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Neural Control and Modulation of Swimming Speed in the Larval Zebrafish

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SUMMARY

Vertebrate locomotion at different speeds is driven by descending excitatory connections to central pattern generators in the spinal cord. To investigate how these inputs determine locomotor kinematics, we used whole-field visual motion to drive zebrafish to swim at different speeds. Larvae match the stimulus speed by utilizing more locomotor events, or modifying kinematic parameters such as the duration and speed of swimming bouts, the tail-beat frequency, and the choice of gait. We used laser ablations, electrical stimulation, and activity recordings in descending neurons of the nucleus of the medial longitudinal fasciculus (nMLF) to dissect their contribution to controlling forward movement. We found that the activity of single identified neurons within the nMLF is correlated with locomotor kinematics, and modulates both the duration and oscillation frequency of tail movements. By identifying the contribution of individual supraspinal circuit elements to locomotion kinematics, we build a better understanding of how the brain controls movement.

INTRODUCTION

An important role of the nervous system is the control of locomotion in order to successfully navigate the environment. In the vertebrate brain and spinal cord, this complex task requires the selection of appropriate motor microcircuits to match the demands of any given situation, resulting in smooth and efficient movement. Critical subcortical pathways for the initiation and control of locomotion via the basal ganglia are conserved throughout the vertebrate lineage both anatomically and functionally (Grillner et al., 2013). These regions are linked to form a control pathway in the brain with output in the spinal cord where locomotor central pattern generators (CPGs) reside. One such motor structure is the mesencephalic locomotor region (MLR), an area where electrical stimulation can initiate locomotion, as first demonstrated in cats nearly 50 years ago, and which functions across locomotor modalities, including walking, flying, and swimming (Cabelguen et al., 2003; Kashin et al., 1974; Shik et al., 1966; Steeves et al., 1987). From this region, signals are conveyed to glutamatergic reticulospinal (RS) cells located in the mid- and hindbrain. These RS neurons are strategically located in the pathway, where visual, postural, and other sensory inputs important for selection of appropriate motor programs are thought to converge (Haehnel et al., 2012; Kohashi and Oda, 2008; Sato et al., 2007). RS neurons excite spinal CPGs (Buchanan and Grillner, 1987; Deliagina et al., 2002; Jordan, 1998) by activating NMDA receptors essential to initiate rhythmic locomotion (Hägglund et al., 2010; McDearmid and Drapeau, 2006; Roberts et al., 2008). This sequence of activation comprises the control or descending pathway for locomotion.

To investigate how neurons in the descending pathway generate commands that produce different speeds of locomotion and how these commands are modulated by relevant sensory inputs, we focused on the RS step in the pathway, which serves as the conduit between the brain and the spinal cord at a critical junction for sensorimotor integration. In the larval zebrafish, the RS population consists of around 300 neurons, many of which are individually identifiable (Kimmel et al., 1982). The activity of this optically accessible population has been linked with locomotion in response to a variety of sensory stimuli (Huang et al., 2013; Kimura et al., 2013; Koyama et al., 2011).

One of these innate sensory-driven locomotor behaviors is the optomotor response (OMR) (Bilotta, 2000; Neuhauss et al., 1999), in which larvae respond to whole-field visual motion (Maaswinkel and Li, 2003; Orger et al., 2000) by swimming and turning to maintain a stable position with respect to their visual environment (Portugues and Engert, 2009). In a survey of RS activity in response to visual stimuli driving the OMR (Orger et al., 2008), the most prominent group activated by visual stimulation that specifically elicits forward-directed locomotion was found in the nucleus of the medial longitudinal fasciculus (nMLF), a cluster of RS cells in the midbrain which extends dendrites toward retino-recipient areas, and projects its axons to the spinal cord

(Gahtan et al., 2005; Kimmel et al., 1982; Wang and McLean, 2014 [this issue of *Neuron*]). This structure is known to be multimodal and is active in response to a variety of stimuli as well as during spontaneous swimming, and is further believed to be implicated in a broad range of intensities of locomotion (Sankrithi and O'Malley, 2010).

In this study we aim to characterize the different kinematic parameters that are dynamically modulated during swimming at different speeds. Larvae swim in units called "bouts," where each individual bout is characterized by a discrete number of tail oscillations that propel the larva through the water. We show that different speeds of locomotion are accomplished not only by changing the speed of these oscillations, but also through a dynamic interplay between the locomotor gait, and the duration, intensity, and rate of movement episodes. A quantitative description of the behavior gives us a starting point to step backward through the circuit and ask how the upstream activity in the RS cells, specifically the nMLF, relates to these kinematic parameters and contributes to this modulation. We observe correlations between activity in identified nMLF neurons and both the visual stimulus and the specific behavioral elements we identify as signatures of changing locomotor speed. We use stimulation and ablation of these cells to assess their necessity and sufficiency in modulating the various behavioral parameters. With in vivo two-photon calcium imaging in an awake, behaving, minimally invasive preparation, we present evidence for selective locomotor modulation by identified neurons. This study allows us to dissect the nature of activity in descending inputs that are important in controlling the speed of locomotion in an intact behaving animal.

RESULTS

Modulation of Locomotor Activity in Response to Whole-Field Visual Motion

In response to optomotor gratings moving at speeds from 0 to 40 mm/s, larval zebrafish adjust their locomotor speed to maintain their position relative to the moving grating. Relevant kinematic parameters were measured in an effort to quantitatively describe this behavioral response. Freely swimming 6-day-post-fertilization (dpf) wild-type (WT) larvae were individually presented with sinusoidal striped patterns moving at different speeds from below, while high-speed video was acquired (Figure 1A). Analysis of the raw video (Figure 1B; Experimental Procedures) allowed us to calculate relevant kinematic variables (Figures 1C–1I). We first confirmed that larvae increase their average swim speed as grating speed increases (Figures 1C and 1D). Over the course of a trial lasting several seconds (Figure 1C), they were able to match grating speeds up to 20 mm/s, but their speed plateaus for gratings moving at faster velocities (Figure 1D).

Larvae swim intermittently in what has been described as a beat-and-glide mode. This includes a bout period when active swimming is performed and the tail is oscillating, followed by an interbout period of varying duration when the larva is not actively swimming, but is either coasting or stationary. A close look at the instantaneous swimming speed (Figure 1C) revealed the cyclic nature of the intermittent swimming style in the peaks and troughs of each line.

We next analyzed individual bouts and interbouts and their contribution to average swimming speed. We observed an increase in average distance per bout with grating speed (Figure 1E). Some of this could be accounted for by the lengthening of bout duration as grating speed increased within the range 0–10 mm/s (Figure 1F), whereas the increase beyond 10 mm/s is accompanied by a rise in the average tail-beat frequency (TBF), which was only modulated for bouts elicited by a grating moving faster than 10 mm/s (Figure 1G). A faster grating led larvae not only to modulate their swim bouts, but also to elicit them more often: an interbout duration of 1 s for a stationary grating became 200 ms by the time the grating moved faster than 10 mm/s (Figure 1H). The latency of the motor response from the initiation of the grating motion was also modulated by grating speed (Figure 1I). We saw a significant decline in latency as the speed of the grating increased, indicating that a faster grating elicited a locomotor response more quickly.

From these data we can identify relevant kinematic variables that are dynamically changing in freely swimming larvae over the range of grating speeds tested. Changes in bout duration, interbout duration, and latency appear to contribute at slower speeds, while changes in TBF are the major contributor at faster speeds. Despite this variety of factors that determine swimming speed, the larva is able to maintain a tight correlation of its own swimming speed with that of moving gratings up to 20 mm/s.

Larval Swim Bouts Cluster into Fast and Slow Types

Having determined that larvae swim faster when presented with faster OMR stimuli, we wanted to know whether they do so by continuously modulating a single type of bout or whether, as for many vertebrates, they are able to engage distinct gaits to locomote at different speeds. In the first scenario, we expect bouts to be distributed continuously throughout parameter space. Alternatively, if locomotor output is organized discretely and different types of bout are recruited, we expect the kinematic parameters across the entire bout population to cluster into two or more distinct groups.

For slow stimuli trials, the bouts formed a single cluster in a space defined by head yaw, mean TBF, rostral bend amplitude, and maximum TBF (Figures 2A and S1 available online). As the grating speed increased, the original cluster shifts progressively in this space, indicating a modulation of the slow swim bout. In addition, a second cluster emerged such that for fast-moving grating trials, two distinct distributions with minimal overlap were observed (Figure 2B). Based on these clusters, we categorized each bout as either a slow bout or a fast bout (Experimental Procedures). We plotted the density of bouts in parameter space defined by selected kinematic variables as quantified in our assay (Figures 2C and S2). To assess the consistency of the categorization, we used four different kinematic parameters and found agreement in all cases (Figure 2D). The fraction of fast bouts elicited by a drifting grating changes continuously from ~4% for slow-moving stimuli to ~50% for stimuli moving at 20 mm/s or faster.

To test whether the kinematic parameters of these two different types of bouts vary with stimulus speed, we repeated the analysis of Figure 1 for slow and fast bouts, respectively (Movie S1). Both bout types showed a progressive modulation of speed and distance (Figures 2E–2G) in response to different

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