

## Description of Additional Supplementary Files

### File Name: Supplementary Movie 1

**Description: VNC imaging volume.** 2-photon z-stack illustrating horizontal sections across the dorsal-ventral extent of the VNC. GCaMP6s (cyan) and tdTomato (red) are expressed throughout the nervous system (*GMR57C10>GCaMP6s; tdTomato*). Imaging depth is indicated on the top-left.

### File Name: Supplementary Movie 2

**Description: Horizontal VNC imaging.** 2-photon imaging of a single horizontal section of the VNC in a fly that walks and grooms. GCaMP6s (cyan) and tdTomato (red) are expressed throughout the nervous system (*GMR57C10>GCaMP6s; tdTomato*). Shown are synchronized raw fluorescence images (**top-left**),  $\% \Delta F/F$  images (**top-right**), behavior video images (**bottom-left**), and forward, sideways, and rotational velocities of the animal (**bottom-right**). Air puff delivery is indicated by a red box above the behavior video images. Registration was performed using  $\gamma = 0$  and  $\lambda = 500$ . Movie is 4x faster than real-time.

### File Name: Supplementary Movie 3

**Description: Coronal VNC imaging.** 2-photon imaging of a single coronal section of the VNC in a fly that walks. GCaMP6s (cyan) and tdTomato (red) are expressed throughout the nervous system (*GMR57C10>GCaMP6s; tdTomato*). Shown are synchronized raw fluorescence images (**top-left**),  $\% \Delta F/F$  images (**top-right**), behavior video images (**bottom-left**), and forward, sideways, and rotational velocities of the animal (**bottom-right**). Registration was performed using  $\gamma = 50$  and  $\lambda = 10,000$ . Movie is 4x faster than real-time.

### File Name: Supplementary Movie 4

**Description: Coronal cervical connective imaging.** 2-photon imaging of a single coronal section of the cervical connective in a fly that walks. GCaMP6s (cyan) and tdTomato (red) are expressed throughout the nervous system (*GMR57C10>GCaMP6s; tdTomato*). Shown are synchronized raw fluorescence images (**top-left**),  $\% \Delta F/F$  images (**top-right**), behavior video images (**bottom-left**), and forward, sideways, and rotational velocities of the animal (**bottom-right**). Registration was performed using  $\gamma = 50$  and  $\lambda = 5,000$ . Movie is 4x faster than real-time.

### File Name: Supplementary Movie 5

**Description: Motion and deformation correction.** 2-photon imaging of a single horizontal section of the VNC in three different animals during behavior. Data are from Supplementary Fig. 4 (Fly 1:  $\lambda = 500$  and  $\gamma = 0$ ; Fly 2:  $\lambda = 1000$  and  $\gamma = 4$ ; Fly 3:  $\lambda = 300$  and  $\gamma = 4$ ). (**top**) Raw, unregistered, fluorescence images. (**middle**) Spatially down sampled transformation vector field used to register raw images. (**bottom**) Registered fluorescence images. Images for flies 1 and 2 are 512 x 512 pixels. Images for fly 3 are 256 x 256 pixels.

### File Name: Supplementary Movie 6

**Description: Semi-automated behavioral classification.** Illustration of semi-automated grooming and walking annotations used for **Figure 2**. Grooming and walking ROIs are shown (white boxes). A behavioral descriptor is indicated on the top-left.

**File Name: Supplementary Movie 7**

**Description: Coronal cervical connective imaging of dorsal Moonwalker Ascending Neurons.** 2-photon imaging of a single coronal section of the cervical connective in a behaving fly. GCaMP6s (cyan) and tdTomato (red) are expressed in MANs (*MAN>GCaMP6s; tdTomato*). Raw fluorescence images of the left and right dMANs are presented and outlined by ROIs (**top-left**). These images are used to calculate  $\% \Delta R/R$  traces for each neuron (**top-right**). Corresponding behavior videography (**bottom-left**) and forward, sideways, and rotational velocities of the animal (**bottom-right**) are shown.

**File Name: Supplementary Movie 8**

**Description: Behavioral responses associated with dorsal Moonwalker Ascending Neuron activity events.** Three example behaviors (rows) produced at the onset of dMAN fluorescence events for three flies (columns). Red squares indicate the onset time of each fluorescence event ( $t = 0$  s). Movie is 3x slower than real-time.

**File Name: Supplementary Movie 9**

**Description: Coronal cervical connective imaging of Moonwalker Descending Neurons.** 2-photon imaging of a single coronal section of the cervical connective in a behaving fly. GCaMP6s (cyan) and tdTomato (red) are expressed in MDNs (*MDN-1>GCaMP6s; tdTomato*). Raw fluorescence images of the left and right MDNs are presented and outlined by ROIs (**top-left**). These images are used to calculate  $\% \Delta R/R$  traces for each neuron (**top-right**). Corresponding behavior videography (**bottom-left**) and forward, sideways, and rotational velocities of the animal (**bottom-right**) are shown.

**File Name: Supplementary Movie 10**

**Description: Behavioral responses associated with Moonwalker Descending Neuron activity events.** Three example behaviors (rows) produced at the onset of MDN fluorescence events for three flies (columns). Red squares indicate the onset time of each fluorescence event ( $t = 0$  s). Movie is 3x slower than real-time.

**File Name: Supplementary Movie 11**

**Description: Coronal cervical connective imaging of A1 neurons.** 2-photon imaging of a single coronal section of the cervical connective in a behaving fly. GCaMP6s (cyan) and tdTomato (red) are expressed in A1 neurons (*A1>GCaMP6s; tdTomato*). Raw fluorescence images of the left and right A1 neurons are presented and outlined by ROIs (**top-left**). These images are used to calculate  $\% \Delta R/R$  traces for each neuron (**top-right**). Corresponding behavior videography (**bottom-left**) and forward, sideways, and rotational velocities of the animal (**bottom-right**) are shown.

**File Name: Supplementary Movie 12**

**Description: Behavioral responses associated with left A1 neuron activity events.** Three example behaviors (rows) produced at the onset of A1 fluorescence events for three flies (columns). Red squares indicate the onset time of each fluorescence event ( $t = 0$  s). Movie is 3x slower than real-time.

**File Name: Supplementary Movie 13**

**Description: Behavioral responses associated with right A1 neuron activity events.** Three example behaviors (rows) for each of three flies (columns) generated at the onset of right A1 neuron fluorescence events. Red squares indicate the time of each fluorescence event ( $t = 0$  s). Movie is 3x slower than real-time.

**File Name: Supplementary Movie 14**

**Description: Behaviors and neural activity in *Act88F:Rpr* and control animals.** Horizontal section imaging data from the prothoracic neuromere of *Act88F:Rpr* animals (**left**, *Act88F:Rpr/elav-GAL4; GCaMP6s/+;*), or control animals (**right**, *+/elav-GAL4; GCaMP6s/+;*). 2-photon imaging data are not registered.

**File Name: Supplementary Movie 15**

**Description: Coronal and horizontal section imaging of A1 neurons in the VNC.** A comparison of coronal (left), or horizontal (right) section imaging of the cervical connective in the same animal. GCaMP6s (cyan) and tdTomato (red) are expressed in A1 neurons (*A1>GCaMP6s; tdTomato*). Raw fluorescence images (**top**) are presented alongside corresponding behavior video images (**bottom**).

**File Name: Supplementary Movie 16**

**Description: Long-term behavior in animals used to image dMAN activity.** Behavior movies for three flies (*MAN>GCaMP6s; tdTomato*) during the first imaging experiment (0 min) as well as 20 min and 40 min afterwards.

**File Name: Supplementary Movie 17**

**Description: Long-term behavior in animals used to image MDN activity.** Behavior movies for three flies (*MDN>GCaMP6s; tdTomato*) during the first imaging experiment (0 min) as well as 20 min and 40 min afterwards.

**File Name: Supplementary Movie 18**

**Description: Long-term behavior in animals used to image A1 activity.** Behavior movies for three flies (*A1>GCaMP6s; tdTomato*) during the first imaging experiment (0 min) as well as 20 min and 40 min afterwards.