Supplemental Experimental Procedures

Fly Stocks

Drosophila were raised in sparse cultures on standard cornmeal agar medium supplemented with rehydrated potato flakes (Carolina Biological Supply), and kept on a 12-h light, 12-h dark cycle at 25° C. All imaging and electrophysiology experiments were performed on female flies 1-3 days post-eclosion. Flies for all optogenetics experiments were raised on food supplemented with all-trans retinal (all-trans-retinal was prepared as a 35 mM solution in ethanol, and 100 ml of this solution was mixed into a layer of rehydrated potato flakes approximately 0.5 cm deep in a standard 6-oz culture bottle). Descriptions of all fly stocks used in the study are listed in the Table of Transgenes. The genotypes used, by figure, are listed in the Table of Genotypes. Both of these tables are appended to the end of this section.

Electrophysiology

Whole-cell patch clamp recordings were performed as previously described (Wilson et al., 2004), with some modifications. Flies were cold-anesthetized and fixed to the underside of a custom-milled steel platform (0.001" thickness). The fly was mounted with its ventral side facing up, using UV-cured glue (KOA 300, KEMXERT). The ventral head and anterior thorax were partly inserted through a hole in the platform. Thus, both the ventral head and a small part of the ventral thorax (from the neck connective to the base of the mesothoracic legs) were visible and accessible from above. The top side of the platform, and thus also the exposed parts of the head and thorax, were continually perfused with oxygenated saline. In all experiments except for those in Figure 7, all six legs were glued to the holder with UV-cured glue. A small hole was manually dissected in the cuticle of the ventral thorax to expose the prothoracic neuromeres, and the perineural sheath was gently removed with fine forceps to expose neuronal cell bodies.

The saline which perfused the preparation contained: 103 mM NaCl, 3 mM KCl, 5 mM TES, 8 mM trehalose, 10 mM glucose, 26 mM $NaHCO_3$, 1 mM NaH_2PO_4, 1.5 mM $CaCl_2$, and 4 mM $MgCl_2$ (pH 7.1, osmolality adjusted to 270-275 mOsm). The saline was bubbled with 95% O2/5% CO_2 and was perfused at ~2-3 ml/min. Recordings were performed at room temperature. Cell bodies were visualized using an infrared LED (Smartvision) and a 40× water-immersion objective on an upright compound microscope equipped with a fluorescence attachment (Olympus BX51F).

Whole-cell patch-clamp recordings were targeted to GFP-labeled cell bodies in the prothoracic region of the VNC. The internal patch pipette solution contained (in mM): 140 potassium aspartate, 10 HEPES, 1 EGTA, 4 MgATP, 0.5 Na_3GTP, 1 KCl, and 13 biocytin hydrazide (pH 7.2, osmolarity adjusted to ~265 mOsm). Although there were other VNC neurons labeled by each Gal4 line (Figure S3A), it was easy to distinguish the target neurons from the other neurons by the characteristic and reliable positions of their cell bodies, as well as their intrinsic properties: recorded neurons in each class had a characteristic input resistance, resting membrane potential, and spike waveform. We were able to reliably record from midline local and projection neurons by targeting the most ventral cell bodies along the midline in each GAL4 line. We targeted intersegmental neurons based on their large cell body size and characteristic position. Typical positions of target neuron cell bodies are indicated in Figure S3A.

All recordings were made in current-clamp mode using an Axopatch 200B amplifier. Data were low-pass filtered at 5 kHz before they were digitized at 10 kHz by a 16 bit A/D converter (National Instruments, USB-6343), and acquired in Labview. Stable recordings were typically maintained for 1-2 hours. A small hyperpolarizing current (approximately -5 to -10 pA) was injected to compensate for the depolarizing seal conductance (Gouwens and Wilson, 2009). Analysis of electrophysiology data was performed with custom scripts written in MATLAB and Python.

For all bristle recordings displayed, the second-most distal bristle on the posterior surface of the femur (Figure S5A) was manually clipped with fine forceps to approximately 25% of its full length. We chose this bristle because it is among the largest on the prothoracic leg (Hannah-Alava, 1958), and we found it more difficult to record spikes from smaller bristles. To record bristle neuron signals, a glass recording pipette was inserted over the cut bristle tip. The recording electrode was filled with a high K^+ saline that was identical to the external saline except for the concentrations of NaCl (9 mM) and KCl (121 mM). This solution was designed to mimic the high K^+ concentration of the mechanoreceptor lymph (Grunert and Gnatzy, 1987; Thurm and Kuppers, 1980), and has previously been shown to effectively preserve the bristle's trans-epithelial potential and mechanosensory responses (Kernan et al., 1994). Bristle recordings were band-pass filtered using a 2^{nd} order Butterworth filter with cutoff frequencies of 100 and 400 Hz. In all bristle recordings, the recorded spike amplitude was greater at high firing rates (e.g., Figure 2A).

Mechanical and Optogenetic Stimulation

Bristles were mechanically stimulated with a closed-loop piezoelectric actuator (Physik Instrumente P-841.60, 90 µm travel range, with E-509.S1 sensor/piezo servo-control module). The bristle recording electrode consisted of a glass capillary which was mounted on the actuator with a custom-milled aluminum holder, which held the capillary firmly in place with a

set screw. A recording wire was fixed to the interior of the pipette with a rubber gasket (Axon Instruments) and connected to the headstage by 6 inches of flexible shielded wire. Extracellular bristle signals were acquired in zero-current (I=0) mode with an Axon 200B patch-clamp amplifier, and digitized at 10 kHz. The same basic configuration was used for recording bristle neuron spikes (Figure 2 and Figure 3) and also for simply deflecting the bristles without recording from the bristle neurons (Figures 4-7); the only difference was that in the latter configuration the recording wire was not inserted into the glass capillary.

Insect bristles are directionally selective— they respond most strongly to deflection in a particular direction, which depends on the asymmetric orientation of the hair socket (Burrows, 1996; Corfas and Dudai, 1990). For all bristle recordings in this study, we restricted the stimulus to movement in the bristle's preferred direction, i.e. the direction that reduced the acute angle between the bristle and the cuticle.

Optogenetic stimuli were delivered to the leg with a fiber optic cannula (0.22 NA, Thorlabs) coupled to a green LED driver (530 nm; Smartvision S-30). We used a fiber optic cannula with a 50 µm core (Thorlabs) in all experiments except the GCaMP imaging experiments in Figure 2C-G, where we used a cannula with a 200 µm core. For the experiments in Figures 2A, 2C-G, 4A, 5A, and 6A, the stimuli were 100 ms light pulses. For the experiments in Figures 4D-E and 5D-E, the light stimuli were 500 ms light pulses, and in Figure 6C, 200 ms and 500 ms stimuli were used. All light stimuli were delivered at 5 kHz with a 40% duty cycle, and the LED was powered at 40% of its maximum output. The diameter of the effective light spot at the sample, measured by recording from a bristle and sequentially moving the fiber optic away from the recording site, was approximately 200 µm, encompassing 20-80 bristles, depending on the location on the leg (Hannah-Alava, 1958). Although some LexA lines had expression in central neurons in the VNC (Figure S2), these cells were not directly stimulated by focal illumination of the leg. We verified that axons of passage were not activated by the stimuli we used in these experiments (Figure S6B).

Mechanical and optogenetic stimuli were generated in Labview and sent to the amplifier at 5 kHz using a separate analog output DAQ (National Instruments 9263). The positions of the recording/stimulation electrode and the fiber optic were controlled with separate servo-controlled XYZ translation stages (Thorlabs) and custom Labview software. The fly's leg, as well as the mechanical and optical probes, were visualized with a camera positioned below the stage (Point Grey Firefly) coupled to a 50× air objective (Olympus). The camera and objective were also mounted on servo-controlled translation stages, to visualize the stimulus probes and the surface of the fly's leg.

Calcium Imaging

The experimental preparation for calcium imaging was essentially the same as for electrophysiological recordings, except that the sheath was left intact and the fly's esophagus and crop were removed to prevent movement. GCaMP6f was expressed in all neurons under Gal4/UAS control, and Chrimson was expressed specifically in leg bristle neurons under LexA/LexAOp control (genotype: *UAS-GCaMP6f/R38b08-LexA;R57c10-Gal4/LexAop-Chrimson::TdTomato*). The optogenetic stimulus was centered on the femur/tibia joint of the fly's left leg (for details see Mechanical and Optogenetic Stimulation, above).

Images were acquired in framescan mode on a custom built two-photon microscope using ScanImage 3.8 software (Pologruto et al., 2003), with excitation light at 925 nm. Each trial comprised 53 frames (512×512 pixels) imaged at 1.93 Hz, scanning from top the left of each frame to bottom right, with 5 stimuli delivered at 4 sec intervals. A single trial was captured at each z-plane, starting at z=0 µm at the ventral surface of the VNC, and progressing dorsally to z =-400 µm, in 10 µm steps. There was a 30 sec gap between each trial. We observed spontaneous neural activity throughout the course of the experiment, indicating that the fly remained healthy and responsive.

Within each trial, all pixels from the frame containing the light artifact from the LED stimulus were set to the baseline intensity. A Gaussian low-pass filter of 5×5 pixels was then applied, and data from each trial were aligned in the *xy* plane on a frame-by-frame basis using efficient subpixel motion registration (Guizar-Sicairos et al., 2008). ROIs were manually segmented within each imaging plane to identify individual neuronal cell bodies. ROIs in adjacent *z*-planes with greater than 50% *xy* overlap were considered to be part of the same neuron. For each neuron that spanned multiple *z* planes, only the largest 2-D ROI (within a single *z*-plane) was included for subsequent analysis. Neurons with a cell body area of greater than 49 μm^2 (~8 µm diameter) were classified as motor neurons.

Calcium signals (ΔF/F) were measured as changes in fluorescence (ΔF) normalized to the fluorescence during the baseline period (F, average of the 4 lowest-intensity frames in each trial.). Cross correlation of stimulus and cellular calcium signal vectors was performed for each imaging trial (n = 53 frames) using the *xcov* function in MATLAB, and normalizing by the standard deviations of the stimulus and cellular calcium signal vectors. For all analyses in Figure 2, we define the stimulus correlation as the mean of the sample correlations across two lag values (+1, +2 frames). Values for the correlation between the stimulus waveform and the ΔF/F waveform were typically less than 0.5 because the stimulus was brief (<1 frame

every 4 sec), while calcium signals typically persisted across multiple frames, either due to sustained activity or the intrinsically slow kinetics of GCaMP.

To compare whether the response of a neuron was correlated with the bristle stimulus at a level above chance, we carried out a permutation test. We again computed the cross-correlation of stimulus and cellular calcium signals, with the difference that individual time points of the stimulus vector were randomly shuffled. This procedure was repeated 1000 times for each neuron to obtain a null distribution of correlation values (Figure 2F, bottom histogram); the confidence intervals indicated in Figure 2 were calculated from this null distribution. The correlation threshold varied slightly depending on whether we shuffled the stimulus vectors (0.19), cellular calcium signal vectors (0.15), or both stimulus and cellular calcium signal vectors (0.16). Overall, the three shuffling procedures produced qualitatively similar results, but we selected the most stringent threshold as a conservative estimate of the number of neurons in the prothoracic VNC responding to bristle stimulation.

Paired Whole-cell Recordings

In Figure 7, we targeted paired whole-cell recordings to distinct central neuron types by labeling both cell populations with GFP (genotype: *pJFRC7-20XUAS-IVS-mCD8::GFP;R13d11-Gal4/R18g08-Gal4* or *R69c05-Gal4*). During the recording, the fly's activity was recorded at 30 fps with a video camera (Point Grey Firefly) equipped with a compact long-working-distance magnifying lens (Infinistix 94 mm/1.00x) mounted under the recording stage. Fly movement was computed as the sum of absolute pixel intensity differences across adjacent video frames, normalized to the peak value in the corresponding experiment. For the activity traces in Figure 7, movement was computed for a region of interest that encompassed the fly's prothoracic leg, though the fly's other legs and abdomen occasionally entered this region.

Pharmacology

Drugs were bath applied via the saline perfusate. Tetrodotoxin (TTX) was prepared as a concentrated stock solution in sodium citrate, CGP54626 was prepared as a concentrated stock solution in dimethyl sulfoxide, picrotoxin was prepared as a concentrated stock solution in aqueous NaCl (140 mM), and methyllycaconitine (MLA) was prepared as a stock solution in water. For the midline local neurons and the midline projection neurons (Figures 5 and 6), 10 μM picrotoxin was sufficient to block inhibitory responses. For the intersegmental neurons (Figure 4), 100 μM picrotoxin and 50 μM CGP54626 was required to block inhibition. The requirement for CGP54626 implies a role for $GABA_B$ receptors (Wilson and Laurent, 2005), and the need for a higher concentration of picrotoxin suggests a role for GluCl receptors (Liu and Wilson, 2013).

Central neuron responses to bristle stimulation were completely eliminated after blocking voltage-gated sodium channels with TTX (1 μM), as we would expect if they depended on spikes in bristle neurons. The same effect was observed with the nicotinic acetylcholine receptor antagonist methyllycaconitine (MLA, 1 μM). This latter result implies that bristle neurons are cholinergic, like most insect mechanoreceptor neurons (Burrows, 1996). This result is contrary to previous reports suggesting that histamine is the bristle neuron neurotransmitter (Buchner et al., 1993; Melzig et al., 1996; Melzig et al., 1998). Iontophoresis of histamine into the neuropil evoked no response in intersegmental neurons, while acetylcholine iontophoresis evoked large depolarizing responses (data not shown). In addition, histamine receptor antagonists (100 μM pyrilamine and 200 μM cimetidine) did not have a reliable effect on the responses of intersegmental neurons to mechanical stimulation of femur bristles, whereas these responses were blocked by the nicotinic antagonist methyllycaconitine (MLA, 1 μM). Bath application of histamine (1 mM) increased the input resistance of intersegmental neurons, suggesting a neuromodulatory effect.

Immunohistochemistry and Anatomy

Immunohistochemistry was performed using established methods (Wilson and Laurent, 2005). Brains and VNCs were dissected and fixed for 15 min at room temperature in 4% paraformaldehyde, then rinsed in phosphate buffered saline (PBS) and incubated in blocking solution (5% goat serum in PBS + X% Triton X-100 [PBST]). They were then incubated in blocking solution with primary antibodies for 24 hours at room temperature, followed by washing in PBST and incubation in blocking solution containing secondary antibodies for 24 hours at room temperature. Samples were rinsed with PBST, mounted in Vectashield, imaged on an Olympus FV1200 confocal, and analyzed in Fiji. To reconstruct the morphology of single neurons (Figures 3A and S3B), we manually traced the skeleton of each biocytin-filled neuron using the Simple Neurite Tracer plugin in Fiji. We used the Fill out command to generate a three-dimensional volume of the neuron, which was subsequently converted to a z-projection (Figures 3A and S3B).

To visualize the morphology of each biocytin-filled neuron in the context of the surrounding neuropil, the primary antibody solution contained mouse nc82 (1:50, Developmental Studies Hybridoma Bank), and the secondary antibody solution contained Alexa Fluor 568 conjugated to streptavidin (1:1000, Life Technologies) and Alexa Fluor 633 goat anti-mouse (1:250, Life Technologies), again in blocking solution. For anti-GABA immunostaining, the primary antibody solution contained mouse nc82 (1:40, Developmental Studies Hybridoma Bank), rat anti-CD8 (1:50 Life Technologies), and rabbit anti-GABA (1:100, Sigma), and the secondary antibody solution contained Alexa Fluor 633 goat anti-mouse (1:250,

Life Technologies), Alexa Fluor 568 goat anti-rabbit (1:250, Life Technologies), and Alexa Fluor 488 goat anti-rat (1:250, Life Technologies). For anti-DvGluT immunostaining, the primary antibody solution contained rabbit DvGluT (1:5000, gift of A. DiAntonio, (Daniels et al., 2008)) and rat anti-CD8 (1:50 Life Technologies), and the secondary antibody solution contained Alexa Fluor 568 goat anti-rabbit (1:250, Life Technologies), and Alexa Fluor 488 goat anti-rat (1:250, Life Technologies). For anti-ChAT immunostaining, the primary antibody solution contained mouse ChAT4B1 (1:100, Developmental Studies Hybridoma Bank, (Takagawa and Salvaterra, 1996)) and the secondary antibody solution contained was Alexa Fluor 633 goat anti-mouse (1:250, Life Technologies).

In Figure 3A, femur bristles were dye-filled using established techniques for labeling thoracic bristles (Kays et al., 2014), with slight modifications. Briefly, female flies were decapitated and glued to insect pins, with the prothoracic legs glued in an extended position. A single femur bristle was plucked with fine forceps from the leg of each fly, and flies were fixed overnight in 4% paraformaldehyde in 0.2 M carbonate-bicarbonate buffer at 4° C. Bristles sockets were filled with DiI dye (Life Technologies; 32 µg/µl in ethanol) using a micromanipulator-mounted pipette, during which time the flies were immersed in 0.2 M carbonate-bicarbonate buffer for 72 hours at room temperature, with the dye-filled bristle socket resting above the buffer. VNCs were then dissected and imaged as described above.

Table of Transgenes

Genotype	Purpose	Source
R38b08-LexA (attp40)	Leg bristle neurons/ Mechanosensory neurons innervating chemosensory bristles	(Jenett et al., 2012)
R48a07-LexA (attp40)	Trochanter/Femur Hair plates, unknown cells in the distal tarsus	(Jenett et al., 2012)
0203-LexA (III)	Femur/coxa campaniforms, some femoral chordotonal neurons	(Gohl et al., 2011), generated by J.C.T. using InSITE swap into 0203-Gal4 (PBac{IS. -LexA}0203)
iav-LexA (VK00013)	Femoral and tibial chordotonal organs	(Shearin et al., 2013)
R13d11-Gal4 (attp2)	Intersegmental neurons	(Jenett et al., 2012)
R18g08-Gal4 (attp2)	Midline projection neurons	(Jenett et al., 2012)
R69c05-Gal4 (attp2)	Midline local neurons	(Jenett et al., 2012)
nsyb-Gal4 (attp2), also known as (R57c10-Gal4)	Pan-neuronal	(Jenett et al., 2012)
ChAT-Gal4.7.4 (II)	Cholinergic neurons	(Salvaterra and Kitamoto, 2001)
pJFRC7-20XUAS-IVS-mCD8::GFP (attp2)	GFP for patching and confocal imaging	(Pfeiffer et al., 2010)
pJFRC7-20XUAS-IVS-mCD8::GFP (attp40)	GFP for patching and confocal imaging	(Pfeiffer et al., 2010)
pJFRC15-13XLexAop2-mCD8::GFP (attp2)	GFP for confocal imaging of peripheral neurons	(Pfeiffer et al., 2010)
13XLexAop2-IVS-Syn21-Chrimson::tdT-3.1-p10-F8 (VK00005)	For optogenetic mechanoreceptor stimulation	gift of Barret Pfeiffer and David Anderson (Klapoetke et al., 2014; Pfeiffer et al., 2010; Pfeiffer et al., 2012)
13XLexAop2-IVS-Syn21-Chrimson::tdT-3.1-p10-F8 (su(Hw)attP5)	For optogenetic mechanoreceptor stimulation	gift of Barret Pfeiffer and David Anderson (Klapoetke et al., 2014; Pfeiffer et al., 2010; Pfeiffer et al., 2012)
20XUAS-IVS-GCaMP6f (attP40)	Genetically-encoded calcium indicator	(Chen et al., 2013)

Table of Genotypes

Figure 1A	ChAT-Gal4.7.4/ JFRC7-20XUAS-IVS-mCD8::GFP (attp40)
Figure 1C	R38b08-LexA (attp40)/+; pJFRC15-13XLexAop2-mCD8::GFP (attp2)/+ R48a07-LexA (attp40)/+; pJFRC15-13XLexAop2-mCD8::GFP (attp2)/+ 0203-LexA/pJFRC15-13XLexAop2-mCD8::GFP (attp2) iav-LexA(VK00013)/pJFRC15-13XLexAop2-mCD8::GFP (attp2)
Figure 2A	R38b08-LexA (attp40)/+; 13XLexAop2-IVS-Syn21-Chrimson::tdT-3.1-p10-F8 (VK00005)/+
Figure 2C-G	R38b08-LexA (attp40)/20XUAS-IVS-GCaMP6f (attP40); R57c10(nSyb)-Gal4 (attp2)/ 13XLexAop2-IVS-Syn21-Chrimson::tdT-3.1-p10-F8 (VK00005)
Figure 3	pJFRC7-20XUAS-IVS-mCD8::GFP (attp40);R13d11-Gal4 (attp2) pJFRC7-20XUAS-IVS-mCD8::GFP (attp40);R69c05-Gal4 (attp2) pJFRC7-20XUAS-IVS-mCD8::GFP (attp40);R18g08-Gal4 (attp2)
Figure 4A-B	R38b08-LexA (attp40)/ 13XLexAop2-IVS-Syn21-Chrimson::tdT-3.1-p10-F8 (su(Hw)attP5); R13d11-Gal4 (attp2)/ pJFRC7-20XUAS-IVS-mCD8::GFP (attp2)
Figure 4D-E	pJFRC7-20XUAS-IVS-mCD8::GFP (attp40)/ 13XLexAop2-IVS-Syn21 Chrimson::tdT-3.1-p10-F8 (su(Hw)attP5);R13d11-Gal4 (attp2)/ iav-LexA (VK00013)
Figure 5	R38b08-LexA (attp40)/ 13XLexAop2-IVS-Syn21-Chrimson::tdT-3.1-p10-F8 (su(Hw)attP5); R69c05-Gal4 (attp2)/ pJFRC7-20XUAS-IVS-mCD8::GFP (attp2)
Figure 6	R38b08-LexA (attp40)/ 13XLexAop2-IVS-Syn21-Chrimson::tdT-3.1-p10-F8 (su(Hw)attP5); R18g08-Gal4 (attp2)/ pJFRC7-20XUAS-IVS-mCD8::GFP (attp2)
Figure 7	pJFRC7-20XUAS-IVS-mCD8::GFP; R13d11-Gal4/R18g08-Gal4 pJFRC7-20XUAS-IVS-mCD8::GFP; R13d11-Gal4/R69c05-Gal4
Figure S1	R38b08-LexA (attp40)/+; pJFRC15-13XLexAop2-mCD8::GFP (attp2)/+ R48a07-LexA (attp40)/+; pJFRC15-13XLexAop2-mCD8::GFP (attp2)/+ 0203-LexA/pJFRC15-13XLexAop2-mCD8::GFP (attp2) iav-LexA(VK00013)/pJFRC15-13XLexAop2-mCD8::GFP (attp2)
Figure S2	R38b08-LexA (attp40)/+; pJFRC15-13XLexAop2-mCD8::GFP (attp2)/+ R48a07-LexA (attp40)/+; pJFRC15-13XLexAop2-mCD8::GFP (attp2)/+ 0203-LexA/pJFRC15-13XLexAop2-mCD8::GFP (attp2) iav-LexA(VK00013)/pJFRC15-13XLexAop2-mCD8::GFP (attp2)
Figure S3	pJFRC7-20XUAS-IVS-mCD8::GFP (attp40);R13d11-Gal4 (attp2) pJFRC7-20XUAS-IVS-mCD8::GFP (attp40);R69c05-Gal4 (attp2)

	pJFRC7-20XUAS-IVS-mCD8::GFP (attp40);R18g08-Gal4 (attp2)
Figure S4	pJFRC7-20XUAS-IVS-mCD8::GFP (attp40);R13d11-Gal4 (attp2) pJFRC7-20XUAS-IVS-mCD8::GFP (attp40);R69c05-Gal4 (attp2) pJFRC7-20XUAS-IVS-mCD8::GFP (attp40);R18g08-Gal4 (attp2)
Figure S5	pJFRC7-20XUAS-IVS-mCD8::GFP (attp40);R13d11-Gal4 (attp2) pJFRC7-20XUAS-IVS-mCD8::GFP (attp40);R69c05-Gal4 (attp2) pJFRC7-20XUAS-IVS-mCD8::GFP (attp40);R18g08-Gal4 (attp2)
Figure S6	pJFRC7-20XUAS-IVS-mCD8::GFP (attp40);R13d11-Gal4 (attp2) pJFRC7-20XUAS-IVS-mCD8::GFP (attp40);R69c05-Gal4 (attp2) pJFRC7-20XUAS-IVS-mCD8::GFP (attp40);R18g08-Gal4 (attp2) 47c08-LexA (attp40)/ 13XLexAop2-IVS-Syn21-Chrimson::tdT-3.1-p10-F8 (su(Hw)attP5); R13d11-Gal4 (attp2)/ pJFRC7-20XUAS-IVS-mCD8::GFP (attp2)
Figure S7	R38b08-LexA (attp40)/ 13XLexAop2-IVS-Syn21-Chrimson::tdT-3.1-p10-F8 (su(Hw)attP5); R18g08-Gal4 (attp2)/ pJFRC7-20XUAS-IVS-mCD8::GFP (attp2)
Table S1	R48a07-LexA (attp40)/ 13XLexAop2-IVS-Syn21-Chrimson::tdT-3.1-p10-F8 (su(Hw)attP5); R13d11-Gal4 (attp2)/ pJFRC7-20XUAS-IVS-mCD8::GFP (attp2) R48a07-LexA (attp40)/ 13XLexAop2-IVS-Syn21-Chrimson::tdT-3.1-p10-F8 (su(Hw)attP5); R69c05-Gal4 (attp2)/ pJFRC7-20XUAS-IVS-mCD8::GFP (attp2) R48a07-LexA (attp40)/ 13XLexAop2-IVS-Syn21-Chrimson::tdT-3.1-p10-F8 (su(Hw)attP5); R18g08-Gal4 (attp2)/ pJFRC7-20XUAS-IVS-mCD8::GFP (attp2) pJFRC7-20XUAS-IVS-mCD8::GFP (attp40)/ 13XLexAop2-IVS-Syn21-Chrimson::tdT-3.1-p10-F8 (su(Hw)attP5);R18g08-Gal4 (attp2)/ iav-LexA (VK00013) pJFRC7-20XUAS-IVS-mCD8::GFP (attp40)/ 13XLexAop2-IVS-Syn21-Chrimson::tdT-3.1-p10-F8 (su(Hw)attP5);R69c05-Gal4 (attp2)/ iav-LexA (VK00013) pJFRC7-20XUAS-IVS-mCD8::GFP (attp40)/ 13XLexAop2-IVS-Syn21-Chrimson::tdT-3.1-p10-F8 (su(Hw)attP5);R13d11-Gal4 (attp2)/ 0203-LexA pJFRC7-20XUAS-IVS-mCD8::GFP (attp40)/ 13XLexAop2-IVS-Syn21-Chrimson::tdT-3.1-p10-F8 (su(Hw)attP5);R18g08-Gal4 (attp2)/ 0203-LexA pJFRC7-20XUAS-IVS-mCD8::GFP (attp40)/ 13XLexAop2-IVS-Syn21-Chrimson::tdT-3.1-p10-F8 (su(Hw)attP5);R69c05-Gal4 (attp2)/ 0203-LexA

Table S1

	R13d11-GAL4 (intersegmental)	R69c05-GAL4 (local)	R18g08-GAL4 (projection)	Motor neurons (control)
R38b08-LexA (bristles)	excitation: entire ipsilateral leg (n > 10), blocked by 1 µM mla	excitation: distal ipsilateral leg (n > 10), blocked by 1 µM MLA inhibition: proximal ipsilateral leg (n > 10) blocked by 10 µM picrotoxin	excitaiton: ipsilateral and contralateral legs (n > 10), blocked by 1 µM MLA inhibition: ipsilateral and contralateral legs (n > 10), blocked by10 µM picrotoxin	
iav-LexA (chordotonal)	inhibition: prothoracic ipsilateral femur (n=9), blocked by 100 µM picrotoxin and 50 µM CGP54626	no response to peripheral light stimuli on ipsilateral leg (n=2)	no response to peripheral light stimuli on ipsilateral leg (n=3)	excitation: prothoracic ipsilateral femur (n= 2)
R48a07-LexA (hair plates	no response to peripheral light stimuli on ipsilateral leg (n=2)	no response to peripheral light stimuli on ipsilateral leg (n=2)	no response to peripheral light stimuli on ipsilateral leg (n=2)	excitation: prothoracic ipsilateral femur (n=2), blocked by 1 µM TTX in 1 prep
0203-LexA (campaniform)	no response to peripheral light stimuli on ipsilateral leg (n=2)	no response to peripheral light stimuli on ipsilateral leg (n=2)	no response to peripheral light stimuli on ipsilateral leg (n=2)	excitation: prothoracic ipsilateral femur (n = 5), blocked by 1 µM MLA in 2 preps

Table S1, related to Figures 4-6. Table of mechanoreceptor/central neuron connectivity

This table provides a summary of all the pairwise combinations of mechanoreceptor and central neurons we tested for functional connectivity. For each combination, Chrimson was driven in peripheral mechanoreceptors under the control of LexA (rows), while central neurons were labeled with mCD8::GFP under the control of GAL4 (columns). Whole-cell recordings were targeted to central neurons, and a spot on the leg was stimulated with light through an optical fiber (see Methods for details). The spot was moved during the experiment so that the entire leg was ultimately illuminated. For cases in which we did not observe a postsynaptic response, we confirmed that optogenetic stimulation was working by recording light-evoked activity from unlabeled motor neurons, which were identified based on their large, lateral cell bodies. Thus, we are confident that these negative results are not due to inefficacy of our optogenetic methods, but rather due to lack of connectivity.

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