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Supplemental Information

**A central somatotopic map of the fly leg
supports spatially targeted grooming**

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Examples of bristle axons

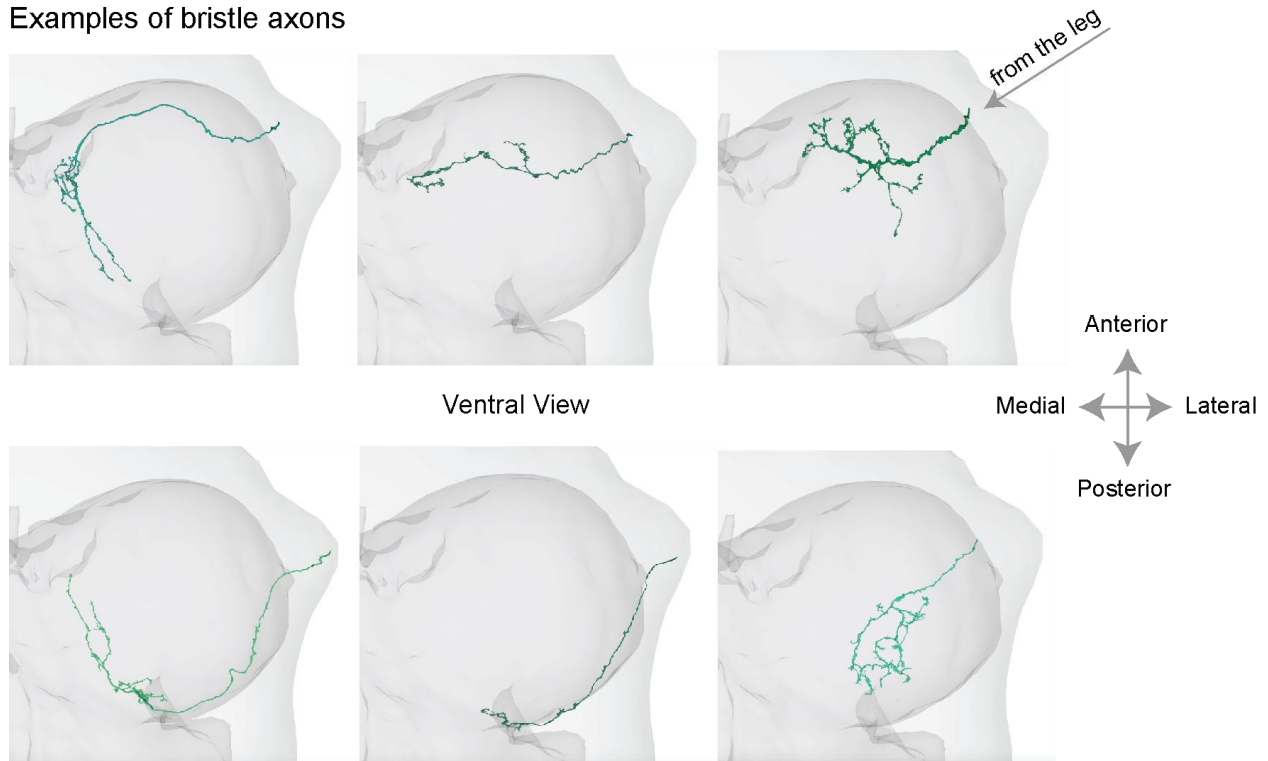


Figure S1. Bristle axons vary in morphology. Related to Figure 1 and Figure 2. Individual bristle axon morphologies. Three bristle axons that branch anteriorly (top row), and three that branch posteriorly (bottom row). Axons that cross the anterior to posterior border (left), axons that do not cross (middle), and axons that project closer to the center of the left leg neuromere (right).



Figure S2. GFP expression of bristle neurons driven by coexpression of different transcription factors in the larval leg imaginal disc, leg, and VNC. Related to Figure 1. A) For each line, only bristle cells that express a specific transcription factor will be labeled with GFP. **B)** Example genetic cross. **C)** Shown are maximum intensity projections of cells in the larval leg imaginal disc LexAop-mCD8::GFP(green) and an antibody against phalloidin (magenta). Bristle neurons in the leg and VNC were labeled with mcd8::GFP (green) and an antibody against the neuropil marker bruchpilot (magenta), green arrows indicate a sample of labeled bristle neurons. From left to right: all bristle neurons labeled by R38B08-LexA alone, bristle neurons that coexpressed *hedgehog* (hh), *midline* (mid), *decapentaplegic* (dpp), *dachshund* (dac), *rotund* (rn), and *apterous* (ap) during metamorphosis.

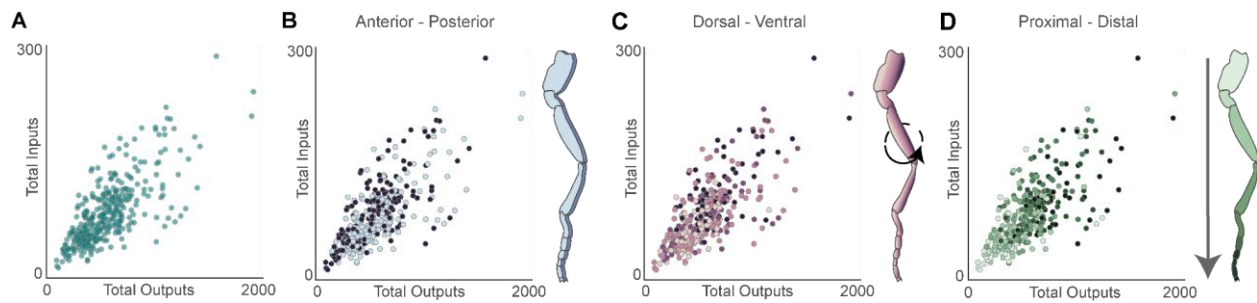


Figure S3. Synaptic input and output counts do not vary somatotopically. Related to Figure 2. A) Number of input and output synapses for each reconstructed bristle axon (teal). Colored by the predicted spatial location on the leg along the **B)** anterior-posterior axis ($r^2=4.64e-05$), **C)** dorsal-ventral axis ($r^2=0.05$), **D)** proximal-distal axis ($r^2=0.30$).

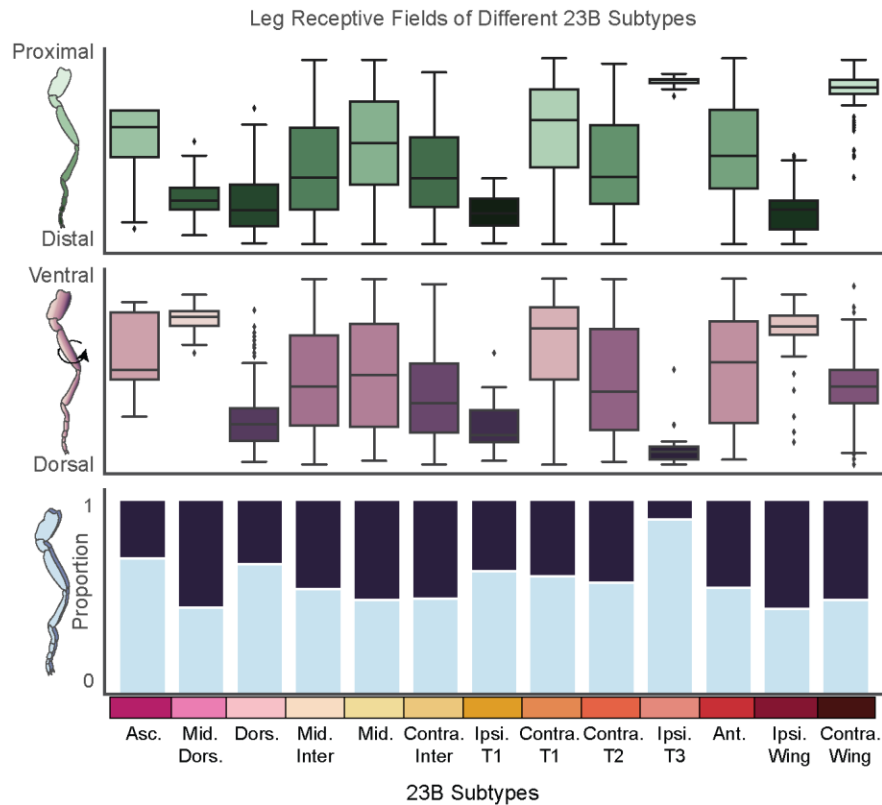


Figure S4. Leg receptive fields for different 23B subtypes. Related to Figure 4. A) Receptive fields along the P/D axis (top), D/V axis (middle), A/P axis (bottom, anterior:light blue, posterior: dark blue) for different 23B subtypes. Individual points represent input synapses from bristle axons and the y axis represents where on the leg each presynaptic bristle axon originates. The order of 23B subtypes along the x axis is consistent across all three plots. For all box plots, center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range.

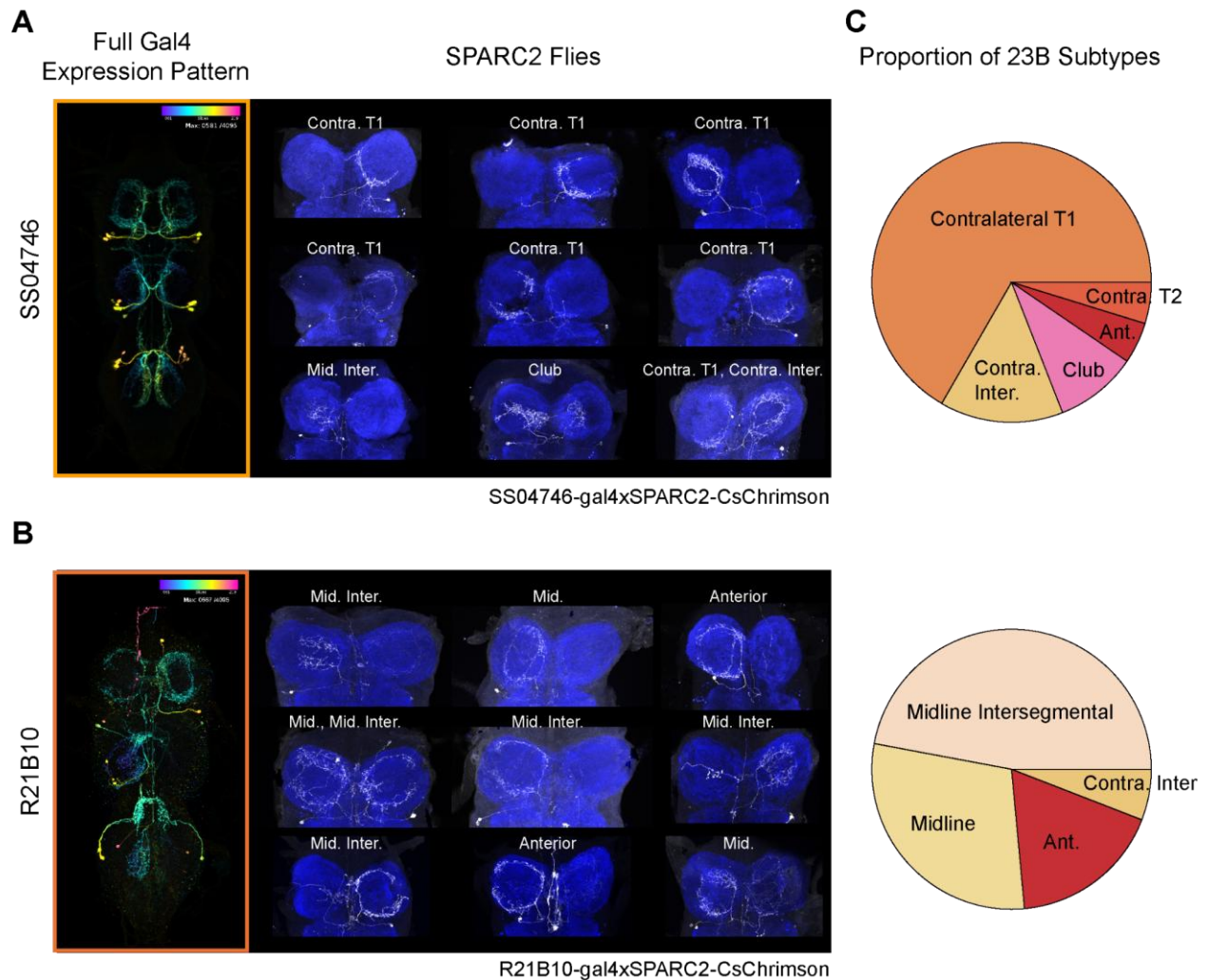


Figure S5. Experimental lines SS04746 and R21B10 label different 23B subtypes. Related to Figure 5. **A)** Full Gal4 VNC expression of SS04746 from the Janelia library (left). Example VNCs from sparsified line SS04746-gal4xSPARC2-CsChrimson (right). **B)** Full Gal4 VNC expression of R21B10 from Janelia FlyLight⁷⁶ (left). Example VNCs from sparsified line R21B10-gal4xSPARC2-CsChrimson (right). In all SPARC2 experiments 23B neurons in the VNC were labeled with mcd8::GFP (white) and an antibody against the neuropil marker bruchpilot (blue). Each neuron was classified by where in the VNC the axon projected to. **C)** Proportion of different 23B subtypes in SS04746 (n=21) and R21B10 (n=17).

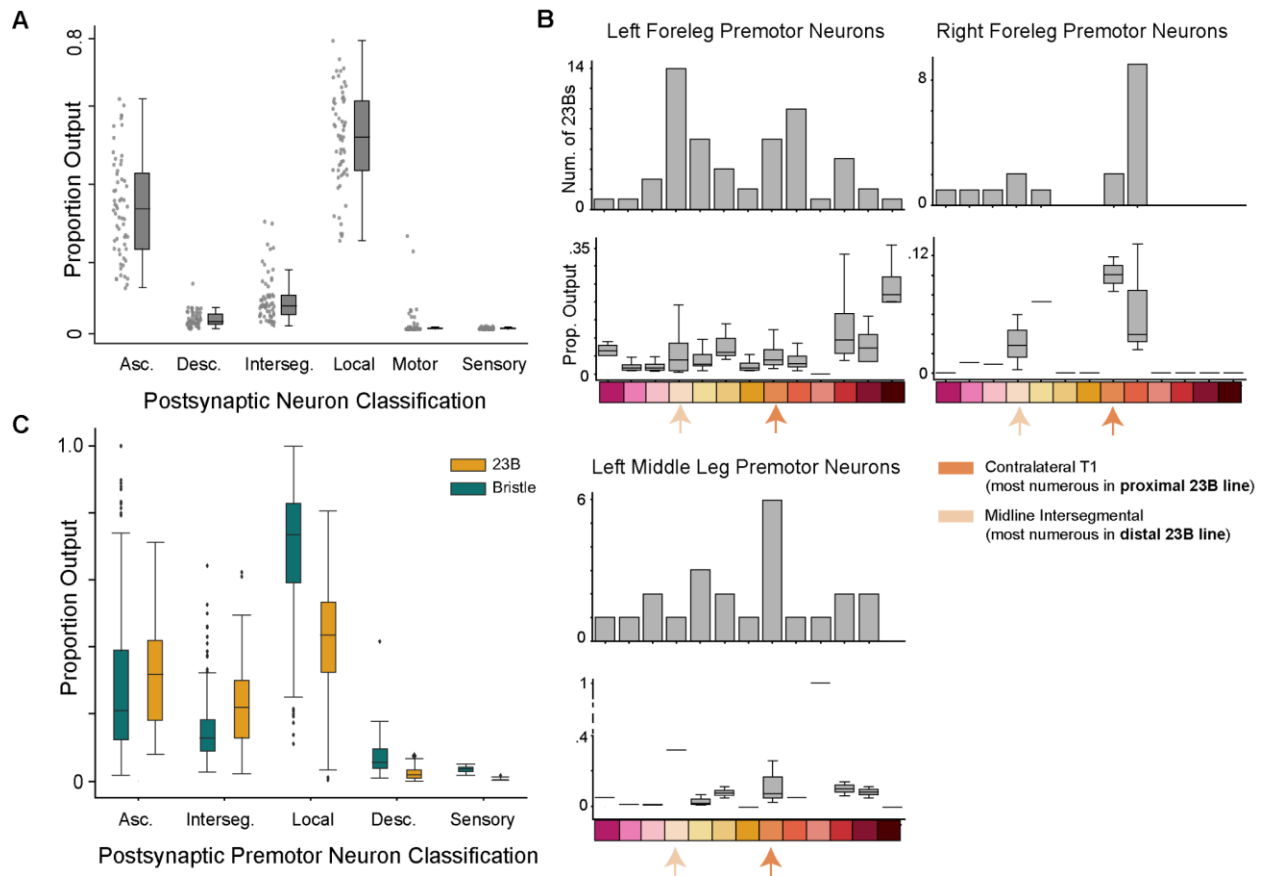


Figure S6. 23B subtypes connectivity onto premotor neurons in T1L, T1R, and T2L. Related to Figure 6. **A)** Proportion of 23B output connectivity onto different neuron classes in the VNC. **B)** 23B subtype connectivity onto premotor pools for the left front leg (T1L), right front leg (T1R), and the left middle leg (T2L). The bar graph represents the number of 23B neurons of each subtype that contact any premotor neurons within each leg neuropil. Boxplots represent the proportion of 23B output synapses onto premotor neurons within each leg neuropil. Color bars represent different 23B subtypes, from left to right: Ascending, Midline Dorsal, Dorsal, Midline Intersegmental, Midline, Contralateral Intersegmental, Ipsilateral T1, Contralateral T1, Contralateral T2, Ipsilateral T3, Anterior, Ipsilateral Wing, and Contralateral Wing. Arrows indicate the most prominent subtype in the *proximal-sensing* (SS04746) and *distal-sensing* (R21B10) grooming lines. **C)** Proportion of total premotor output onto different classification types; Asc: ascending, Desc: descending, Interseg: intersegmental. Color denotes the presynaptic partner either 23B (orange) or bristle neuron (teal). For all box plots, center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range.

Name	Genotype	Figure Reference
dac-Gal4 (imaginal disc)	w[1118]; dac-Gal4 / +; UAS-mcd8::GFP / +	Figure 1C and Figure S2
rn-Gal4 (imaginal disc)	[1118]; +; rn-Gal4 / UAS-mcd8::GFP	Figure 1C and Figure S2
dac-Gal4 (adult leg and VNC)	w[1118]; dac-Gal4 / UAS-flp;LexAop>stop>mcd8::GFP / R38B08-LexA	Figure 1C and Figure S2
rn-Gal4 (adult leg and VNC)	w[1118] ; LexAop>stop>mcd8::GFP / UAS-flp; rn-Gal4 / R38B08-LexA	Figure 1C and Figure S2
R38B08-LexA (imaginal disc, adult leg, and VNC)	w[1118]; R38B08-LexA / +; LexAop-mcd8::GFP / + (no expression)	Figure S2
hh-Gal4 (imaginal disc)	w[1118]; +; hh-Gal4 / UAS-mcd8::GFP	Figure S2
mid-Gal4 (imaginal disc)	w[1118]; mid-Gal4 / +; UAS-mcd8::GFP / +	Figure S2
dpp-Gal4 (imaginal disc)	w[1118]; +; dpp-Gal4 / UAS-mcd8::GFP	Figure S2
ap-Gal4 (imaginal disc)	w[1118]; ap-Gal4 / +; UAS-mcd8::GFP / +	Figure S2
hh-Gal4 (adult leg and VNC)	w[1118]; hh-Gal4 / UAS-flp; LexAop>stop>mcd8::GFP / R38B08-LexA	Figure S2
mid-Gal4 (adult leg and VNC)	w[1118]; mid-Gal4 / UAS-flp; LexAop>stop>mcd8::GFP / R38B08-LexA	Figure S2
dpp-Gal4 (adult leg and VNC)	[1118] ; LexAop>stop>mcd8::GFP / UAS-flp; dpp-Gal4 / R38B08-LexA	Figure S2
ap-Gal4 (adult leg and VNC)	w[1118]; ap-Gal4 / UAS-flp; LexAop>stop>mcd8::GFP / R38B08-LexA	Figure S2
R21B10-Gal4	P{UAS-phiC31}attP18 / w[1118]; TI{20XUAS-SPARC2-S-Syn21-CsChrimson::tdTomato-3.1}CR-P40	Figure 5 and Figure S5
Ss04746 split Gal4	w[1118]; P{y[+t7.7] w[+mC]=R77C10-p65.AD}attP40 / P{y[+t7.7] w[+mC]=20XUAS-IVS-CsChrimson.mVenus}attP40 ; P{y[+t7.7] w[+mC]=VT026010-GAL4.DBD}attP2 / +	Figure 5 and Figure S5
Empty-SpGal4 (Control)	w[1118]; P{y[+t7.7] w[+mC]=p65.AD.Uw}attP40 / P{y[+t7.7] w[+mC]=20XUAS-IVS-CsChrimson.mVenus}attP40; P{y[+t7.7] w[+mC]=GAL4.DBD.Uw}attP2 / +	Figure 5
Sparsified R21B10-Gal4	P{UAS-phiC31}attP18 / w[1118]; TI{20XUAS-SPARC2-S-Syn21-CsChrimson::tdTomato-3.1}CR-P40; R21B10-GAL4 / +	Figure S5
Sparsified ss04746-Gal4	P{UAS-phiC31}attP18 / w[1118]; P{y[+t7.7] w[+mC]=R77C10-p65.AD}attP40/ TI{20XUAS-SPARC2-S-Syn21-CsChrimson::tdTomato-3.1}CR-P40; P{y[+t7.7]	Figure S5

	w[+mC]=VT026010-GAL4.DBD}attP2 / +	
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Table S1. *Drosophila melanogaster* genotypes used for experiments. Related to Figure 1, Figure 5, Figure S2 and Figure S5. All genotypes used for experiments in Figure 1 and Figure 5 along with the shorthand name and specific figure references.

Fly Transgene	Gene	Type	Spatial Expression	Spatial Extent on the leg
hh-GAL4	Hedgehog (hh)	Signaling molecule	Posterior leg	Femur, tibia, tarsal segments 1-5
mid-Gal4	Midline (mid)	Transcription factor	Ventral leg	Ventral femur
dpp-GAL4	Decapentaplegic (dpp)	Signaling molecule	Dorsal leg	Dorsal: Coxa, femur, tibia, tarsal segments (1-5)
dac-GAL4	Dachshund (dac)	Transcription factor	Proximal leg	Femur, Tibia, Tarsal segments 1-2 (segments 3 & 4 have one neuron labelled)
rn-GAL4	Rotund (rn)	Transcription factor	Distal leg	Tarsal Segments 1-5
ap-GAL4	Apterous (ap)	Transcription factor	Very distal leg	Tarsal Segments 4-5

Table S2. Spatial expression of different genes in the adult fly leg. Related to Figure 1 and Figure S2. Spatial expression patterns and their spatial extent on the leg for fly transgenes that exhibited spatial patterning in the adult leg and VNC.