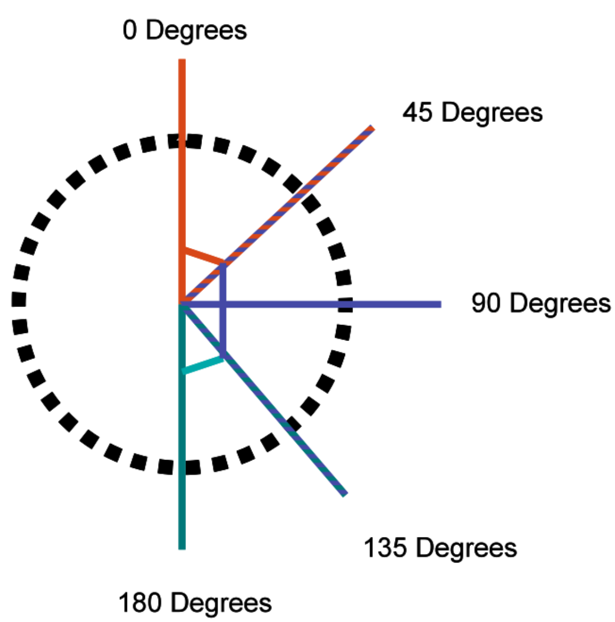

Figure 1 | Hypothesis. Animals will step more consistently at trained angles and within 45 degrees of the trained angle. Hypothesized forward trained performance is shown in red, hypothesized sideward trained performance is shown in blue, and hypothesized backward trained performance is shown in green.

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pothesis, because the large majority of rats show stepping in their trained direction. However, data also reveals some interesting features that were not predicted by previous research or accounted for in the original hypothesis.

Methods

Twenty-two female Sprague Dawley rats (20 weeks old) underwent complete spinal cord transection at the T12-L1 intersection. They were trained and tested in subsequent weeks. Training was conducted by implementing a combination of three training paradigms: manual step-training, epidural stimulation, and pharmacological (quipazine) injection. Rats were trained to step on a treadmill in three separate groups: seven rats were forward trained at 0 degrees (FT), seven rats were sideward trained at 90 degrees (ST), and eight rats were backward trained at 180 degrees (BT). Training occurred over a five week period, 20 minutes per day, and testing was conducted at seven weeks post injury[7-9].

All rats were tested to walk while receiving epidural stimulation and quipazine injection without manual support from humans (physically alternating each limb forward in a walking motion). All rats, regardless of training, were tested to walk on a treadmill that was rotated in the clockwise direction from 0 to 180 degrees. During testing, video was continuously recorded. Video was recorded using four cameras, two on each side of the rat at 45 degrees and 135 degrees with respect to the direction of motion.

Video data was analyzed as the rats walked in all directions, particularly at: 0 degrees, 0-45 degrees transition, 45 degrees, 45-90 degrees transition, 90 degrees, 90-135 degrees transition, 135 degrees, 135-180 degrees transition, and at 180 degrees (for the purpose of this paper transition data is not presented). VirtualDubMode software (open source software) was used to view the videos.

Specifically, the number of steps taken by the right leg (lead leg) at a given angle was quantified. Stepping results at each angle were analyzed in two ways. First, at each angle rats were divided by whether they could show a stepping response (2 or more steps) or a lack of stepping response (less than 2 steps). Secondly, at each angle the percentage of rats from each group were calculated by whether they could step consistently (stepping continuously with 9-10 steps) or non-consistently (stepping discontinuously and between 0-8 steps).

Results

Rats from each group were able to step (2 or more steps) in the direction that they were trained. 100% of FT rats stepped in the forward direction, 100% of sideward-trained rats stepped in the sideward direction, and 62% of backward-trained rats stepped in the backward direction. In addition, 72% of FT rats were able to consistently take 9-10 steps at 0 degrees. 57% of ST rats were able to consistently take 9-10 steps at 90 degrees. 25% of BT rats were able to consistently take 9-10 steps at 180 degrees (Figures 2, 3, and 4).

Moreover, the rats were also able to step consistently at angles other than their trained angle. 100% of ST rats (Figure 3) and 88% of BT rats (Figure 4) stepped consistently for 9-10 steps at 0 degrees. 14% of FT rats (Figure 2) and 50% of BT rats (Figure 4) stepped consistently for 9-10 steps at 90 degrees. 14% of both FT and ST rats stepped consistently at 180 degrees (Figure 3).

Regardless of training, all rats performed better during testing within the more forward facing angles. Taking 90 degrees as exactly in the middle of backward and forward stepping, the more forward-facing

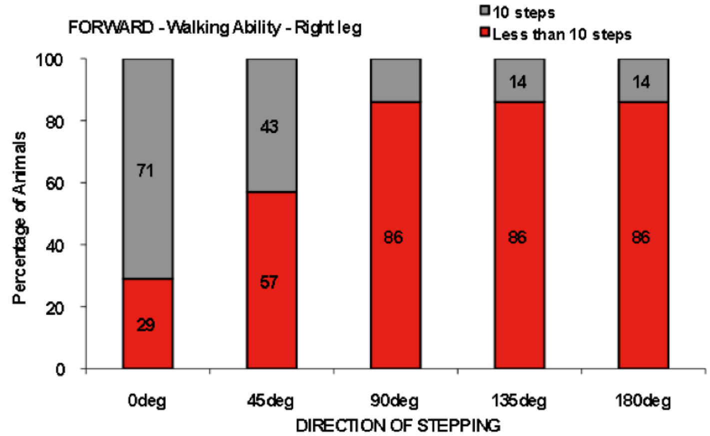

Figure 2 | Forward-trained rats (FT) - Walking Ability - Right Leg. Percentage of FT rats that step consistently (9-10 steps) or non-consistently (0-8 steps) at each angle. FT rats stepped more consistently overall at forward-facing (0 and 45 degrees) angles.

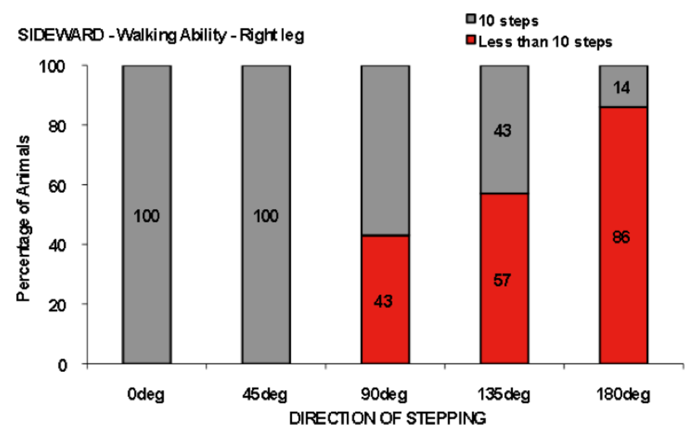

Figure 3 | Sideward-trained rats (ST) – Walking Ability – Right Leg. Percentage of ST rats that step consistently (9-10 steps) or non-consistently (0-8 steps) at each angle. ST rats stepped more consistently overall at forward-facing (0 and 45 degrees) angles.

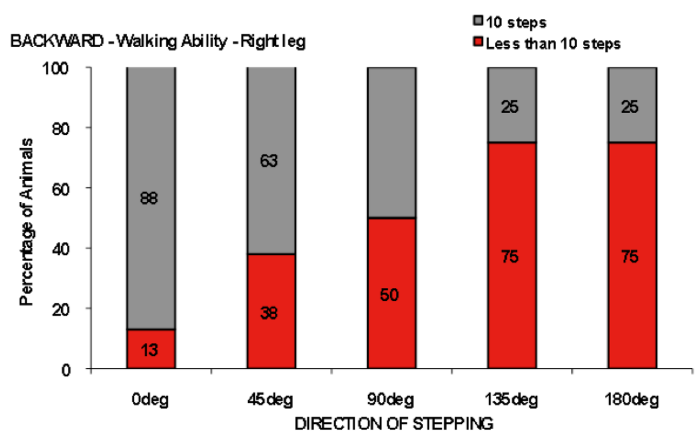

Figure 4 | Backward-trained rats (BT) – Walking Ability – Right Leg. Percentage of BT rats that step consistently (9-10 steps) or non-consistently (0-8 steps) at each angle. BT rats stepped more consistently overall at forward-facing (0 and 45 degrees) angles.

angles are defined as 0 and 45 degrees. Of FT rats, an average of 58% stepped 9-10 times in the forward range while only 14% stepped 9-10 times in the backward facing range (135 degrees and 180 degrees). 100% of ST rats stepped 9-10 times at 0 and 45 degrees, while an average of

	0, 45 degrees (forward-facing)	135, 180 degrees (backward-facing)
FT	58%	14%
ST	100%	29%
BT	76%	26%

Figure 5 | Forward-facing vs. backward-facing angles. Average percentage of rats per group that stepped consistently at either forward-facing or backward-facing angles. Regardless of the trained angle, each group stepped more consistently at the forward-facing (0 and 45 degrees) angles.

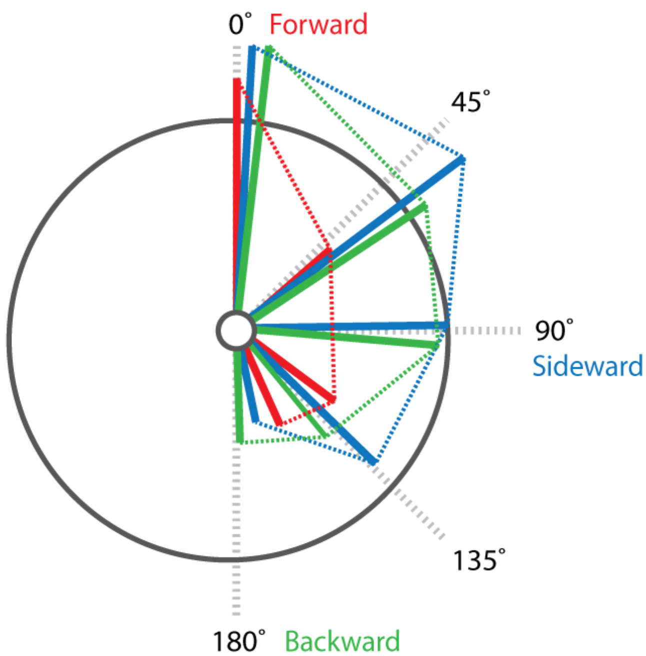

Figure 6 | Overall results. Sideward-trained rats showed the most overall consistent stepping. Forward-trained is shown in red, sideward-trained is shown blue, and backward-trained is shown in green.

only 29% of these ST rats stepped 9-10 times at 135 and 180 degrees. An average of 76% of BT rats stepped 9-10 times at 0 and 45 degrees, while only 26% of them stepped 9-10 times at 135 and 180 degrees (Figure 5).

An overall observation of all stepping angles revealed that ST rats showed the most consistent stepping, followed by BT rats and FT rats. All groups of rats demonstrated the ability to consistently step at all angles (Figure 6).

Discussion

The results of this current study show that rats can be trained in specific locomotor stepping tasks. Specifically, spinal transected rats trained to forward step, sideward step, or backward step, are able to step in their trained direction. As expected, FT rats stepped consistently in the forward direction, ST rats stepped consistently in the sideward direction, and BT rats stepped consistently in the backward direction. These results are consistent with past results and the current hypothesis[3].

Interestingly, it was observed that training in one direction was accompanied by unique stepping patterns in other directions. In some cases, stepping was more consistent at angles different from the trained angle. The ST and BT rats, for example, stepped more consistently at 0 degrees than at their trained angles of 90 and 180 degrees, respectively. In particular, ST rats stepped even more consistently at 0 degrees than did the FT rats. This demonstrates that rats trained specifically and only in one task stepped more consistently in a similar task, even though they had never trained in that task. This however was not true for the FT animals. The FT rats stepped less consistently as the angle shifted away from 0 degrees, thereby stepping more consistently in the forward direction than in either of the other two directions. In fact, all the rats, regardless of training, stepped more consistently at forward facing angles, 0 and 45 degrees. An explanation for this phenomenon is still unclear. It is possible that the animals stepped most consistently while facing forward because of the common nature of forward stepping. Also, there could be physiological factors that may support such consistent stepping in the untrained directions observed in the ST and BT groups.

To elaborate further, the concept of multifunctional circuits is explained[10]. Multifunctional circuits are neuronal networks in the spinal cord that bring about more than one rhythmic motor pattern. The leech, a non-vertebrate commonly used to study spinal neuronal circuitry, has been shown to have a common neuronal circuitry that controls the rhythmic motor tasks of both swimming and crawling[11]. Depending upon the sensory information received, the neuronal pool in this circuitry is modulated and activates multi-functional muscles that ultimately produce one, but never both, of either swimming or crawling behaviors. There is also evidence from studies with human infants that suggests that a common neuronal spinal circuitry most likely controls stepping in different directions based on environmental need. These multifunctional circuits can be modulated by neuromodulators, supraspinal input, or sensory feedback to perform the necessary task[12].

A similar system could theoretically exist to explain the stepping behavior of rats. This data has demonstrated that training in one direction can lead to consistent stepping in different directions. The FT rats, ST rats, and BT rats stepped consistently in all directions. Like the swimming and crawling multifunctional circuitry of the leech, there may be a multifunctional circuitry that exists in rats for forward, sideward, and backward stepping. It is possible then, that the sensory feedback from stepping in one direction modulates the neuronal spinal circuitry that exists to power stepping in different directions. Specifically, training in one direction may strengthen the entire spinal circuitry dedicated to stepping, by activating and recruiting neurons. Consequently, strengthening the spinal circuitry by training to step in one direction may increase the ability to step in different directions.

Interestingly, the percentage of rats that stepped consistently at different angles than their trained angle varied among the three groups. ST rats stepped most consistently in different directions, followed by BT rats and FT rats. It is not clear why training to sideward step or backward step led to more consistent stepping in different directions than training to forward step. Yet, based upon these results, it is reasonable to infer that certain locomotor training tasks can influence the strength of a neuronal spinal circuitry more than others.

The real world application of this result is that humans with spinal cord injury can consider training in more than one similar task for motor recovery. This seems counterintuitive, because recovery from any injury is most commonly achieved by training in the specific task that pertains to the injury. However, this study is unique in that it is the first study to examine the consequence of training spinal rats in different stepping directions. Based on the data, one can speculate that training in the sideward and backward directions might facilitate stepping in the forward direction. This study is just a beginning in understanding multifunctional circuits and training after spinal cord injury, and more research is necessary to gain a concrete understanding of locomotor recovery after spinal cord injury.

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