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Ji Ye Kwon April 12, 2012

Investigating the co-activation of autonomic and motor coordination within locomotion

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Abstract

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Spinal cord injuries (SCI) sever communication and interconnections between the autonomic system and the locomotive system. Due to the uncoupling of these two systems, the ability of the blood pressure and the respiratory system to adapt to movement is essentially gone. In this research, the autonomic interaction with locomotion was analyzed in neonatal rodents. The entire circuitry required to generate locomotion is housed within the upper lumbar spinal cord, and the circuitry is termed the central pattern generator. This region is also where sympathetic preganglionic neurons (SPNs) controlling hindlimb function are also located. I hypothesized that the SPNs housed within the upper lumbar cord (L1-L2) contained intraspinal neural elements that connect to and modify locomotor circuits. To test this hypothesis, electrophysiological and pharmacological experiments were performed in the isolated neonatal rodent spinal cord maintained *in vitro*. In two different experimental paradigms, sacral dorsal root stimulation and bath-application of serotonin (5-HT) with N-methyl-D-aspartate (NMDA) induced locomotor-like activity in the spinal cord, reported electroneurographically from motor axons exiting lumbar ventral root L2. By electrically stimulating the motor ventral roots that also contain SPN axons during ongoing locomotion, the influence of the SPNs was explored. For sacral dorsal root stimulation-evoked locomotion, simultaneous stimulation on a sacral dorsal root and a L2 ventral root increased the burst amplitude, showing that axons in the L2 ventral root can feedback and amplify motor output. However, such dual stimulation did not alter the locomotor rhythm. However, L2 ventral root stimulation during pharmacologically induced locomotion was able to alter the locomotor rhythm. Stimuli were shown to be able to ‘restart’ a locomotor cycle. Both observations demonstrate that axons contained within the L2 ventral root also have intraspinal connections onto the locomotor CPG. As motor axons are not able to do this, I assert that the axons are from SPNs. Overall these findings support an existing link between the autonomic and somatic circuits, independent of brain commands. Further research will advance the understanding of sympathetic preganglionic neurons and their effect on the locomotor central pattern generator. This will provide useful insight for advancement in neuroprosthetic devices for SCI patients.

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INTRODUCTION

Spinal cord

The spinal cord is a part of the central nervous system (CNS). It is a multifaceted CNS structure that serves as a critical center for neuronal circuits that integrate and coordinate complex sensory, motor, and autonomic functions[7]. The basic anatomy of the spinal cord is shown in Figure 1. The spinal cord is comprised of the grey matter and white matter is organized into the cervical, thoracic, lumbar, and sacral segments. The white matter, which contains axon tracts, surrounds the gray matter, which contains the neuronal cell bodies divided into anatomical layers called laminae. Lamina IX is part of the ventral horn and contains the motor neurons. These motoneurons send axons to exit the spinal cord via ventral roots. The ventral horn and intermediate zone of the spinal cord contain neural elements associated with motor output. These neurons receive input from both sensory systems as well as signals from the brain. Sensory information enters the spinal cord through dorsal roots and either synapse onto spinal neurons or project rostrocaudally through the medium of white matter[1].

Each segment of the spinal cord has a spinal nerve that exits which contains the sensory, motor, and autonomic nerve fibers involved in body functions[7]. The lumbar portion of the spinal cord contains the greater number of neurons required for the hind limb functions[7]. The spinal cord also houses the entire neuronal network that organizes locomotion: the locomotor central pattern generator[9]. The exact location and interneuronal makeup of the locomotor central pattern generator (CPG) has not been identified in mammals, but studies have found that L1-L2 spinal segments are the site of the core neural network for locomotion[3].

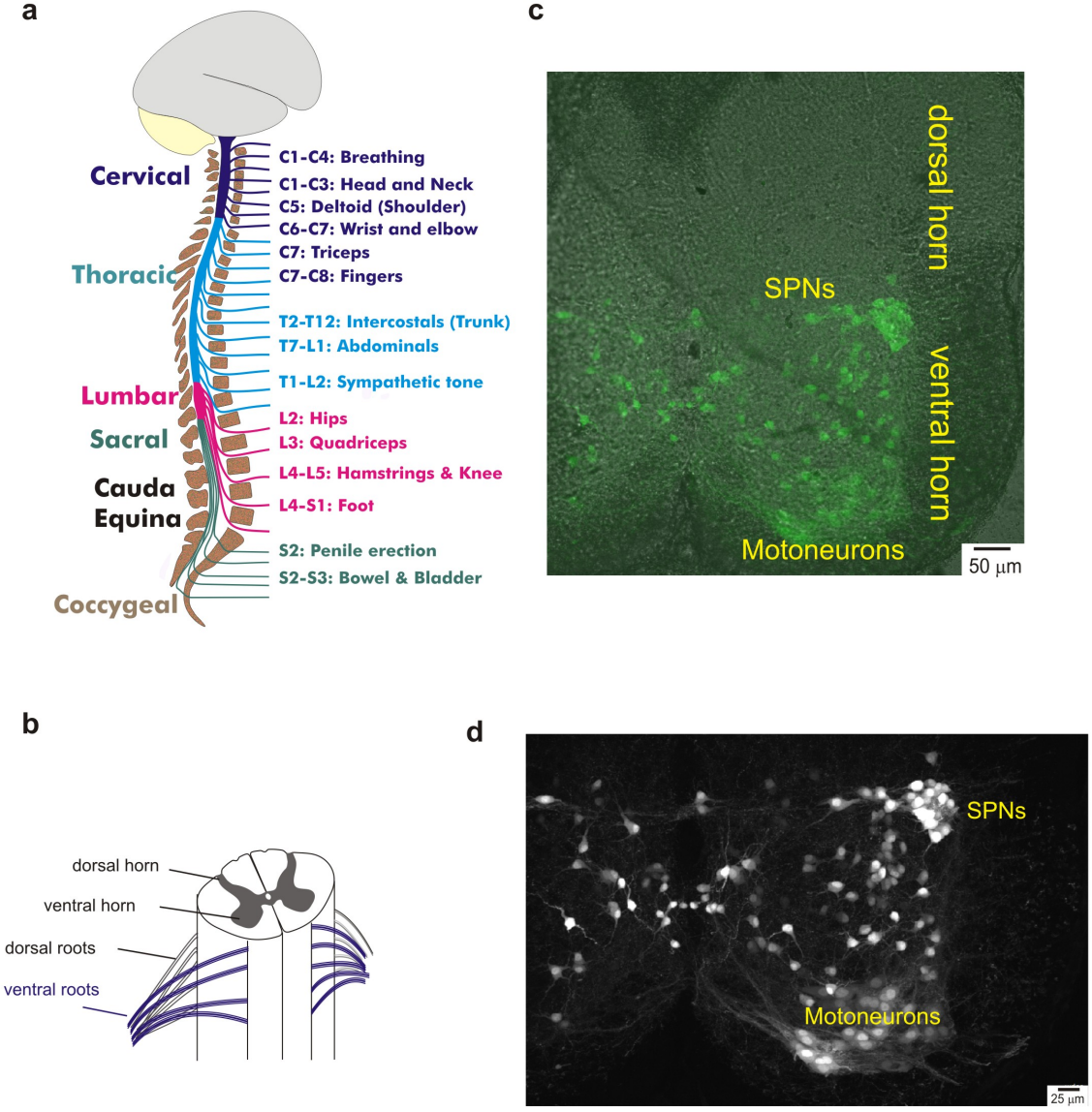

Figure 1:Basic anatomy of the spinal cord

(a) The spinal cord is housed within the vertebral column and divided into several regions specialized to control different regions of the body. (b) Schematized cross section of the spinal cord identifying the sensory dorsal horn gray matter where sensory afferents enter via dorsal roots and the ventral horn gray matter containing motoneurons whose axon exits via ventral roots. (c) A genetic marker labels motoneurons and sympathetic preganglionic neurons located in the ventral half of the spinal cord. (d) Same image as in A in grayscale with dorsal horn omitted.

Spinal cord injuries

Spinal cord injuries (SCIs) occur when trauma impairs the function of the body below the level of injury. SCIs disconnect the communication between the brain and the neurons below the injury. The spinal circuitry and neurons below the injury may still retain their functional abilities, but the control and modification of them via descending commands from the brain is lost. Due to this disconnect, various connections between functional circuitries that exert complex and tunable control of bodily sensation or movement generation are lost. One of the interconnections commonly lost in SCI is between the autonomic system and the motor system. This disconnect is characterized by autonomic dysreflexia, bladder-sphincter dyssynergia, sexual dysfunction, and motor control dysfunction. With the regard to locomotion, the ability of the blood pressure and the respiratory system to adapt to varying metabolic demands of movement is essentially gone. The emphasis of this research project is on the interaction of the autonomic sympathetic nervous system with the locomotive system will lead to an understanding of and development of methods for recovering the lost locomotor function. The intact functions of neuronal circuitries provide hope of restoring the operation of the spinal cord for SCI patients.

Somatic and autonomic nervous system

The nervous system is split into the central nervous system and the peripheral nervous system. The central nervous system consists of the brain and the spinal cord, while the peripheral nervous system consists of neurons, ganglia, and nerves. The peripheral nervous system is further divided into the somatic system and the autonomic system. The somatic system is responsible for the voluntary control of body movements and carrying motor and sensory information. On the other hand, the autonomic system is responsible for the involuntary actions

with its control of smooth muscle and is also responsible for the subconscious movements with its control of breathing, circulation, and digestion.

Commonly, the motor and autonomic nervous system subdivisions have been studied separately in spinal cord injury studies. This is most likely due to the divisions in the nervous system, with the somatic nervous system to be associated with the voluntary movements and the autonomic nervous system to be associated with involuntary actions. However, it was proved that recruitment of locomotor and cardiorespiratory (autonomic) responses were not due to separate brain circuits, but rather were generated from common brain structures including the hypothalamus[5]. This means that locomotion and autonomic sympathetic nervous system functions are tightly linked in the brain. Typically, an increase in the autonomic cardiorespiratory drive is correlated with locomotor onset. The increased intensity of locomotion was matched with increased limb perfusion; the autonomic nervous system regulates the blood pressure and respiration to adapt to the animal's movements[1]. The paralleled system ensures sufficient supply of oxygen and nutrients to meet the metabolic demands of movement. This essential coordination of hindlimb and cardiorespiratory activity is lost after spinal cord injury and leads to devastating aberrant motor control syndromes characteristic of their uncoupling including autonomic dysreflexia, bladder-sphincter dyssynergia, sexual dysfunction, and motor control dysfunction. Re-coupling motor and autonomic nervous system is imperative for regaining lost functionality of locomotion and for treating spinal cord injuries.

Evidence of autonomic interactions with the locomotor CPG in spinal cord has been demonstrated previously. In rabbits, the sympathetic outflow was recorded from the phrenic nerve. This study demonstrated that the respiratory information, transmitted by the phrenic nerve, was in conjunction with limb movements[21]. In cats, sympathetic efferent activity from the

phrenic, cardiac, and cervical nerves was phase coupled to the hindlimb, forelimb, and trunk muscle efferents during induced locomotion[18].

The L1-L2 ventral roots contain axons of L1-L2 sympathetic preganglionic neurons, and these are the ones that control hindlimb sympathetic function in rat and mouse. Interestingly, the same L1-L2 spinal segments contain the core neural network for generating locomotion[3]. This anatomical coexistence may help coordinate the known strong interrelationship between spinal autonomic and locomotor systems. Thus, an integrative study of the parallel systems within the spinal cord seemed of upmost importance to investigate.

Locomotor pattern generator network

The locomotor central pattern generator (CPG) is responsible for the rhythmic behavior in the act of moving, such as in the actions of walking, running, swimming, or slithering. This specialized network of neurons is capable of autonomously generating rhythms. It works to coordinate the movement of the muscles to propel the animal in its environment[1]. The CPG acts as an oscillator between the right and left sides of the body, and between flexors and extensors; this alternating rhythm can be initiated by the descending commands from the brain or by sensory stimuli as part of an escape response[7]. This motor output with its alternation of bursts can be recorded from the right and left lumbar L2 ventral roots, as it has been shown that activity from these roots correspond to activity in flexors[10]. As the hindlimb locomotor CPG is fundamentally composed of left-right and flexor-extensor alternation, the circuitry must include reciprocal inhibitory connections between left and right hindlimbs, and between flexors and extensors (Figure 2.a). For example, if the left side hip flexors were active, hip flexors on the right side would be inhibited. This mutual inhibition is responsible for the production of alternating patterns. A ‘half-center’ organization of mutual inhibitory interactions has been

hypothesized to account for the CPG's role in the alternation between flexors and extensors in a limb and in the left-right alternation in flexors and extensors between corresponding limbs[14].

The neurons comprising the locomotor CPG can generate coordinated locomotor activity without commands from the brain or sensory feedback[9]. Aside from brain, alternating left-right activity can be initiated in the isolated spinal cord by delivering neuromodulators like serotonin (5-HT) that are normally released from the brain[17]. Stimulation of afferents in the sacral dorsal roots has also been reported to generate a locomotor-like mother rhythm[22]. The resulting motor efferent output behaviors have been studied and identified. Recent unpublished work in the Hochman lab suggests that part of this measured rhythmic output actually arises from SPNs, as an autonomic efferent pattern generator whose sympathetic preganglionic axons exit L2 ventral roots and is recruited by sacral visceral C fiber afferents[1].

Locomotor-like activity (LLA) in the isolated neonatal rodent spinal cord is a powerful model system

An isolated spinal cord can be maintained *in vitro* and elicited to display this rhythmic alternating activity by pharmacological induction (bath applied neuroactive molecules)[11] or by electrical stimulation of the sensory afferent-containing dorsal roots[12]. In activating this rhythmic alternation via various neurotransmitters and excitatory amino acids, the activity is recorded and measured by ventral roots. The motor output from the lumbar ventral root L2 corresponds to the activity in flexor muscles and the reciprocal inhibition resulting in left-right rhythms[11]. The induced locomotion in the isolated spinal cord was termed locomotor-like activity (LLA). The LLA spatiotemporally resembles the locomotion of the intact hindlimb and is thought to provide a signature for activation of the locomotor circuitry[11]. Bath application of different combinations of drugs has an excitatory effect on the CPG. Applying both serotonin (5-HT) and N-methyl-D-

aspartate (NMDA) induces LLA in neonatal rodents. This combination generates stable and long lasting rhythmic activity. In addition to 5-HT/NMDA, other neurochemicals such as dopamine and noradrenaline have produced rhythmic motor patterns for several hours[6,22].

Electrical stimulation of the sacral and lumbar dorsal roots is another method of inducing LLA. The electrical stimulus recruits the sensory afferents that subsequently activate the spinal hindlimb locomotor CPG in the neonatal rodent spinal cord[1,22]. This method provides the ability to access the motor system by using preexisting, natural feedback pathways. The resulting alternating left-right rhythmic motor pattern can be recorded from the lumbar L2 ventral roots. Stimulating the sacral dorsal roots has been shown to depend on activation of sacral cord relay neurons projecting rostrally into the lumbar segments[1].

This induced locomotion can be maintained *in vitro* in rat and mouse spinal cords. As locomotor CPG is present at birth, the isolated intact neonatal rodent spinal cord maintained *in vitro* is a powerful model system to effectively study the properties of locomotion. Viability can be maintained by the O_2 supplied to an artificial cerebral spinal fluid (aCSF). This prevents hypoxia and maintains the life of the spinal cord for several hours[19]. The extracellular medium could be easily manipulated by introducing drugs into the aCSF bath. Electrical stimulation could also be easily manipulated by the suction electrode placement on the small, isolated spinal cord.

The sympathetic SPNs are housed in thoracic and upper lumbar (L1 and L2) spinal cord. By electrically stimulating the upper lumbar ventral roots containing autonomic efferent axons, back-propagating action potentials travel into the cell bodies and possibly also the dendrites and intraspinal axons of sympathetic preganglionic neurons (SPNs). If SPNs have local axons that synapse on other neurons, other spinal neurons may be recruited following ventral root

stimulation. If the SPNs contribute to activation of the locomotion, stimulating the ventral L2 roots may modify properties of the observed locomotor output. An important complication to such an experimental design is that L2 ventral root stimulation also recruits the predominantly flexor motor axons that also exit this root. Therefore, if L2 ventral root stimulation does alter locomotion, the technically much more difficult selective stimulation on SPNs in the sympathetic chain will ultimately be required to unambiguously identify actions as arising from SPNs.

Experiments are proposed to test coupling between the autonomic and somatic systems

The proposed experiments were performed to test the hypothesis that SPNs can modify induced locomotion thereby supporting a coupling between autonomic and somatic circuits independent of descending brain commands. Experimental paradigms that test this coupling involve two separate experimental approaches. The first involves electrically activating LLA by stimulating the sensory afferents entering sacral dorsal roots. The effect of simultaneous L1/L2 lumbar ventral root stimulation on the observed LLA was tested. The second involves generating long-lasting LLA with bath applied 5-HT and NMDA. Here again I tested effects of L1/L2 lumbar ventral root stimulation on the observed LLA. Any observed alterations in either experimental paradigm would support a link between the autonomic and motor systems during locomotor activity. For example, expected alterations would be 'resetting' or changing locomotor frequency. Sensory afferent stimuli can reset or entrain locomotor rhythms[8]. Resetting occurs when the stimulus changes the duration of an ongoing extensor or flexor burst such that there is a delay or advancement of future bursts which occur at the same frequency as before. Entrainment occurs when the locomotor frequency is made to follow the frequency of the sensory stimulus.

Ultimately, a method is sought to recouple coordination of the autonomic and motor circuits. The coupling and control of the interconnected locomotion and autonomic system will be useful insight for spinal cord injured individuals. In seeking this, the responses and control of arterial pressure, respiratory activity, and locomotion will be better understood.

METHODOLOGY

All procedures were approved by the Emory University Institution Animal Care and Use Committee and were in compliance with the National Institutes of Health guidelines for animal care.

Experiments were performed using spinal cords of postnatal day zero (P0) to postnatal day 4 (P4) mice and rats. Animals were decapitated and eviscerated. The animals were then placed in a recording chamber containing a bath of ice-cold (4° C) oxygenated (95% O_2 /5 % CO_2) artificial cerebral spinal fluid (aCSF) which contained in mM: 128 NaCl, 1.9 KCl, 1.2 KH_2PO_4, 26 $NaHCO_3$, 2.4 $CaCl_2$, 1.3 $MgSO_4$, and 10 glucose at pH of 7.4[8]. The spinal cords were quickly exposed to the oxygenated aCSF by a ventral vertebrectomy. The spinal cords were then isolated with the roots intact and removed from the vertebral column. The isolated cords were pinned and stabilized ventral side up with insect pins. The isolated cords were superfused with the oxygenated aCSF for 40 min at room temperature before starting the experiments.

Dual stimulation of the sacral dorsal and lumbar ventral roots

Stimulation suction electrodes and recording suction electrodes were placed onto the roots in all the experiments. The glass suction electrodes averaged a tip diameter of 30 μm. Glass suction electrodes were applied to a lumbar L2 ventral root and a sacral S1-S4 dorsal root for stimulation and the opposite L2 ventral root for recording. In some cases, recordings were also

collected from the lumbar L1 ventral root on the same side as the stimulated lumbar L2 ventral root. This was to show the maintenance of rhythmic, alternating left-right bursts during ventral root stimulation. For most experiments, the data was digitally amplified (10,000x), low-pass filtered (3 kHz) and high pass filtered (300 Hz), and recorded by Digidata 1320A, Axon Instruments.

The rhythmic, alternating bursts were induced by electrically stimulating the sacral dorsal roots. The stimulation of the sacral dorsal roots generated the locomotor-like activity and served as the control. Trains of stimuli between 0.4 and 2 Hz were used at intensities of 100-200 μA and durations of 100-200 μs. There was a resting period between the trains of stimulus for sufficient recovery of the motoneuron depolarization occurring throughout the stimulation trains[16]. Coupled to the repetitive stimulation of the sacral dorsal root, another glass suction electrode was applied to a lumbar L2 ventral root for stimulation (Figure 2.b). Two different stimulation protocols were tested. The first protocol was to stimulate the dorsal root between 0.4 and 2 Hz, used at intensities of 100-200 μA and durations of 100-200 μs. Additionally a ventral L2 root was stimulated at 50 Hz, 5 pulses every 10 seconds, used at intensities of 50-200 μA and durations of 50-200 μs. The second stimulation protocol was to stimulate the dorsal root between 0.4 and 2 Hz, used at intensities of 100-200 μA and durations of 100-200 μs. Additionally, a ventral L2 root was stimulated between 0.2 and 1 Hz, with simultaneous stimuli pulses as the dorsal stimuli, used at intensities of 50-200 μA and durations of 50-200 μs

Pharmacological induction

In certain experiments, locomotor-like activity was evoked by the application of serotonin (5-HT) and N-methyl-D-aspartate (NMDA) to the isolated spinal cord (Zhong et al. 2006). The concentrations of 10-25 μM 5-HT and 5 μM NMDA were applied to the oxygenated

aCSF bath. The neurochemicals were obtained from Sigma-Aldrich, and the stock solutions of 10-100 mM were added to the bath according to the desired concentration. The induced locomotor-like activity from the NMDA/5-HT was coupled to electrical stimulation of the L2 ventral root (Figure 2.c). The stimulus trains to the ventral L2 root was between 0.2 and 50 Hz, used at intensities of 50-200 μA and durations of 50-200 μs.

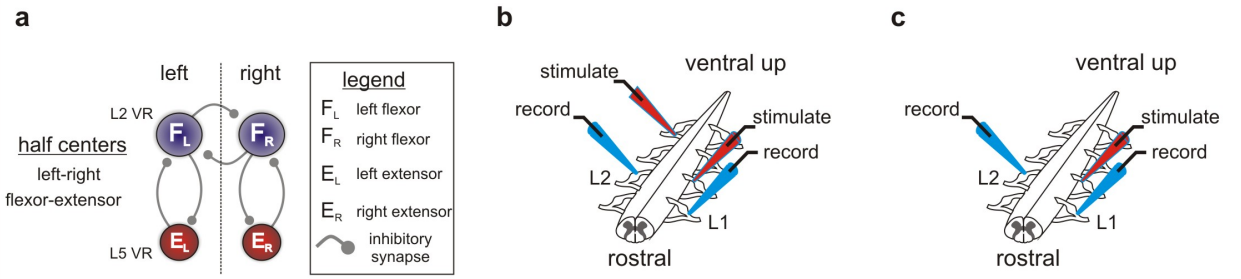

Figure 2: Schematics of the proposed organization of the spinal locomotor central pattern generator and general recording configurations

(a) The spinal locomotor central pattern generator is thought to have a half center organization characterized by receiprocal inhibitory connections between left and right and between flexors and extensors. (b) Isolated spinal cord preparation with stimulating both dorsal sacral and ventral L2. (c) Isolated spinal cord preparation with 5-HT/NMDA application and stimulation.

Analysis

All data was recorded by a Digidata 1320A, Axon Instruments. The recordings were analyzed by Clampfit 10.2 software (Molecular Devices) while locomotor cycle based analyses were exported to a specialty written MATLAB program called SpinalMOD (Spinal Motor Output Detector; http://userwww.service.emory.edu/~shochm2/SpinalMOD.html). SpinalMOD was used to quantify and compare rhythmic locomotor patterns. By opening the files collected with pCLAMP, burst duration was visually evaluated. Data were then rectified and filtered for burst identification. After the onset and offset of bursts were

identified, the burst intensity, burst duration, bursting waveforms, and phase response curves were calculated and exported to CorelDRAW format.

RESULTS

During dorsal root stimulation evoked LLA, ventral stimulation does not alter locomotor rhythm

Locomotor-like activity evoked by sacral dorsal root stimulation was characteristically alternating and rhythmic between the right and left L2 ventral roots. The occurrence of a burst on the same side ventral root was prompt, and a corresponding silent period on the opposite root with subsequent bursting coordinated with quiescence on the previous side (left-right alternation) was evident in the 7 out of 11 experiments using the stimulus-induced rhythmic pattern as a control. Stimulation with pulses at the amplitude of 200 μA, duration of 200 μs, and frequency of 2 Hz most reliably induced LLA. Stimulating the ventral root alone at the same amplitude, duration, and frequency as the sacral dorsal root stimulus did not evoke this alternating LLA (n=2). Sporadic activity during ventral root stimulation was occasionally observed, but a rhythmic right-left pattern was never seen (Figure 3). Stimulus pulses with amplitudes ranging from 50-200 μA, duration of 50-200 μs, and frequency of 0.4-50 Hz were not sufficient for recruitment of lumbar motor rhythmogenesis.

Dual stimulation of the sacral dorsal and lumbar ventral roots with the protocol of stimulating the ventral root at 5 pulses every 10 seconds at 50 Hz was conducted in 5 experiments, using the sacral dorsal root stimulation as the control. There was no obvious change in the onset of LLA or in the intensity of the burstlets when the ventral root was stimulated at 5 pulses at 50 Hz every 10 seconds with intensities of 50-200 μA and durations of 50-200 μs (n=5).

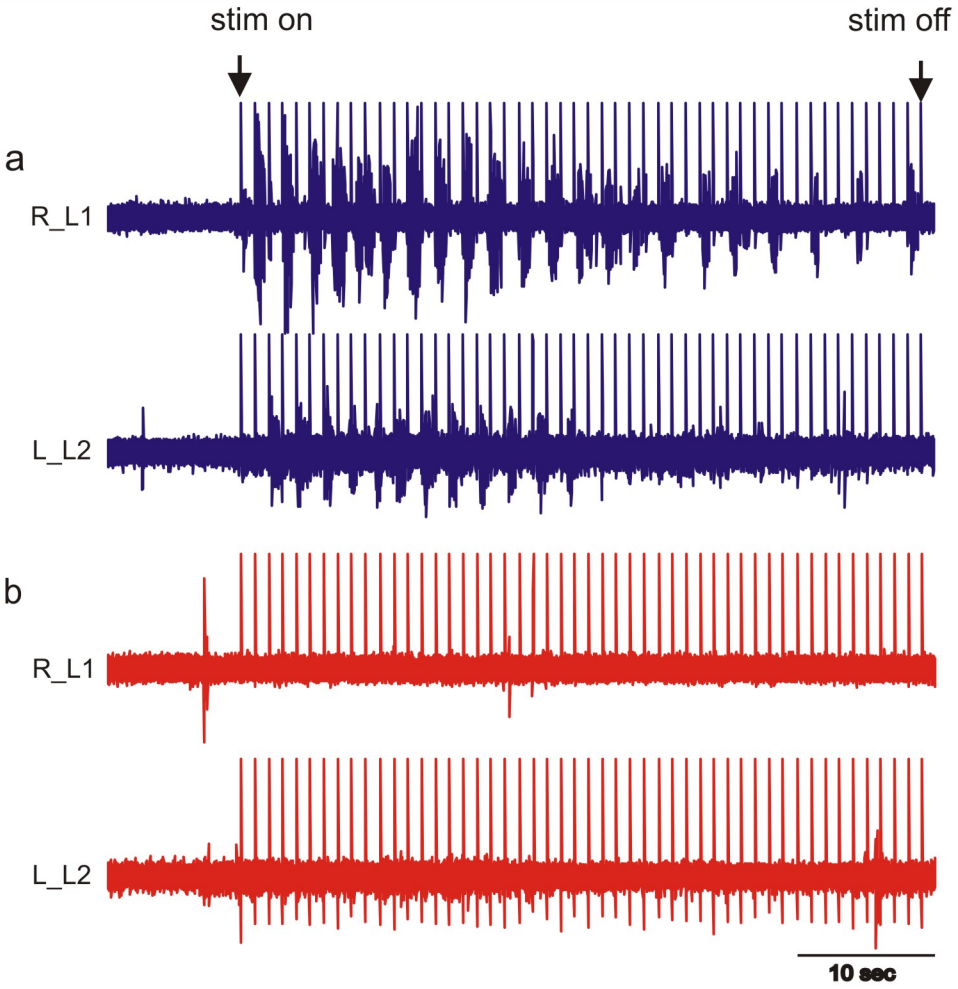

Figure 3: Ventral root stimulation on its own was not able to activate locomotion.

(a) Raw ventral root electroneurograms showing activity following dorsal sacral stimulation at 1 Hz with 100 μA and 100 μs. This electrical stimulation evoked rhythmic bursting from the right and left ventral lumbar roots. (b) Ventral lumbar root stimulation at 1 Hz with 100 μA and 100 μs did not evoke rhythmicity. The large regular pulses shown in the recordings are stimulus artifacts.

In comparison, the ventral and dorsal roots were stimulated simultaneously at the frequency of 1 Hz and 2 Hz. This provided evidence of a contribution of ventral root stimulation to the observed LLA in one experiment. Specifically, the addition of the ventral stimulation at the same amplitude, duration, and frequency as the dorsal stimulus increased burst amplitude by

22% (of the largest observed burst compared to the mean of the largest burst amplitudes before and after ventral root stimulation) on the same side of the stimulated root (Figure 4).

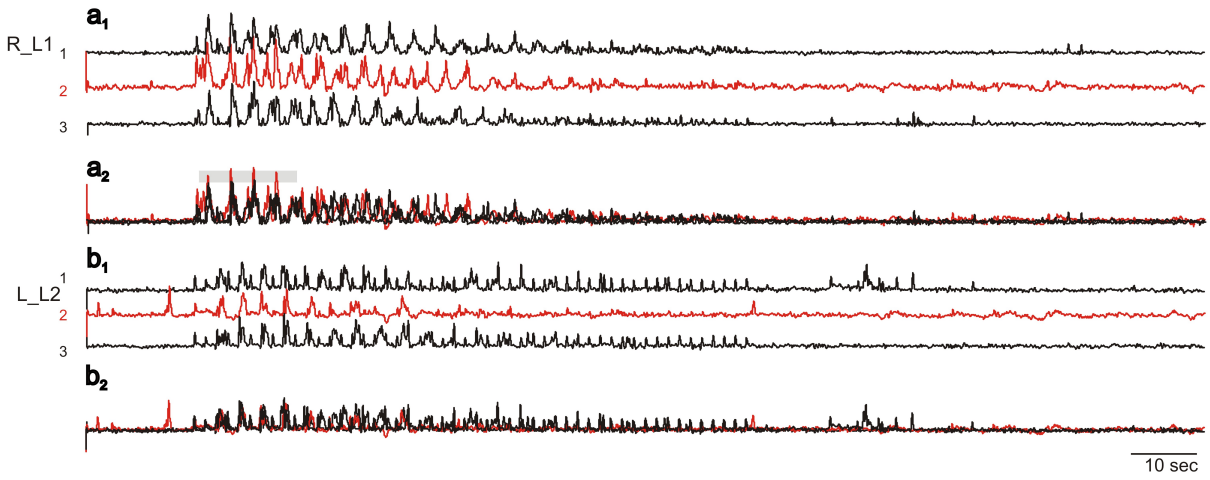

Figure 4: Combined stimulation of dorsal and ventral roots increases burst amplitude on the stimulated side. Shown are recordings of three separate events from the right (a) and left (b) L2 ventral roots with stimulation episodes separated (a_1 and b_1) or overlayed (a_2 and b_2). A right sacral dorsal root was stimulated at 1 Hz in episodes 1 and 3 (in black) and the right L2 ventral root was also stimulated at 1 Hz in episode 2 (in red). Motor activity was low-pass filtered and stimulus artifact removed to better disclose amplitude differences. Note that burst amplitude is larger in the right ventral L1 root, which is on the same side as the stimulated ventral root. This is clearly seen in the overlaid episodes in a_2 (highlighted area). In contrast, no amplitudes changes were seen on the side opposite to ventral root stimulation (b_2).

In summary, the electrical stimulus acting on the lumbar ventral root appeared more effective at a lower continuous frequency compared to a pattern of a train of stimuli at higher frequency delivered less frequently. Nevertheless, stimulating the lumbar ventral roots containing autonomic efferents was not able to alter LLA frequency but only modulate amplitude, suggesting a lack of interaction at the level of the circuitry that generates the rhythm.

During neurochemically-evoked LLA, ventral root stimulation resets on-going rhythm

With the application of 5-HT/NMDA, the locomotor CPG was activated. The ongoing activity is seen as highly regular alternating left-right oscillatory bursting in recorded L2 ventral roots. This pharmacologically induced LLA was seen in 5 experiments out of 8 (e.g. figure 6). With the introduction of electrical stimulation via ventral L2, the burst organization was temporarily modified as detailed below.

Unlike during dorsal root stimulation, ventral root stimuli (50-200 μA, 50-200 μs) delivered at 50 Hz (5 pulses per 10 seconds; n=5) evoked changes in the on-going chemically-induced locomotor rhythm. In particular, the introduction of ventral root stimuli classically 'reset' the locomotor rhythm (n=4/5; Figure 5). As expected, the specific effect on the rhythm (shortening or lengthening an ongoing burst) depended on the timing of the stimulation in the locomotor cycle. When the ventral stimulus was delivered in the middle of the flexion period on the same side of the spinal cord, the flexion was flexor phase was aborted and the burst duration increased on the opposite side. This was a classic example of resetting. It was also observed that when the stimulus was delivered at the onset of the left phase, the subsequent burst on the opposite site was weakened. Overall, ventral root stimuli delivered at frequencies of 1-50 Hz were capable of having an effect on LLA while frequencies below 1 Hz did not evoke such a response.

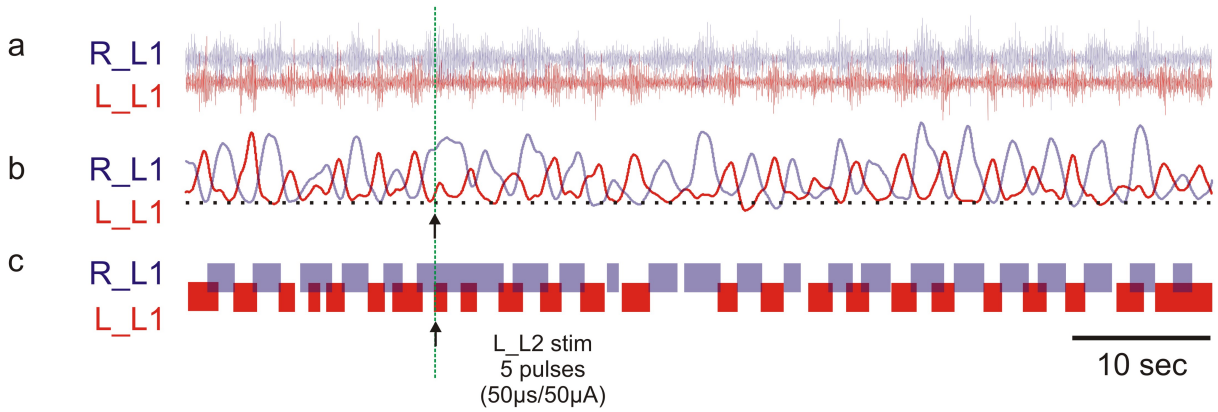

Figure 5: Ventral stimulation modifies 5-HT/NMDA induced locomotion
(a) A raw ventral root electroneurogram of the right and left ventral L1 was obtained, and it showed rhythmic bursting evoked by 10 µM 5-HT and 5 µM NMDA. It also showed the effect from stimuli on the left lumbar L2 ventral root delivered as 5 pulses. Arrows and vertical dashed line denotes time of stimulation. (b and c) Low-pass filtered signal (b) and bars depicting burst duration (c) helped visualize the effect of stimulation of burst amplitude and duration. Note that after the first stimulus delivered during the onset of the flexion phase of the same side, the flexion period terminated and bursting on the opposite side was prolonged.

It was also suggestive from one experiment that continuous stimulation of the ventral L2 root alone is sufficient in depolarizing the axons. This was seen with stimuli delivered at 1.0 Hz or faster (200 µA and 200 µs; Figure 6). The individual pulses of ventral stimulation at 50 Hz did not yield such results of depolarizing the axons. This depolarization from ventral stimulation during 5-HT/NMDA induced LLA was in accordance with the depolarization due to the addition of ventral stimulation during dorsal root stimulation. In the experiment shown in Figure 6, the high-pass filter was set lower to demonstrate that the increased burst amplitude was associated with a DC depolarization. The DC depolarization is an indication of a direct depolarization of the axons in the recorded ventral root and demonstrates that activity in ventral root axons can

provide a positive feedback amplification of motor output. The ventral root stimulation in both types of LLA demonstrated ventral root axons were able to directly affect the motor output.

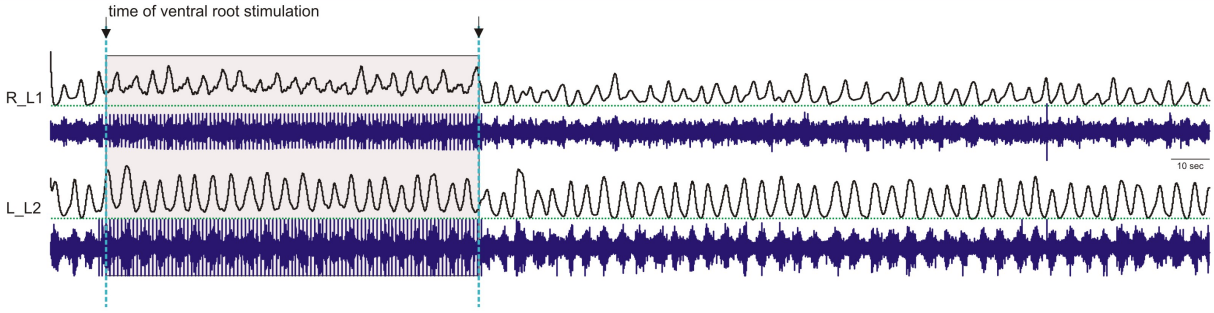

Figure 6: Ventral root stimulation during 5-HT/NMDA locomotion depolarizes ventral root axons on both sides.
The right L2 ventral root was stimulated at 1 Hz (200μs/200μA) during the period outlined by the arrows and vertical dashed lines. Both right L1 and left L2 roots are shown with raw electroneurographic activity below and low-pass filtered activity above. Note that for both roots there was evidence of a tonic depolarization as shown in the low-pass filtered records. This is seen as a depolarizing shift in the baseline (green horizontal line denotes baseline before onset of stimulation).

DISCUSSION

Previous work in the Hochman laboratory demonstrated that SPNs with axons exiting L1 and L2 ventral roots are rhythmically active during hindlimb locomotion. These SPNs are known to control hindlimb function and are located exactly in the spinal cord region where the locomotor CPG is thought to be located. These experiments tested the hypothesis that SPNs may be part of the locomotor CPG and therefore sought to examine the effect of stimulating SPNs during ongoing locomotion. Observed effects would support the coupling between spinal autonomic and somatic circuits in the control of locomotion. The tactic chosen was to stimulate the axons of SPNs in the L1 or L2 ventral roots during ongoing locomotion and look for changes. While hip flexor motoneurons also exit these ventral roots, there is no evidence that motoneurons are part of the locomotor CPG. Two different experimental approaches were used to activate a LLA; (i) repetitively stimulating the sensory afferents or (ii) bath applying 5-HT and NMDA. In

both approaches of the experimental paradigms, the effects of the L1/L2 lumbar ventral root stimulation on the observed LLA were studied.

Compared to the pharmacologically induced LLA, the rhythmicity from dorsal root stimulation was observed over a more limited frequency range. Also, the left-right rhythm was less dependable as the occurrence of burst doublets was observed. Nevertheless, LLA evoked by both 5-HT/NMDA and dorsal root stimulation exhibited the reciprocal alternation between left-right roots.

Similarities and differences in the effects of L1/L2 lumbar ventral root stimulation were seen between the LLA evoked by sensory afferent stimulation vs. neurochemically. In both cases ventral stimulation at low frequency was able to increase burst amplitude on the same side of the stimulated root. This was an indication that activity in ventral root axons can provide a positive amplification of motor output. However, stimulation of lumbar ventral roots containing autonomic efferents was not able to modify the locomotor rhythm, suggesting a lack of interaction at the level of the circuitry that generates the rhythm.

In contrast, L1 or L2 ventral root stimulation during drug-evoked LLA clearly altered the ongoing rhythm of locomotion. The ventral stimulation 'reset' the rhythm of the bursts. Depending on the timing of the ventral stimulation in the locomotor cycle, the locomotor rhythm was either shortened or lengthened for a short period of time after the stimulus. It was known that resetting occurs when the sensory afferent is stimulated, but this experiment suggested that autonomic efferents could also reset on-going locomotion. The observed alteration in the locomotor rhythm suggests that these output elements of the sympathetic system also contain intraspinal elements that are linked to the locomotor CPG.

The alteration of the rhythm of locomotion, the alteration of the burst amplitude, and the direct depolarization due to ventral root stimulation advocated for the existence of the coupling between the autonomic and somatic system. The alterations to the locomotor rhythm are suggestive of the sympathetic system also having intraspinal elements that link and influence the locomotor CPG. A more selective stimulation of the SPNs in the sympathetic chain is required to definitively identify the SPNs as the modulator of the alterations in the locomotion.

This research was to investigate more fully the interaction of the somatic and autonomic system in generating rhythmic patterns in the spinal cord of neonatal rodents. The characterization presented in this novel study showed that stimulating lumbar ventral roots influenced the existing motor rhythm. This work provided further insight of how the two divisions of the nervous system interact to generate and modulate rhythmic movement. The modulation of locomotor rhythms by ventral root stimulation indicates that the autonomic system is an ideal target for therapies for recovery following spinal cord injury. This research and future studies concentrating on the interplay of the somatic and autonomic systems within the spinal cord will ultimately lead to more specific and improved prosthetics for SCI patients.

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