Supplementary figures

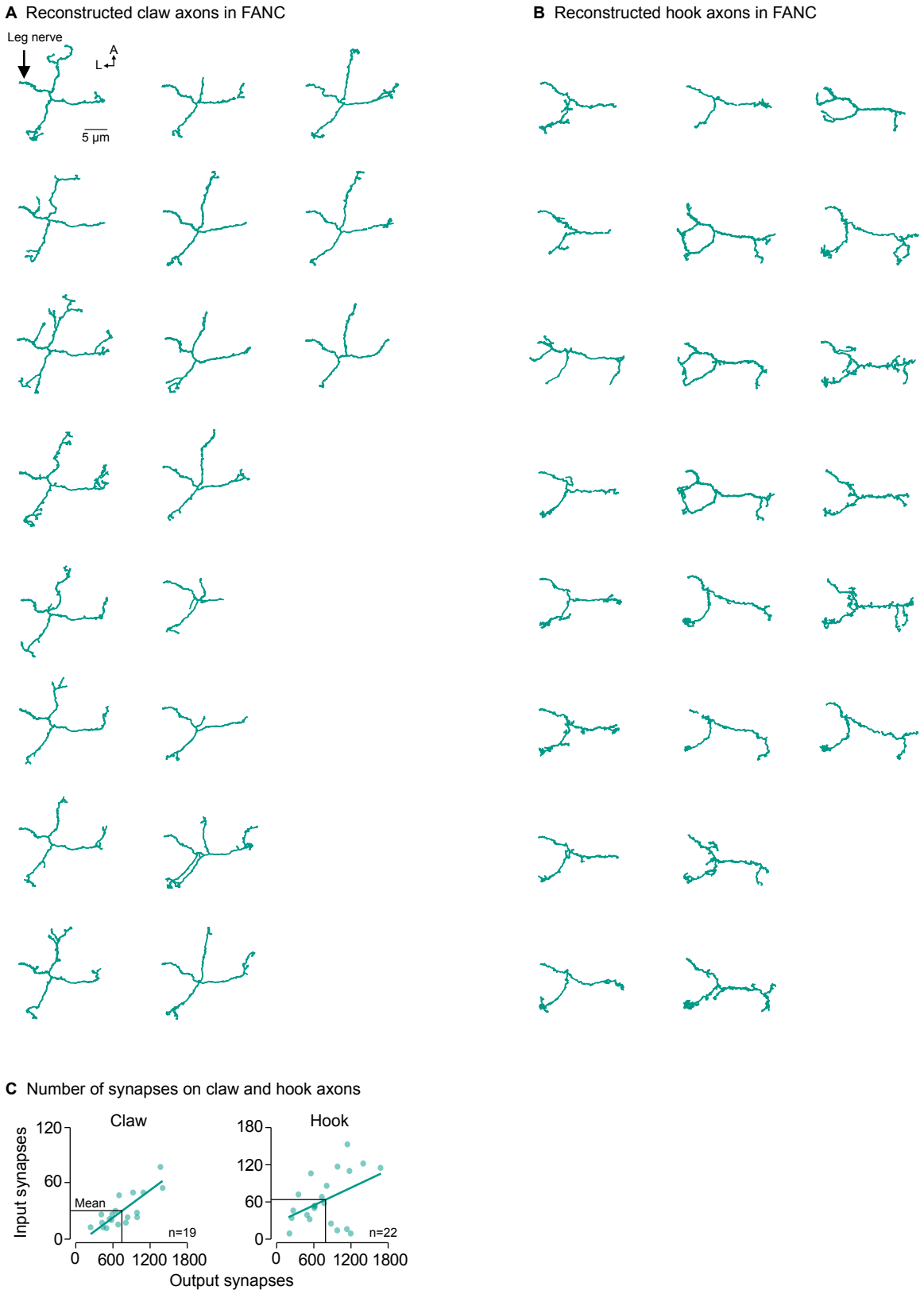

Figure S1.

(A) Top view of reconstructed claw axons in the left front leg neuromere of the FANC connectome. A: anterior; L: lateral.

(B) Top view of reconstructed hook axons in the left front leg neuromere of the FANC connectome. View as in (A).

(C) Number of input and output synapses for individual claw and hook axons. Black lines indicate the mean. Green lines indicate linear fits. n: number of axons.

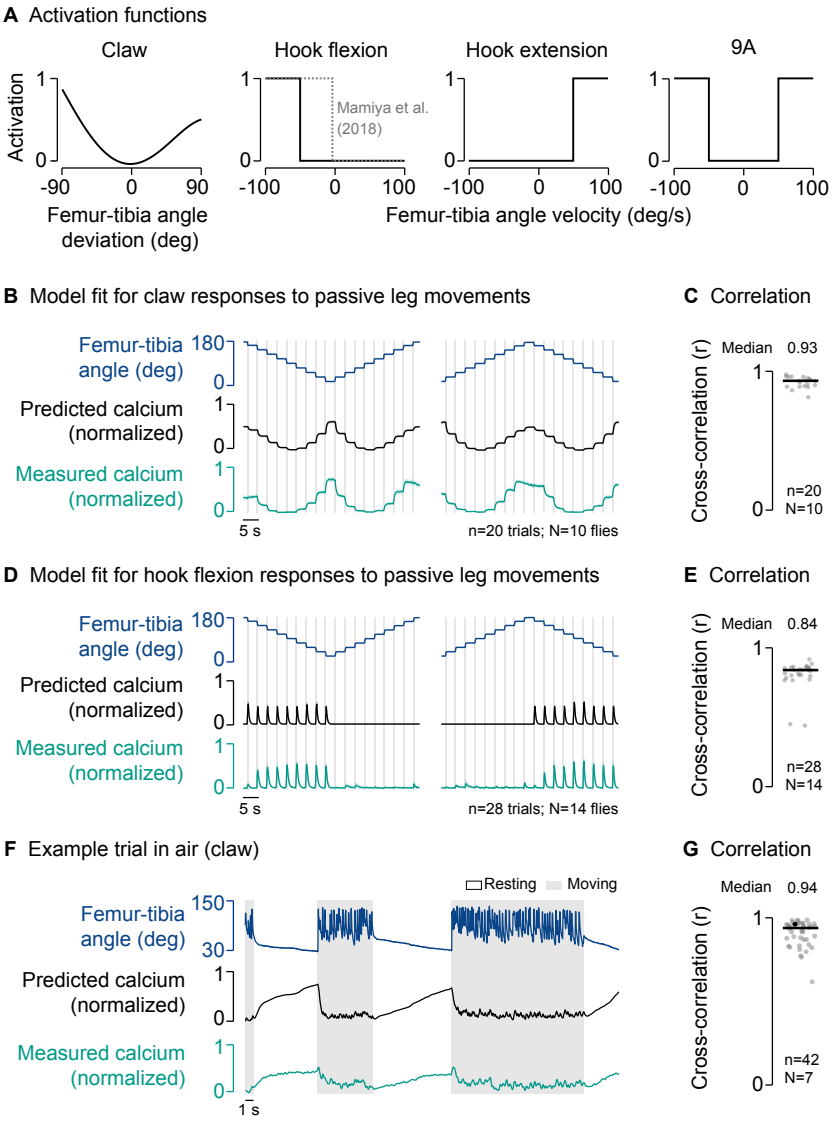

Figure S2.
(A) Activation functions for claw, hook flexion, hook extension, and 9A neurons.
(B) Measured and predicted (fitted) calcium signals of claw axons in response to applied ramp-and-hold movements of the femur-tibia joint. Experimental data from Mamiya et al. (2018). Lines show mean of animal 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) Example trial of two-photon calcium imaging of claw axons and behavior tracking without the treadmill.
(G) Same as (C) but for claw axons imaged without the treadmill. The black dot marks the trial shown in (F).

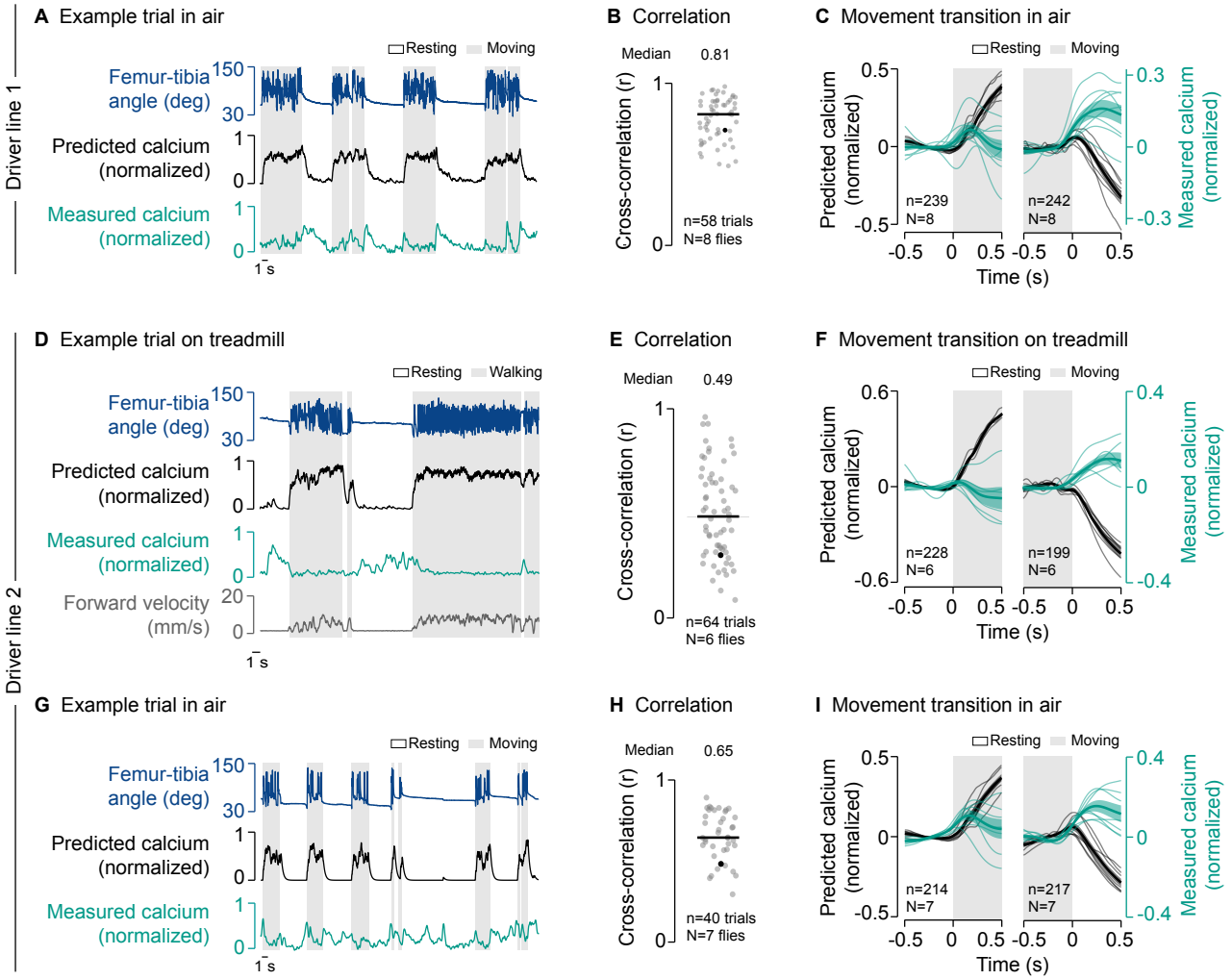

Figure S3.

(A) Example trial of two-photon calcium imaging of hook flexion axons 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) 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) 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.

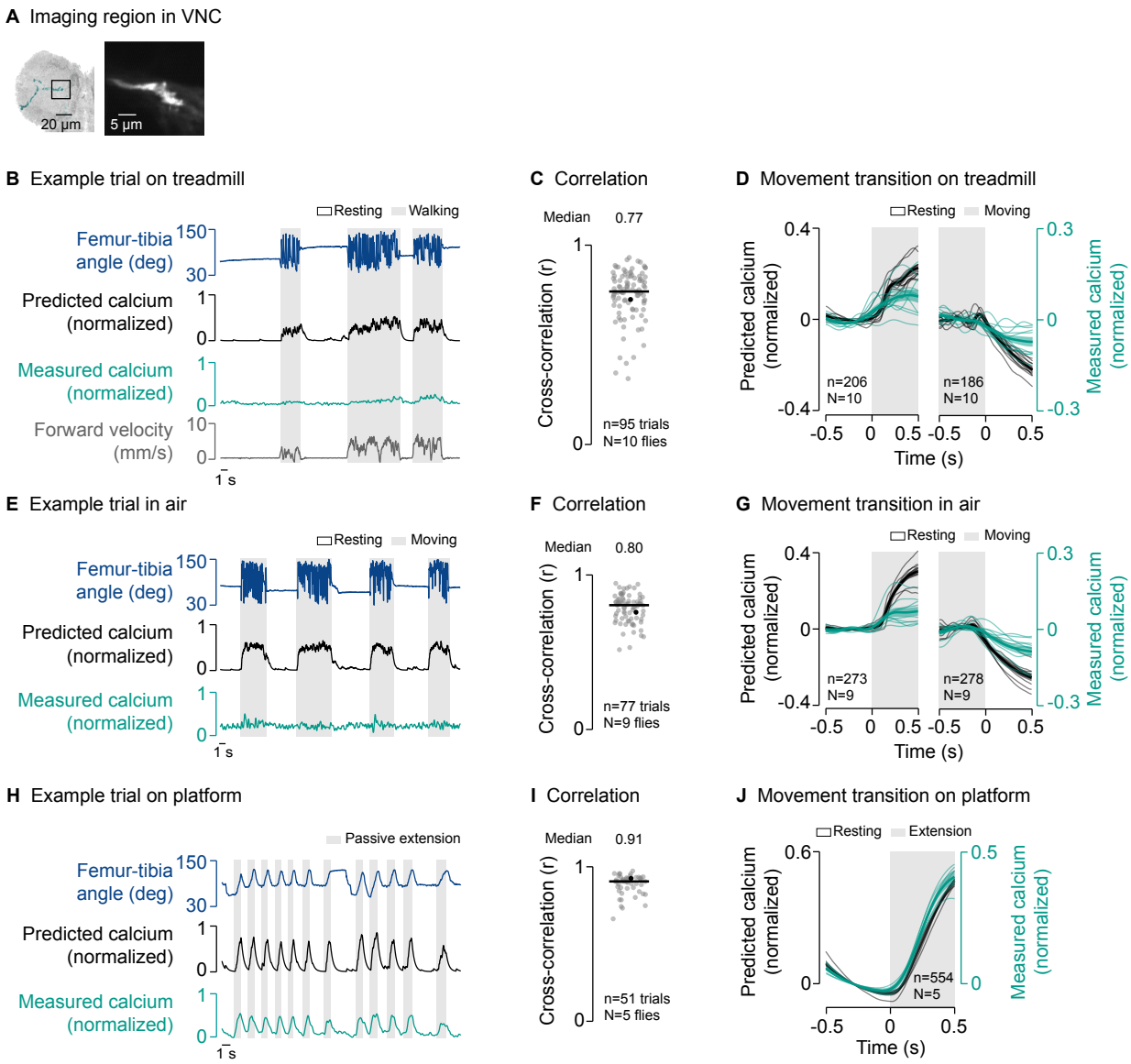

Figure S4.

(A) Left: Confocal image of hook extension axons in the VNC. 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 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 and behavior tracking on the platform.

(I) Same as (C) but for hook extension axons imaged on the platform.

(J) Same as (D) but for hook extension axons imaged on the platform.

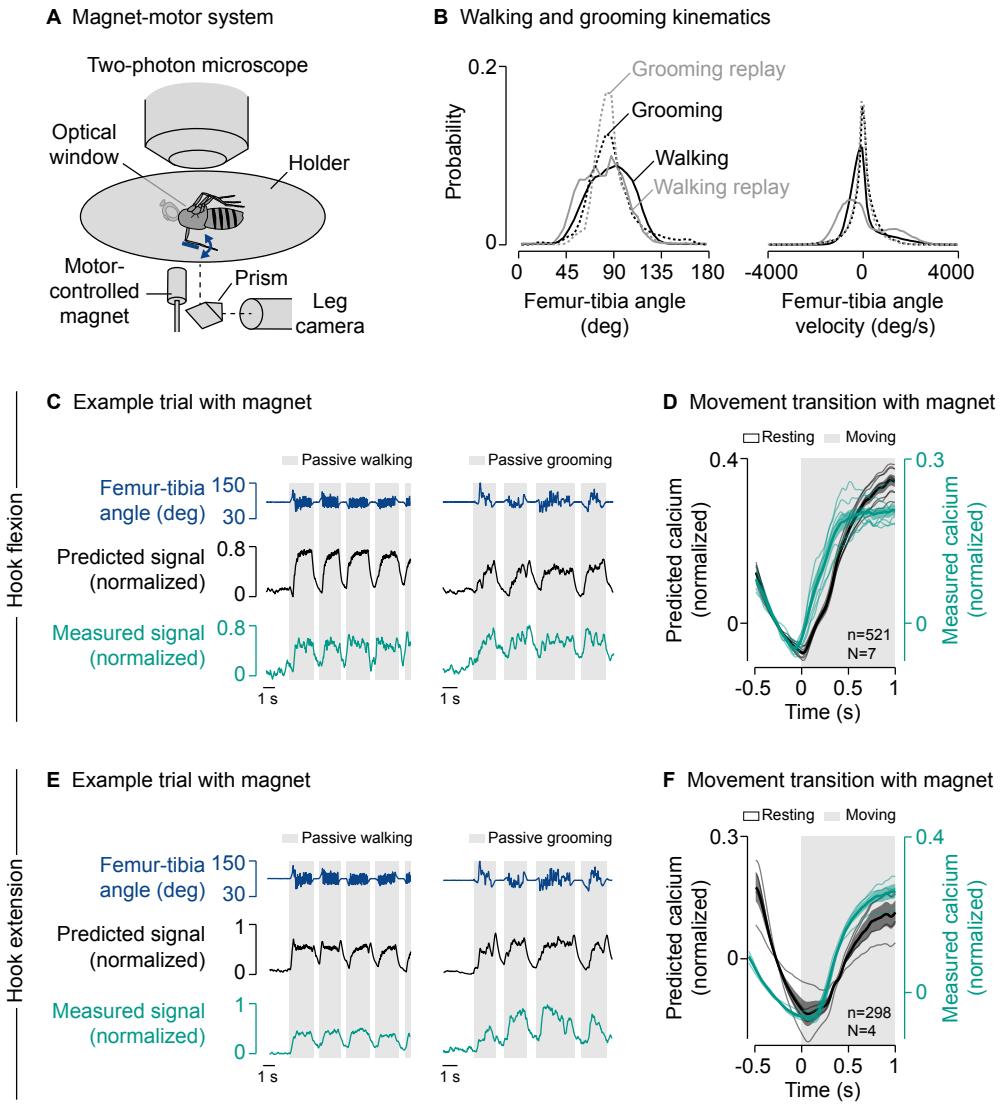

Figure S5.

(A) Experimental setup for two-photon calcium imaging from VNC neurons and leg tracking of a front leg of 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 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) Same as (C) but for hook extension axons.

(F) Same as (D) but for hook extension axons.

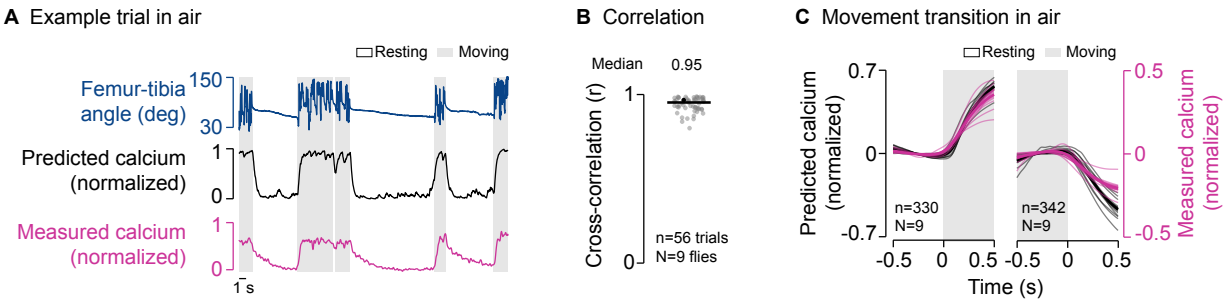

Figure S6.
(A) Example trial of two-photon calcium imaging of 9A neurons and behavior tracking without the treadmill.
(B) Cross-correlation coefficient between predicted and measured calcium signals per trial at a time lag of zero. The black line shows the median. The black dot marks the trial shown 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.

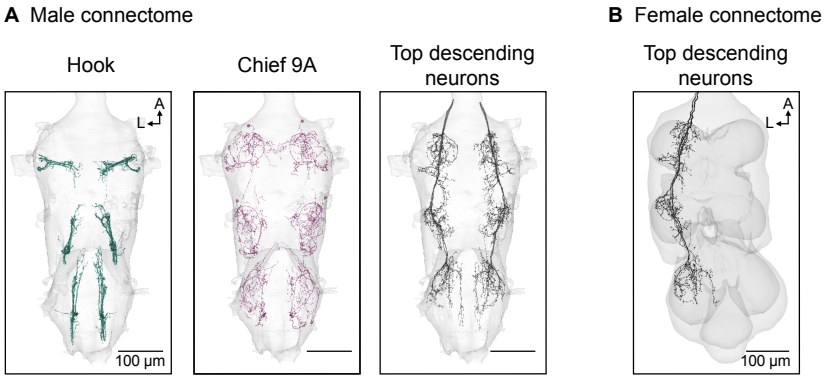

Figure S7.
(A) Hook axons, chief 9A neurons presynaptic to hook axons, and the top two descending neurons presynaptic to the chief 9A neurons in the male VNC connectome (MANC). A: anterior; L: lateral.
(B) Top two descending neurons presynaptic to the chief 9A neuron in the female VNC connectome (FANC). A: anterior; L: lateral.

Supplementary videos

Video S1
Claw axons, hook axons, the chief 9A neuron presynaptic to hook axons, and the top two descending neurons presynaptic to the chief 9A neuron 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 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.