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

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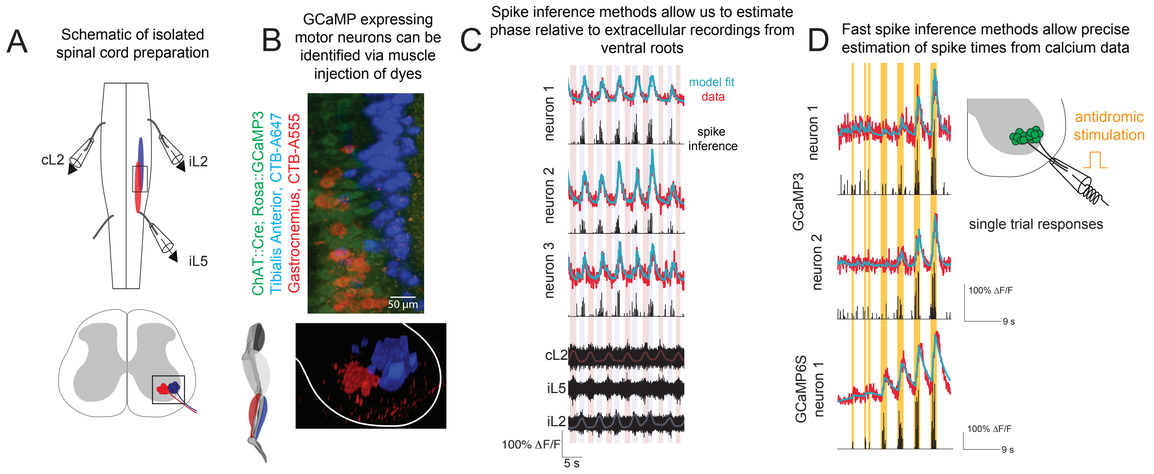
Large-scale optical imaging provides a means for studying how neural circuits can generate patterned motor output

How does a neural circuit generate behavioral output? Neural circuits in the spinal cord are necessary for the production of patterned motor neuron activity during walking. This pattern is complex: to control the hindlimb of a mouse, hundreds of motor neurons must be precisely activated in order to contract each muscle at the correct moment during the step cycle. Interneuronal networks in the spinal cord can generate this pattern without any external inputs, but the means by which this is achieved is unknown. In this project, we have developed large-scale optical imaging techniques to interrogate the activity of spinal neurons during locomotor-like network activity in order to address three questions:
1) How similar is drug-induced fictive locomotion to real locomotion?
2) How much variance exists within a motor pool during locomotion?
3) How do different molecularly defined subtypes of interneurons in the spinal cord drive motor neurons to fire appropriately?



Network activity during fictive locomotion is stationary

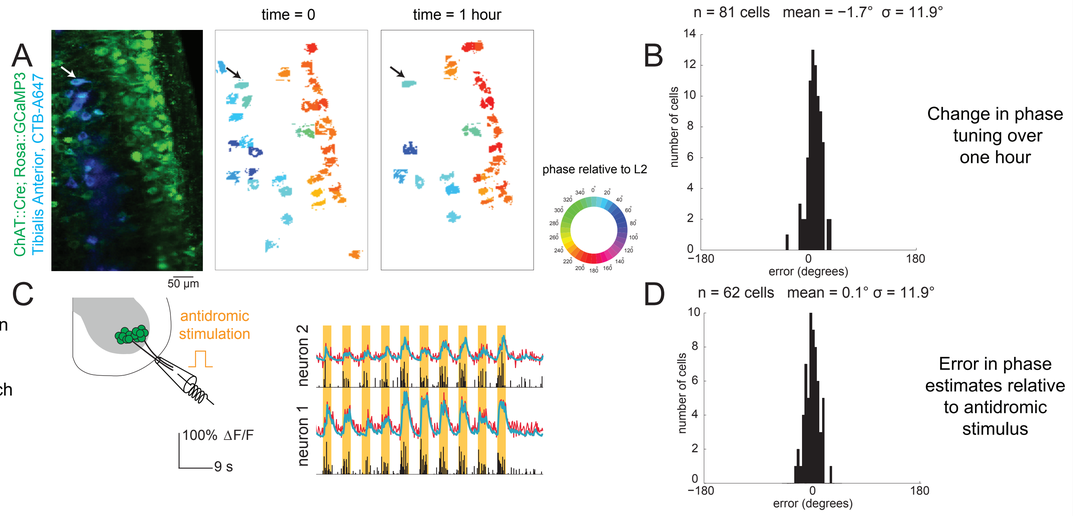
Rationale: There are nearly 2000 motor neurons that innervate muscles in each hindlimb of the mouse. In order to functionally characterize nearly all of these neurons in a single preparation, we must pool data across imaging fields registered to a fixed electrical recording obtained throughout the entire experiment. This approach requires that: 1) phase must be stable over time, 2) our estimates of phase must be accurate.

Phasic tuning of individual motor neurons is stable over hours

Change in phase tuning over one hour

Antidromic stimulation allows us to easily test our ability to estimate phase in each experiment

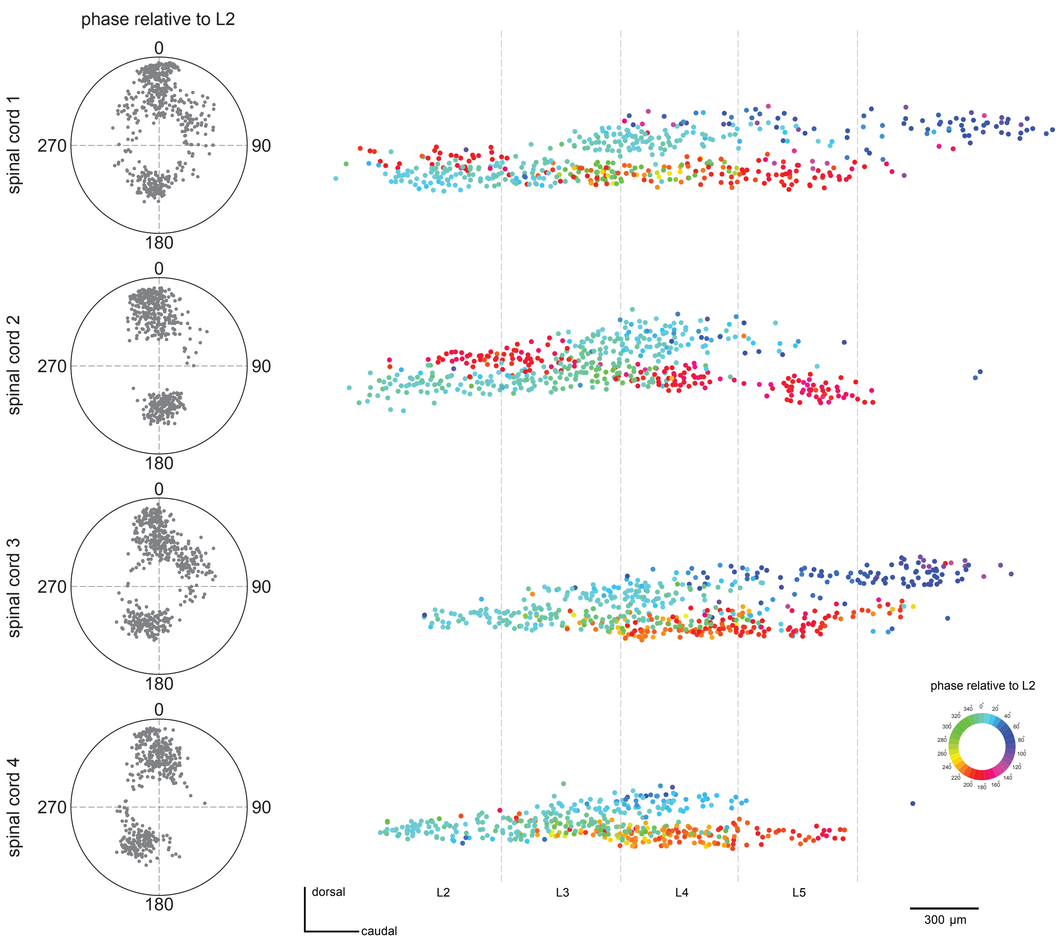
Error in phase estimates relative to antidromic stimulus



Main findings: Antidromic stimulation of ventral roots lets us drive motor neurons to fire in arbitrary patterns. By driving motor neurons to fire in a periodic pattern, we can quantify the quality of our phase estimation method in every experiment. In general, phase estimation is accurate to within 15 degrees. Similarly, measuring phase across the same neurons at different times during the experiment verifies that phase is stable over time.

How are individual motor neurons recruited to fire during fictive locomotion?

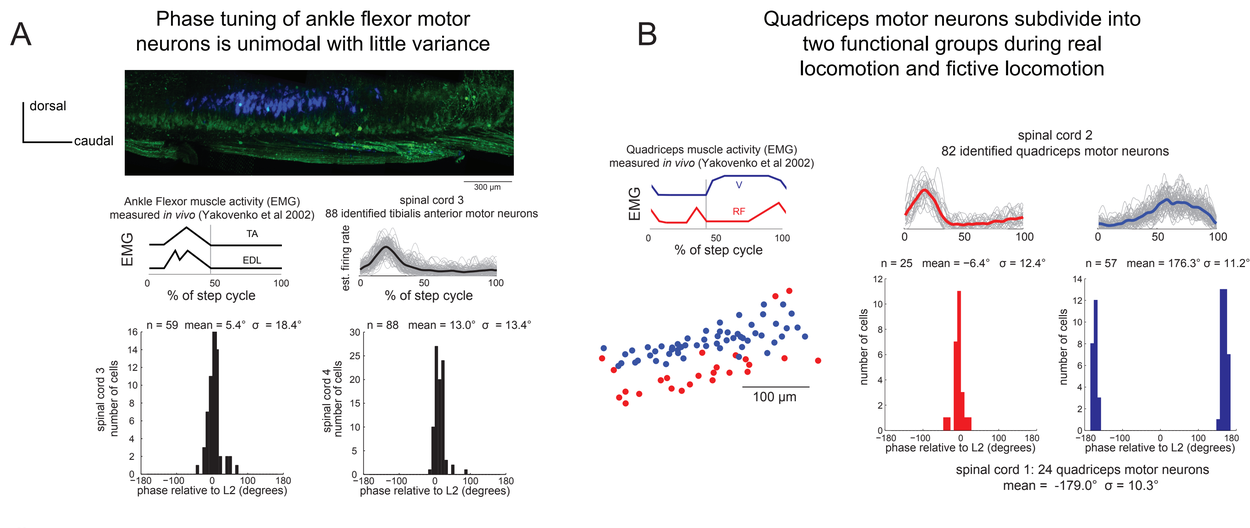
Rationale: While alternation between rostral and caudal lumbar segments has been observed during fictive locomotion in the mouse (e.g. between L2 and L5), it is not known how much complexity exists in the phasic tuning of individual motor neurons. For example, is fictive locomotion a single network state or a group of similar outputs? Does structure exist beyond simple flexor-extensor alternation? How much variance exists across an individual lumbar segment?

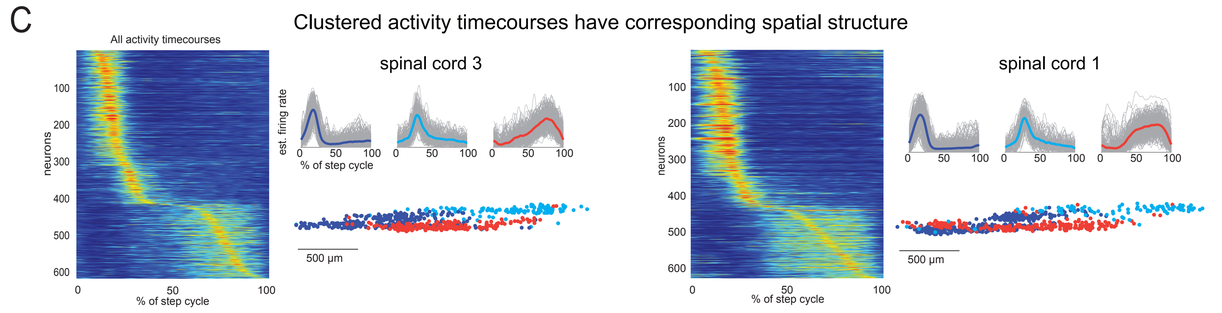


Main findings: The observed network activity is remarkably consistent between preparations. In addition, the phasic tuning of individual motor neurons during fictive locomotion is more complex than simple rostrocaudal, flexor-extensor alternation as there are flexor and extensor motor neurons intermingled at each lumbar segment.

How much variance in activity exists within identified motor pools?

Rationale: The recruitment patterns of hindlimb muscles during locomotion have been well characterized via EMG studies. However, it is unclear how the firing patterns of every motor neuron within an individual pool contribute to the observed muscle activity timecourse. Our approach of imaging most hindlimb projecting motor neurons allows us to address two questions:
1) How much variance exists in the phasic tuning of each motor neuron in a single pool?
2) How do these activity patterns compare to EMG waveforms observed in identified muscles?



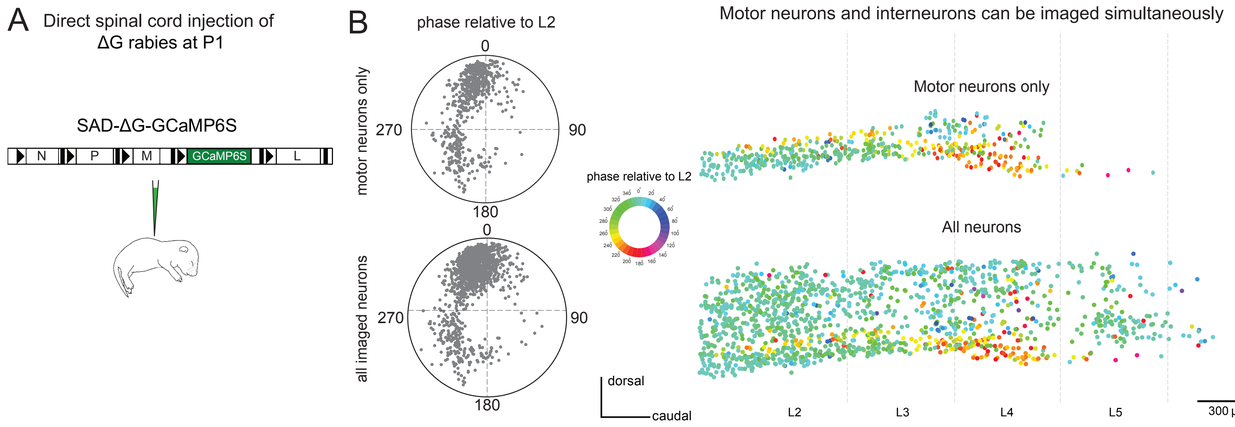


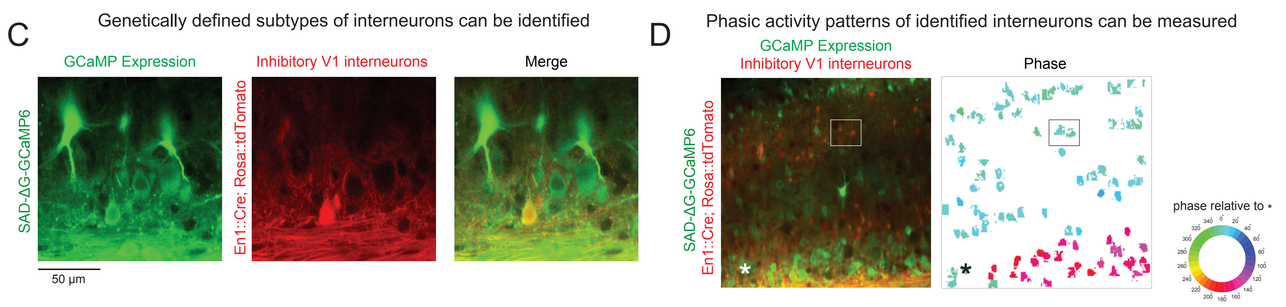
Main findings: The firing pattern of neurons in ankle flexor and quadriceps motor pools resembles the firing patterns recorded with EMG electrodes during walking behavior. Additionally, there is no detectable variance in the phase tuning of an individual pool. This is remarkable because hindlimb motor pools span more than a millimeter of length in the lumbar spinal cord and are adjacent to pools that fire out of phase.

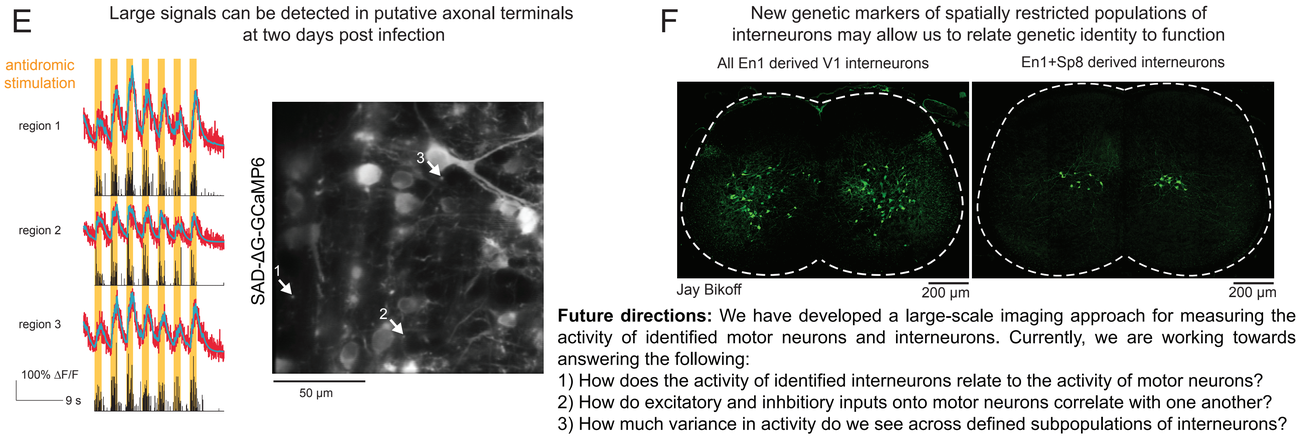
How do spinal interneuron subtypes generate motor output?

Rationale: Interneurons in the spinal cord are molecularly and anatomically diverse. Genetic ablation studies have demonstrated that specific molecularly defined subtypes (named V0-V3) are necessary for generating robust fictive locomotor activity. However, the contributions of these distinct subtypes in generating key elements of fictive locomotor output are poorly understood. In this work, we extended our large-scale imaging methods towards the analysis of genetically accessible groups of interneurons.

Approach: Two days following injection of glycoprotein-deleted rabies containing GCaMP6S into the spinal cord, GCaMP expression is bright enough to image motor neurons, interneurons, and axonal terminals onto motor neuron somata. The use of En1 mice that express red fluorescent protein in the axonal processes of V1 derived inhibitory interneurons will allow us to simultaneously characterize the tuning of a motor pool, as well as synaptic inputs arising from V1 interneurons.







Future directions: We have developed a large-scale imaging approach for measuring the activity of identified motor neurons and interneurons. Currently, we are working towards answering the following:
1) How does the activity of identified interneurons relate to the activity of motor neurons?
2) How do excitatory and inhbitiory inputs onto motor neurons correlate with one another?
3) How much variance in activity do we see across defined subpopulations of interneurons?

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