Supporting Information

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SI Experimental Procedures

Zebrafish Lines and Care. All experimental protocols were approved by the animal research ethical committee, Stockholm. Two transgenic zebrafish lines were used. In the one line (Chx10:GFP), the expression of green fluorescent protein (GFP) was driven in V2a interneurons by the promoter of the transcription factor Chx10. Double in situ hybridization of GFP and Chx10 has shown that the Chx10:GFP transgenic line only marks the V2a interneurons (1). V2a interneurons have been previously described as circumferential ipsilateral descending (CiD) neurons (1, 2). In the other transgenic line (GlyT2:GFP), GFP is expressed under the control of the promoter of the glycine transporter 2 gene (3). All experiments were carried out in 4- to 5-d-old transgenic larval zebrafish at room temperature (~22 °C).

Backfilling of Motoneurons. Motoneurons were backfilled in anesthetized larval zebrafish with 0.03% tricaine methanesulfonate (MS222; Sigma-Aldrich) in extracellular solution containing (in mM): 134 NaCl, 2.9 KCl, 1.2 $MgCl_2$, 2.1 $CaCl_2$, 10 Hepes, 10 glucose (pH 7.8; osmolarity 290 mOsm) by applying a small amount of tetramethylrhodamine dextran (3000 MW; Invitrogen) on the skin of the animal. An etched tungsten wire was then inserted through the skin of the animal to inject the dye into the muscles. The animals were left to recover for 1–2 h and to allow the retrograde transport of the tracer to motoneuron somata.

Laser Ablation of GFP-Labeled Interneurons. Larval zebrafish were first anesthetized and then transferred to the ablation chamber, embedded in 1.5% low-melt agarose containing and covered with zebrafish extracellular solution containing 0.03% MS-222. The chamber was then placed under the confocal microscope (LMS 510; Carl Zeiss). In most experiments 15 of the ~50 visible GFP-labeled V2a interneurons (~30%) per segment, chosen randomly without any consideration of their dorso–ventral position, were photoablated individually using a two-photon laser (wavelength 800 nm) over 10 segments in the midbody region of the animal. This paradigm was chosen because ablation of 30% of V2a neurons over 10 segments produced a significant effect for the measured parameters while the treated fish were still viable. When >50% V2a interneurons were ablated, most animals failed to recover within 1 h after the ablation. When V2a interneurons were ablated in only one or two segments, no significant changes in the measured parameters were detected. In some experiments, we ablated 5 V2a interneurons (~10%) per segment to investigate proportion-dependent effects. Similarly, ~15% (8 of 50) per segment of the dorsally located V2a interneurons were ablated to test how they affect the peak and steady-state swimming frequencies. As a control, we also photoablated 15 GFP-labeled GlyT2 inhibitory interneurons per segment over 10 segments to test the effects of cell ablation per se. After ablation, the fish were removed from the agarose and left to recover from the anesthesia for 1–2 h and until they could swim freely in the dish before the start of recording of swimming activity. Control fish were embedded in agarose alongside the ablated fish, but were not subjected to cell photoablation.

Zebrafish Preparations. The fish were anesthetized and then pinned down using tungsten pins placed through the notochord in a Sylgard-lined recording chamber. The skin was removed from both sides of the body with fine forceps before the fish were paralyzed with 6.25 μM α-bungarotoxin (Sigma-Aldrich) for 10 min. The larvae were placed dorsal side up to monitor left–right alternating activity during swimming or the rostro–caudal propagation of activity. The preparation was then perfused with extracellular solution for 15 min before the start of the experiment.

Electrophysiology. Extracellular recordings were performed from the motor nerves running through the intermyotomal clefts from two segments either opposite to each other or along the rostro–caudal axis of the fish. Swimming activity was induced by electrical stimulation or by application of *N*-methyl-D-aspartate (NMDA). For electrical stimulation, a glass electrode was placed at the level of the otic vesicle and current stimulation (1 ms, 5–150 μA) was delivered every 5 min. Extracellular glass suction electrodes used for recording and stimulation electrodes were pulled from borosilicate glass and broken down to the desired tip diameter (15–25 μm) and fire polished. The stimulation intensity was gradually increased until a bout of swimming activity could be reliably elicited. The control stimulation threshold was set to the minimum intensity that induced swimming activity. The threshold stimulation ranged between 5 and 20 μA in control preparations.

Data Acquisition and Analysis. Extracellular signals were amplified with a differential AC amplifier (A-M Systems), filtered with low- and high-cutoff frequencies, digitized at 10 kHz with an A/D converter (Molecular Devices) and recorded on a personal computer using pClamp software (Molecular Devices). Data analysis was performed using correlation analysis in Spike2 (Cambridge Electronic Design) (4–6). From the peak times of these correlation traces, period and phase values of the motor pattern were calculated. The locomotor cycle duration was determined as the time between two consecutive peaks of the autocorrelation. The left–right phase was determined as the time between 0 and the center peak of the cross-correlation from a right recording versus a left recording and then expressed as percentages of the locomotor period. The rostro–caudal delay was determined as the time between 0 and the center peak of the cross-correlation between caudal and rostral recording. The phase lag was then calculated by dividing the rostro–caudal delay by the number of segments separating the recording electrodes and was then expressed as percentage of the cycle duration. The peak frequency was determined as the mean of the two highest swimming frequencies (at the onset of the swimming episode) and the steady state as the mean of three to seven subsequent cycles with stable frequencies. The analysis of the cumulative distribution of the swimming frequency and the Kolmogorov–Smirnov test were done using Matlab. All values are given as mean ± SEM. Unless otherwise stated, the significance of differences of means between experimental groups and conditions was analyzed using Student's *t* test. Means were considered statistically significant at *P* values of <0.05.

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