Supporting Information

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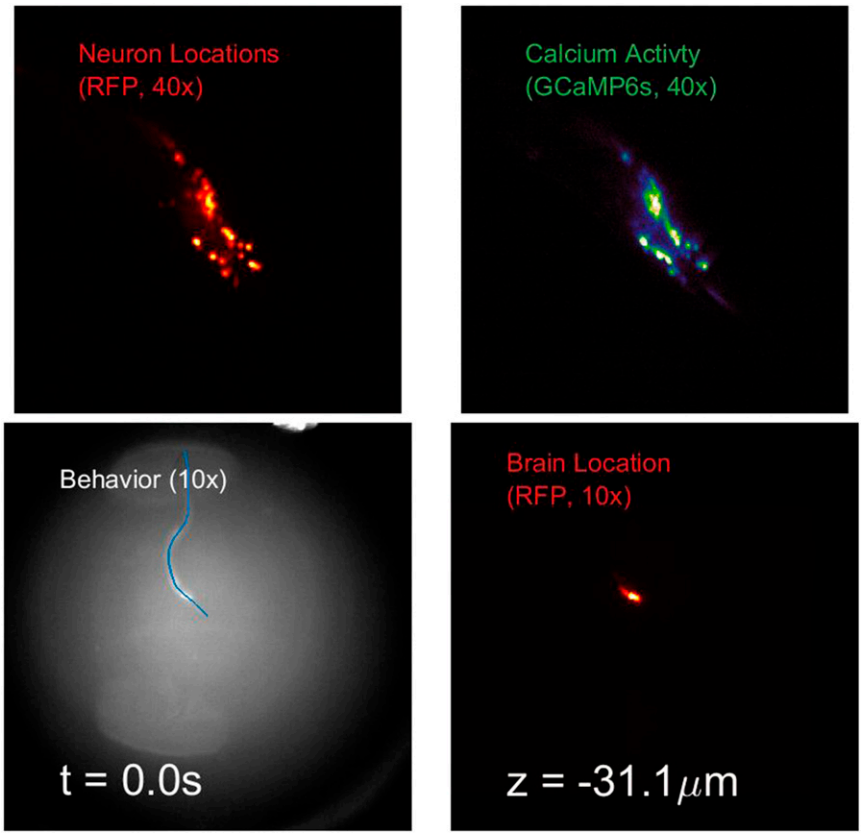
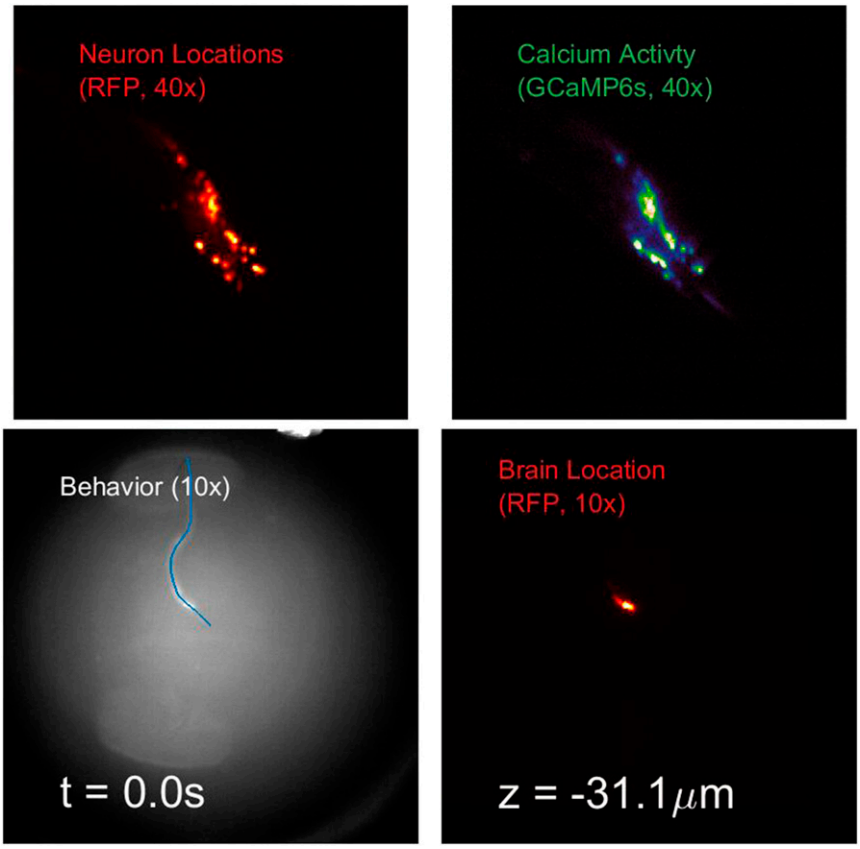


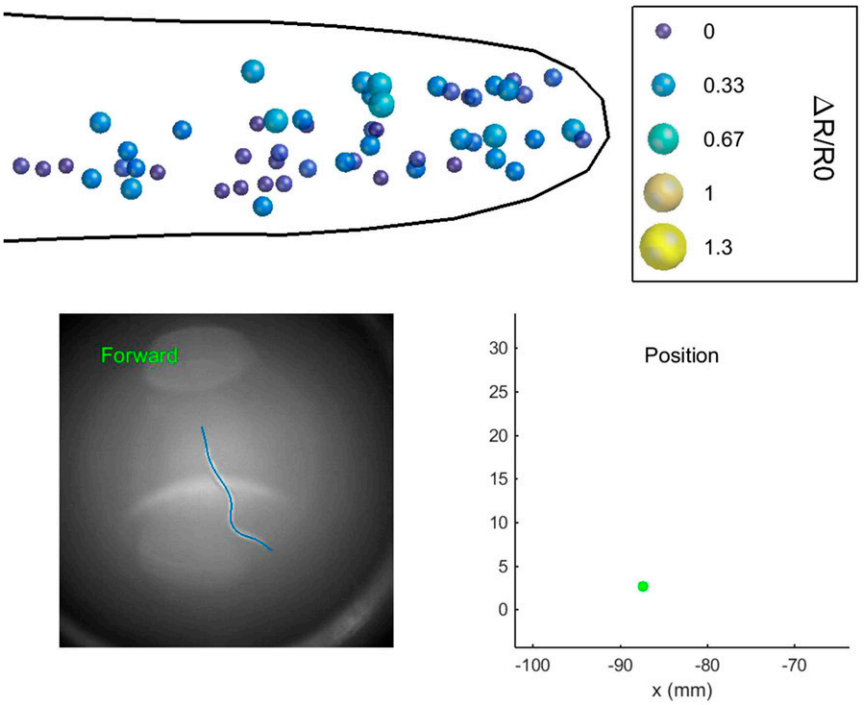
Movie S1. Four simultaneously recorded video streams of worm 3 are shown as the animal crawls freely. The location of the neurons, calcium activity, and animal behavior are all visible. The animal expresses RFP and GCaMP6s in the nuclei of neurons. The top two panels show red- and green-channel fluorescent images (respectively) that are recorded through the 40× objective. The objective translates through the volume of the worm tracing the path of a triangle wave in *z* with frequency 3 Hz. As the objective scans through the worm's brain the fluorescent images go from dark (outside the worm) to bright (inside the worm) to dark again (outside the worm) giving the appearance of a 6-Hz flicker. The locations of individual neuronal nuclei are visible in the upper left panel (field of view is 150 μm wide). Fluorescence from the calcium indicator GCaMP6s is visible in the upper right panel (false color: blue is low, yellow is high; field of view is 150 μm wide). The lower two panels show the animal's behavior imaged through the 10× objective. The collective fluorescence of all of the neurons in the animal's head is visible in the lower right panel (field of view is 1.3 mm). This fluorescent image is used for real-time feedback to keep the head centered within the field of view. Dark-field images of the animal's posture and behavior are visible in the lower left panel. A blue line showing the animal's centerline is added to the images.

Movie S1



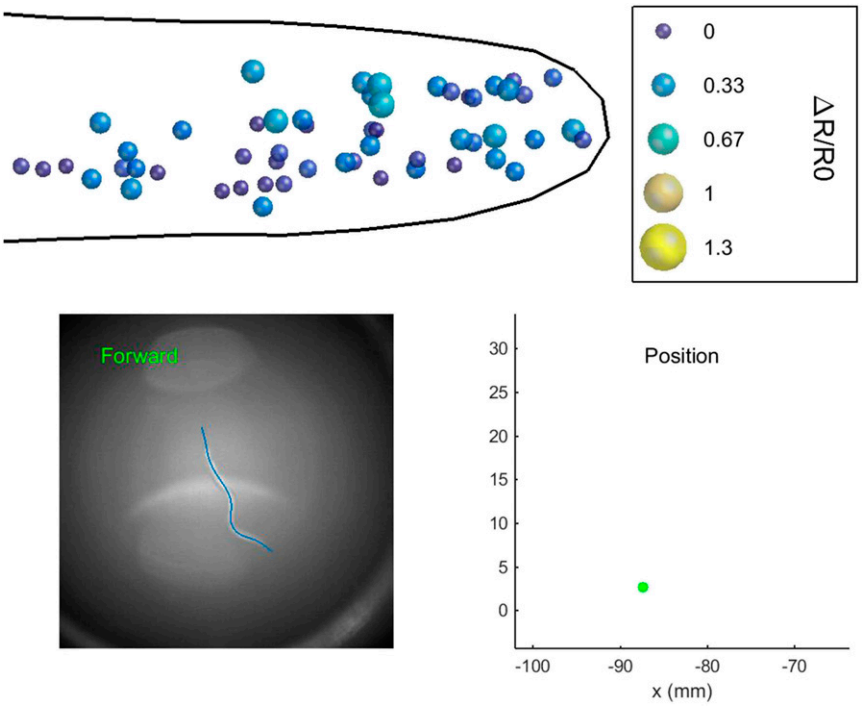
Movie S2. Same as Movie S1, but playback is a quarter speed.

Movie S2



Movie S3. Whole-brain neural activity of individual neurons is visualized during animal behavior. Each sphere corresponds to the location of a recorded neuron. Each neuron's size and color is indicative of calcium activity within the neuron and corresponds to percent change from baseline of the ratio *R* of GCaMP6s fluorescence intensity to RFP fluorescence intensity. A cartoon showing the outline of the head of the animal is marked in black. The animal's position in the imaging arena is shown in the lower right. The animal's behavior is shown in the lower left with the worm's centerline indicated in blue. The instantaneous classification of the worm's behavior based on an eigenworm analysis is indicated in the upper left. Worm 1 is shown. Video plays in real time.

Movie S3



Movie S4. Same as Movie S1, but playback is sped up fivefold.

Movie S4

Dataset S1. Neural signal from each of the worms used in this study, including four worms expressing pan neuronal GCaMP6s (strain AML14) and one control worm expressing pan neuronal GFP (strain AML18)

Dataset S1

The first four columns show the time, center-of-mass position of the worm in *xy* in the reference frame of the arena, and the behavior of the worm. Each column after that shows the signal from a given neuron, represented as the fractional change from baseline of the ratios of the green- and red-channel fluorescence intensity, $\Delta R/R_0$ after accounting for photobleaching. In the behavior column, −1 indicates reverse motion, 0 indicates pausing, 1 indicates forward motion, and 2 indicates corresponds to turns. Nan's and blanks are inserted when the behavior or neural signal could not be found at that time point.

Dataset S2. Worm 1; posture information; *x* coordinate

Dataset S2

The coordinates of the worm's 102-point centerline are provided at ∼60 Hz for the duration of the recording. *xy* coordinates for the worm centerline are in the reference frame of the image. Each row corresponds to a different point along the centerline, while each column refers to a different time point. Note that centerlines are recorded asynchronously from neural activity, although both are recorded with synchronized clocks.

Dataset S3. Worm 1; posture information; *y* coordinate

Dataset S3

The coordinates of the worm's 102-point centerline are provided at ∼60 Hz for the duration of the recording. *xy* coordinates for the worm centerline are in the reference frame of the image. Each row corresponds to a different point along the centerline, while each column refers to a different time point. Note that centerlines are recorded asynchronously from neural activity, although both are recorded with synchronized clocks.

Dataset S4. Worm 1; posture information; time

Dataset S4

The coordinates of the worm's 102-point centerline are provided at ∼60 Hz for the duration of the recording. *xy* coordinates for the worm centerline are in the reference frame of the image. Each row corresponds to a different point along the centerline, while each column refers to a different time point. Note that centerlines are recorded asynchronously from neural activity, although both are recorded with synchronized clocks.

Dataset S5. Worm 2; posture information; *x* coordinate

Dataset S5

The coordinates of the worm's 102-point centerline are provided at ∼60 Hz for the duration of the recording. *xy* coordinates for the worm centerline are in the reference frame of the image. Each row corresponds to a different point along the centerline, while each column refers to a different time point. Note that centerlines are recorded asynchronously from neural activity, although both are recorded with synchronized clocks.

Dataset S6. Worm 2; posture information; *y* coordinate

Dataset S6

The coordinates of the worm's 102-point centerline are provided at ∼60 Hz for the duration of the recording. *xy* coordinates for the worm centerline are in the reference frame of the image. Each row corresponds to a different point along the centerline, while each column refers to a different time point. Note that centerlines are recorded asynchronously from neural activity, although both are recorded with synchronized clocks.

Dataset S7. Worm 2; posture information; time

Dataset S7

The coordinates of the worm's 102-point centerline are provided at ~60 Hz for the duration of the recording. *xy* coordinates for the worm centerline are in the reference frame of the image. Each row corresponds to a different point along the centerline, while each column refers to a different time point. Note that centerlines are recorded asynchronously from neural activity, although both are recorded with synchronized clocks.

Dataset S8. Worm 3; posture information; *x* coordinate

Dataset S8

The coordinates of the worm's 102-point centerline are provided at ~60 Hz for the duration of the recording. *xy* coordinates for the worm centerline are in the reference frame of the image. Each row corresponds to a different point along the centerline, while each column refers to a different time point. Note that centerlines are recorded asynchronously from neural activity, although both are recorded with synchronized clocks.

Dataset S9. Worm 3; posture information; *y* coordinate

Dataset S9

The coordinates of the worm's 102-point centerline are provided at ~60 Hz for the duration of the recording. *xy* coordinates for the worm centerline are in the reference frame of the image. Each row corresponds to a different point along the centerline, while each column refers to a different time point. Note that centerlines are recorded asynchronously from neural activity, although both are recorded with synchronized clocks.

Dataset S10. Worm 3; posture information; time

Dataset S10

The coordinates of the worm's 102-point centerline are provided at ~60 Hz for the duration of the recording. *xy* coordinates for the worm centerline are in the reference frame of the image. Each row corresponds to a different point along the centerline, while each column refers to a different time point. Note that centerlines are recorded asynchronously from neural activity, although both are recorded with synchronized clocks.

Dataset S11. Worm 5; posture information; *x* coordinate

Dataset S11

The coordinates of the worm's 102-point centerline are provided at ~60 Hz for the duration of the recording. *xy* coordinates for the worm centerline are in the reference frame of the image. Each row corresponds to a different point along the centerline, while each column refers to a different time point. Note that centerlines are recorded asynchronously from neural activity, although both are recorded with synchronized clocks.

Dataset S12. Worm 4; posture information; *y* coordinate

Dataset S12

The coordinates of the worm's 102-point centerline are provided at ~60 Hz for the duration of the recording. *xy* coordinates for the worm centerline are in the reference frame of the image. Each row corresponds to a different point along the centerline, while each column refers to a different time point. Note that centerlines are recorded asynchronously from neural activity, although both are recorded with synchronized clocks.

Dataset S13. Worm 4; posture information; time

Dataset S13

The coordinates of the worm's 102-point centerline are provided at ~60 Hz for the duration of the recording. *xy* coordinates for the worm centerline are in the reference frame of the image. Each row corresponds to a different point along the centerline, while each column refers to a different time point. Note that centerlines are recorded asynchronously from neural activity, although both are recorded with synchronized clocks.

Dataset S14. GFP control worm (AML 18); posture information; *x* coordinate

Dataset S14

The coordinates of the worm's 102-point centerline are provided at ~60 Hz for the duration of the recording. *xy* coordinates for the worm centerline are in the reference frame of the image. Each row corresponds to a different point along the centerline, while each column refers to a different time point. Note that centerlines are recorded asynchronously from neural activity, although both are recorded with synchronized clocks.

Dataset S15. GFP control worm (AML 18); posture information; *y* coordinate

Dataset S15

The coordinates of the worm's 102-point centerline are provided at ~60 Hz for the duration of the recording. *xy* coordinates for the worm centerline are in the reference frame of the image. Each row corresponds to a different point along the centerline, while each column refers to a different time point. Note that centerlines are recorded asynchronously from neural activity, although both are recorded with synchronized clocks.

Dataset S16. GFP control worm (AML 18); posture information; time

Dataset S16

The coordinates of the worm's 102-point centerline are provided at ~60 Hz for the duration of the recording. *xy* coordinates for the worm centerline are in the reference frame of the image. Each row corresponds to a different point along the centerline, while each column refers to a different time point. Note that centerlines are recorded asynchronously from neural activity, although both are recorded with synchronized clocks.

Other Supporting Information Files

SI Appendix (PDF)