Brainstem circuits help zebrafish get into the swim of things

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In this issue of Neuron, Berg et al.[1] investigate the functional contribution of two molecularly distinct subpopulations of spinal-projecting midbrain neurons in adult zebrafish, shedding light on mechanisms regulating properties of locomotion such as speed and bout duration.

Central to the need for rapid adaption to ever-changing environmental conditions, animals can perform large repertoires of locomotor behaviors, ranging from slow exploratory maneuvers to fast escape responses that together are critical to finding food or surviving predator attacks. This behavioral flexibility relies on the brain's ability to select appropriate motor programs and coordinate body and appendage muscles (limbs, wings, or fins) to move at the most relevant speed. Thus, identifying brain circuits that control speed is a fundamental step in understanding how the nervous system sets and adjusts movement velocity in general and during locomotion in particular. Recent studies have combined the targeting of genetically defined subpopulations of spinal neurons with electrophysiology and behavior analysis to reveal the modular organization of spinal circuits recruited in a speed-dependent manner in zebrafish[2] and to uncover the molecular identity of spinal neurons that control specific ranges of locomotor speed in mice[3]. Although these results illustrate the existence of dedicated circuits engaged in speed regulation within the spinal cord, spinal circuits require inputs from descending projection neurons to generate and pattern locomotor activity. Yet, to what extent spinally projecting brain neurons differentially encode and control speed parameters is poorly understood. Electrical stimulation experiments in the brain have long identified brainstem regions that can reliably trigger locomotor movements, with graded speed and duration elicited as a function of stimulation intensity and duration. The mesencephalic locomotor region (MLR), located at the interface between the midbrain and the pons, represents the most studied and evolutionarily conserved brainstem region implicated in locomotor control[4]. Recent studies demonstrated that glutamatergic neurons in the MLR's main anatomical subdivisions could differentially modulate slow and fast movements[5]. In addition, a subset of MLR neurons projects to medulla neurons regulating high-speed locomotion in mice[6], suggesting that at least one pathway for speed control in the brainstem relies on midbrain inputs to the caudal medulla as an intermediate signaling center for movement execution. Whether direct input channels from the midbrain to the spinal cord exist to possibly control different locomotor speed regimes is, however, unclear. Moreover, the detailed anatomical, molecular, and physiological organization of circuits encoding speed in the brainstem remains elusive, hindering our understanding of mechanisms modulating specific features of speed control between the brain and the spinal cord.

In this issue of Neuron, Berg et al.[1] make a big step forward in this direction by combining anatomical tracing, calcium imaging, electrophysiology, and 2-photon ablation of targeted neurons in juvenile and adult zebrafish to ask how speed-modulating circuits in the midbrain are organized. The authors begin by mapping the spatial organization of brainstem neurons projecting to the spinal cord using retrograde tracer injections. They identify four clusters of spinal projection neurons along the rostro-caudal axis of the brainstem reticular formation and focus their efforts on the most rostral population located in the midbrain, referred to as the nucleus of the medial longitudinal fasciculus (nMLF) (Figure 1). This group of descending neurons has recently been implicated in visually driven locomotor responses across a large spectrum of speed regimes in larval zebrafish[7], suggesting that they may retain the ability to modulate locomotor speed. Next, the authors analyze the neurotransmitter phenotype of nMLF neurons as an entry point to dissect their putative functional diversity. They find that all nMLF neurons express the vesicular glutamate transporter vGlut2 and the transcription factor Pitx2. In contrast, nMLF neurons differ in vGlut1 transporter expression, with only lateral nMLF neurons expressing vGlut1 in addition to vGlut2 (Figure 1). These results demonstrate that glutamatergic nMLF neurons spatially cluster in the midbrain based on distinct gene expression profiles.

To test whether the two nMLF subpopulations differentially contribute to movement modulation in adult zebrafish, the authors first determine the recruitment profile of nMLF neurons during locomotion using 2-photon calcium imaging and video recording of spontaneous tail movements in head-fixed juvenile or adult fish. Medial vGlut2$^+$ nMLF cells were active during both low (slow speed) and large (fast speed) amplitude tail movements, while lateral vGlut1$^+$ neurons were active in movements composed of mixed low/large amplitude locomotor movements. These results point to differential encoding of locomotor speed in the two nMLF subpopulations. To further document these properties, the authors use an ex vivo preparation with paired patch clamp and nerve root recordings, allowing for precise monitoring of spontaneous fictive swimming speed. They find that medial vGlut2$^+$ neurons fired tonically during both slow and fast fictive swimming with spike frequency increasing during fast swimming, while lateral vGlut1$^+$ neurons were only active during fast swimming. Because this preparation is devoid of external inputs, these data indicate that speed modulation is encoded within the nMLF in the absence of sensory feedback. Further analysis reveals that each of the two subpopulations was active at the onset and throughout the portion of swimming that matched their speed-dependent recruitment profile, suggesting possible roles in initiation and maintenance of the speed regime during slow or fast locomotion. However, given the many different spinal-projection neurons in the zebrafish brainstem, the importance of nMLF neurons in speed control remained to be determined. To assess the contribution of nMLF subpopulations during locomotion, the authors perform 2-photon ablations of either medial or lateral nMLF neurons and quantify swimming activity parameters in the absence of each nMLF subpopulation. They find that eliminating medial nMLF neurons reduced slow exploratory swimming velocity, duration of locomotor episodes, and the probability to generate swimming bouts. In contrast, the ablation of lateral vGlut1$^+$ neurons had no effect on these parameters. To specifically test the contribution of nMLF neurons during fast swimming, the authors use acoustic stimuli to induce fast escape responses. In this context, the ablation of each nMLF subpopulation affected escape response velocity, with fish unable to produce fast swimming (>30Hz) without medial vGlut2$^+$

neurons and displaying a smaller swimming frequency range upon lateral vGlut1$^+$ nMLF ablation. These results suggest that medial nMLF neurons are critical to produce both slow and fast swimming, while lateral nMLF neurons are important to reach the highest speed regime during fast locomotor episodes.

Together, the results by Berg et al.[1] offer high-resolution insight into how speed locomotor parameters are controlled by different brainstem descending projection neurons in zebrafish. The findings set the stage to further investigate the principles of speed regulation based on these entry points. One question raised by this study is how descending speed signals are integrated within post-synaptic circuits in the spinal cord and brainstem. In larval spinal cord, nMLF connections are not restricted to a particular motor neuron subtype[8]. Even though the present study does not specify the nature of contacted motor neurons in adult fish, nMLF might provide excitatory drive to most motor neurons irrespective of their speed-dependent recruitment profiles, whose net effect likely depends on a combination of nMLF firing frequency (tonic drive) and intrinsic properties of recipient motor neurons (input resistance, firing threshold) rather than input alone. Additionally, nMLF inputs might also influence specific spinal interneurons tasked to set motor neuron firing frequency at different speeds[2]. Furthermore, nMLF neurons collateralize to specific regions of the brainstem and might thereby influence other neurons critical for locomotion. Relevant in this context, a recent study revealed the functional coupling between the zebrafish MLR and medulla Chx10$^+$ V2a neurons during locomotion[9]. Whether MLR and the more rostral nMLF neurons act in concert to control locomotion through overlapping or distinct brainstem targets is currently unclear, yet the abovementioned loss-of-function experiments suggest that the two regions do not have a redundant role. Given this dichotomy, it will be interesting to ask whether nMLF population homologs exist in rodents and what their function might be. A key remaining question is also how nMLF neurons achieve their tuning across the speed regime. Projection mapping to the nMLF region reveals visual and vestibular system inputs, among others, and that nMLF neurons interconnect across the midline (Figure 1), suggesting that nMLF neurons integrate inputs from multiple streams and perhaps align activity bilaterally. A recent study in larva also revealed that brainstem cranial relay neurons form preferential connections to the most lateral nMLF neurons[10], providing evidence of how speed tuning differences between the two nMLF populations might be achieved. This pathway might play a critical role in the amplification of Mauthner cell-related sensory signals to promote the transition between the initial large tail bend response (Mauthner-dependent) and the subsequent fast swimming response necessary for successful overall escape responses, provided it remains functional in adult zebrafish. A more detailed mapping of how specific nMLF subpopulations are embedded into broader circuitry will no doubt disentangle these possibilities. Given the fast pace of discovery, we can be confident that the promising combination of patterned optogenetics with imaging or electrophysiology in genetically tractable neuronal populations and at single-cell resolution will provide answers in the foreseeable future. Such future experiments will generate circuit models that can be probed across species and developmental dimensions to assess their conservation, persistence, and possibly plasticity.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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Figure 1. Functional organization of speed-modulating circuits in the adult zebrafish brainstem

Ob, olfactory bulb; Te, telencephalon; OT, optic tectum; Cb, cerebellum; Med, medulla; SC, spinal cord; MB, midbrain; HB, hindbrain; nMLF, nucleus of the medial longitudinal fasciculus; RN, red nucleus; MLR, mesencephalic locomotor region; M, Mauthner; RF, reticular formation.