Supplemental information

Presynaptic inhibition selectively suppresses leg proprioception in behaving *Drosophila*

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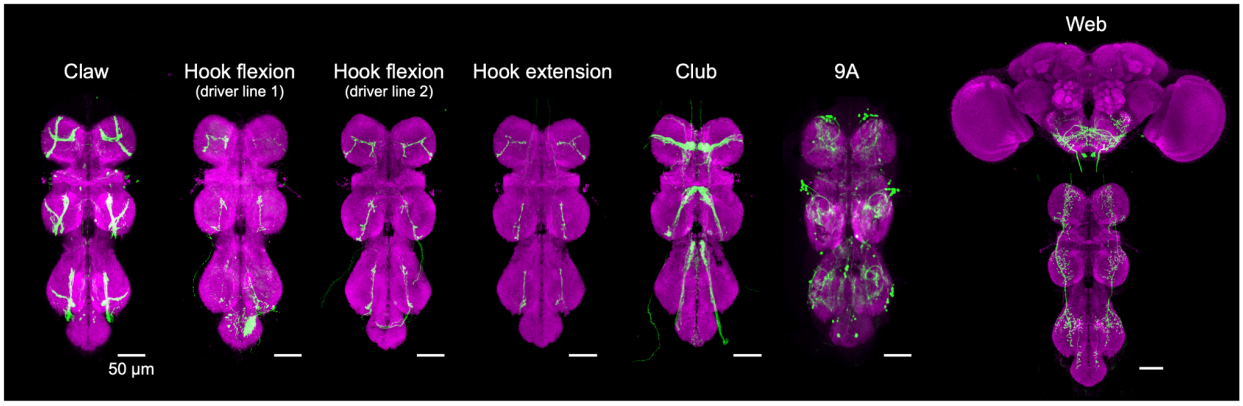

Figure S1. Related to Figures 2-5
Confocal images showing the expression patterns of the GAL4 and split-GAL4 driver lines used to label FeCO neurons, 9A neurons, and web neurons. Green: GFP or mVenus; magenta: neuropil stain (nc82). Genotypes are listed in the table of genotypes. Images of the claw line, the hook flexion line 1, and the web line are from FlyLight.

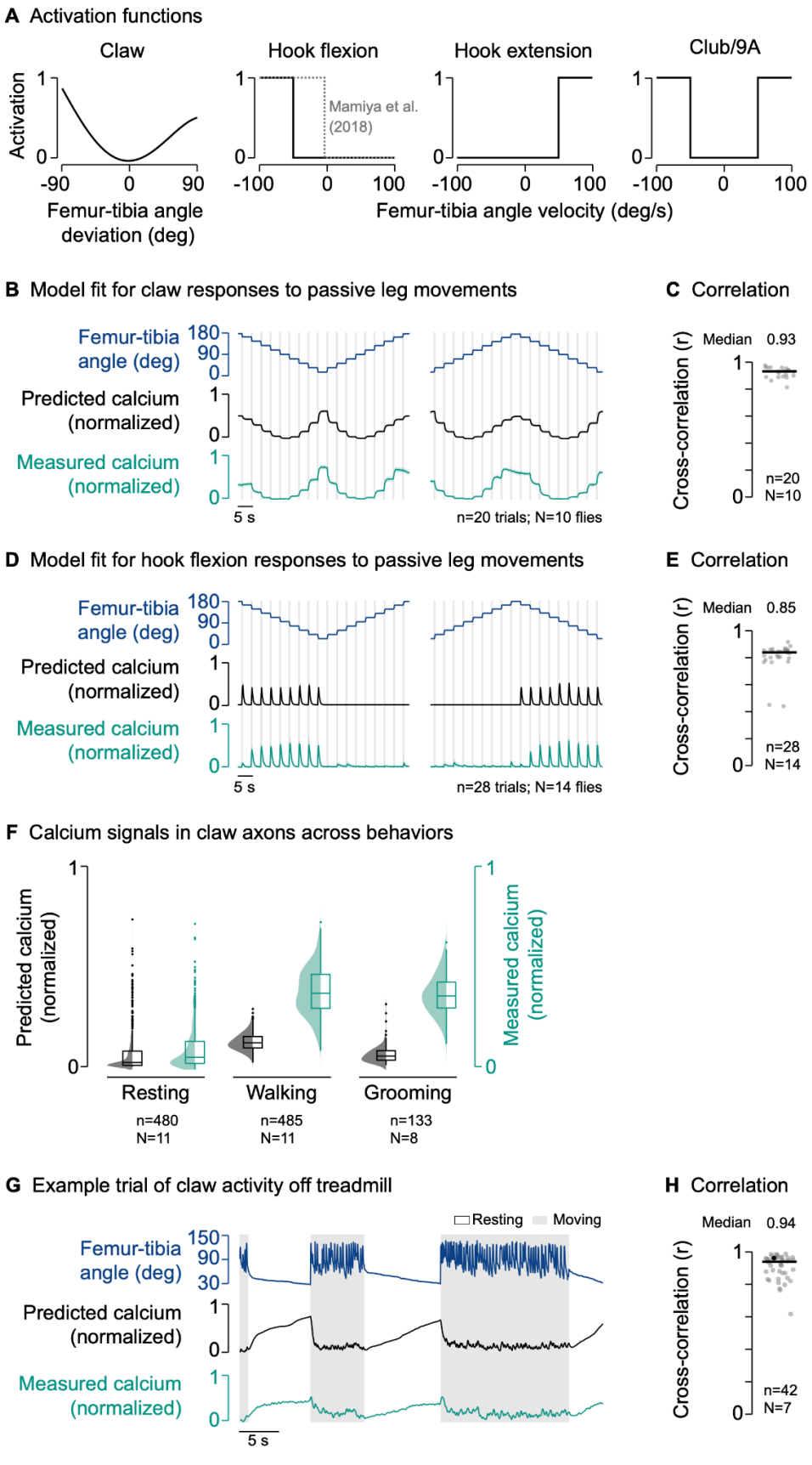
A Activation functions
Claw
Hook flexion
Hook extension
Club/9A
Activation
Mamiya et al. (2018)
Femur-tibia angle deviation (deg)
Femur-tibia angle velocity (deg/s)
B Model fit for claw responses to passive leg movements
Femur-tibia angle (deg)
Predicted calcium (normalized)
Measured calcium (normalized)
5 s
n=20 trials; N=10 flies
C Correlation
Cross-correlation (r)
Median 0.93
n=20
N=10
D Model fit for hook flexion responses to passive leg movements
n=28 trials; N=14 flies
E Correlation
Median 0.85
n=28
N=14
F Calcium signals in claw axons across behaviors
Resting
Walking
Grooming
n=480
N=11
n=485
N=11
n=133
N=8
G Example trial of claw activity off treadmill
Resting
Moving
H Correlation
Median 0.94
n=42
N=7

Figure S2. Related to Figure 2

(A) Activation functions for claw, hook flexion, hook extension, and club and 9A neurons.

(B) Measured and predicted (fitted) calcium signals of claw axons in response to applied ramp-and-hold movements of the femur-tibia joint. Experimental data from Mamiya et al. (2018). Lines show mean of animal means, shadings show standard error of the mean. n: number of trials (10 trials per ramp-and-hold stimulus, totalling 20 trials for both stimuli); N: number of flies.

(C) Cross-correlation coefficient between predicted and measured calcium signals per trial at a time lag of zero. The black line shows the median. n: number of trials; N: number of flies.

(D) Same as (B) but for hook flexion axons.

(E) Same as (C) but for hook flexion axons.

(F) Median predicted and measured calcium signals in claw axons during resting, walking, and grooming. Bouts are ≥1 s in duration. Distributions show kernel density estimations. n: number of behavioral bouts; N: number of flies. Note that the predicted calcium signals during walking and grooming appear weak because they are normalized to the maximum predicted value in the dataset, which occurred during an outlier resting bout in which the leg was extremely flexed.

(G) Example trial of two-photon calcium imaging of claw axons in the neuromere of the left front leg and behavior tracking without the treadmill.

(H) Same as (C) but for claw axons imaged without the treadmill. The black dot marks the trial shown in (G).

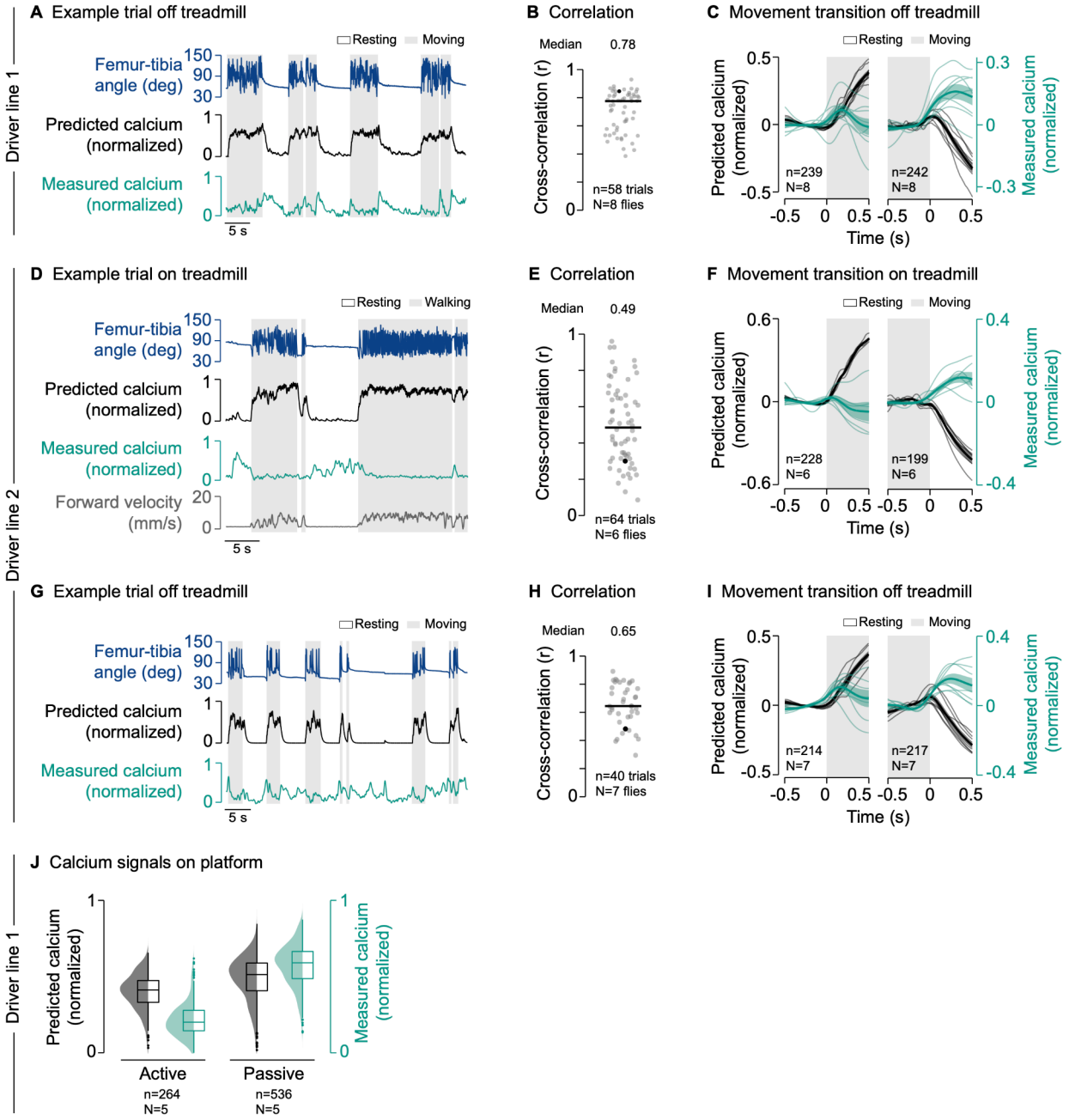

Figure S3. Related to Figure 3

(A) Example trial of two-photon calcium imaging of hook flexion axons in the neuromere of the left front leg and behavior tracking without the treadmill.

(B) Cross-correlation coefficient between predicted and measured calcium signals per trial at a time lag of zero. The black line shows the median. The black dot marks the trial shown in (A). n: number of trials; N: number of flies.

(C) Predicted and measured calcium signals aligned to the transitions into and out of movement. Signals are baseline subtracted (mean from -0.5 to 0 s). Thin lines show animal means, thick lines show mean of means, shadings show standard error of the mean. n: number of transitions; N: number of flies.

(D) Example trial of two-photon calcium imaging of hook flexion axons (second driver line) in the neuromere of the left front leg and behavior tracking on the treadmill.

(E) Same as (B) but for hook flexion axons (second driver line) imaged on the treadmill.

(F) Same as (C) but for hook flexion axons (second driver line) imaged on the treadmill. Movement includes walking and grooming.

(G) Example trial of two-photon calcium imaging of hook flexion axons (second driver line) in the neuromere of the left front leg and behavior tracking without the treadmill.

(H) Same as (B) but for hook flexion axons (second driver line) imaged without the treadmill.

(I) Same as (C) but for hook flexion axons (second driver line) imaged without the treadmill.

(J) Median predicted and measured calcium signals during active and passive movement bouts on the platform. Bouts are ≥0.5 s in duration. Distributions show kernel density estimations. n: number of movement bouts; N: number of flies.

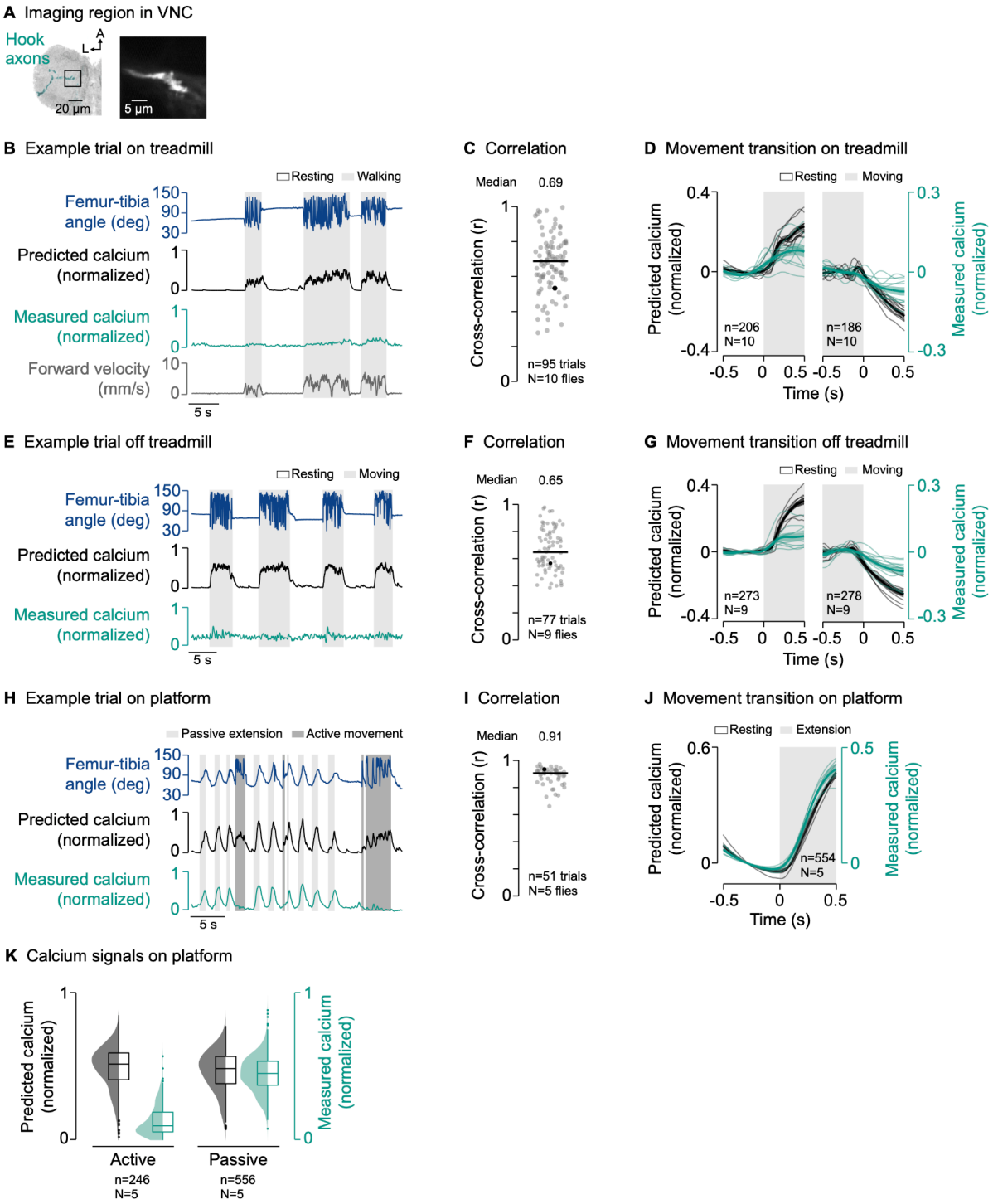

Figure S4. Related to Figure 3

(A) Left: Confocal image of hook extension axons in the neuromere of the left front leg. The black box indicates the imaging region. Green: GFP; gray: neuropil stain (nc82). A: anterior; L: lateral. Right: Mean tdTomato signal within the imaging region during an example trial.

(B) Example trial of two-photon calcium imaging of hook extension axons in the neuromere of the left front leg and behavior tracking on the treadmill.

(C) Cross-correlation coefficient between predicted and measured calcium signals per trial at a time lag of zero. The black line shows the median. The black dot marks the trial shown in (B). n: number of trials; N: number of flies.

(D) Predicted and measured calcium signals aligned to the transitions into and out of movement. Movement includes walking and grooming. Signals are baseline subtracted (mean from -0.5 to 0 s). Thin lines show animal means, thick lines show mean of means, shadings show standard error of the mean. n: number of transitions; N: number of flies.

(E) Example trial of two-photon calcium imaging of hook extension axons and behavior tracking without the treadmill.

(F) Same as (C) but for hook extension axons imaged without the treadmill.

(G) Same as (D) but for hook extension axons imaged without the treadmill.
(H) Example trial of two-photon calcium imaging of hook extension axons in the neuromere of the left front leg and behavior tracking on the platform.
(I) Same as (C) but for hook extension axons imaged on the platform. Active movements were excluded for the cross-correlation.
(J) Same as (D) but for hook extension axons imaged on the platform.
(K) Median predicted and measured calcium signals during active and passive movement bouts on the platform. Bouts are ≥0.5 s in duration. Distributions show kernel density estimations. n: number of movement bouts; N: number of flies.

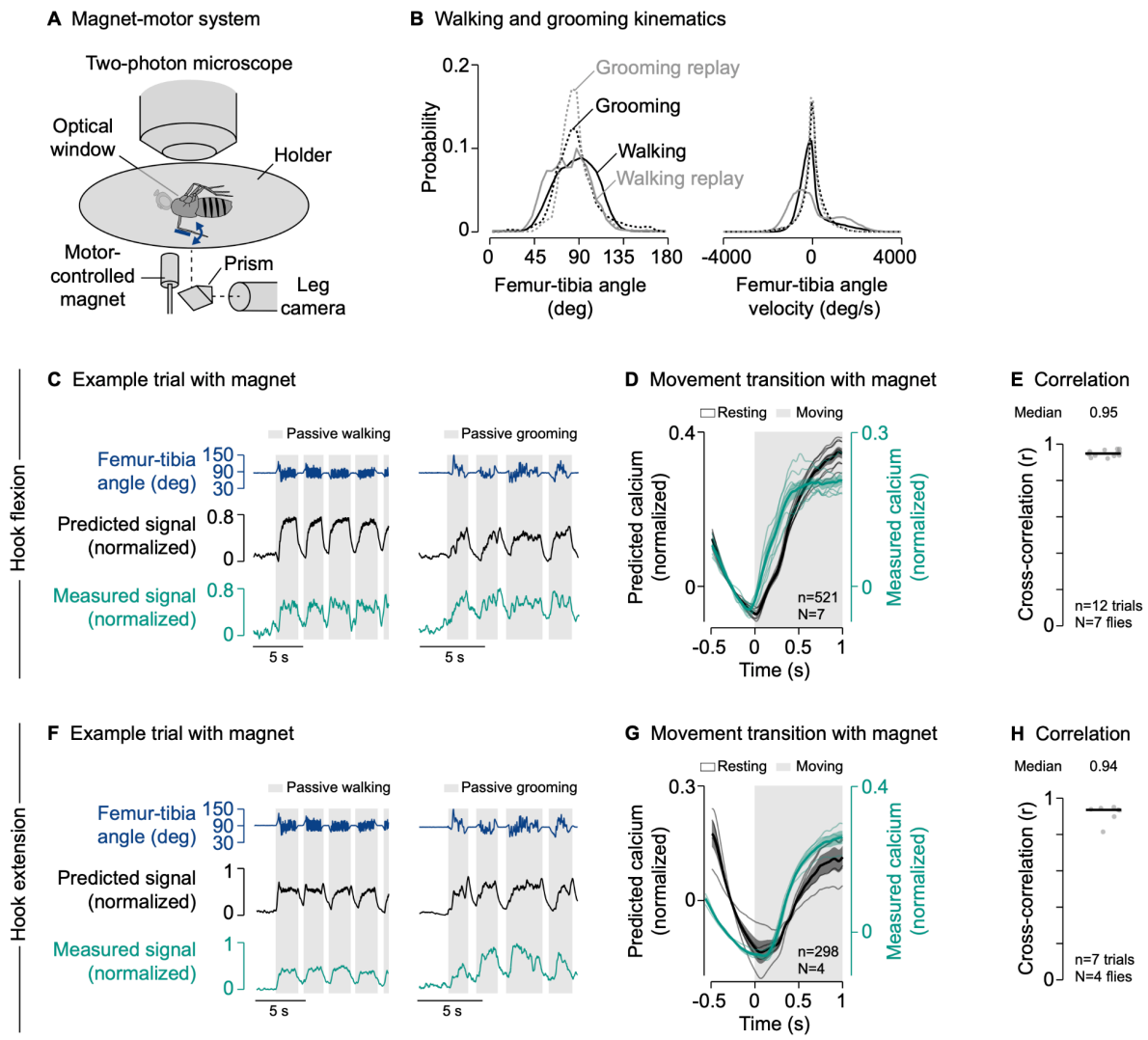

Figure S5. Related to Figure 3
(A) Experimental setup for two-photon calcium imaging from neurons in the neuromere of the left front leg and leg tracking in a tethered fly. All joints except for the femur-tibia joint of a front leg are fixated. The femur-tibia joint is passively moved via a motor-controlled magnet.
(B) Probability distributions of walking and grooming kinematics recorded in the hook flexion neuron dataset and the walking and grooming kinematics used for passive replay with the setup shown in (A).
(C) Example trial of two-photon calcium imaging of hook flexion axons in the neuromere of the left front leg and behavior tracking with the magnet-motor system.
(D) Predicted and measured calcium signals aligned to the transition into passive movement. Movement includes passive walking and passive grooming. Signals are baseline subtracted (mean from -0.5 to 0 s). Thin lines show animal means, thick lines show mean of means, shadings show standard error of the mean. n: number of transitions; N: number of flies.
(E) Cross-correlation coefficient between predicted and measured calcium signals per trial at a time lag of zero. The black line shows the median. Trials are either walking or grooming replay. n: number of trials; N: number of flies.
(F) Same as (C) but for hook extension axons.
(G) Same as (D) but for hook extension axons.
(H) Same as (E) but for hook extension axons.

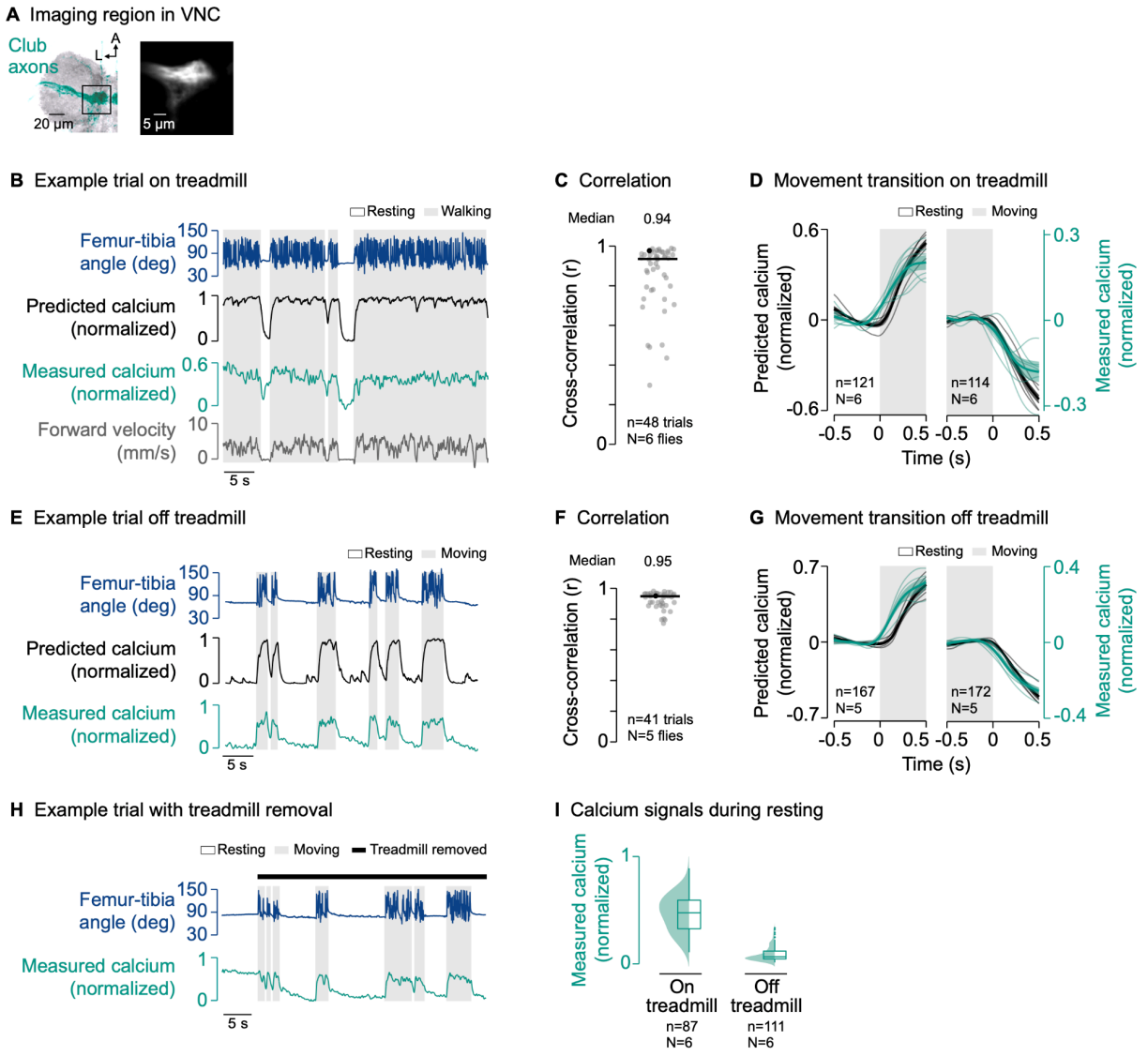

Figure S6. Related to Figure 3

(A) Left: Confocal image of club axons in the neuromere of the left front leg. The black box indicates the imaging region. Green: GFP; gray: neuropil stain (nc82). A: anterior; L: lateral. Right: Mean tdTomato signal within the imaging region during an example trial.

(B) Example trial of two-photon calcium imaging of club axons in the neuromere of the left front leg and behavior tracking on the treadmill.

(C) Cross-correlation coefficient between predicted and measured calcium signals per trial at a time lag of zero. The black line shows the median. The black dot marks the trial shown in (B). n: number of trials; N: number of flies.

(D) Predicted and measured calcium signals aligned to the transitions into and out of movement. Movement includes walking and grooming. Signals are baseline subtracted (mean from -0.5 to 0 s). Thin lines show animal means, thick lines show mean of means, shadings show standard error of the mean. n: number of transitions; N: number of flies.

(E) Example trial of two-photon calcium imaging of club axons in the neuromere of the left front leg and behavior tracking without the treadmill.

(F) Same as (C) but for club axons imaged without the treadmill.

(G) Same as (D) but for club axons imaged without the treadmill.

(H) Example trial of two-photon calcium imaging of club axons in the neuromere of the left front leg and behavior tracking. In this example, the treadmill was lowered (“removed”) about 5 s into the trial so the fly’s legs could not touch it.

(I) Median calcium signals during resting bouts on and off the treadmill. Bouts are ≥1 s in duration. Distributions show kernel density estimations. n: number of movement bouts; N: number of flies.

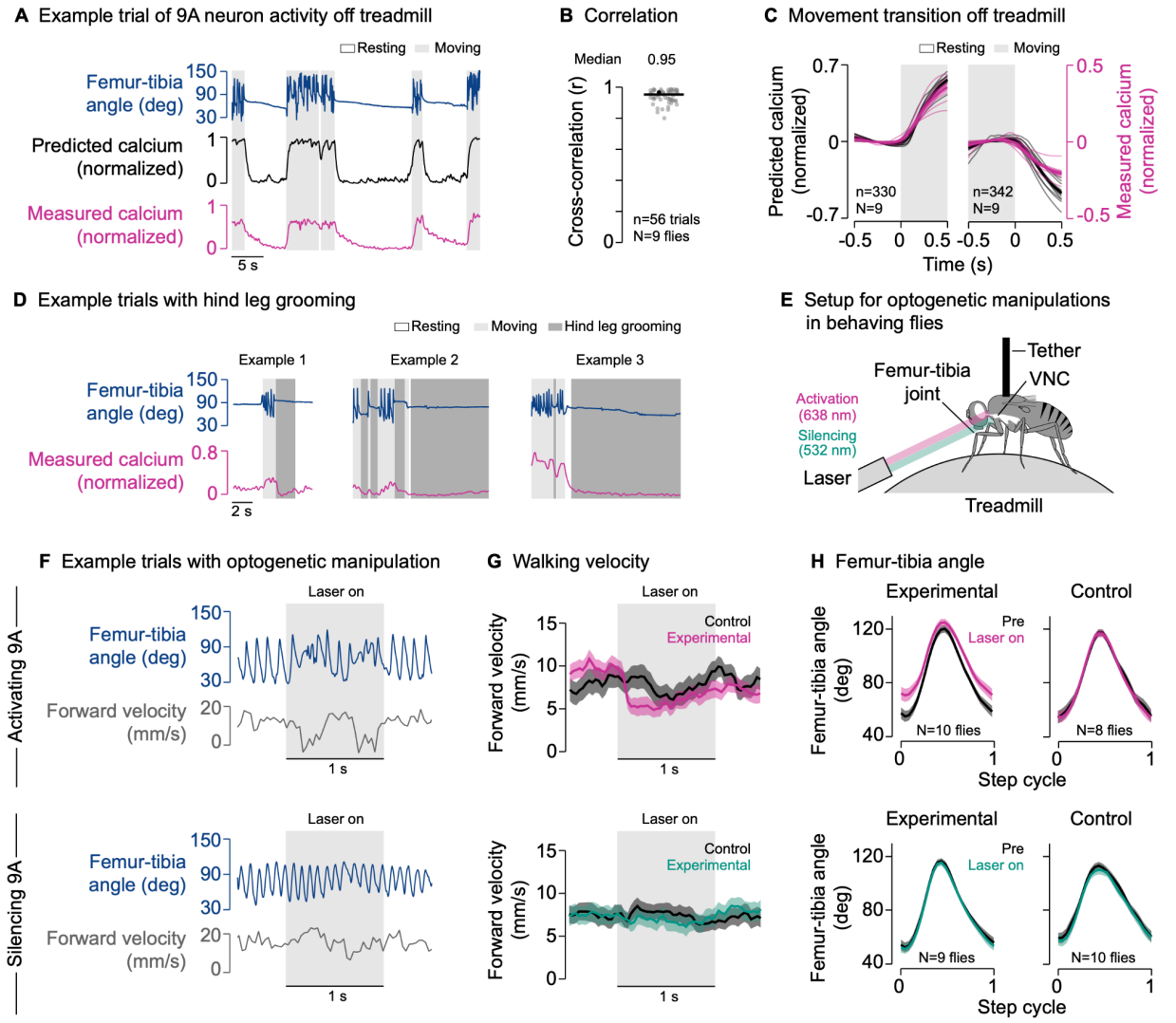

Figure S7. Related to Figure 4

(A) Example trial of two-photon calcium imaging of 9A neurons in the neuromere of the left front leg and behavior tracking without the treadmill.

(B) Cross-correlation coefficient between predicted and measured calcium signals per trial at a time lag of zero. The black line shows the median. The black dot marks the trial shown in (A). n: number of trials; N: number of flies.

(C) Predicted and measured calcium signals aligned to the transition into and out of movement. Signals are baseline subtracted (mean from -0.5 to 0 s). Thin lines show animal means, thick lines show mean of means, shadings show standard error of the mean. n: number of transitions; N: number of flies.

(D) Examples of two-photon calcium imaging of 9A neurons in the neuromere of the left front leg during hind leg grooming.

(E) Experimental setup for optogenetic manipulations of neurons in the neuromere of the left front leg of tethered flies walking on a treadmill. A red laser is used for optogenetic activation (via CsChrimson), a green laser is used for optogenetic silencing (via GtACR1). The fly's wings are trimmed.

(F) Example trials of optogenetic activation (top) and silencing (bottom) of 9A neurons in experimental flies walking on the treadmill.

(G) Average forward velocity of experimental and control flies during the 2 s trials. Thick lines show means of animal means, shadings show 95% confidence intervals of animal means. In experimental flies, 9A neurons are activated (top) or silenced (bottom). Control flies have the same genetic background but lack GAL4 expression.

(H) Femur-tibia angle of the left front leg of experimental and control flies normalized in time to the step cycle. Thick lines show means of animal means, shadings show 95% confidence intervals of animal means. Pre indicates steps recorded in the 0.5 s prior to laser onset. Laser on indicates steps recorded during the 1 s laser stimulation. In experimental flies, 9A neurons are activated (top) or silenced (bottom). Control flies have the same genetic background but lack GAL4 expression.

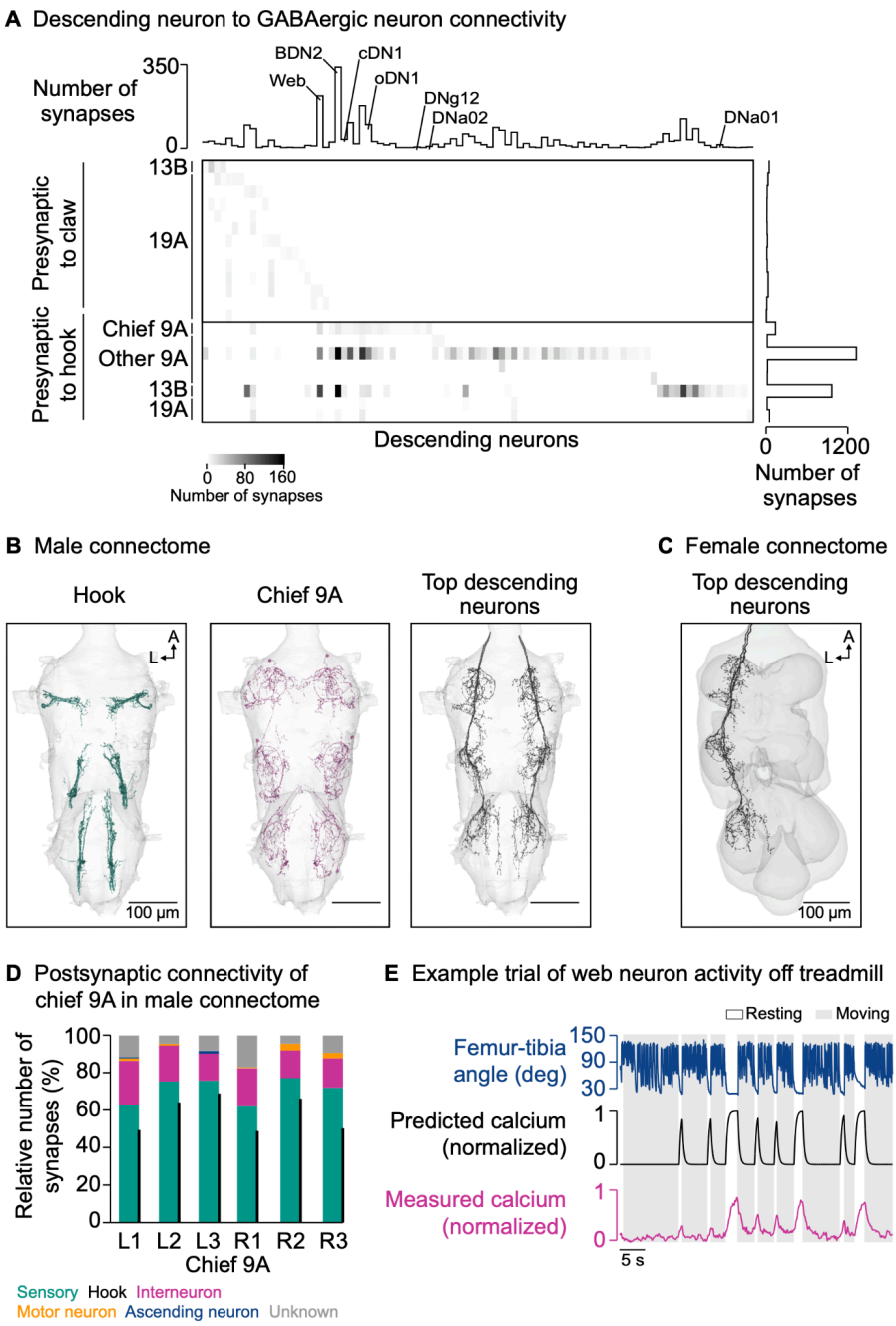

Figure S8. Related to Figure 5

(A) Connectivity of descending neurons with GABAergic neurons presynaptic to claw and hook axons. The grayscale heatmap indicates the number of synapses between neurons (connection strength). BDN2, cDN1, and oDN1 promote walking (Sapkal et al. 2023). DNa01 and DNa02 promote turning (Yang et al. 2023). DNg12 promotes grooming (Guo et al. 2022).

(B) Hook axons, chief 9A neurons presynaptic to hook axons, and the top two descending neurons presynaptic to the chief 9A neurons in the male VNC connectome (MANC). A: anterior; L: lateral.

(C) Top two descending neurons presynaptic to the chief 9A neuron in the female VNC connectome (FANC). A: anterior; L: lateral.

(D) Outputs of chief 9A neurons onto different neuron types (MANC connectome). Black bars indicate output onto hook axons. L: left side of the VNC; R: right side of the VNC; 1: front leg neuromere; 2: middle leg neuromere; 3: hind leg neuromere.

(E) Example trial of two-photon calcium imaging of the web neuron in the neuromere of the left front leg and behavior tracking without the treadmill.

Video S1
Neurons of interest in the FANC connectome.

Video S2
Example trials of two-photon calcium imaging of claw axons and behavior tracking on the treadmill and without the treadmill. Videos are sped up 2x. The measured calcium signal is based on the ratio of GCaMP to tdTomato. For this video, GCaMP images were low-pass filtered using a moving average filter with a time window of 0.2 s. tdTomato images are not shown.

Video S3
Example trials of two-photon calcium imaging of hook flexion axons and behavior tracking on the treadmill, without the treadmill, and on the platform. Videos are sped up 2x. The measured calcium signal is based on the ratio of GCaMP to tdTomato. For this video, GCaMP images were low-pass filtered using a moving average filter with a time window of 0.2 s. tdTomato images are not shown.

Video S4
Example trials of two-photon calcium imaging of club axons and behavior tracking on the treadmill, without the treadmill, and when the treadmill is removed during the trial. Videos are sped up 2x. The measured calcium signal is based on the ratio of GCaMP to tdTomato. For this video, GCaMP images were low-pass filtered using a moving average filter with a time window of 0.2 s. tdTomato images are not shown.

Video S5
Example trials of two-photon calcium imaging of 9A axons and behavior tracking on the treadmill, without the treadmill, and on the platform. Videos are sped up 2x. The measured calcium signal is based on the ratio of GCaMP to tdTomato. For this video, GCaMP images were low-pass filtered using a moving average filter with a time window of 0.2 s. tdTomato images are not shown.

Video S6
Example trials of two-photon calcium imaging of web axons and behavior tracking on the treadmill and without the treadmill. Videos are sped up 2x. The measured calcium signal is based on the ratio of GCaMP to tdTomato. For this video, GCaMP images were low-pass filtered using a moving average filter with a time window of 0.2 s. tdTomato images are not shown.

Table S1. Neurons of interest in the FANC connectome

Name in this study	Description	Neurotransmitter	ID
Claw	Sensory axon in T1L	ACH	648518346500641203
Claw	Sensory axon in T1L	ACH	648518346498453780
Claw	Sensory axon in T1L	ACH	648518346499722309
Claw	Sensory axon in T1L	ACH	648518346483783343
Claw	Sensory axon in T1L	ACH	648518346496335735
Claw	Sensory axon in T1L	ACH	648518346486653999
Claw	Sensory axon in T1L	ACH	648518346474593218
Claw	Sensory axon in T1L	ACH	648518346500681907
Claw	Sensory axon in T1L	ACH	648518346477981653
Claw	Sensory axon in T1L	ACH	648518346484687181
Claw	Sensory axon in T1L	ACH	648518346489569900
Claw	Sensory axon in T1L	ACH	648518346487912272
Claw	Sensory axon in T1L	ACH	648518346508752447
Claw	Sensory axon in T1L	ACH	648518346514361543
Claw	Sensory axon in T1L	ACH	648518346481759933
Claw	Sensory axon in T1L	ACH	648518346490596093
Claw	Sensory axon in T1L	ACH	648518346488815453
Hook	Sensory axon in T1L	ACH	648518346480125925
Hook	Sensory axon in T1L	ACH	648518346476657526
Hook	Sensory axon in T1L	ACH	648518346481857725
Hook	Sensory axon in T1L	ACH	648518346514448583
Hook	Sensory axon in T1L	ACH	648518346480666625
Hook	Sensory axon in T1L	ACH	648518346509569667
Hook	Sensory axon in T1L	ACH	648518346507233352
Hook	Sensory axon in T1L	ACH	648518346494933426
Hook	Sensory axon in T1L	ACH	648518346494932914
Hook	Sensory axon in T1L	ACH	648518346494264434
Hook	Sensory axon in T1L	ACH	648518346481856445
Hook	Sensory axon in T1L	ACH	648518346489572037
Hook	Sensory axon in T1L	ACH	648518346501288024
Hook	Sensory axon in T1L	ACH	648518346477034696
Hook	Sensory axon in T1L	ACH	648518346494933170
Hook	Sensory axon in T1L	ACH	648518346486753761
Hook	Sensory axon in T1L	ACH	648518346496729980

Hook	Sensory axon in T1L	ACH	648518346494071307
13B	Interneuron in T1L presynaptic to claw	GABA	648518346484847261
19A	Interneuron in T1L presynaptic to claw	GABA	648518346531401754
19A	Interneuron in T1L presynaptic to claw	GABA	648518346472653065
19A	Interneuron in T1L presynaptic to claw	GABA	648518346488555913
19A	Interneuron in T1L presynaptic to claw	GABA	648518346498429489
19A	Interneuron in T1L presynaptic to claw	GABA	648518346502916595
19A	Interneuron in T1L presynaptic to claw	GABA	648518346488659278
19A	Interneuron in T1L presynaptic to claw	GABA	648518346521506809
19A	Interneuron in T1L presynaptic to claw	GABA	648518346518741215
19A	Interneuron in T1L presynaptic to claw	GABA	648518346465091957
19A	Interneuron in T1L presynaptic to claw	GABA	648518346494217863
19A	Interneuron in T1L presynaptic to claw	GABA	648518346499593822
19A	Interneuron in T1L presynaptic to claw	GABA	648518346501344355
19A	Interneuron in T1L presynaptic to claw	GABA	648518346491096865
3A	Interneuron in T1L presynaptic to claw	ACH	648518346499860364
Chief 9A	Interneuron in T1L presynaptic to hook	GABA	648518346496946148
9A	Interneuron in T1L presynaptic to hook	GABA	648518346479847574
9A	Interneuron in T1L presynaptic to hook	GABA	648518346479837078
9A	Interneuron in T1L presynaptic to hook	GABA	648518346498002535
9A	Interneuron in T1L presynaptic to hook	GABA	648518346479879156
9A	Interneuron in T1L presynaptic to hook	GABA	648518346467364359
9A	Interneuron in T1L presynaptic to hook	GABA	648518346486716621
13B	Interneuron in T1L presynaptic to hook	GABA	648518346502572199
19A	Interneuron in T1L presynaptic to hook	GABA	648518346479427282
19A	Interneuron in T1L presynaptic to hook	GABA	648518346494008718
8A	Interneuron in T1L presynaptic to hook	GLUT	648518346488868849
1A	Interneuron in T1L presynaptic to hook	ACH	648518346488991501
8B	Intersegmental ascending neuron presynaptic to hook	ACH	648518346489674348
8B	Intersegmental ascending neuron presynaptic to hook	ACH	648518346479174095
18B	Intersegmental ascending neuron presynaptic to hook	ACH	648518346490380810
18B	Intersegmental ascending neuron presynaptic to hook	ACH	648518346473080420
22A	Interneuron in T1L presynaptic to hook	ACH	648518346518548566
Hook	Sensory axon presynaptic to hook	ACH	648518346480125925
Hook	Sensory axon presynaptic to hook	ACH	648518346494933170

Hook	Sensory axon presynaptic to hook	ACH	648518346481857725
Hook	Sensory axon presynaptic to hook	ACH	648518346476657526
Hook	Sensory axon presynaptic to hook	ACH	648518346494933426
Hook	Sensory axon presynaptic to hook	ACH	648518346490237960
Hook	Sensory axon presynaptic to hook	ACH	648518346496671612
Hook	Sensory axon presynaptic to hook	ACH	648518346514448583
Hook	Sensory axon presynaptic to hook	ACH	648518346501288024
Hair plate	Sensory axon presynaptic to hook	ACH	648518346499992716
Unknown	Neurite in T1L presynaptic to hook	N/A	648518346494610152
Web	Descending neuron presynaptic to chief 9A	N/A	648518346478690132
BDN2	Descending neuron presynaptic to chief 9A	N/A	648518346459693060
Descending neuron	Descending neuron presynaptic to chief 9A	N/A	648518346476980936
Descending neuron	Descending neuron presynaptic to chief 9A	N/A	648518346459681796
oDN1	Descending neuron presynaptic to chief 9A	N/A	648518346504806022
Descending neuron	Descending neuron presynaptic to chief 9A	N/A	648518346485765060
Descending neuron	Descending neuron presynaptic to chief 9A	N/A	648518346479290513
DNa02	Descending neuron presynaptic to chief 9A	N/A	648518346478550356
Descending neuron	Descending neuron presynaptic to chief 9A	N/A	648518346475284321
Descending neuron	Descending neuron presynaptic to chief 9A	N/A	648518346475392384
Descending neuron	Descending neuron presynaptic to chief 9A	N/A	648518346484448003
DNg12	Descending neuron presynaptic to chief 9A	N/A	648518346498347057
Descending neuron	Descending neuron presynaptic to chief 9A	N/A	648518346496855192
cDN1	Descending neuron presynaptic to chief 9A	N/A	648518346520017233
Descending neuron	Descending neuron presynaptic to chief 9A	N/A	648518346494165305
Descending neuron	Descending neuron presynaptic to chief 9A	N/A	648518346487779095
Descending neuron	Descending neuron presynaptic to chief 9A	N/A	648518346490241884
Descending neuron	Descending neuron presynaptic to chief 9A	N/A	648518346477810925
Descending neuron	Descending neuron presynaptic to chief 9A	N/A	648518346496600109

IDs are from FANC CAVE materialization version 840, timestamp 2024-01-17T08:10:01.179472.

Table S2. Neurons of interest in the MANC connectome

Name in this study	Description	Neurotransmitter	ID or group name
Hooks	Sensory axons from hook neurons in all leg neuropils	ACH	SNpp38
Chief 9A	Interneuron in T1L presynaptic to hook	GABA	100513
Chief 9A	Interneuron in T2L presynaptic to hook	GABA	13157
Chief 9A	Interneuron in T3L presynaptic to hook	GABA	14517
Chief 9A	Interneuron in T1R presynaptic to hook	GABA	165560
Chief 9A	Interneuron in T2R presynaptic to hook	GABA	12443
Chief 9A	Interneuron in T3R presynaptic to hook	GABA	12804
Web	Descending neuron presynaptic to chief 9A in T1L, T2L, T3L	GABA	10107 (DNxl041)
Web	Descending neuron presynaptic to chief 9A in T1R, T2R, T3R	GABA	10103 (DNxl041)
Descending neuron	Descending neuron presynaptic to chief 9A in T1L, T2L, T3L	ACH	10093 (DNxl058)
Descending neuron	Descending neuron presynaptic to chief 9A in T1R, T2R, T3R	ACH	10339 (DNxl058)

IDs are from MANC version 1.0.

Table S3. Neurons of interest in the FlyWire connectome

Name in this study	Description	Neurotransmitter	ID or group name
Web	Descending neuron presynaptic to chief 9A in T1L, T2L, T3L	GABA	720575940636656632 (DNg74_b)
Web	Descending neuron presynaptic to chief 9A in T1R, T2R, T3R	GABA	720575940627087646 (DNg74_b)

IDs are from FlyWire public release version 783.