Supporting Information

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SI Text

Quantification of Bursting Patterns

Signals were amplified 100× with Grass P15 amplifiers with a bandwidth of 3 Hz and 10 kHz (Grass Technologies), further amplified 50× with Cygnus FLA-01 amplifiers (Cygnus Technologies), digitized by Axon Instruments DigiData 1200 series, (Molecular Devices) and recorded on a computer using AxoScope 9 software (Molecular Devices). Burst parameters were analyzed as described in detail previously (1). Briefly, each episode of activity consisted of three to four bursts/cycles. Each cycle was divided into 100-ms bins until the start of the next cycle, and whether a muscle was active or not during each bin was determined. Histograms were then constructed by determining the proportion of times a muscle was active in each 100-ms bin preceding and subsequent to the 0 time point for multiple cycles, and expressed as a percentage of the total number of cycles analyzed.

1. Landmesser LT, O'Donovan MJ (1984) Activation patterns of embryonic chick hind limb muscles recorded in ovo and in an isolated spinal cord preparation. *J Physiol* 347:189–204.

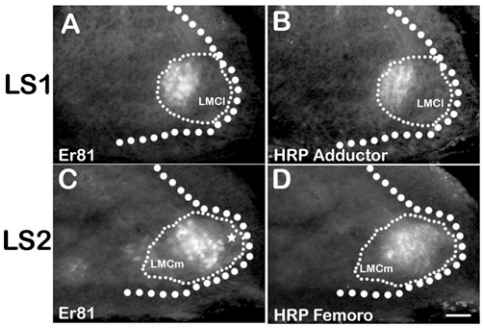

Fig. S1. Er81 expression and HRP retrograde labeling shows that it is an appropriate marker for adductor and femorotibialis motoneurons. (*A*) At lumbosacral (LS)1 the transcription factor is expressed by medially located adductor motoneurons (medial to lateral motor column, LMC_l), which were retrogradely labeled from the adductor muscle of at stage (St) 31 chicken embryo (*B*). (*C*) At LS2, Er81 is expressed by the more laterally located femorotibialis motoneurons (lateral to LMC_m), which were retrogradely labeled from the femorotibialis muscle (*D*). The most lateral motoneurons that belong to the sartorius pool (star in *C*) do not express Er81. The entire lateral motor column (LMC_l and LMC_m) is enclosed by the thin dotted line. The thick dotted line outlines the lateral edge of the gray matter. (Scale bar, 30 μm.) Dorsal is up, lateral is right.

Table S1. Contributions of lumbar spinal nerves 1–3 to the sartorius and femorotibialis muscles in control embryos and those stimulated with channelrhodopsin 2 (ChR2) at twice the normal frequency

Injected LS nerve	Contribution to sartorius	Contribution to femorotibialis
Control (n = 4)		
LS1	4/4	4/4
LS2	4/4	4/4
LS3	0/4	4/4
Light stimulated (n = 6)		
LS1	6/6	3/6
LS2	3/6	6/6
LS3	0/6	6/6

Following injection of either Di-I or Di-Asp into one of the three spinal nerves contributing to the crural plexus in isolated spinal cord preparations, and transport of the dye to the nerve endings, the contribution of each nerve to the sartorius or femorotibialis muscles was determined from frozen sections cut transversely to the limb. Data are presented for control embryos and those that had experienced twice the normal frequency of spontaneous bursting by in ovo light activation of ChR2