Large-scale optical imaging reveals structured network output in isolated spinal cord

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1 Abstract

Isolated neonatal mouse spinal cord contains intact recurrent neural circuits that can generate ordered patterns of periodic population activity (termed fictive locomotion) following experimentally controlled stimulation. Taking advantage of known genetic entry points into the circuit, ablation experiments have demonstrated that specific interneuronal subtypes are necessary to produce and maintain fictive locomotion. However, much remains uncharacterized including the precise structure of fictive locomotion at single-cell resolution, the amount of variance across groups of individual motor neurons that share common muscle targets, and the robustness of phase tuning to network perturbations. In this study, we measured motor neuron activity using large-scale, cellular resolution calcium imaging across hundreds of retrogradely identified motor neurons. Spike inference methods for phase estimation were developed, and were validated in each experiment using antidromic stimulation to generate data where ground-truth phase and spiking information were known. Neurons with the same muscle targets fired in phase with one another, while neurons innervating synergist muscles (quadriceps and ankle flexors) had consistently shifted burst times. Neurons innervating antagonist muscles (quadriceps and hamstrings) reliably fired out of phase. Notably, groups of motor neurons that fired asynchronously were found intermingled at each lumbar spinal segment, demonstrating that the recruitment of motor neurons during fictive locomotion is considerably more complex than simple rostrocaudal alternation. In order to assess the robustness of the observed phasic activity to frequency modulation, we lowered the frequency of fictive locomotion through adenosine application. We observed no change in the phase of individual neurons despite a drop in the rhythm frequency. These results reveal complexity in the specificity of motor activation patterns in isolated spinal circuits and set the stage for work that will examine the role of local circuit interneurons in generating motor activity.

2 Supporting Information

This work presents an optical method for characterizing large volumes of neurons in the mouse spinal cord with a view towards understanding the specific circuit mechanisms involved in generating precise motor activity. Below are three novel contributions made towards this goal.

Large-scale, calcium imaging of identified motor neurons

Calcium imaging has been used for the study of the spinal cord in the past, but the use of synthetic dye has limited such studies to local regions around the dye injection site (e.g. Kwan et al., 2010). By taking advantage of transgenic mice expressing the genetic calcium indicator GCaMP3 (Zariwala et al. 2012), we were able to image hundreds of motor neurons with identified muscle targets during fictive locomotion. As the rhythmic network activity persisted for many hours following the application of rhythmogenic drugs, we could obtain

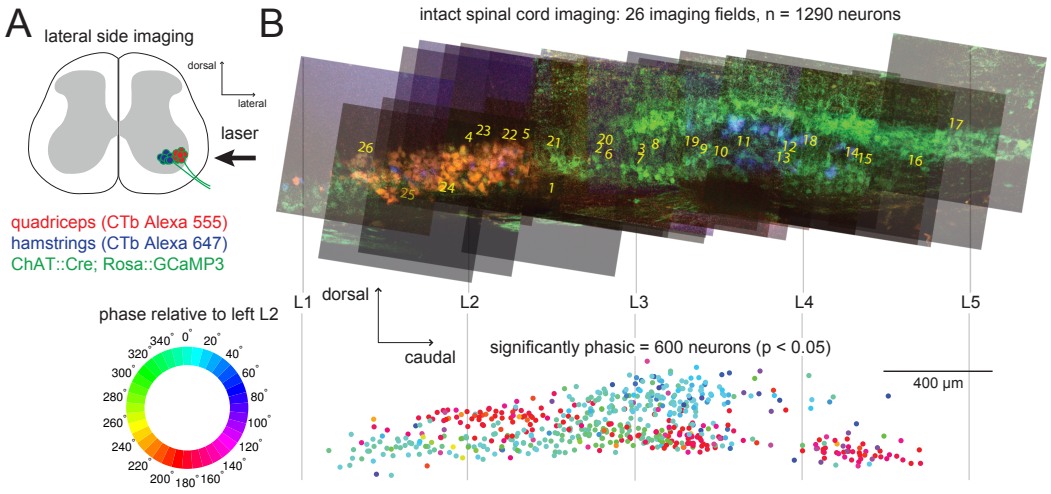

Fig. 1: **A.** An intact spinal cord expressing GCaMP3 was imaged from the lateral side following retrograde tracer (CTb) injections into quadriceps (red) and hamstrings (blue). **B.** *Top:* Motor neurons (green) with identified muscle targets are clustered into pools. *Bottom:* Hundreds of motor neurons were rhythmically active during fictive locomotion. Phasic tuning for each cell was computed relative to an extracellular recording at lumbar segment 2 (L2).

data from 20-30 imaging fields that each contained 20-50 neurons. Phasic tuning was estimated for each neuron relative to electrical recording data that was obtained from a fixed location during all imaging trials, allowing us to generate maps of phasic activity that contained hundreds of identified motor neurons (see Figure 1). These same neurons were often imaged multiple times to assess rhythm stability and to quantify changes after experimental manipulations. Future work will incorporate the use of various genetic mutants to acutely test how different interneuron types generate motor neuron firing dynamics.

Phase estimation and spike train deconvolution

The large volumes of data generated by this approach necessitated the development of an automated approach for data analysis. Following cell centroid detection, the process of optimizing the spatial filters containing individual neurons, spike train deconvolution, and subsequent phase estimation were carried out automatically. Spike train deconvolution was performed using a fast nonnegative deconvolution algorithm (Vogelstein et al., 2010). This algorithm was then used to initialize another deconvolution algorithm that used a more complex model of calcium dynamics that included terms to model fluorescence drift, non-instantaneous transient rise time, and a nonlinear relationship between fluorescence and spike frequency. One feature of this preparation is the fact that motor neurons can be antidromically activated via their ventral roots to fire in specific spiking patterns. Such data was acquired during each experiment and allowed us to characterize the nonlinearity between indicator fluorescence and spiking in every experiment, and to test the accuracy of other model parameter estimates needed for spike inference.

Phase/frequency independence

Genetic ablation of a subset of inhibitory interneurons (Gosgnach et al., 2006), as well as the application of adenosine (Witts et al., 2012), have each been shown to lower the frequency of motor neuron firing without coarsely perturbing phase at the segmental level. However, this has not been demonstrated at the level of individual neurons. In this study, we mapped the phase of hundreds of motor neurons before, during, and after the application of adenosine—which reduces rhythm frequency by about 30%. Across conditions, the phasic tuning of nearly all active neurons remained stable, suggesting that network homeostatic mechanisms maintain phase. Further work may elucidate these mechanisms and explain how gait speed is controlled during locomotion.