Origin and circuitry of spinal locomotor interneurons generating different speeds.

Henrik Boije and Klas Kullander

Department of Neuroscience, Uppsala University, Box 593, 751 24 Uppsala, Sweden

Correspondence: klas.kullander@neuro.uu.se, henrik.boije@neuro.uu.se

Words (maintext): 2460

Figures: 2

Running title: Locomotor circuits governing speed

Keywords: Central Pattern Generator, interneuron, locomotion, mouse, zebrafish

Abstract

The spinal circuity governing the undulatory movements of swimming vertebrates consist of excitatory and commissural inhibitory interneurons and motor neurons. This locomotor network generate the rhythmic output, coordinate left/right alternation, and permit communication across segments. Through evolution, more complex movement patterns have emerged, made possible by sub-specialization of neural populations within the spinal cord. Walking tetrapods use a similar basic circuitry, but have added layers of complexity for the coordination of intralimbic flexor and extensor muscles as well as interlimbic coordination between the body halves and fore/hindlimbs. While the basics of these circuits are known there is a gap in our knowledge regarding how different speeds and gaits are coordinated. Analysing subpopulations among described neuronal populations may bring insight into how changes in locomotor output are orchestrated by a hard-wired network.

Introduction

The earliest characterization of neurons was based on soma position, morphology of dendrites, and axonal projections. Whereas these parameters are still crucial today, our ambition of understanding sub-specialization force us to include several more such as electrophysiological properties, connectivity, and transcriptional profiles (Figure 1A). Recent studies combining these parameters have made breakthroughs regarding categorizing neural subpopulations within the spinal cord, helping us to disentangle how regulation of different movement patterns may be organized by hard-wired neuronal circuits [1,2].

The specification of neurons in the spinal cord is highly dependent on their position along the dorsoventral and rostrocaudal axis. Antiparallel dorsoventral gradients of BMP and Shh during development is translated into various transcriptional networks conveying the competence to form particular sets of neurons [3-5]. Some years ago, Alaynick and colleagues published a summary of the molecular diversity within the spinal cord known at the time; twelve progenitor domains, which differentiate into 23 neuronal subtypes [6,7]. Sub-specialization along the rostrocaudal axis has primarily been focused on motor neurons where the evolution of movable appendixes has, through Hox-genes, generated specialized segments innervating fins, wings and legs (Figure 1B) [8]. Hox6 and Hox10 defines the brachial and lumbar motor neurons, respectively, while Hoxc9 defines the thoracical segments by supressing Foxp1 at non-limb levels [8]. Lateral motor neurons in mice express Lhx1 and direct their axons via induction of EphA4 to dorsal extensor muscles, while medially located motor neurons express Isl1 and target flexor muscles by activation of EphB1 guiding the axons [9]. Of note, several axon guidance signals are integrated, where Netrin-1, which was shown to attract laterolateral motor columns while repelling mediolateral motor columns, coordinate its actions with A- and B-class ephrin ligands[10]. In skates, a cartilaginous fish that perform pelvic fin walking, the motor neurons innervating pectoral and pelvic fins were also segregated into medial Isl1, EphB1 positive and lateral Lhx1, EphA4 positive pools [11]. This suggests a common molecular program for segregating fin- and limb-innervating motor neurons that existed before vertebrates sat foot on land. Furthermore, a variant Hox-gene pattern, along with an inability to produce or respond to retinoic acid, underlies motor neuron specification for digit innervation [12].

Movement in our evolutionary ancestors relied on a basic spinal network governing the frequency output, left-right alternation, and coordinating activity between segments. The formation of fins and limbs, flexor and extensor muscles, and digits, are all examples of evolutionary steps increasing the ability to move at the cost of more complex regulation. These advances have spurred the divergence of neuronal populations and new techniques, combined with ambitious undertakings, have recently revealed a much richer heterogeneity than previously described within the spinal cord. Correlating animal behaviour with network activity and cellular characterization should provide a better understanding of how the locomotor network is formed and how it operates. This review will discuss how and why different gait and speed modules may have evolved and put them in context regarding recent progress in the categorization of new neuronal subpopulations within the spinal cord.

Moving at different speeds

Evolutionary adaptation to the environment has given rise to considerable variation in modes of animal locomotion. Different strategies for locomotion include undulation in snakes and fish, two-legged locomotion in primates and kangaroos, and four-legged locomotion in a variety of tetrapods. Each strategy poses its own challenges to provide a range of speeds suitable for foraging, long-distance travel, escape and hunting. Here we will mainly focus on different swim speeds in zebrafish and trot/gallop in four-legged animals with the purpose of discussing emerging concepts in how interneurons participate in locomotor pattern formation and gait adjustment upon speed change.

In the zebrafish, undulating movements starts within the first day of development as a rhythmic coiling behaviour. Four to five day old larvae swim in a “burst and glide fashion”, regulating their speed by altering the length of the active swim burst period and the inactive interburst period [13]. At first glance, this regulation seems straightforward and could provide a variety of speeds. However, kinematic analysis in 6 and 7 day old larvae revealed thirteen basic swim patterns, which, when used in various combinations, can produce the desired locomotive behaviour [14]. As for varying speed, stimulation and ablation of the multifunctional descending motor nucleus of the medial longitudinal fasciculus resulted in various swim speeds. Image analysis in the same structure found that calcium activity

correlated with bout duration and maximum tail beat frequency. During slow bouts, larvae perform different numbers of oscillatory cycles at the same tail beat frequency, resulting in longer or shorter bouts, whereas for fast bouts larvae swim with different tail beat frequencies over a fixed duration [15].

In adult fish, undulatory swimming is generated by the backward sequential activation of segmental myotomes resulting in a bend [16]. The bending wave travels down the body, which together with the caudal fin, push against the water to produce forward thrust. The timing of muscle activation and the muscle strain cycle that underlies body bending needs to be coordinated, and as a general rule, increased speed is achieved through increased frequency of the tail beat. Notably, the anterior muscles are active for a large part of the locomotor cycle whereas posterior muscle activity duration decreases towards the tail, especially in fish with a pronounced caudal fin generating most of the thrust (Figure 2A-C).

The production of different swim patterns would presumably require various neural circuits to recruit the most purposeful combination of muscle activation. An important question is whether varied movement speeds are produced through continuous frequency changes or through altered movement patterns. Forward locomotion driven by individual strokes in larval zebrafish seems to be controlled by a continuum [17,18], whereas for bursts of activity, a discrete switch in gait and frequency can be found [15,19].

Four-legged animals increase their speed using different strategies depending on the gait. Mammalian tetrapod gaits are characterised by diverse footfall patterns, such that trot and gallop have their own typical sequence in which the four legs are coordinated [20]. Transition pattern analysis in dog revealed a modular organization of locomotion at the behavioral level with three identified modules that cooperate for stable interlimb coordination pattern during walk, trot or gallop [21]. To increase speed during trot, stride frequency is gradually increased until the point where the gait transitions to gallop or bound [22]. Smaller animals, such as the mouse, also display a full repertoire of gaits [23], but then the transition point between trot and gallop occurs at lower speeds and higher stride frequencies. However, stride frequency, stride length and running speed at the trot-gallop switch point correlate with the body mass between different species, even when comparing mice and horses that differ in bodyweight by a factor of 10^4 [22]. After the gait switch,

galloping ensues with a nearly constant stride frequency; instead increased speed is achieved by an increase in stride length (Figure 2D,E).

Why do tetrapods simply not increase the frequency of gait? The selected speed and stride frequency optimize the performance of muscle-tendon units that function as springs; more than half the energy used to lift and reaccelerate the centre of mass during each stride can be recovered from the elastic energy stored during the previous stride [24]. The factor limiting stride length during trot is the length of each leg from the point of ground contact to a virtual hinge at the upper end of each limb pair (Figure 2C). During gallop, the virtual hinge is positioned between the fore and hindlimb pairs resulting in increased length of the moving leg and body together (Figure 2D). In addition, the push from fore and hindlimb pairs are synchronised, and the additional engagement of axial muscle groups, yield increased force towards the ground. This, together with skeletal biomechanical rebound forces and reduced energy consumption[25], explain the gain occurring after the switch to gallop.

These basic differences in gait would predict differences in the spinal interneurons involved in coordinating trot or gallop. Since trot coordinating interneurons should have characteristics allowing them to efficiently oscillate at different frequencies, spinal interneurons involved in coordinating gallop should be prone to remain at the same frequency. Moreover, trot interneurons on the left and right sides at the same segment of the spinal cord should fire in alternation whereas gallop interneurons should fire in synchrony.

Sub-specialization within the locomotor network

Motor neurons, the final common pathway, receive abundant instructive signals from spinal cord interneurons. Correlating sub-specialization of motor neurons with the sub-specialization of interneurons may help us to understand the structure of the underlying networks. The adult zebrafish locomotor network has a modular structure where slow, intermediate, and fast muscles are innervated by three different motor neuron pools, organised in a somatotopic fashion, which are recruited at these three different speeds (Figure 1A) [26]. Functional properties such as spike threshold, filtering properties, and firing patterns of the different motor neuron pools dictate their recruitment in a graded manner.

The origin of excitation in the zebrafish locomotor network, the V2a interneurons, can be subdivided into the same three speed categories based on their connections and ability to recruit motor neurons [27,28]. Also here, their action potential thresholds were graded in a manner that reflects their activity during swimming. Furthermore, the commissural excitatory V0v population, can also be divided into sub-classes whose recruitment correlates to slow, intermediate, and fast swimming speeds [29]. This modularity is evident already in larvae, where red muscles are used for slow swimming and white muscles are recruited for faster swimming [30]. Combined, these studies reveal a modular structure of the zebrafish locomotor network where circuits are sequentially recruited during acceleration. There is some evidence of a similar modularity in the mammalian spinal cord but the added complexity of the networks makes these modules more challenging to disentangle [31].

While motor neurons are the actuators of the activity encoded by interneurons the question remains how the previously described functional characterization correlates to morphological and molecular properties? The V2a interneurons in both zebrafish and mouse can be broadly divided into two classes based on morphology; cells with both ascending and descending axons, and cells with only descending axons [32,33]. In both species there was a bias for the bifurcating subtype in the more anterior segments revealing a difference in specification along the rostrocaudal axis. Chx10, a canonical marker for V2a neurons, was found to be downregulated in the bifurcating subtype in mice, providing a molecular tool for describing the functional difference found between the two classes [32]. Furthermore, single cell RNA sequencing revealed that both these V2a subtypes could be segregated into the same eleven subtypes based on their transcriptional profiles. Genetic signatures were used to classify V2a interneurons as cervical or lumbar and although the molecularly defined subtypes were present in both regions the ratios were skewed indicating a difference in fate composition along the rostrocaudal axis [32].

Inhibitory neurons play a major role in coordinating the locomotor output. Over one-third of the inhibitory interneurons in the mouse ventral spinal cord belongs to the V1 population, marked by the homeodomain transcription factor En1, which are classically divided into three subtypes. Transcript expression studies revealed 19 transcription factors, whose combinatorial expression, along with soma position, connectivity and electrophysiological properties, could be used to define some 50 subtypes of V1 interneurons [34]. Analysis of segregation along the rostrocaudal axis suggest that V1

interneurons display similar limb- versus thoracic-specific population differences as motor neurons [35]. These variances in V1 subpopulations along the length of the spinal cord rely on the Hox-dependent origin where Hoxc9 is a key factor [35]. There seem to be different inhibitory microcircuits for the motor pools innervating hip, ankle, and foot musculature, revealing more submodules that need to be coordinated at different speeds [34]. This shows, not surprisingly, that sub-specialization of motor neurons at limb levels is accompanied by an increased sub-specialization of interneurons.

Conclusions

The heterogeneity of interneurons in the V1 and V2a spinal progenitor domains presumably extend to other spinal interneuron populations, suggesting that the locomotor networks may be vastly more complex than previously appreciated. We may need to incorporate more than two hundred subpopulations of ventral interneurons to fully interpret and understand locomotor coordination. The presence of such vast number of interneuron subpopulations allow for the presence of compartmentalized microcircuits that can be recruited for diverse motor patterns, and perhaps, a workable strategy could be to identify such functional microcircuits. One strategy has been to reconstruct modules in large scale neural recordings, which has been done in Aplysia, supporting the idea that detecting neural modularity will be efficient in efforts trying to reduce the complexity of the brain [36,37]. Another possibility would be to use single cell RNA-sequencing to reconstruct the differentiation trajectory of defined progenitor pools. Analysing the change in the transcriptional network when cells are committed to particular fates over time, would allow us to pinpoint when subpopulations start to diverge. This has, to some extent, already been done for motor neurons [38]. With the assumption that the individual components of microcircuits emerge at similar time points, it could be possible to add time of birth as a parameter for disentanglement of locomotor circuits.

It may also be worthwhile to consider what properties and expected functions of the interneurons that would be required to regulate switches between speeds or gaits. As mentioned above, mammalian trot coordinating interneurons would be expected to alternate between sides and to oscillate at different frequencies following acceleration, whereas gallop interneurons would be stable in the same frequency regardless of speed and

fire in synchrony. The cells should also have a morphology that allow communication with the speed modules within and between segments (Figure 1B). Moreover, since analysis of interlimb modifications suggest that coordination is controlled during the swing phase but not the stance phase, one would assume that interneurons driving such modification should peak in their activity during the swing phase [21]. Computational models suggest that commissural interneurons and long propriospinal neurons provide the necessary homolateral and diagonal control and would be the main targets for adjustment of gaits [39]. Interestingly, the dmrt3 positive dI6 neurons fulfils some of these criteria and although the exact role of these interneurons remains to be established, mutations in the dmrt3 gene results in aberrant coordination in both mice and horses [40].

The complexity is likely less pronounced in the zebrafish spinal cord, making it a simpler starting point for identifying new subpopulations within the existing ones. The future holds promise with the use of new methods such as next generation sequencing, allowing high-throughput analysis of single cell transcriptomes, and connect it to soma position, connectivity, and functionality in order to understand the sub-specialization within the spinal cord. Correlating this information to animal behaviour should bring us closer to understand how neural circuits generate variable locomotor outputs.

Acknowledgements

Work in the laboratory of the authors are financed by grants from the Swedish Research Council, the Swedish Brain Foundation and the Swedish Foundation for Cooperation in Research and Higher Education. Kjell och Märta Beijers foundation.

Annotated references

••Bikoff et al. 2016
By correlating transcriptional profile with soma position, connectivity and electrophysiology the authors show that inhibitory microcircuits are organized to control motor pools that innervate different muscle groups in hip, ankle and foot.

•Jung et al. 2018
By comparing motor neurons innervating fins in fish and limbs in mice the authors reveal a conserved transcriptional network underlying sub-specialization of motor neurons.

••Hayashi et al. 2018
The authors describe sub-populations among V2a interneurons based on morphology, connectivity, transcriptional profiles and functionality. Sub-specialization of this cardinal class of spinal interneurons varies along the rostrocaudal axis.

••Ampatzis et al. 2014
Here, the authors reveal modular structured microcircuits recruited at slow, intermediate, and fast locomotion. Muscles, motor neurons, and excitatory V2a interneurons can all be divided into these three speed modules.

•Jung et al. 2014
The study describe the Hox-code responsible for sub-specialization of motor neurons at limb innervating levels. Expression of Hoxc9 repress Foxp1 at non-limb levels thereby affecting the specification of the lateral motor column.

••Sweeney et al. 2018
This article describe the sub-specialization of the inhibitory V1 interneurons along the rostrocaudal axis. The authors reveal that increased sub-specialization of motor neurons at limb levels is accompanied by a similar expansion of V1 populations driven by Hox-genes.

•Sagner et al. 2018
Through single cell transcriptomics the authors describe the differentiation trajectories of motor neurons. The work reveal the changes in the regulatory networks driving sub-specialization during development.

References

1. Frigon A: **The neural control of interlimb coordination during mammalian locomotion**. *J Neurophysiol* 2017, **117**:2224-2241.
2. Ziskind-Conhaim L, Hochman S: **Diversity of molecularly defined spinal interneurons engaged in mammalian locomotor pattern generation**. *J Neurophysiol* 2017, **118**:2956-2974.
3. Kutejova E, Sasai N, Shah A, Gouti M, Briscoe J: **Neural Progenitors Adopt Specific Identities by Directly Repressing All Alternative Progenitor Transcriptional Programs**. *Dev Cell* 2016, **36**:639-653.
4. Oosterveen T, Kurdija S, Alekseenko Z, Uhde CW, Bergsland M, Sandberg M, Andersson E, Dias JM, Muhr J, Ericson J: **Mechanistic differences in the transcriptional interpretation of local and long-range Shh morphogen signaling**. *Dev Cell* 2012, **23**:1006-1019.
5. Zagorski M, Tabata Y, Brandenberg N, Lutolf MP, Tkacik G, Bollenbach T, Briscoe J, Kicheva A: **Decoding of position in the developing neural tube from antiparallel morphogen gradients**. *Science* 2017, **356**:1379-1383.
6. Alaynick WA, Jessell TM, Pfaff SL: **SnapShot: spinal cord development**. *Cell* 2011, **146**:178-178 e171.
7. Lu DC, Niu T, Alaynick WA: **Molecular and cellular development of spinal cord locomotor circuitry**. *Front Mol Neurosci* 2015, **8**:25.
8. Jung H, Mazzoni EO, Soshnikova N, Hanley O, Venkatesh B, Duboule D, Dasen JS: **Evolving Hox activity profiles govern diversity in locomotor systems**. *Dev Cell* 2014, **29**:171-187.
9. Kao TJ, Law C, Kania A: **Eph and ephrin signaling: lessons learned from spinal motor neurons**. *Semin Cell Dev Biol* 2012, **23**:83-91.
10. Poliak S, Morales D, Croteau LP, Krawchuk D, Palmesino E, Morton S, Cloutier JF, Charron F, Dalva MB, Ackerman SL, et al.: **Synergistic integration of Netrin and ephrin axon guidance signals by spinal motor neurons**. *Elife* 2015, **4**.
11. Jung H, Baek M, D'Elia KP, Boisvert C, Currie PD, Tay BH, Venkatesh B, Brown SM, Heguy A, Schoppik D, et al.: **The Ancient Origins of Neural Substrates for Land Walking**. *Cell* 2018, **172**:667-682 e615.
12. Mendelsohn AI, Dasen JS, Jessell TM: **Divergent Hox Coding and Evasion of Retinoid Signaling Specifies Motor Neurons Innervating Digit Muscles**. *Neuron* 2017, **93**:792-805 e794.
13. Buss RR, Drapeau P: **Synaptic drive to motoneurons during fictive swimming in the developing zebrafish**. *J Neurophysiol* 2001, **86**:197-210.
14. Marques JC, Lackner S, Felix R, Orger MB: **Structure of the Zebrafish Locomotor Repertoire Revealed with Unsupervised Behavioral Clustering**. *Curr Biol* 2018, **28**:181-195 e185.
15. Severi KE, Portugues R, Marques JC, O'Malley DM, Orger MB, Engert F: **Neural control and modulation of swimming speed in the larval zebrafish**. *Neuron* 2014, **83**:692-707.
16. Altringham JD, Ellerby DJ: **Fish swimming: patterns in muscle function**. *J Exp Biol* 1999, **202**:3397-3403.
17. McLean DL, Fan J, Higashijima S, Hale ME, Fetcho JR: **A topographic map of recruitment in spinal cord**. *Nature* 2007, **446**:71-75.

18. McLean DL, Fetcho JR: **Using imaging and genetics in zebrafish to study developing spinal circuits in vivo**. *Dev Neurobiol* 2008, **68**:817-834.
19. Budick SA, O'Malley DM: **Locomotor repertoire of the larval zebrafish: swimming, turning and prey capture**. *J Exp Biol* 2000, **203**:2565-2579.
20. Nauwelaerts S, Aerts P, Clayton H: **Spatio-temporal gait characteristics during transitions from trot to canter in horses**. *Zoology (Jena)* 2013, **116**:197-204.
21. Maes L, Abourachid A: **Gait transitions and modular organization of mammal locomotion**. *J Exp Biol* 2013, **216**:2257-2265.
22. Heglund NC, Taylor CR, McMahon TA: **Scaling stride frequency and gait to animal size: mice to horses**. *Science* 1974, **186**:1112-1113.
23. Lemieux M, Josset N, Roussel M, Couraud S, Bretzner F: **Speed-Dependent Modulation of the Locomotor Behavior in Adult Mice Reveals Attractor and Transitional Gaits**. *Front Neurosci* 2016, **10**:42.
24. Taylor CR: **Force development during sustained locomotion: a determinant of gait, speed and metabolic power**. *J Exp Biol* 1985, **115**:253-262.
25. Fukuoka Y, Fukino K, Habu Y, Mori Y: **Energy evaluation of a bio-inspired gait modulation method for quadrupedal locomotion**. *Bioinspir Biomim* 2015, **10**:046017.
26. Ampatzis K, Song J, Ausborn J, El Manira A: **Pattern of innervation and recruitment of different classes of motoneurons in adult zebrafish**. *J Neurosci* 2013, **33**:10875-10886.
27. Ampatzis K, Song J, Ausborn J, El Manira A: **Separate microcircuit modules of distinct v2a interneurons and motoneurons control the speed of locomotion**. *Neuron* 2014, **83**:934-943.
28. Eklof-Ljunggren E, Haupt S, Ausborn J, Dehnisch I, Uhlen P, Higashijima S, El Manira A: **Origin of excitation underlying locomotion in the spinal circuit of zebrafish**. *Proc Natl Acad Sci U S A* 2012, **109**:5511-5516.
29. Bjornfors ER, El Manira A: **Functional diversity of excitatory commissural interneurons in adult zebrafish**. *Elife* 2016, **5**.
30. Buss RR, Drapeau P: **Activation of embryonic red and white muscle fibers during fictive swimming in the developing zebrafish**. *J Neurophysiol* 2002, **87**:1244-1251.
31. Bellardita C, Kiehn O: **Phenotypic characterization of speed-associated gait changes in mice reveals modular organization of locomotor networks**. *Curr Biol* 2015, **25**:1426-1436.
32. Hayashi M, Hinckley CA, Driscoll SP, Moore NJ, Levine AJ, Hilde KL, Sharma K, Pfaff SL: **Graded Arrays of Spinal and Supraspinal V2a Interneuron Subtypes Underlie Forelimb and Hindlimb Motor Control**. *Neuron* 2018, **97**:869-884 e865.
33. Menelaou E, VanDunk C, McLean DL: **Differences in the morphology of spinal V2a neurons reflect their recruitment order during swimming in larval zebrafish**. *J Comp Neurol* 2014, **522**:1232-1248.
34. Bikoff JB, Gabitto MI, Rivard AF, Drobac E, Machado TA, Miri A, Brenner-Morton S, Famojure E, Diaz C, Alvarez FJ, et al.: **Spinal Inhibitory Interneuron Diversity Delineates Variant Motor Microcircuits**. *Cell* 2016, **165**:207-219.
35. Sweeney LB, Bikoff JB, Gabitto MI, Brenner-Morton S, Baek M, Yang JH, Tabak EG, Dasen JS, Kintner CR, Jessell TM: **Origin and Segmental Diversity of Spinal Inhibitory Interneurons**. *Neuron* 2018, **97**:341-355 e343.

36. Brownstone RM, Stifani N: **Unraveling a locomotor network, many neurons at a time**. *Neuron* 2015, **86**:9-11.
37. Bruno AM, Frost WN, Humphries MD: **Modular deconstruction reveals the dynamical and physical building blocks of a locomotion motor program**. *Neuron* 2015, **86**:304-318.
38. Sagner A, Gaber ZB, Delile J, Kong JH, Rousso DL, Pearson CA, Weicksel SE, Melchionda M, Mousavy Gharavy SN, Briscoe J, et al.: **Olig2 and Hes regulatory dynamics during motor neuron differentiation revealed by single cell transcriptomics**. *PLoS Biol* 2018, **16**:e2003127.
39. Danner SM, Shevtsova NA, Frigon A, Rybak IA: **Computational modeling of spinal circuits controlling limb coordination and gaits in quadrupeds**. *Elife* 2017, **6**.
40. Andersson LS, Larhammar M, Memic F, Wootz H, Schwochow D, Rubin CJ, Patra K, Arnason T, Wellbring L, Hjalm G, et al.: **Mutations in DMRT3 affect locomotion in horses and spinal circuit function in mice**. *Nature* 2012, **488**:642-646.

Figures and figure legends

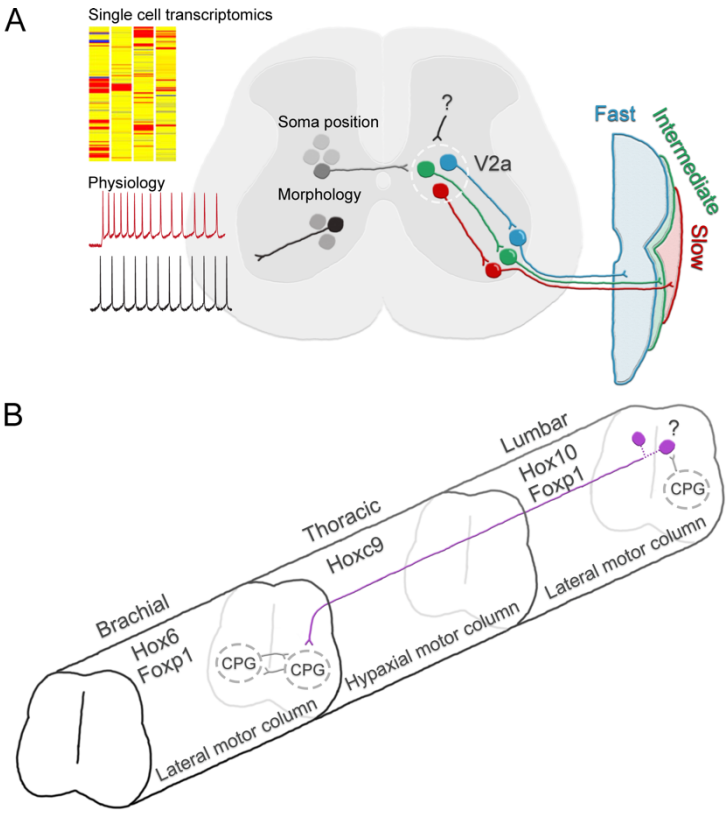

Figure 1. Sub-specialization of neural populations. A) V2a interneurons, motor neurons, and muscles form three distinct speed modules in the zebrafish spinal cord. Evolutionary conserved programs guide the innervation of limbs while motor neuron soma position regulates sensory input. B) Hox-genes encode sub-specialization along the rostrocaudal axis where limb innervating regions display an increased diversification. Identifying interneuron populations coordinating intrasegmental output is critical for our understanding of how speed and gaits are orchestrated.

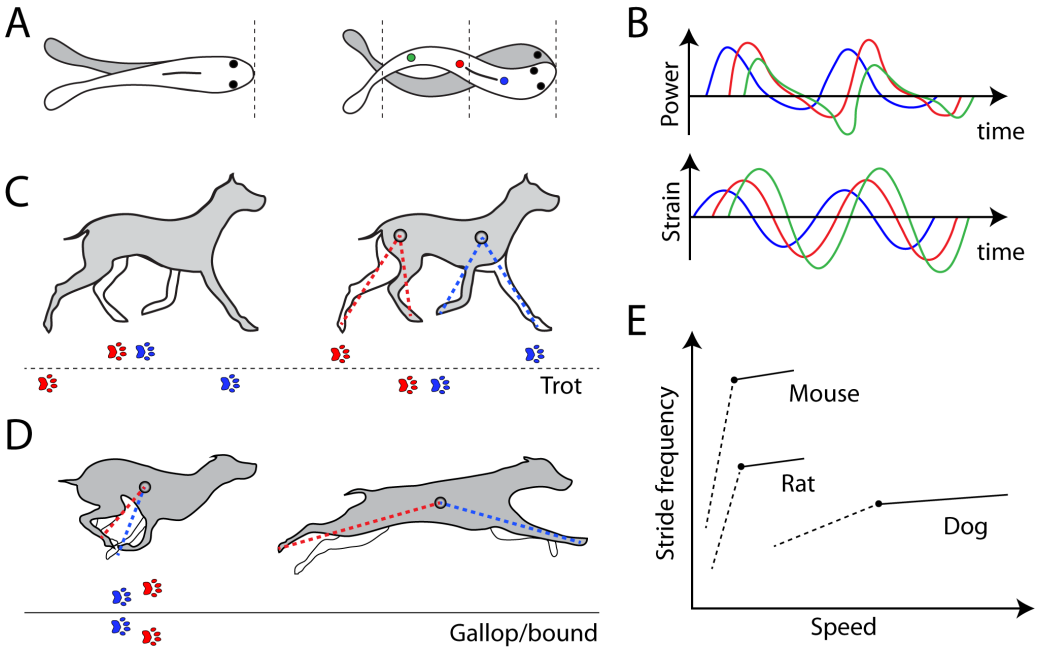

Figure 2. Schematic view over fish and tetrapod locomotion. A) Undulating movements in a generic fish body during slow (left) and faster speeds (right). Stippled lines indicate start and stop of one moving wave. B) Schematic graphs of the power (top) and the strain (bottom) measured in slow muscles at three different positions in the anterioposterior axis of the body as indicated in (A). Note that the intermediate position generates the highest power whereas the posterior position generates the highest strain (modified from Altringham et al., 1999). C, D) Footfall patterns in fore limbs (blue) and hind limbs (red) as well as schematic hinge positions during trotting (C) and gallop/bound (D). E) Schematic graph showing speed and stride frequency relationships during trot (stippled line) and gallop/bound (black line). The dots indicate the transition point between trot and gallop/bound (modified from Heglund 1974).