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Activity-Dependent Plasticity of Spinal Locomotion: Implications for Sensory Processing

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Abstract

The lumbosacral spinal cord of mammals contains the neural circuitry capable of generating full weight-bearing locomotion of the hindlimbs without any supraspinal input. One or more interventions, *e.g.*, pharmacological, epidural stimulation, and/or locomotor training, however, are necessary to gain access to and modulate the properties of this circuitry, and to facilitate recovery of full weight-bearing locomotion after spinal cord injury.

Keywords

rehabilitation; epidural stimulation; pharmacological interventions; spinal learning; locomotor training; sensorimotor plasticity

INTRODUCTION

It is clear that the lumbosacral spinal cord can regain the ability to generate some level of weight-bearing locomotion after a complete spinal cord transection, and that spinal training and learning is an underlying phenomenon that enables this recovery. Supplemental interventions, however, are needed to effectively train the spinal cord in some mammals. We describe how epidural stimulation and pharmacological manipulations can be used to modulate the properties of the spinal circuitry so that sensory input associated with weight bearing can control locomotion over a range of speeds, loads, and direction of stepping. The results from the experiments that have provided the basis for this concept suggest that the lumbosacral spinal circuitry that can generate full weight-bearing locomotion can be used to better understand the neural substrates for more integrative concepts such as perception, cognition, learning, and decision-making.

DOES REPETITIVE NEUROMUSCULAR ACTIVITY FACILITATE SPINAL LEARNING?

Lovely *et al.* (23) published the first quantitative evidence that was based on a controlled experiment of a locomotor learning phenomenon occurring within the spinal locomotor

circuitry. In this experiment a group of adult female cats with a complete low thoracic spinal cord transection were trained to step on a treadmill and their stepping ability was compared to a group of spinal cats that were not trained to step. These results showed that a much higher percentage of adult spinal cord transected cats could perform full weight-bearing stepping than previously thought and that step training resulted in marked improvements in the locomotor capacity of the hindlimbs. Subsequently Barbeau and Rossignol (2) also reported good stepping ability in step-trained adult chronic spinal cats, but it was not clear to what extent the locomotion could be attributed to training since all cats received step training at some stage of the experiment. In neither of these studies was the observed locomotor ability associated with step training recognized as a potential “learning” phenomenon.

Shortly thereafter several kinds of experiments triggered the realization that the spinal cord was capable of learning and that this was task specific (9,10). It became obvious that the improved stepping associated with step training occurred as a result of the repetitive activity of those spinal locomotor circuits that actually generated the load-bearing stepping, since spinal cats that were trained to stand bilaterally learned to stand but could not step as well as even those spinal cats that were not trained at all. Furthermore, the spinal cats could be trained to stand on a single leg, demonstrating that the reinforced pathways were the ones that were being activated. For example, after 30 min of daily stand training on the same leg for up to 12 weeks, the spinal cats showed a clear “preference” for standing on the trained leg when there were attempts to place the hindlimbs symmetrically on a force plate. This asymmetric preference also was manifested in the EMG patterns of the hindlimb muscles. Furthermore, immunohistochemical analysis of glutamic acid decarboxylase (GAD₆₇) levels, the enzyme associated with the synthesis of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), associated with flexor motoneurons were asymmetric (29). The GAD₆₇ levels associated with motoneurons presumed to be innervating knee flexor muscles based on their size, shape, and location in the spinal cord were higher on the stand-trained vs. the non-trained side.

In another type of experiment, we asked whether there was some evidence of short-term memory within the spinal sensorimotor circuitry. We placed an obstacle in front of the paw of a spinal cat while stepping on a treadmill and found that even after a single step cycle in which the paw was tripped, the trajectory of the ipsilateral hindlimb was already modified in a way that “anticipated” the presence of the obstacle on the subsequent step (12). The combination of these experiments as well as others (16) led us to the realization that the spinal cord not only could learn, but that the circuitry was smart, *i.e.*, it had the ability to recognize a specific pattern of sensory input and make appropriate decisions that would be consistent with continuing successful locomotion.

Another indicator that the spinal plasticity manifested as improved stepping ability was a learning phenomenon was suggested by the following experiment. Complete spinal cats were step trained for 12 weeks and then not trained for the next 12 weeks. After about 6 weeks without training there was no significant degradation in stepping ability, but after 12 weeks there was little or no successful stepping ability remaining (12). In addition, stepping ability was recovered much more rapidly with retraining than with the initial training period. Thus, it appeared at this stage that a motor skill could be improved with practice and that the skill could be lost if not practiced, *i.e.*, perhaps the spinal circuitry can learn and then forget.

IS IMPROVED MOTOR PERFORMANCE DUE TO TRAINING A SPECIFIC NEUROMOTOR PATHWAY?

One could take the view that even though learning can occur within the spinal circuitry, the spinal circuitry is limited to learning how to step forward and to stand. It seems, however, that

the spinal circuitry can learn other skills that it is “taught”. For example, the spinal circuitry can learn to avoid shock by controlling foot position. Buerger and Chopin (6) initially demonstrated this phenomenon in adult rats within a few hours after a complete mid-thoracic spinal cord transection in which the spinal rats learned to control the position of the paw while suspended in a sling. When the paw was extended below some threshold level, it received an electrical shock. After several minutes of this position-dependent shock, the spinal circuitry learned to avoid the shock by keeping the leg in a more flexed position, *i.e.*, above the threshold level. When a yoked rat received the same shock patterns, *i.e.*, the shocks were unrelated to paw position, no learning occurred. Grau and colleagues (19) have performed several variations of the experiment described above in attempts to understand the mechanisms of this instrumental learning paradigm. These later experiments have demonstrated further evidence of spinal learning, clearly satisfying the criteria for instrumental learning. It also seems more clear and definitive that this learning is NMDA-mediated and the evidence is consistent with brain-derived neurotrophic factor (BDNF) playing a critical role in this plasticity (Fig. 1) (3, 18,19).

Spinal learning also has been examined using a model in which animals are presented with a shock to the tibial nerve to induce an H-reflex and the animals receive a reward for either modulating the amplitude up or down over a period of days and thousands of shocks. These were some of the first experiments to demonstrate that while learning occurs in the spinal circuitry the source of the learning stimulus is dependent on supraspinal descending systems and, more specifically, at least in part, by the corticospinal tract (30). This learning phenomenon has been found to be associated with modulation of GABAergic synapses associated with the motoneurons (25). It was shown that the up-modulation of the H-reflex can correct the asymmetry in locomotion in rats after a mid-thoracic unilateral dorsal column lesion (30). Bigbee *et al.* (3) compared spinal rats that were tested based on an instrumental learning paradigm for their ability to learn a position-sensitive test for the paw in response to a shock to the paw after 7 weeks of training to stand on one leg, training to step bilaterally, or not trained (Fig. 2). There were no differences in the learning potential between non-trained and step-trained rats, but learning the paw position task was impaired significantly in both the ipsilateral and contralateral hindlimbs of the unilaterally stand-trained rats. Interestingly, in rats that were trained to increase the amplitude of the H-reflex, several characteristics of the stepping pattern were modified (30).

STRATEGIES TO FACILITATE SPINAL LEARNING

Pharmacological agents and epidural stimulation have been used successfully in complete spinal animals to facilitate stepping during training. A question arises as to whether these interventions can facilitate long-term changes that promote improved locomotion and raises the issue as to whether any learning-related phenomenon was due to the direct effect of the intervention, independent of the specific pathways that were activated repetitively. To address this issue, complete spinal mice were trained on a treadmill to step using quipazine, a 5-HT agonist, to facilitate stepping on a daily basis. The stepping performance of these mice was compared to those that only received quipazine daily. Stepping ability was enhanced in the mice receiving training plus quipazine, but no improvement was observed in the mice that only received quipazine (7,15). Further experiments using quipazine to facilitate stepping demonstrated the importance of the intrinsic variability between steps as a factor in how well the spinal cord learns to step (Figs. 3 and 4). These results indicate that improvement in locomotion was a function of the repetitive activation of the sensorimotor pathways, and occurred only as a result of the facilitation of the pathways activated during training in the presence of quipazine.

Another example of the specificity of spinal circuitry adaptation was demonstrated in rats that received a moderate spinal cord contusion injury and then trained to run in a voluntary activity wheel (13). The rats that were allowed to run in a voluntary wheel performed more poorly in stepping on a treadmill belt than similarly injured rats that were not exposed to the activity wheel. It was speculated that running in a voluntary wheel with a wire grid surface represented a significantly different sensorimotor experience than stepping on a flat surface. When the surface of the activity wheel was replaced with a solid surface, the rats exposed to the activity wheel stepped better on the treadmill than rats that were not exposed to the activity wheel. These results demonstrate an even further level of specificity of the sensorimotor pathways that are trained than was evident in the stand versus step-trained cats as described above.

The extensive amount of data derived from animal experimentation raises obvious questions as to whether similar phenomena can be observed in humans with a severe spinal cord injury. These studies have led to an increasing number of efforts to apply activity-based rehabilitation interventions. Compared to weight-supported locomotor training, there are some obvious advantages for individuals with a spinal cord injury to maintain, and perhaps gain, locomotor function by working on a stationary bicycle that is equipped with the capability of stimulating flexor and extensor muscles of the knee and ankle in synchrony with the movement of the pedals. This type of exercise can be important in maintaining muscle mass, in minimizing the onset of the metabolic syndrome associated with Type II diabetes, and in maintaining the musculoskeletal system so that step training eventually can be initiated with less likelihood of sustaining a musculoskeletal injury once training begins. Cycling exercise also is likely to stimulate a general increase in cardiopulmonary function. Another advantage is the lesser effort and equipment demands of the bicycle compared to the treadmill. Bicycle training also is much more practical because the requirements for therapy assistance are less, and perhaps none, depending on the severity of the injury. A disadvantage is that the movement differs significantly from that of stepping and, therefore, given the specificity of learning the motor task that is practiced, the degree of transfer of improved flexor-extensor coordination on the bicycle to stepping may be limited. In addition, cycling does not incorporate a significant level of practice for postural maintenance.

A practical strategy may be to begin rehabilitation using a bicycle and then proceed to actual load-bearing stepping as the neuromuscular system regains and adapts to elevated functional levels. Thus, a critical question is “to what degree can one gain load-bearing stepping capability by practicing on a bicycle?” The experiments with complete spinal rats suggest that the improvements in stepping are similar after being trained on a motorized bicycle and trained to step on a treadmill. The difficulty with this conclusion is that it is not feasible to train adult complete spinal rats to step without some other means of facilitating the excitability of the spinal circuitry, *e.g.*, pharmacologically or by tonic epidural stimulation (12). This is unlike the human in that even complete spinal cord injured individuals can be trained on a treadmill so that the level of coordination and the amplitude of recruitment of motor units within a given motor pool can be improved significantly with practice, even in individuals that were injured years earlier (11). There have been similar results in spinal trained cats (9,10). These results demonstrate that a significant potential for plasticity can be preserved for long periods after a spinal cord injury.

DOES THE AMOUNT AND TIME OF PRACTICE MATTER?

It is reasonably obvious that the amount of practice will determine the level of improvement in stepping after a severe spinal cord injury. The dose-response curve, however, is very poorly defined and it is highly unlikely that this relationship is linear. A direct attempt to address this issue was made by Cha *et al.* (8). Five-day old rats were completely spinalized and at about one month of age one group of these rats began training on a robotic device that moved the

legs over a treadmill belt in a fixed, step-like trajectory for 100 cycles a day (Fig. 5). A second group was trained in the same way but for 1000 cycles per day. A third group was not trained. After 6 weeks of training, the rats trained for 1000 steps were able to step with greater consistency than those trained for 100 steps, suggesting that “more is better”. Other efforts have been made to identify the minimum amount of practice needed to produce improved stepping, but this has been made largely on an ad hoc basis by comparing results across experiments. Significant improvement in stepping of adult spinal mice administered quipazine prior to each training session can occur with as little as 15 min of step training per day, 5 days/week for 5 weeks (15). Thirty min of step training per day, 7 days/week for 6 weeks has been successful in adult spinal rats that have received quipazine and/or epidural stimulation prior to each training session (21).

After a moderate contusion injury at T9, post-injury wheel running of mice given access to wheels for either 3 or 7 days/week were compared (13). Open field behavior observed for 15 weeks showed a significant linear increase in motor score ratings across groups. The hindlimb kinematics of treadmill walking revealed that both wheel-running groups improved stepping ability compared with sedentary controls and those that ran 7 days/week demonstrated more successful stepping on almost all criteria of locomotor quality. The data also suggested that the improvement in function could have been related to changes in serotonin fibers immediately caudal to the injury epicenter. These data, in general, suggest that progressive recovery of locomotor-related behavior can occur spontaneously over a period of weeks but the level and rate of recovery is further improved if locomotion is practiced 3 days/week and even more if practiced 7 days/week.

Surgical cord isolation of the lumbosacral segments of the spinal cord has proven to be a useful model in dissecting the effects of activity level on the neuromuscular system. This model of spinal cord isolation involves a complete spinal cord transection at both a mid-thoracic and a lower sacral level combined with bilateral deafferentation between the transection sites. In this preparation the motoneuron-muscle connectivity is maintained and, therefore, is fundamentally different from muscle denervation with respect to the effects on the muscle as well as the motoneurons. Those lumbosacral neurons that have the capability of central pattern generation will continue to receive input but it will be from other interneurons within the lumbosacral segments. While this amount of symmetric continuity within the segments is capable of generating action potentials in motoneurons, this occurs rarely (28). Thus, we consider this model to provide virtual electrical silencing of the spinal motoneurons and, therefore, a relatively effective means of examining the role of neuromuscular activity in defining neural pathway properties. After 6–8 weeks of spinal cord isolation motoneurons innervating the lower limb muscles had a lower rheobase and a higher spike afterhyperpolarization amplitude and input resistance compared to control rats (5). Also, more motoneurons demonstrated a frequency-relationship consistent with the activation of persistent inward currents. Thus, after spinal cord injury the motoneuron properties tend to become like those observed in more excitable motoneurons. It is also interesting that the properties of the motoneurons after spinal cord isolation did not differ from those observed after spinal cord transection alone. This would suggest that the predominant factors in preserving normal motoneuron properties are derived from supraspinal input.

PHARMACOLOGICAL INTERVENTIONS TO FACILITATE SPINAL LEARNING: THE GABAERGIC SYSTEM

After a complete spinal cord injury, step or stand training can markedly change the physiological and biochemical state of multiple neurotransmitter-modulator systems, and thereby change the pharmacological properties of the sensorimotor pathways that generate stepping and standing. Pharmacological as well as biochemical data show that task-specific

adaptive changes occur in spinal neuronal circuits of spinal cats trained to stand or step (12, 26,29). Administration of modest doses of the glycine receptor antagonist strychnine that did not affect locomotion in step-trained spinal cats, significantly improved the stepping ability of poorly stepping non-trained or stand-trained spinal cats (12). Similarly, the stepping ability of poorly stepping spinal cats can be dramatically improved by administration of the GABA_A receptor antagonist bicuculline. The improvement of stepping upon the administration of strychnine or bicuculline may occur by facilitating neuronal excitation by blocking the abnormally high levels of general inhibition resulting from a complete spinal cord injury.

Non-trained spinal rats have increased levels of the GABA synthetic enzyme GAD₆₇ and glycine and GABA_A receptors in the lumbar spinal cord, whereas step-trained spinal rats have near-normal levels (12,29). In agreement with this, GAD₆₇ protein levels in lumbar spinal cord extracts were higher than normal in spinal cats that were step trained for 2 months and then not trained for 5 months. After just one week of retraining, however, there was a significant reduction in GAD₆₇ protein levels (29). In step-trained cats, the GAD₆₇ levels around motoneurons were inversely correlated with stepping ability. Training spinal cats to bear weight also appears to reduce GABA signaling. Cellular mapping of GAD₆₇ also suggests that the spinal cord of both stand-trained and non-trained spinal cats have selectively higher inhibitory potential compared to step-trained animals. These findings suggest that the inability of stand-trained spinal animals to step is closely linked to an elevated level of inhibition of flexor motor pools. GABA-mediated actions in the spinal cord include pre-synaptic inhibition of primary afferent fibers and post-synaptic inhibition of interneurons, sensory neurons, and motoneurons (1).

The specificity of GABAergic changes associated with the specific type of training emphasizes the concept that the activity in the neural networks of the lumbar spinal cord is, to a large extent, determined by the pattern of activity that the hindlimbs experience. Based on a detailed ultrastructural analysis of the synaptic boutons on the some of tibialis anterior motoneurons in young adult rats that were spinalized at a mid-thoracic level at postnatal day 5, there was a relative increase in the number of inhibitory relative to excitatory synapses, but this ratio was normalized in spinal rats that were trained to step (22). This defect was of a greater magnitude in the gamma motoneurons than the alpha motoneurons. How these changes are related to increased inhibitory and/or excitatory synaptic activity, and in which neural circuits this is manifested, remains unclear.

Spinal cord injury also may have an effect on non-neuronal cells in the spinal cord. For example, compared to control rats, spinal rats showed elevated immunostaining of the GABA_A receptor γ -2 subunit in astroglial cells, *i.e.*, cells positive for the cellular marker glial fibrillary acidic protein (GFAP), in the ventral horn regions surrounding retrogradely labeled soleus and tibialis anterior motoneurons (4). This elevated level returns toward nearly undetectable control levels after 6 weeks of step training.

Administration of 5-HT, its precursor 5-hydroxytryptophan (5-HTP), or the 5-HT agonists quipazine and m-chlorophenylpiperazine (m-CPP), are effective in improving locomotion in spinal cats (20,26) and rats (14,23). Although descending 5-HT axons are eliminated by a complete spinal cord injury, the receptors remain largely intact and functional, although their levels are modified. Consistent with these pharmacological studies, autoradiographic receptor-binding studies showed elevated levels of α 1-NA, α 2-NA, and 5-HT_{1A} receptors in selected laminae of the lumbar spinal cord segments of spinal cats at 15–30 days post-injury, which gradually returned to control values (17,27). These changes in receptor density and the type of receptor could possibly change the excitability level of different types of neurons contributing to the locomotor circuitry.

USING THE SPINAL CIRCUITRY TO MODEL INTEGRATIVE PRINCIPLES OF THE BRAIN

A major limitation in understanding how the brain processes multiple sources of sensory information, makes decisions regarding the appropriate motor response to a given pattern of sensory information, and even in determining what the critical sensory information is which leads to a given simple or more complex motor response is very poorly understood. Part of the reason for this is the lack of technical capability to monitor a sufficient number of physiological events during routine motor behaviors. Different strategies historically have been used to circumvent this limitation, but all are based on the concept of reducing the complexity of brain function by eliminating some of its components, either surgically or pharmacologically, or by reducing the functional capacity of the system using anesthetics or other experimental perturbations. Another approach to gain simplicity has been to study more evolutionary simpler animals and even the isolation of neuronal networks of these animals. It seems increasingly apparent that the complete spinal animal can be used for understanding how the nervous system perceives complex patterns of sensory information, how it interprets these inputs, makes decisions, and executes online elegant levels of motor control. For example, the complete spinal model demonstrates that all of these complex functions can be carried out by the spinal circuitry. As the evidence above suggests, the spinal cord can perceive and recognize very complex patterns of sensory information and based on this information it can make appropriate decisions to sustain locomotion. In response to the specific patterns of sensory information received, the spinal cord can step in the appropriate direction and it can learn and forget. More importantly all of these phenomena occur *in vivo* in completely non-anesthetized preparations with no supraspinal input.

The spinal circuitry can no longer be perceived as simply a relay system between the brain and the motor pools. Instead, there are features of the spinal circuitry that function remarkably similar to functional circuits within the brain. Based on these observations, it is becoming increasingly apparent that much can be learned about how the normal nervous system, *i.e.*, the brain and spinal cord, functions as well as how to optimize the recovery of function after a spinal cord injury.

PRINCIPLES OF SENSORIMOTOR PLASTICITY

Based on many of the observations regarding the recovery of motor function after a spinal cord injury, it seems timely to determine the degree to which one can formulate some general principles as to the nature of this recovery. It appears that the following principles can be formulated:

1. The changes in the synaptic efficacy associated with improved sensorimotor function represent a phenomenon that occurs among neural networks throughout the nervous system.
2. The execution of a motor task by the spinal circuitry in absence of supraspinal input is defined by the efficacy of those synapses that translate a sensory profile to a given combination of motor pools.
3. The efficacy of the synaptic events within an integrated sensorimotor circuit is a function of the frequency and/or pattern of activity of the circuitry that is involved with that specific task.
4. This efficacy and predictability of excitation of those synapses within that network that generates a motor task defines the accuracy and predictability of performing the given motor task.

CONCLUSIONS

Those neural pathways directly involved in a motor task seem to be selectively reinforced with repetitive activity.

Reinforcement of these neural pathways is manifested as enhanced recruitment within a motor pool and improved coordination among those motor pools that generate a practiced motor task.

The improved motor performance after a complete spinal cord transection is in large part a manifestation of spinal learning.

While there are multiple cellular, extracellular, and intercellular changes that are activity dependent, it remains unclear which of these changes are critical for spinal learning to occur.

Multiple neural growth factors are highly activity dependent in the spinal cord and may play a critical role in learning and other adaptive processes.

The implications of the activity-dependent plasticity that has been identified in the learning of a motor event by the spinal circuitry may be providing important clues as to how plasticity can be directed among circuits within the brain.

The functional strategies of the spinal locomotor circuitry related to sensory perception, recognition, and decision-making suggest that the sensorimotor pathways of the lumbosacral spinal cord could serve as a powerful *in vivo* model for understanding basic principles of sensory processing, perceiving and recognizing complex patterns, learning and in understanding the mechanisms of activity-dependent adaptations in the central nervous system.

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Fig. 1.

Correlation between mean response duration during the first 10 min of training and mRNA levels for BDNF (A), CaMKII (B), CREB (C), and synapsin I (D). There was a significant positive correlation for all the molecular systems examined except synapsin I. (Reprinted from Gómez-Pinilla F, Huie JR, Ying Z, Ferguson AR, Crown ED, Baumbauer KM, Edgerton VR, Grau JW. BDNF and learning: Evidence that instrumental training promotes learning within the spinal cord by up-regulating BDNF expression. *Neuroscience*. 2007; 148:893–906. Copyright © 2007 Elsevier. Used with permission.)

Fig. 2.

Instrumental learning in Non-Tr, Step-Tr, and Stand-Tr rats after 7 weeks of step training or unilateral hindlimb stand training. The flexion duration (A) and the number of flexion responses (C) for each 1 min bin over 30 min of testing are shown, as are the overall average test flexion durations (B) and response numbers (D). For flexion duration, Non-Tr and Step-Tr groups were not significantly different, while the Stand-Tr flexion duration was significantly lower relative to both the Step-Tr and Non-Tr groups (B). The average number of flexion responses was not different between groups (D). Data are mean $\pm$ SEM. *, significantly different at $p < 0.05$.

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Fig. 3.

Soft robotic control schematics on how the semi-active control paradigm for step training is implemented. A moving window (thin interrupted line) bounds the desired trajectory (thick solid line) of the mouse limb during stepping. Within the window, the robotic arm allows the mouse to vary its movement. However, when the neural control desired trajectory falls outside of the window, the robot will experience a convergent velocity field, that actively returns the mouse's limbs back within the window. This type of soft control is thought to approximate the "assist as needed" approach used by experienced therapists. (Reprinted from Cai LL, Courtine G, Fong AJ, Burdick JW, Roy RR, Edgerton VR. Plasticity of functional connectivity in the adult spinal cord. *Phil. Trans. Roy. Soc. Lond. B: Biol. Sci.* 2006; 361:1635–46. Copyright © 2006 Royal Society Publishing. Used with permission.)

Fig. 4.

Schematic of a semi-active fixed-trajectory paradigm for step training where the desired limb trajectory (thick solid line) is bounded by both inner and outer boundaries (thin interrupted line) is shown. The actual trajectory that the neural circuits might induce is allowed to vary within the boundary. However, once the trajectory falls outside of the boundary, the robot will actively bring it back within the boundaries. The black line with periodic dots illustrates the potential positions that the intrinsic neural control might choose to generate for any given bin time. The probability that the neural control would move the limb to the exact position defined by the thick solid line, representing a fixed trajectory, is highly unlikely. As a result, theoretically, the neural control system is continuously disrupted by the fixed trajectory paradigm. This fixed trajectory, therefore, does not allow the neural control circuitry to respond to any of its intrinsic activation patterns, but rather forces the intrinsic circuitry to continuously respond to external perturbations. This strategy for control would seem to unnecessarily disrupt the spinal circuitry and in the process minimize or even preclude the intrinsic circuitry from interpreting relevant proprioceptive information required to generate a solution (*i.e.*, make choices) and, thus, presumably prevent the circuitry from meaningful learning phenomena. (Reprinted from Cai LL, Courtine G, Fong AJ, Burdick JW, Roy RR, Edgerton VR. Plasticity of functional connectivity in the adult spinal cord. *Phil. Trans. Roy. Soc. Lond. B: Biol. Sci.* 2006; 361:1635–46. Copyright © 2006 Royal Society Publishing. Used with permission.)

Fig. 5.

Training amount determines the responsiveness to hindlimb weight bearing. Mean stance duration (A), mean swing duration (B), mean step length (C), and mean step height (D) are shown across different levels of weight bearing for stepping tests at $8 \text{ cm} \cdot \text{sec}^{-1}$ performed after 4 weeks of training. A “55”, “65”, and “75” indicates that the data are significantly different from 55%, 65%, and 75% body weight support (BWS), respectively. For the 1000-step group in (A): the 55% BWS data are significantly different from the data at 75%, 85%, and 95% BWS; the 65% BWS data are significantly different from the data at 85% and 95% BWS; the 75% BWS data are significantly different from the data at 95% BWS. For the 100-step group in (A), the 55% BWS data are significantly different from the 65%, 85%, and 95% BWS data. For the 1000-step group in (B): the 55% and 65% BWS data are significantly different from the 85% and 95% BWS data, and the 75% BWS data are significantly different from the 95% BWS data. Boxes, significant difference between the 1000-step and 100-step groups. Values are mean $\pm$ SEM calculated from 13 rats in each group. BWS is body weight support. (Reprinted from Cha J, Heng C, Reinkensmeyer DJ, Roy RR, Edgerton VR, de Leon RD. Locomotor ability in spinal rats is dependent on the amount of activity imposed on the hindlimbs during treadmill training. *J. Neurotrauma* 2007; 24:1000–12. Copyright © 2007 Mary Ann Liebert, Inc. Used with permission.)