Phase-Dependent Effects of Spinal Cord Stimulation on Locomotor Activity

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***Abstract*—This paper examines how electrical stimulation of the spinal cord can modulate the output of the central pattern generator (CPG) for locomotion. Application of discrete current pulses to a single spinal segment was shown to affect multiple parameters of an ongoing locomotor pattern in an *in vitro* spinal cord. For any given stimulus, the effects on frequency, duration, and symmetry of locomotor output were strongly dependent on the phase at which stimulation was applied within the CPG cycle. Additionally, most stimuli had an immediate impact and evinced no effects on subsequent cycles. The most dramatic changes were seen when stimulation was applied during motor bursting: stimuli applied to the ipsilateral spinal hemicord increased the burst length, while stimuli applied to the contralateral spinal hemicord decreased the burst length. Smaller changes were observed when stimulating during delays between motor bursts. Thus, phasic stimulation was shown to influence the behavior of the CPG and spinal locomotion circuits on a cycle-by-cycle basis. This work represents the first step toward our ultimate goal of developing a neuroprosthetic device to restore locomotion after a severe spinal cord injury.**

***Index Terms*—Central pattern generator, lamprey, locomotion control, neural interface, neuroprosthesis, phase-dependent response, phase reset, spinal cord injury, spinal cord stimulation.**

I. Introduction

CENTRAL pattern generators (CPGs) are specialized groups of neurons that produce rhythmic, patterned outputs to control motor systems. In vertebrates, the CPG for locomotion is located in the spinal cord, where sensory inputs are integrated and used to modulate the CPG output to ensure production of smooth motor trajectories in response to mechanical perturbations [1]. Typically, descending connections from the brain are required to guide, adapt, and initiate locomotion, but the brain is not needed for the CPG to produce the basic motor output [2], [3].

In many cases, a severe spinal cord injury located above the lumbar spinal cord will leave the locomotor CPG circuits intact but damage the descending fiber tracts from the brain [4]. In the absence of descending input, the CPG circuits remain inactive, causing paralysis [5]. Recently, it has been shown that epidural stimulation of the lumbar spinal cord is sufficient to reactivate the central pattern generator in completely paralyzed human subjects [4], [5], in rats [6], and in cats [7], [8], when the spinal cord injury is located above this area. However, to date these techniques have provided only coarse control over CPG activity. In order to guide useful locomotion, it will be necessary to achieve cycle-by-cycle control of the activated CPG.

The long-term goal of this project is to develop an implantable neuroprosthetic device that can monitor the activity of the CPG, muscles, and limbs, and apply the appropriate stimulation patterns to the spinal cord to dynamically control locomotion in a paraplegic patient. In this paper, we present data from *in vitro* experiments on an animal model of spinal cord injury and show that the activity of spinal locomotor circuits can be modulated on a cycle-by-cycle basis by applying discrete pulses of electrical stimulation to the spinal cord at defined phases of the CPG cycle.

The lamprey has been used as a model organism for studying the spinal control of locomotion for over 25 years. Lampreys are eel-like aquatic vertebrates that propel themselves through the water by producing a traveling wave of muscle excitation that propagates from the head, through their 100 spinal segments, to the tail [9]. Despite their relatively simple nervous systems, lampreys' CPGs have been shown to incorporate many of the same features as the CPGs of limbed vertebrates [10], and to have the basic neuronal circuitry found in more complex spinal cords [9], suggesting that findings in the lamprey may generalize to other species.

In a healthy lamprey, the propagating activity has a wavelength equal to the body length, producing a constant phase delay of 3.6° between each pair of segments [2], [11]. Within a given spinal segment, the activity of muscles on the left side of the body is 180° out-of-phase with the activity of muscles on the right side. Conveniently, both of these activation patterns are observed in the lamprey spinal cord *in vitro*, after the cord is excised from the body and chemically activated [11]. This so-called "fictive swimming" can be recorded from the ventral roots, and reliably represents the expected motor output.

Under normal conditions, ventral root activity in the lamprey is approximately symmetric, with each side of the spinal cord bursting for approximately 40% of the locomotor cycle [11]. However, as we demonstrate here, when an electrical stimulus is applied, the normal pattern of ventral root bursting may be altered in a variety of ways, including changes to burst sym-

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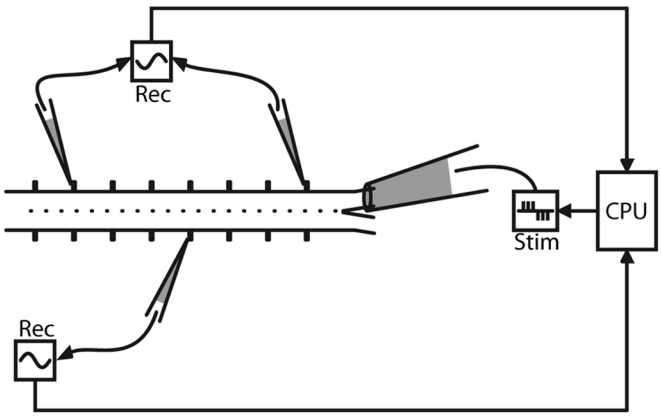

Fig. 1. Experimental setup: a custom-designed stimulator applied electrical pulses to the spinal hemicord via a suction electrode, while fictive motor output was captured by suction electrodes positioned on the ventral roots. Exact placement of electrodes varied between experiments to optimize signal quality.

metry, burst duration, and cycle period. The exact changes depend strongly on the phase of locomotion at which stimulation is applied, but also on the shape and size of the stimulus waveform. In an intact animal, inducing a specific change in the pattern of bursting will alter the motor output, thereby affecting behavior [12]. The results of this study indicate that by building a "library" of achievable modifications to the bursting rhythm, it should be possible to guide locomotion on a fine scale and on a cycle-by-cycle basis.

Much is known about the anatomy and physiology of the central pattern generator in lamprey, and many models describing the various cell types, inputs, and outputs of the system can be found in the literature [9], [13]–[19]. However, these models primarily focus on the segmental rhythm-generating network and do not include details of the many ascending, descending, and intersegmental fibers [12], [20]–[23] (see [24]–[26] for some counter-examples). Although we limit our stimuli to a single spinal segment, our electrodes surround an entire spinal hemicord (Fig. 1; Section II-B), likely generating a very complicated spatiotemporal charge distribution. Therefore, instead of attempting to compute the effects of stimulation on specific spinal neurons, we have decided to treat the entire spinal cord as a "black box." Besides simplifying the analysis, there is another reason for taking this approach: we intend to use the experimental paradigm described here in future studies on more advanced vertebrates, and detailed models of spinal CPG circuits do not exist for these systems. Additionally, because the primary long-term goal of this work is to develop a functional neuroprosthesis, we are prioritizing study of the practical input/output relationships over physiological mechanisms or spinal neuroanatomy.

II. Methods

A. Surgical Procedures

A total of sixteen lamprey (*Petromyzon marinus* ammocoetes, a stable juvenile form of the animal similar to the adult), obtained from a commercial collector (Sidney Morkert, Ludington, MI), were used for these experiments. Each animal was rapidly decapitated and eviscerated, and the skin and musculature were carefully removed to preserve the motor nerves [27]. Beginning caudal to the gills, the next 25–50 segments of the spinal cord were dissected and placed in a saline-filled chamber cooled to 9 °C. Fictive swimming was then induced by bath application of 0.125–0.50 mM D-glutamate.

B. Experimental Protocol

Motor activity was recorded with unipolar suction electrodes (glass capillaries pulled to a fine diameter and filled with saline solution) placed on the ventral roots exiting from the spinal cord. In the lamprey, the peripheral nerves are not mixed, so all activity in the output nerve is from motoneurons—the signals acquired contain compound action potentials from large motor axons. Recordings were amplified by low-noise differential amplifiers (Model P15, Grass-Telefactor, West Warwick, RI) and digitized (VCOM16, WinTron Technologies, Howard, PA) for storage and analysis.

In six experiments, motor activity was recorded from three locations along the spinal cord: two electrodes were positioned on one side of the cord near the rostral and caudal ends, while a third electrode was positioned between them on the other side of the cord (Fig. 1). In six other experiments, it was only possible to obtain recordings from one location on each side of the cord. Finally, the spinal cords of four animals failed to recover from the stress of dissection and could not produce any regular bursting pattern; data from these lampreys were excluded from the analysis.

Once fictive swimming was established, the spinal cord was prepared for electrical stimulation. Approximately one segment at the rostral or caudal end of the spinal cord was cut along the midline using a dissecting scalpel, and a few millimeters of one of the resulting two hemicords was taken into a large suction electrode (Fig. 1) so that electrical stimuli could be applied unilaterally. Brief (8–40 msec), unipolar, biphasic, square-wave, cathodic-first stimuli (2–12 μA) were delivered via a custom constant-current stimulator constructed from commercially available components. The stimulator was designed to communicate over a serial bus with a personal computer. Custom interface software allowed for programming the stimulator with different stimulation parameters, including positive pulse duration (PPD), negative pulse duration (NPD), inter-pulse interval (IPI), positive pulse amplitude (PPA), negative pulse amplitude (NPA), and number of pulses per burst (NPB) [28].

Every experiment consisted of 1–16 different "sessions," each of which used a different stimulation waveform. Within a session, the stimulus waveform was held constant, and after recording approximately 100 cycles of unperturbed (control) activity, 50–100 stimuli were delivered to the spinal cord at random times to provide an even distribution of stimuli throughout the locomotor CPG cycle. To ensure that the CPG would be able to recover from each stimulus before the next one was applied, the minimum time between stimuli was manually set to an appropriate value based on visual feedback (usually 10–15 s, depending on the stimulus amplitude and the natural burst frequency). The time of each stimulus was recorded by the digitizing hardware and synchronized to the ongoing neural

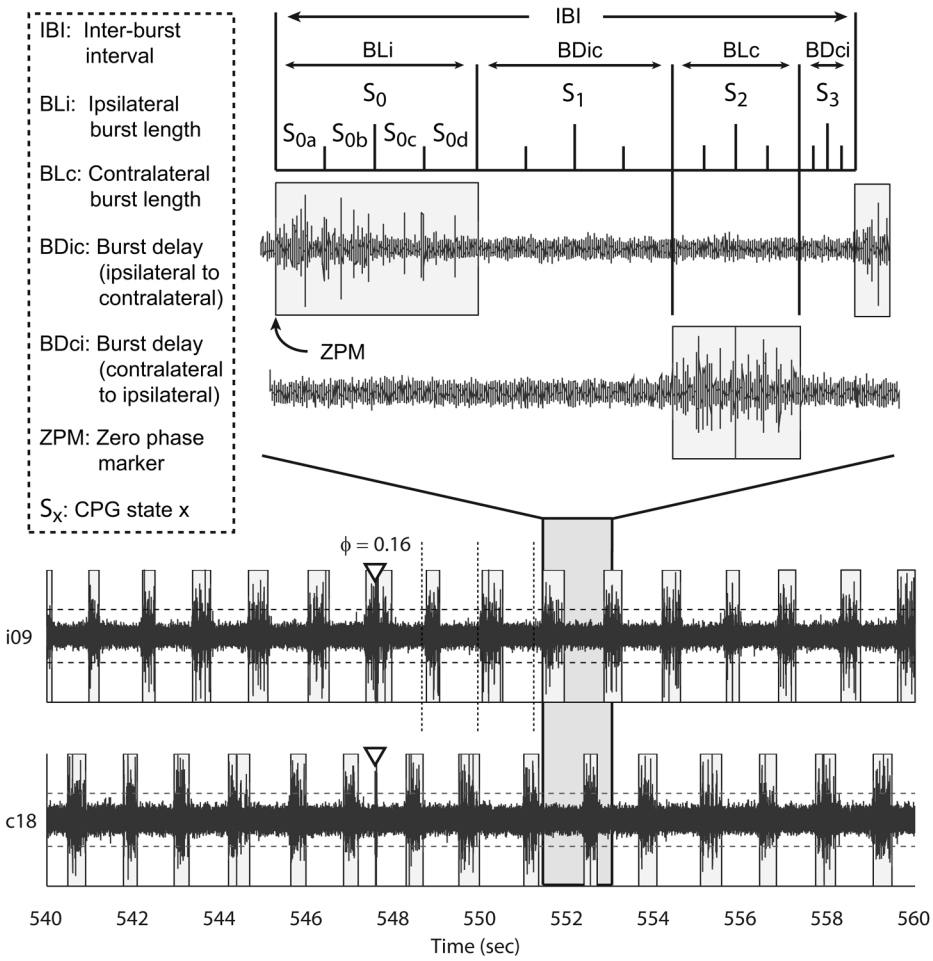

Fig. 2. Amplified, digitized, and high-pass filtered ventral root recordings from ipsilateral spinal segment 9 (i09) and contralateral segment 18 (c18) observed during a typical cycle of fictive locomotion ("ipsilateral" and "contralateral" refer to the placement of the recording electrode relative to the site of stimulation). Spike thresholds are drawn as dashed lines and the detected bursts are highlighted by rectangles. One stimulus is applied to the ipsilateral hemicord at the time indicated by an upside-down triangle at phase $\phi = 0.16$, state S_{0b} (although shown here, the stimulus artifact is removed before analysis). This stimulus resets the CPG rhythm and slightly delays the onset of the next bursts by 7.8% of the IBI (compare to Fig. 4); the expected times of the ZPMs for the subsequent three cycles are shown by vertical dotted lines.

recordings. Overall, data was collected from 102 sessions spanning 12 different animals.

At the start of each experiment, the stimulator was programmed with a standard stimulus waveform ($\mathrm{PPD} = 10$ ms, $\mathrm{NPD} = 10$ ms, $\mathrm{IPI} = 1$ ms, $\mathrm{NPB} = 2$) that was demonstrated to reliably elicit responses in preliminary studies. While visually monitoring the fictive swimming on an oscilloscope, the positive and negative pulse amplitudes were gradually increased from approximately 1 μA until a significant change in cycle period was observed, and these "baseline" parameters were used for the first experimental session of the day. In subsequent sessions, the stimulus amplitude was first varied around the baseline while keeping other stimulus parameters fixed, and then the total charge delivered was kept constant while the PPD, NPD, and NPB were modified. The goal of these manipulations was to find the most effective stimuli for controlling each spinal cord, and although there may be some relationship between the phase-dependent responses and the stimulus parameters, this was not systematically studied in these experiments. Therefore, in the analysis that follows, we only consider the time at which the stimuli are applied and measure the aggregate effects of stimulation using multiple different stimulus waveforms.

C. Analysis

Data analysis was performed off-line, using custom software programs written for MATLAB (The Mathworks, Natick, MA). In these routines, digitized ventral root recordings were first high-pass filtered to remove any slow changes in the baseline level, after which bursts were detected using a simple algorithm. Briefly, the algorithm imposes a threshold to separate neural spikes from background noise, and then groups spikes that occur within a specified interval into "bursts." Bursts that are shorter than a given duration are removed, as are bursts closer together than a preset minimum. All parameters of the algorithm are adjusted manually for each experiment based on the signal-to-noise level of the recordings and on empirical observations of the normal burst lengths, burst frequency, and other burst properties. The onset and offset times of the remaining bursts serve as the basis for all subsequent computations, although the raw data is preserved and used for displaying the results (Fig. 2).

The effects of stimulation were characterized as functions of the "phase" of the CPG and the "state" of fictive locomotion at which they were applied. A particular state was assigned to each

pair of spinal segments (one ipsilateral to the site of stimulation and one contralateral to the site of stimulation) depending on the activity recorded on the corresponding ventral roots. The onset of bursting on the ventral root ipsilateral to the site of stimulation was used to indicate the start of State 0 (S_0), while the end of S_0 was coincident with the offset of bursting (Fig. 2). Between the offset of bursting on the ipsilateral root and the onset of bursting on the contralateral root, the system was considered to be in State 1 (S_1). Bursting on the contralateral root constituted State 2 (S_2), and the interval between the offset of bursting on the contralateral root and the onset of bursting on the ipsilateral root was designated as State 3 (S_3). In many cases, the data had structure on a fine scale within states, so states were evenly divided into four quarters (e.g., S_{0a}–S_{0d}) for most of the statistical computations (Fig. 2).

The phase of the CPG was defined as a real-valued variable ϕ in spherical space $\mathbb{S}^1$, which takes on values in the range $\phi \in [0, 1]$[29]. For convenience, zero phase was chosen as the beginning of S_0. During an experiment, the times at which the system entered S_0 were computed and stored as "zero phase markers" (ZPM). This allowed for a simple expression of the phase ϕ at a given time t

$$\phi = \frac{t - t_{\mathrm{ZPM}}}{T_0} \tag{1}$$

where t_{ZPM} is the time of the most recent zero phase marker and T_0 is the average cycle period observed during unperturbed bursting. Alternative measures of phase were considered, but this proved to be the most robust.

Under normal conditions, in the isolated spinal cord preparation used here, motor activity is directly related to the outputs of the central pattern generator, so the state of fictive locomotion is directly related to the phase of the CPG. However, this need not be the case after an electrical stimulus is applied to the cord, so in the analysis that follows, we attempt to distinguish between stimuli that reset the phase of the CPG and those that just affect the bursting without changing the cycle period. A stimulus was considered to have a resetting effect only if it induced a permanent shift in the expected times of ZPMs, or equivalently, if only the period of the perturbed cycle (and not the period of subsequent cycles) was significantly different from zero (see Section II-D for statistical methods).

In each experimental session, the control data was used to estimate a number of different parameters that characterize the normal bursting, including the mean values and standard deviations of burst length recorded by each electrode (BLc and BLi), the mean delay and standard deviation between bursting observed on pairs of electrodes (BDic and BDci), and the mean cycle period and its standard deviation (also called inter-burst interval, or IBI; see Fig. 2 for illustration and acronym definitions). When a stimulus was applied, its effect on each of these parameters was calculated for the perturbed cycle and two subsequent cycles, and the results were tabulated as a function of phase. Thus, each stimulus contributed one data point to multiple different phase-dependent response (PDR) curves (e.g., Fig. 6). However, only three experimental sessions showed significant effects on any burst parameter in the subsequent cycles, and in those cases, the magnitude of the changes was small, so only data from the perturbed cycle is shown. Some of the weak stimuli tested had no effect on any of the burst parameters in any cycle—these 22 sessions were excluded from further analysis.

D. Statistics

To confirm the statistical significance of the effects of a particular stimulus on locomotor output, data points within a session were first binned according to the state of the CPG when stimulation occurred (S_{0a}–S_{3d}). Then, for each parameter of bursting under study, the mean value was subtracted from each data point and the difference was stored as a measure of the impact of the stimulus; however, differences within less than one standard deviation (σ) from the mean (μ) were discarded. Statistical significance ($\alpha = 0.025$) was based on the *sign test*, with the assumption that the distribution of signs under the null hypothesis is equivalent to that of a binomial random variable with $p = 0.5$. That is, to locate significant increases (decreases) in a given burst parameter, the number of data points X in any single bin greater than $\mu + \sigma$ (less than $\mu - \sigma$) was treated as a binomial random variable b, and significance was assigned for $P(b > X) \leq 0.025$.

Although a standard z-test is also a valid metric for significance in these data, it gave false positives in a few cases where there was a small baseline shift in some burst parameter over the course of the experimental session. Additionally, the z-test statistic was frequently "significant" even though the individual values were distributed both above and below the mean. In our implementation of the sign test, any baseline shift within ± 1 standard deviation of the mean was automatically eliminated, and even if the average change in a burst parameter after stimulation was greater than a standard deviation from the mean, it would only be significant if this effect occurred "most" of the time. That is, our sign test only assigned significance if an effect was sufficiently large *and* sufficiently common. Therefore, we chose to implement a sign test for statistical significance because it was determined to be more conservative.

III. Results

In 63 of the 80 experimental sessions analyzed (22 sessions were excluded from analysis; see Section II-C), stimulation reset the phase of the CPG. These resets were manifested by a significant change in the perturbed cycle period followed by no change in subsequent cycles (i.e., a permanent shift in the expected times of ZPMs). The histogram in Fig. 3 illustrates when stimulation was most effective at resetting the CPG by showing counts of the number of sessions in which stimulation at a particular state had a significant effect on the perturbed cycle period. Although Fig. 3 summarizes data over many different animals, different patterns of stimulation, different electrode locations, and different stimulus amplitudes, two trends are easily seen: IBI was increased when stimulation was applied toward the end of S_0 and decreased when stimulation was applied near the start of S_2. Less commonly, significant effects were apparent when stimulation was applied during S_1 and S_3.

To determine the magnitude of the effects tabulated in Fig. 3, the 50–100 stimuli in each session were first binned by state.

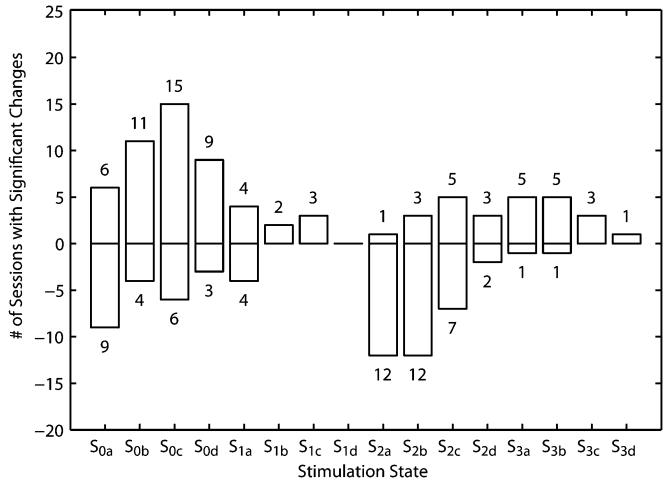

Fig. 3. Histogram of the total number of experimental sessions across all animals in which stimulation at a particular state reset the CPG, manifested as a significant effect (increase or decrease) on the perturbed cycle period. Significant increases are shown as positive deviations, decreases are shown as negative deviations.

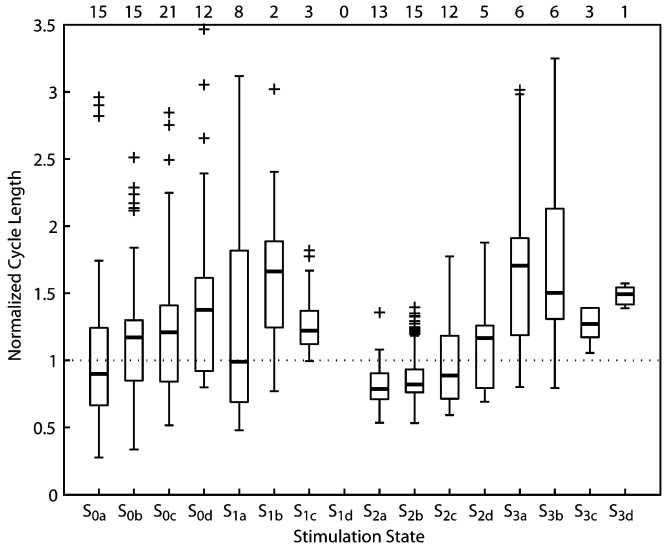

Fig. 4. Box plot of stimulation-induced changes in cycle period across all significant experimental sessions, normalized to the average values observed during control recordings. Note the large changes when stimulation is applied during S_{0c}–S_{1a} and during S_{2a}–S_{2c}. Plotting conventions: thick horizontal lines represent the median values; unfilled rectangles are drawn from the first to the third quartile (25th–75th percentile); vertical lines extend from the quartiles to the maximum and minimum values, excluding outliers; and near outliers are plotted individually $(+)$. Integers above the plot represent the number of sessions that contributed data to the box below; each session contains between 50–100 stimuli.

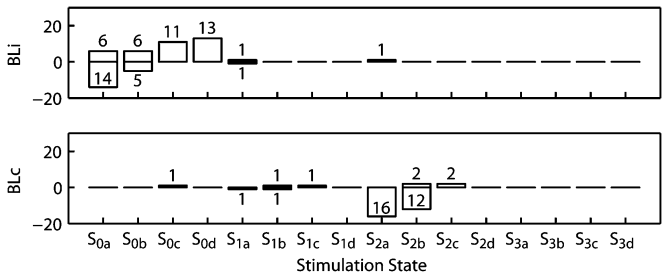

Fig. 5. Histogram of the number of experimental sessions in which stimulation at a particular state reset the CPG and had a significant effect (increase or decrease) on the ipsilateral or contralateral burst length (BLi/BLc). Significant increases are shown as positive deviations, decreases are shown as negative deviations.

Then, because the data was being combined across experimental sessions, each session's data was normalized to the average IBI observed during that session's control bursting. Finally, within a state, the data was combined in all sessions in which stimulating during that state produced a significant effect on cycle period. The resulting median values and ranges of effects are illustrated in Fig. 4. It can be seen that the amplitude of changes in cycle period is continuously modulated by the state of the CPG—there are smooth transitions between the increases in IBI seen for stimulation during ipsilateral bursting and the decreases in IBI seen for stimulation during contralateral bursting. Note that the large range of amplitudes within each bin is partially due to the wide variation in stimulus currents applied in the different experimental sessions. Although the absolute value of stimulation current required to affect the bursting varied between preparations, for any given animal, the magnitude of stimulation effects was proportional to the amount of charge delivered by the electrode (data not shown).

Each phase reset or increase/decrease in cycle period must be caused by one or more changes in burst length or burst delay (Fig. 2). The most common significant effects contributing to changes in cycle period were an increase in ipsilateral burst length (BLi) and a decrease in contralateral burst length (BLc), when stimulation was applied during ipsilateral or contralateral bursting (S_0 or S_2), respectively (Fig. 5). A less common cause was a decrease in the delay between ipsilateral and contralateral bursting (BDic) when stimulation was applied during S_0 (data not shown). Also, in a few cases, the change in cycle period was due to a combination of statistically "insignificant" effects. Note that an increased BLi and decreased BDic act on the IBI in opposite directions, so the net positive change in IBI observed in S_0 (Fig. 4) prescribes the relative magnitude of these effects (also see Fig. 7).

In addition to illustrating the relative frequency of significant effects, Fig. 5 also shows that the effects of stimulation are usually immediate and short-lasting: for example, stimulation applied during ipsilateral bursting (S_0) primarily affects the ipsilateral burst length (BLi)—if the effects persisted or were delayed, the histogram would also show a large number of significant changes to the contralateral burst length (BLc). Furthermore, as described in Section II-C, only three experimental sessions showed effects on any parameter of bursting during the two cycles after stimulation.

The general trends seen across multiple experiments are mirrored closely by individual sessions. Fig. 6 shows data from a typical experimental session in which 100 stimuli were applied to the spinal cord throughout the CPG cycle (note that stimuli are applied at random times and are not always evenly distributed). In this session, as in many sessions, stimulating during most of S_0 extended the ipsilateral burst by a fixed amount of time after stimulation was applied ($\mathrm{BLi} = 1.46\phi + 0.32$ sec, $R^2 = 0.76$, $\phi \in [S_{0a}, S_{0b}]$; two outliers with $\mathrm{BLi} < \mu - \sigma$ were removed from the fitted data). This increase in BLi was uncompensated by other factors and therefore resulted in a roughly

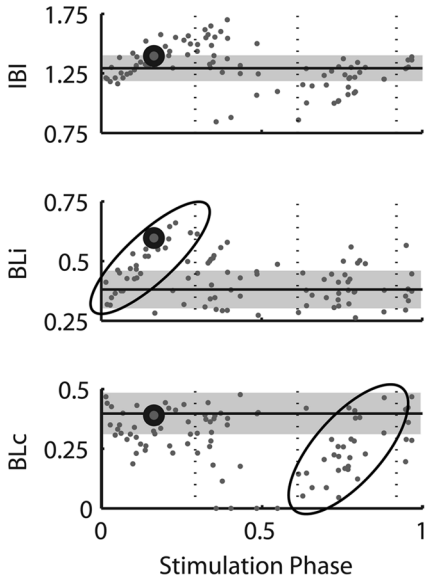

Fig. 6. Phase-dependent response (PDR) plots of data from experimental session #101402. Stimulus parameters were fixed throughout the experiment (PPD = NPD = 10 msec, IPI = 1 msec, PPA = NPA = 2.17μA, NPB = 2). All of the effects of one particular stimulus (shown in the ventral root recordings of Fig. 2) are highlighted with large markers. In all plots, dashed vertical lines indicate the average transition times between states during control bursting, a solid horizontal line is drawn at the mean (control) value for that burst parameter, and gray shading extends one standard deviation in each direction. Ovals highlight regions of significant increases in BLi and significant decreases in BLc.

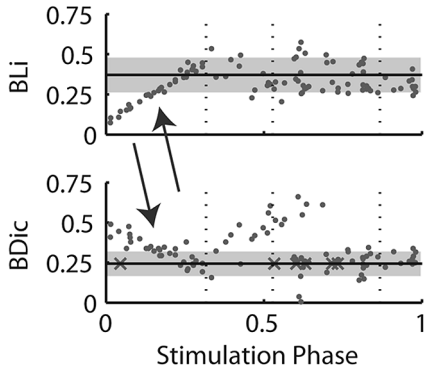

Fig. 7. PDR plots of BLi and BDic data from experimental session #071505. In S_0, the induced changes in these two parameters essentially negate each other (arrows). Stimulus parameters were fixed throughout the experiment (PPD = NPD = 10 msec, IPI = 1 msec, PPA = NPA = $4.34\ \mu$A, NPB = 1). Plotting conventions as in Fig. 6.

equivalent increase in IBI. Stimulating in S_0 did not affect the other burst parameters, but stimulating in the beginning of S_1 extended the BDic (data not shown) and cycle period, accordingly. Finally, stimulating during the beginning of S_2 decreased the contralateral burst length by an amount linearly proportional to the phase (slope = 0.62, $R^2 = 0.40$, $\phi \in S_2$), thereby decreasing the cycle period.

All of the experiments described so far have used stimuli that reset the phase of the CPG, however, not all sessions evinced effects on the cycle period—17 of the 80 sessions analyzed only showed significant changes on the burst parameters (BLi, BLc, BDic, or BDic), without perturbing the CPG rhythm. In many of these experiments (usually associated with low-amplitude stimuli), strong effects on one burst parameter were compensated by another. For example, Fig. 7 shows the data from a typical experimental session in which decreases in the ipsilateral burst length (BLi $= 1.04\phi + 0.10$ s, $R^2 = 0.88$, $\phi \in S_0$) were compensated by increases in the delay between ipsilateral and contralateral bursting (BDic $= -0.57\phi + 0.43$ s, $R^2 = 0.55$, $\phi \in S_0$), so that the contralateral burst occurred approximately at its regularly scheduled time. One possible explanation for the relatively infrequent occurrence of this result is that the experimental design could have inadvertently selected against this kind of data due to our stimulus waveform selection criteria (Section II-B).

IV. Discussion

The control of CPGs using phase-resetting stimuli can be studied using the mathematics of nonlinear oscillators [29], [30]. In this work, we have avoided using the typical formalisms for phase resetting (e.g., phase-response curves, or PRCs) both because there is no observable variable that uniquely represents phase after stimulation, and because the functional output of the motor system is best characterized by studying each parameter separately. However, if it can be determined that the effects of a particular stimulus are consistent with those expected from an instantaneous phase shift, i.e., instantaneous modification of a single burst parameter without any effect on other parameters, PRCs could be used to analyze the response. Because many of our stimuli did act in this way, the PDR plots of inter-burst interval are essentially equivalent to PRCs: Fig. 6(a) shows that stimulation early in the CPG cycle causes a phase delay (increases IBI) while stimulation later in the CPG cycle causes a phase advance (decreases IBI). This type of effect is called a "Type 1" phase-resetting response (see [29] for an in-depth treatment of PRCs and response types), which has also been observed in other studies on stimulating ongoing motor rhythms [31], [32]. Previous work on lamprey spinal cords has shown that both descending modulatory inputs and sensory feedback to the locomotor CPG can elicit phase resets [12], [27]. Indeed, these are believed to be the normal mechanisms by which behavioral modifications are produced in all intact animals [33]–[35]. The foregoing analysis suggests that similar effects can be achieved using external artificial inputs to spinal neurons. That is, we have shown that application of discrete pulses of electrical stimulation to the spinal cord is sufficient to manipulate the output of the locomotor CPG (Fig. 8).

Some likely differences between the natural inputs to the CPG and our artificial inputs are the locations and mechanisms of action. Although these experiments did not allow us to uniquely identify the targets of stimulation, the effects of stimuli recorded at multiple positions along the spinal cord provided some information in this regard. Specifically, stimuli were observed to affect all segments of the spinal cord simultaneously (but the effects diminished with distance from the stimulation electrode) and the impact on any given segment was dependent on the local CPG state. Therefore, it is possible that our stimuli transiently activated intersegmental fibers, which subsequently impinged on the same neural subcircuits within each segment [36]. Moreover, small changes in the electrode position or hemicord dissection could have altered these local targets in the few experimental sessions in which stimulation did not reset the phase.

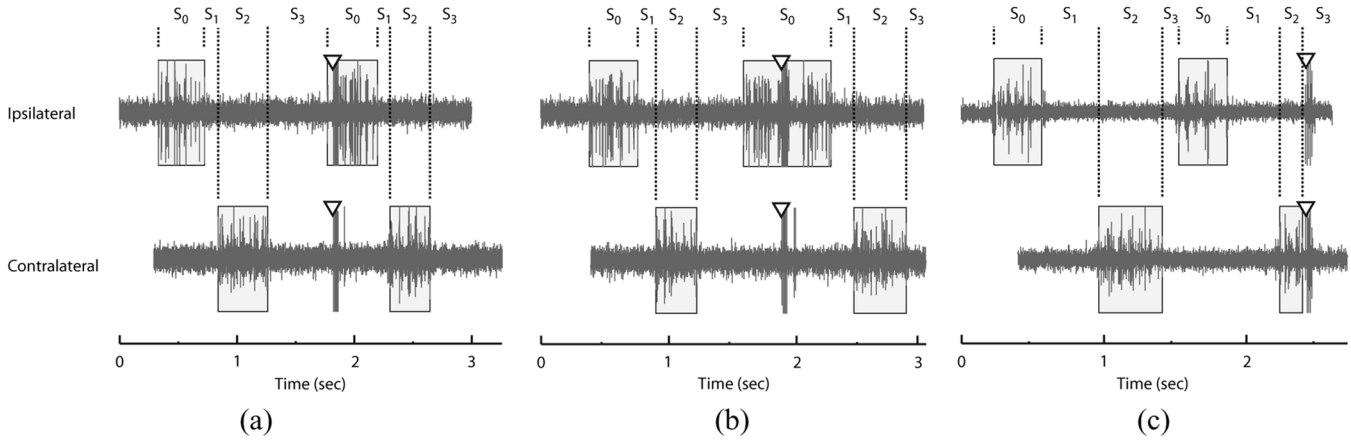

Fig. 8. Examples of how stimulation (arrowheads; the stimulus artifact is removed before analysis but is shown here) can be used to control the locomotor output. (a) Stimulation applied during state S_{0a} does not affect the burst length. (b) Stimulation applied during states S_{0b}–S_{0d} increases the ipsilateral burst length. (c) Stimulation applied during states S_{2a}–S_{2c} decreases the contralateral burst length (note that the burst offset is not coincident with the stimulus pulse because the offset is considered to be the time of the last spike threshold crossing).

The exact targets of stimulation will be addressed in a future study.

Although the PDR curves illustrated in Section III provide essential information for future experiments using *in vitro* lamprey spinal cords with hemicord suction electrodes, stimulation of the spinal cords of higher vertebrates, and even *in vivo* lamprey spinal cords, will not necessarily elicit similarly shaped PDRs (although preliminary data from Jung suggests that intact lampreys with spinal lesions do respond in a similar manner; see [26]). For example, it will be difficult to attach hemicord suction electrodes to intact lampreys, and different electrodes will probably activate different spinal circuits, producing different phase-dependent responses. Furthermore, an implantable prosthetic device based on spinal cord stimulation will almost definitely use more sophisticated electrode technology and require novel placement strategies. Therefore, one of the important contributions of this work is the development of an experimental paradigm that can easily be adapted to any given preparation. That is, we anticipate that the strategy of applying brief electrical stimuli and using the phase-dependent responses to control locomotion on a cycle-by-cycle basis will apply to any particular choice of electrode design, stimulus waveform, or electrode placement. As described in Section I, this was an important factor in our decision to treat the lamprey spinal cord as a "black box."

In a future neuroprosthetic device, the technique outlined above could be combined with other therapeutic options [5], [7], [8], [37]–[41] to allow both coarse and fine control over locomotor output. To achieve this, a set of data would have to be collected for each subject to determine exactly how the CPG will respond to each electrode/stimulus combination. Next, a control strategy would be designed based on these phase-dependent response curves and the desired motor output. Finally, this strategy would be embedded in a microcontroller that would monitor the ongoing state of the locomotor system and apply stimuli when applicable to achieve a desired gait.

For example, if tonic background stimulation (cf. [5], [7], and [8]) was used to provide a basic "on/off" control, there may be asymmetry in the motor output. Assuming that a particular stimulus had a PDR curve like Fig. 6, unilateral weakness could be corrected by supplying stimulation with the proper amplitude during bursting on the weaker side [cf. Fig. 8(b)]. Alternatively, if the bursting was symmetric but the gait speed was too slow, two stimuli could be applied at times appropriate to decrease both the BLi and BLc [using the data in Fig. 6, this would require one electrode on each hemicord; also see Fig. 8(c)]. Of course, depending on the shapes of the PDRs for a given subject, only certain modulations would be possible. Additionally, concerns about electrode biocompatibility, access to critical spinal circuits, side-effects on pain pathways, and other practical considerations would all have to be addressed, but these issues are beyond the scope of this work.

V. Conclusion

We have shown that the activity of spinal locomotor circuits can be modulated on a cycle-by-cycle basis by applying discrete pulses of electrical stimulation to the spinal cord at defined phases of the CPG cycle. This serves as the first step toward our long-term goal of developing an implantable neuroprosthetic device to dynamically control locomotion after spinal cord injury. Our current work focuses on developing a computational framework for evaluating potential control strategies to achieve a particular gait. By iterating the effects of stimulation using measured PDR data, we should be able to estimate the stability of responses to a sequence of stimuli—this is similar to finding stable fixed points in a one-dimensional Poincaré map [42]. Once the simulation architecture is complete, we will begin using *in vitro* lamprey spinal cords under closed-loop control to effectuate arbitrary swimming patterns.

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