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Peeling back the layers of locomotor control in the spinal cord

David L McLean[1] and Kimberly J Dougherty[2]

Vertebrate locomotion is executed by networks of neurons within the spinal cord. Here, we describe recent advances in our understanding of spinal locomotor control provided by work using optical and genetic approaches in mice and zebrafish. In particular, we highlight common observations that demonstrate simplification of limb and axial motor pool coordination by spinal network modularity, differences in the deployment of spinal modules at increasing speeds of locomotion, and functional hierarchies in the regulation of locomotor rhythm and pattern. We also discuss the promise of intersectional genetic strategies for better resolution of network components and connectivity, which should help us continue to close the gap between theory and function.

Addresses
[1] Department of Neurobiology, Northwestern University, Evanston, IL, USA
[2] Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, PA, USA

Corresponding authors: McLean, David L (david-mclean@northwestern.edu) and Dougherty, Kimberly J (kimberly.dougherty@drexelmed.edu)

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Introduction

'It is inessential at present whether the lumbar centres are two in number and situate on opposite sides of the spinal cord; or whether they are four in number and situated in antagonistic pairs on each side of the cord; or whether there are more than four in number.'

T. Graham Brown, 1911 [1]

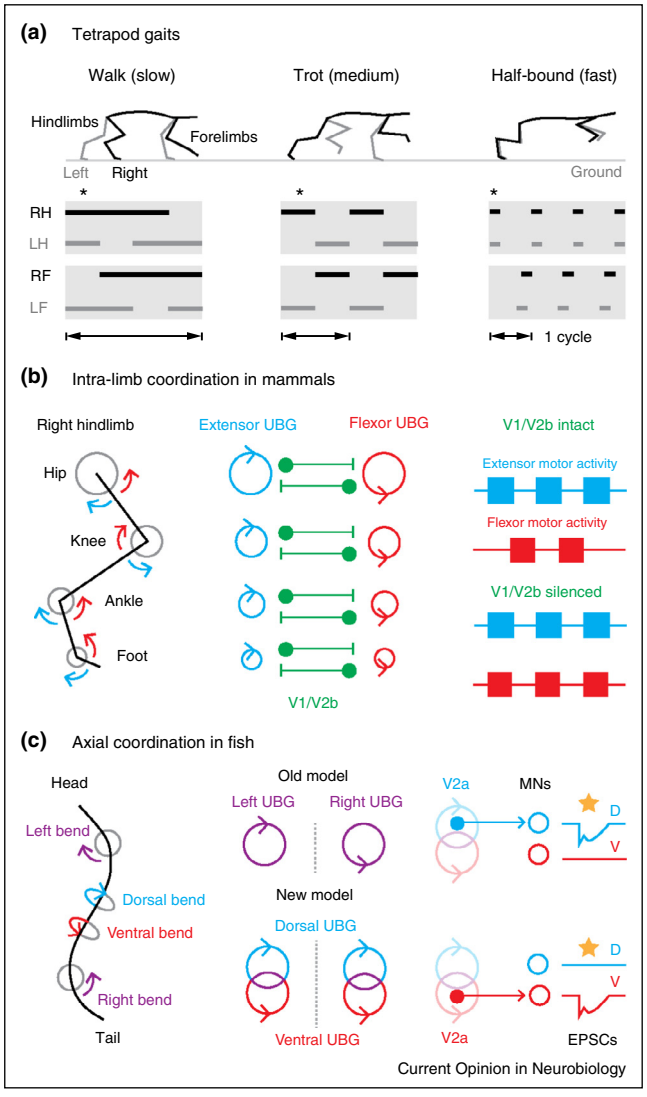
The evidence that networks of neurons within the spinal cord are sufficient to generate locomotion is over a century old. Although this idea remains largely uncontested [2], work since then has led to modifications of the original model Brown put forth to explain his observations, namely the 'half-center' hypothesis. According to this concept, locomotion relies on pools of premotor excitatory interneurons locked in rhythmic alternation by fatigable sources of inhibition. While Brown was understandably less concerned with the number, location or scalability of neuronal half-centers, more recent models have attempted to account for the complexity of motor coordination during locomotion, where muscles are not always purely antagonistic and movements are not always at the same speed. Here, we highlight recent studies testing some of the major predictions arising from past and current models. To this end, we will focus on work using optical and genetic approaches to interrogate the spinal locomotor networks of mice and zebrafish.

Spinal network modularity

In mammals, spinal motor neurons are grouped into functionally and spatially distinct pools according to the muscles they innervate [3]. For locomotion, these pools must be appropriately coordinated within and between two sets of bilaterally paired limbs (Figure 1a). Given the prohibitive complexity of independently controlling motor neurons, theories about premotor control have also invoked a pooled or 'modular' organization, as exemplified by the 'unit burst generator' (UBG) hypothesis of Sten Grillner [4]. According to this idea, motor pools controlling flexor or extensor movements around different joints have their own dedicated UBG made up of interconnected excitatory interneurons, whose purpose is to drive rhythmic motor activity (Figure 1b). The UBG concept deviates from the half-center hypothesis in that reciprocal inhibition is not a prerequisite for rhythmicity, which allows UBGs to change their relative state of coupling (e.g., antagonistic versus synergistic) and provides a basis for variations in limb coordination during locomotion.

A recent paper from the Kiehn lab has tested one of the major predictions of a UBG type organization, namely that motor pools should be able to generate rhythmic activity independently. To do so, Hagglund *et al.* [5•] used transgenic lines of mice in which optogenetic actuators were selectively expressed in spinal glutamatergic neurons. The advantage over past work is that this approach allowed for the targeted and reversible activation and silencing of restricted regions of the spinal cord [6]. Using this method, combined with bulk recordings from ventral roots and more selective recordings from rootlets, the authors demonstrate the independent bursting capability of flexor-related and

Figure 1



Modular control of ipsilateral motor pools in mammals and fish. **(a)** Changes in frequency and coordination between limbs associated with tetrapod locomotion are depicted by kinematic snapshots (upper panels) showing right (R, black) and left (L, grey) hindlimbs (H) and forelimbs (F) during three different gaits. Ground contact for each limb (bottom) is indicated by solid bars and asterisks indicate time points shown in the top panels. **(b)** Articulations around the joints within a limb (left) are divided into extensor (blue arrows) and flexor (red arrows) movements. Intra-limb coordination based on the unit burst generator (UBG) hypothesis (adapted from [4]), likely involving the V1 and V2b ipsilateral inhibitory neurons, is illustrated in the center. Right panel summarizes results testing the involvement of V1/V2b interneurons in flexor–extensor coordination [9•]. **(c)** Top down view of the midline of a fish swimming is presented on the left, where movements are coordinated across (left–right) and along the same side (dorsal–ventral) of the body. In the middle, an older UBG model is compared with an updated one, incorporating independent UBG control of dorsal (blue) and ventral (red) muscles along the same side of the body. Experiments demonstrating the segregation of V2a neurons into dorsal and ventral microcircuits are illustrated on the right [10••]. Yellow stars indicate optogenetic activation of V2a neurons, which evokes electrical/chemical excitatory post-synaptic currents (EPSCs) in either dorsally projecting (D) or ventrally projecting (V) motor neurons (MNs).

extensor-related motor pools in both spatially distant and more closely apposed locations in the lumbar spinal cord.

So how might these independent UBGs be coordinated during locomotion? At least for movements within a limb, flexor–extensor alternation during locomotion has been attributed to the reciprocal actions of ipsilateral sources of inhibition [7]. A recent paper from the Goulding lab has examined the role of ipsilateral inhibitory interneurons in mediating flexor–extensor alternation in the hindlimbs. The work relied on methods to manipulate these populations based on their developmentally derived molecular signatures [8], specifically En1-labeled V1 neurons from the p1 progenitor domain and Gata3-labeled V2b neurons from the p2 progenitor domain. Consistent with the UBG hypothesis, Zhang *et al.* [9•] demonstrate that in the absence of ipsilateral inhibition flexor and extensor motor pools can burst rhythmically, however the pools become synchronized (Figure 1b). The work also demonstrated a functional redundancy in that flexor–extensor alternation is only abolished after silencing both the V1 and V2b populations, suggesting that inhibitory flexor–extensor modules are found in both groups.

While the organization of separate UBGs for control of ipsilateral motor pools was considered a specialization of limb control, recent work has extended this concept to the axial networks controlling swimming in larval zebrafish. Using paired voltage-clamp recordings to compare the relative timing of excitatory and inhibitory synaptic currents during 'fictive' swimming, Bagnall and McLean [10••] reveal that the inputs to motor neurons that innervate either dorsal or ventral trunk musculature along one side of the body are not completely shared. To link these observations to molecularly defined spinal circuitry, the authors drove stochastic expression of a light-gated channel into a major source of ipsilateral premotor excitatory drive, namely Chx10-labeled V2a neurons [11–13,14•]. Consistent with the assessments of network drive, optogenetic activation of sparsely labeled V2a neurons demonstrated the existence of mutually exclusive input patterns (Figure 1c). Although the original formulation of UBGs suggested that left–right alternation represented the minimum functional module in primitive axial networks [4], there is also evidence for separate spinal drive to motor neurons innervating dorsal and ventral trunk muscles in lampreys [15,16]. Taken together, the findings suggest a finer scale modular organization of axial premotor networks than previously appreciated and also provide an early evolutionary template for independent control of musculature on the same side of the body, as proposed by the UBG concept (Figure 1c).

Speed control

When considering the manifestation of spinal network modularity, it is important to remember that motor pools are not uniform in their composition, nor are they likely to