

782 single:  $p=1.0$ ).  $*=p<0.05$ ,  $**=p<0.01$ ,  $***=p<0.001$ . For all box plot figures, middle lines indicate the  
783 median, lower and upper edges of the box indicate quartiles below and above the median, and upper  
784 and lower whiskers indicate the points furthest from the median whose value did not exceed 1.5 times  
785 the first-to-third quartile range above the third quartile or below the first quartile.  
786

### 787 **Figure 7: Interneurons Regulate MSN Activity and Movement State.**

788 **(A)** MSN population fluorescence aligned to all PV calcium event onsets (orange) versus MSN calcium  
789 event onsets (blue) from all PV-Cre animals. MSN population peak fluorescence was significantly  
790 reduced in the 500ms following a PV calcium event (PV post-event: rank-sum test, two-sided,  
791  $\text{ranksum}=2.43\text{e}+12$   $n_{\text{PV}}=237528$  event-neurons,  $n_{\text{MSN}}=20289773$  event-neurons, across all 6 PV  
792 animals and 18 sessions,  $p=1.48\text{e}-06$ ), in comparison to a MSN calcium event (blue). Plots are mean  
793  $\pm$ SEM. **(B)** Same as (A) but for Chat-Cre animals. MSN peak population fluorescence was significantly  
794 elevated in the 500ms following a CHI calcium event (green: Chat-Cre post-event: two-sided, rank-  
795 sum test,  $\text{ranksum}=1.73\text{e}+12$ ,  $p=2.53\text{e}-09$ ,  $n_{\text{MSN}}=22116844$  event-neurons,  $n_{\text{CHI}}=153680$  event-  
796 neurons from 10 sessions in 6 animals), compared to an MSN event (blue). **(C)** Change in coincident  
797 MSN activity (MSN co-activity) following a MSN calcium event (blue), a PV calcium event (orange), or  
798 a CHI calcium event (green) from all animals and normalized to event onset for all three types ( $t=0$ ).  
799 Plots are mean  $\pm$ SEM. A magnified inset demonstrating the change in MSN co-activity probability in  
800 the 100ms following a MSN, PV or CHI calcium event is shown to the right. Following a CHI calcium  
801 event, there is an increase in MSN co-activity, whereas following a PV calcium event, there is a  
802 reduction in MSN co-activity relative to the change in MSN-triggered co-activity (Pairwise two-sided z-  
803 tests, PV-MSN vs CHI-MSN,  $z=-40.8$ ,  $p=0$ ; PV-MSN vs MSN-MSN,  $z=-47$ ,  $p=0$ ; CHI-MSN vs MSN-  
804 MSN,  $z=20.4$ ,  $p=0$ . Bonferroni corrected post-hoc for multiple comparisons; raw proportions: MSN:  
805  $3.52\text{e}-03$ , PV: $2.30\text{e}-03$ , CHI: $5.91\text{e}-03$ ,  $n_{\text{CHI}}=38189238$ ,  $n_{\text{CHI}}=38189238$  event-pairs,  $n_{\text{MSN}}=9644946557$   
806 event-pairs,  $n_{\text{PV}}=48285061$  event-pairs, across all 12 animals and 28 sessions). **(D)** A time series  
807 showing MSN-MSN event co-activity during laser stimulation of PV cells (orange) and CHIs (green)  
808 during laser stimulation in PV-Chrimson mice ( $n=4$  PV-Chrimson mice and  $n=4$  Chat-Chrimson mice).  
809 For reference, the change in MSN-MSN co-activity around random MSN events from all time periods  
810 outside of optogenetic stimulation for all PV-Chrimson and Chat-Chrimson mice is shown in blue. Plots  
811 are mean  $\pm$ SEM. **(E)** Population line plots quantifying the change in the MSN-MSN co-activity upon  
812 optogenetic stimulation of PVs or CHIs in PV-Chrimson and Chat-Chrimson mice. Opto-stim induced  
813 co-activity was compared to the endogenous rate of MSN-MSN co-activity from the non-opto  
814 stimulation periods of the imaging session, indicated by the dashed blue line. Error bars and center  
815 are mean  $\pm$  SEM. During optogenetic stimulation of CHIs MSN-MSN co-activity increased, whereas  
816 during optogenetic stimulation of PVs, MSN-MSN co-activity decreased, relative to the endogenous  
817 MSN-MSN co-activity rate (Pairwise two-sided z-tests,  $n_{\text{CHI}}=2078751$  event-pairs,  $n_{\text{MSN}}=694475807$   
818 event-pairs,  $n_{\text{PV}}=682922$  event-pairs; CHI-MSN vs PV-MSN  $z=78.7$ ,  $p=0$ ; MSN-MSN vs MSN-PV,  $z=-$   
819  $228$ ,  $p=0$ ; MSN-MSN vs CHI-MSN,  $z=19.2$ ,  $p=0$ ,  $n=4$  PV-Chrimson mice and  $n=4$  Chat-Chrimson mice,  
820 Bonferroni corrected post-hoc for multiple comparisons).  $*=p<0.05$ ,  $**=p<0.01$ ,  $***=p<0.001$ . **(F)**  
821 Change in movement speed after peaks in MSN-MSN co-activity events (see supplemental methods),  
822 binned into 500ms windows for analysis. Error bars and center are mean  $\pm$  SEM. Peaks in MSN co-  
823 activity were followed by decreases in speed similar to that observed following CHI events, and CHI  
824 optogenetic stimulation (Friedman test, main effect of time,  $n=2249$  peaks in coactivity,  $X^2(8)=520$ ,  $p<$   
825  $0.001$ ; Tukey's HSD post-hoc, (vs 0-0.5 s) 0.5-1 seconds,  $p=0.030$ , 1.00-1.50 seconds,  $p=9.30\text{e}-07$ ,  
826 1.50-4.50 seconds, all  $p=8.97\text{e}-08$ ; mean ranks: 5.81, 5.54, 5.35, 5.04, 4.84, 4.72, 4.62, 4.52, and  
827 4.56).  
828

### 829 **Online Methods**

#### 831 **Animal Surgery**

832 All animal procedures were approved by the Boston University Institutional Animal Care and Use  
833 Committee. Combined experiments included data from mice expressing Cre recombinase in either  
834 choline acetyltransferase expressing cells (Chat-Cre:  $n=14$ ; GM24Gsat) or parvalbumin (Pvalb)

835 expressing cells (PV-Cre mice: n=14; B6;129P2-Pvalb<sup>tm1(cre)Arbr/J</sup>), 8–12 week old at the start of the  
836 experiments. Both male and female mice were used in this study and breeders were obtained  
837 commercially (Chat-Cre: Mutant Mouse Resource Center, Davis, CA; and PV-cre: Jackson  
838 Laboratory, Maine). One cohort of mice was used for imaging studies without optogenetic laser  
839 illumination (n=6 Chat-Cre mice, and n=6 PV-Cre mice). Mice first underwent surgery for implantation  
840 of a sterilized custom imaging window with an attached guide cannula that was assembled before  
841 surgery. The window/guide assembly consisted of a stainless steel imaging cannula (OD: 0.317 cm,  
842 ID: 0.236 cm, height, 2 mm diameter), fitted with a circular coverslip (size 0; OD: 3mm) adhered using  
843 a UV-curable optical adhesive (Norland Products). The guide cannula (26 gauge; C135GS4; Plastics,  
844 Roanoke, VA) was fixed at a 45° angle and terminated flush to the base of the imaging window. To  
845 access dorsal striatum, the overlying cortical tissue was carefully aspirated away to expose the corpus  
846 callosum as an anatomical guide. The white matter was carefully thinned until the underlying striatal  
847 tissue could be visualized via a surgical microscope. The imaging window was then lowered in place  
848 and centered over the dorsal striatum (AP: +0.5, ML:1.8 mm, DV: -1.6 from brain surface). During the  
849 same surgery, a custom aluminum head-plate was attached to the skull, anterior to the imaging  
850 cannula. Upon complete recovery (14-21 days after surgery), animals were injected with a 1µL cocktail  
851 containing 500 nL AAV9-Syn-GCaMP6f.WPRE.SV40 (titer: 6.6 e12 GC/ml) and 500 nL AAV9-CAG-  
852 flex- tdTomato.WPRE.SV40 virus (titer: 5.1e12 GC/ml) through the attached guide cannula. Virus was  
853 delivered via a 10µL syringe (701N; Hamilton Company, Reno, NV) controlled by a microinfusion  
854 pump (UltraMicroPump3-4; World Precision Instruments, Sarasota, FL) fitted with a 33 gauge infusion  
855 cannula (C135IS4; Plastics, Roanoke, VA).

856  
857 A second cohort of mice was used for the behavioral and network analysis during optogenetic  
858 experiments (n=16 combined: n=4 for each group analysis; PV-Cre mice expressing Chrimson-  
859 tdTomato, Control PV-Cre mice expressing tdTomato without Chrimson; Chat-Cre mice expressing  
860 Chrimson-tdTomato, and Control Chat-Cre mice expressing tdTomato without Chrimson). These  
861 animals were prepared similar to the description above, except that virus infusion for tdTomato or  
862 Chrimson-tdTomato was performed in a separate procedure 10 days prior to window implantation.  
863 Mice were infused with either AAV9-CAG-flex- tdTomato (opto-controls), or AAV9-Syn-flex-Chrimson-  
864 tdTomato (opto-group). To ensure the expression of Chrimson or tdTomato maximally within the  
865 imaging hemisphere, a total volume of 3µL's was delivered at two depths (1.5 µL: AP: +0.5, ML:1.8  
866 mm, DV: -3.5; and 1.5 µL: AP: +0.5, ML:1.8 mm, DV: -2.3). Injections were delivered via pressure  
867 ejection (10-15 psi, 15-20 ms pulses delivered at 0.5Hz) through a glass pipette (diameter: 1.2 mm)  
868 pulled to a sharp point and then broken at the tip to a final inner diameter of ~20µm. The pipette was  
869 lowered over 3 min and allowed to remain in place for 3 min before infusion began. The injection  
870 occurred at a rate of 100 nl/min and 10 min passed before raising the pipette to the second location.  
871 At the conclusion of the second infusion, an additional 10 min delay was introduced before the pipette  
872 was slowly withdrawn over 2-3 minutes. AAV9-Syn-flex-Chrimson was obtained from the University of  
873 North Carolina Vector Core: WPRE.SV40 virus (titer: 5.9e12 GC/ml). Following the window  
874 implantation surgery, and after complete recovery (14-21 days after surgery), 500 nL AAV9-Syn-  
875 GCaMP6f.WPRE.SV40 (titer: 6.6 e12 GC/ml) was infused through the attached guide cannula as  
876 described earlier.

## 877 878 **Animal Habituation**

879 Following surgery and virus infusion (typically about 21-28 days), mice were first handled for several  
880 days before being head fixed to the treadmill/imaging apparatus. Mice were then habituated to running  
881 on the spherical treadmill while head fixed for two weeks, 3-4 days per week, at the same time of day  
882 as subsequent recording sessions. Each animal received at least 6 habituation sessions prior to the  
883 first recording day. The cohort of mice used in the optogenetic studies underwent the same handling  
884 and habituation protocol, but with 4-5 additional sessions. Habituation was performed in the dark with  
885 the same light illumination intensity as it would be for recording sessions. Habituation sessions were  
886 the same duration as an imaging session.  
887

888 **Data Acquisition**

889 Image acquisition with sCMOS cameras:

890 Animals were positioned underneath the microscope, and imaged while freely running on the spherical  
891 treadmill. Image acquisition was performed via custom microscope equipped with a scientific CMOS  
892 (sCMOS) camera (ORCA-Flash4.0 LT Digital CMOS camera C11440-42U; Hamamatsu, Boston, MA).  
893 GCaMP6f fluorescence excitation was accomplished with a 5W LED (LZ1-00B200, 460 nm; LedEngin,  
894 San Jose CA). tdTomato fluorescence excitation was accomplished with a 1000mA LED (LXML-PX02-  
895 0000, 567 nm; Lumileds, San Jose CA). The custom microscope included a Leica N Plan 10X 0.25  
896 PH1 microscope objective lens, a dual band excitation filter (FF01-468/553-25), a dichroic mirror  
897 (FF493/574-Di01-25x36), and a dual band emission filter (FF01-512/630-25; Semrock, Rochester,  
898 NY), and a commercial SLR lens focused to infinity as the tube lens (Nikon Zoom-NIKKOR 80-200mm  
899 f/4 AI-s). The camera when coupled to a 10X objective lens yields an imaging field of view of  
900  $1.343 \times 1.343$  mm with each pixel corresponding to  $1.312 \times 1.312$   $\mu$ m.

901  
902 A custom MATLAB script was used to trigger frame capture and to synchronize image acquisition with  
903 movement. TTL trigger pulses were delivered to the camera using a common I/O interface (USB-6259;  
904 National Instruments, Austin, TX). Image acquisition was performed using HC Image Live (HC Image  
905 Live; Hamamatsu; Boston, MA). The time interval between image frame capture was  $47.0 \pm 0.2$  ms  
906 ( $\sim 21.3$ Hz, mean  $\pm$  standard deviation,  $n=28$  sessions). For each image frame, exposure time was fixed  
907 at 20 ms. Image data were stored as multi-page tagged image file format (mpTIFF's). For a recording  
908 session of 10 mins, approximately 24 GigaBytes of image data were stored, spreading across 6  
909 mpTIFF video files. The acquisition software was configured to buffer all frames in computer RAM to  
910 optimize speed. At the conclusion of the GCaMP6f imaging session, we imaged tdTomato  
911 fluorescence for  $\sim 10$  seconds (50 frames at 20ms or 200ms exposure per frame) to identify GCaMP  
912 expressing interneurons.

913  
914 Optogenetic laser equipment and protocol:

915 A 635 nm 200mW red diode laser (Shanghai Laser Optics and Century Co.) was coupled to the  
916 imaging scope via an optical fiber (BFL48-200, Thor Labs, Newton, NJ) with the fiber tip focused to  
917 illuminate the entire imaging window from above. Laser illumination was under TTL control using the  
918 image acquisition software HC Image Live (HC Image Live; Hamamatsu; Boston, MA). Briefly, a 15ms  
919 pulse was initiated 10ms after "camera readout" concluded, and another image frame capture could  
920 not begin until a minimum of 5ms had passed since the laser illumination TTL pulse ended. The  
921 average sampling rate across optogenetic sessions was slower ( $63.4 \pm 0.4$  ms/sample ( $\pm$  SEM) for  
922 the Chrimson and control sessions) due to the requirements of a 15ms laser pulse in the imaging loop.

923  
924 Prior to each imaging session, laser output was adjusted to 10mW at the height of the imaging  
925 cannula window using a light meter calibrated for 635nm wavelength (PM100D, Thor Labs, Newton,  
926 NJ). Optogenetic recording sessions were identical to other imaging sessions described earlier, but  
927 with 13-23 laser stimulation trials. Laser trials occurred randomly within a session, but each trial was  
928 separated by  $40 \pm 15$  seconds. A laser trial consisted of 100 light pulses, 15ms per pulse, delivered  
929 between imaging exposures (at  $\sim 15$  Hz). All four groups of animals underwent two recording sessions  
930 with 3-7 days between sessions.

931  
932 Movement data acquisition:

933 The spherical treadmill was constructed following the design of Dombeck et al.<sup>51</sup>. Briefly, the treadmill  
934 consisted of a 3D printed plastic housing and a Styrofoam ball supported with air. Movement was  
935 monitored using two computer USB mouse sensors affixed to the plastic housing at the equator of the  
936 Styrofoam ball. Each sensor was mounted 3-4mm away from the surface of the ball, and 78 degrees  
937 apart. The x- and y-surface displacement measured by each sensor was acquired using a separate  
938 computer running a Linux OS (minimal CentOS 6). A simple multi-threaded python script was used to  
939 send packaged  $\langle dx, dy \rangle$  data at 100Hz to the image acquisition computer via a RS232 serial link.  
940 Packaged motion data were received on the imaging computer using a MATLAB script, and

941 synchronized to each acquired frame. Because of a delay in initiating movement sensor data capture,  
942 we excluded the first 5 seconds of the motion data (following interpolation) and imaging data (following  
943 event identification) from the beginning of every recording session in the subsequent analysis.  
944

#### 945 **ROI Calcium Trace Processing and Calcium Event Characterization**

##### 946 Image pre-processing: contrast enhancement, motion correction and baseline subtraction:

947 Image frames underwent several pre-processing steps prior to trace extraction<sup>52</sup>. Briefly, we first  
948 applied homomorphic filtering to each image frame to enhance contrast, and then performed motion  
949 correction using cross-correlation between a given image frame and a reference frame. The reference  
950 frame was updated by sequential addition of each corrected frame. Baseline subtraction was  
951 performed using a two-step process. We first identified the minimum fluorescence for each pixel  
952 across the first 2047 frames of each recording session (~96 seconds), and spatially convolved the  
953 minimum fluorescence throughout the whole image field to determine the background value for each  
954 pixel. We then subtracted this value from each frame. In the second step, we subtracted the average  
955 intensity of pixels with the lowest dynamic range within the first 2047 frames of the recording sessions  
956 from all pixels within a frame, and set the lower pixel value boundary to 0. Each frame was then  
957 converted to a uint8 file format.  
958

##### 959 Region of Interest (ROI) identification and ROI $\Delta F/F$ trace extraction:

960 Several semi-automatic algorithms have been developed for ROI identification<sup>52-54</sup>. However, we found  
961 that these algorithms were not sufficiently robust in identifying all neurons in the striatum. Thus, we  
962 manually selected ROIs based on morphology using a circle with a radius of 6 pixels (7.8 microns),  
963 from single frame representations of the recording session. To identify the PV or CHI cells, we first  
964 processed the tdTomato video as that described in image pre-processing above, but without  
965 background subtraction. We further smoothed each image with a spatial filter (a square spatial  
966 Gaussian filter of width 5 pixels and a standard deviation of 0.8). Images were then thresholded to  
967 reveal the brightest 5% pixels that correspond to PV and CHI cells.  
968

969 ROI Fluorescence was calculated as the average intensity across all pixels in the circle. In instances  
970 when a small number of pixels overlapped between two ROIs, the overlapping pixels were only  
971 assigned to one of the labeled ROIs. We interpolated fluorescence time series, so that consecutive  
972 data were 0.0469 seconds apart, using MATLAB function: `interp1(*, 'pchip')`.  $\Delta F/F$  was calculated as  
973 the fluorescence at each time point minus the mean, and then divided by the mean.  
974

##### 975 Calcium event identification:

976 Two types of calcium events were detected. One type we considered “transient”, with a fast rise and  
977 decay, and others we considered “slow”, with a delayed rise and long decay. Each cell was first  
978 assigned to one of these two categories through manual inspection. We found 1.9% of neurons had  
979 both transient and slow calcium event characteristics. These neurons were excluded from further  
980 analyses. To identify individual calcium events,  $\Delta F/F$  calcium traces were first smoothed using a  
981 moving average of 21 points, scaled by a value of 1.05. We then detrended the  $\Delta F/F$  calcium traces  
982 by subtracting a local minimum value for each time point, defined as the minimum value within a  
983 radius of 500 data points of the smoothed trace, similar to Jia et al<sup>55</sup>. For “transient” calcium events,  
984 we identified all points with fluorescence exceeding 3 standard deviations above the mean computed  
985 from the non-event portion of the trace. We iteratively performed this step, where the points above  
986 threshold were excluded from future iterations. Consecutive points above this threshold were then  
987 grouped as a putative event, plus one data point before the onset. If the addition of the single data  
988 point resulted in two overlapping putative events, they were grouped into one event. Calcium events  
989 overlapping with the start or the end of a recording session were discarded. Finally, only events with  
990 maximum amplitudes exceeding 5 standard deviations of the baseline were considered a calcium  
991 event.  
992

993 For “slow” calcium events, we further smoothed “detrended  $\Delta F/F$ ” with the MATLAB function

994 “sgolayfilt”, with an order 3 polynomial, and 101 data points. We then identified all points using the  
995 same methods as described for transient calcium events, except using a threshold of 2 standard  
996 deviations above the mean.

997

998 Laser induced calcium events, manually identified as those coincident with laser onset have durations  
999 between the transient and the slow calcium events. For these events, we first high-pass filtered the  
1000  $\Delta F/F$  calcium traces at  $\sim 2e-05$  Hz (MATLAB commands “butter” with arguments 2 and 0.0005, and  
1001 then “filtfilt” for zero-phase filtering). We then identified all points using the same methods as  
1002 described for transient calcium events, using a threshold of 2 standard deviations above the mean.

1003

1004 After removal of the first five seconds of the fluorescence traces due to motion artifact as described  
1005 previously, events continuous with the beginning of the shortened session were not considered for  
1006 further analysis.

1007

#### 1008 Calcium event property calculation:

1009 To characterize calcium events from each cell type, we first subtracted the baseline, calculated as the  
1010 mean of the 20 data points before each event onset from the event time series. To estimate area  
1011 under the curve for transient calcium events, we further smoothed these events with a 21 point moving  
1012 average filter before baseline subtraction. Rise time was calculated as the time from calcium event  
1013 onset, the first data point of a calcium event, to the peak. To compute area under the curve, peak  
1014 widths, and calcium event amplitude, we added one extra data point to each event after event  
1015 termination. To compare calcium event properties between high- versus low-speed time periods,  
1016 calcium events were first averaged for each ROI, and then compared across all neurons. ROIs that did  
1017 not have an event in either low or high-speed time period were excluded from the respective statistical  
1018 testing.

1019

#### 1020 Deconvolution of calcium traces:

1021 Deconvolution occurred by labeling the rising phase of a calcium event, including the event maximum  
1022 as ones (active regions), and the rest zeroes. Deconvolved calcium traces were used in Figures 2H,  
1023 4D-E, 5A-C, and 7C-F.

1024

### 1025 **Movement Data Analysis**

#### 1026 Linear velocity calculation:

1027 We first calibrated the spherical treadmill by pinning the two sides of the ball at the equator for  
1028 physical distance, or at the top and the bottom for rotation. Linear velocity in perpendicular X and Y  
1029 directions is calculated as:

1030

1031

$$X = \frac{L - R \cos \theta}{\cos(\frac{\pi}{2} - \theta)}$$

1032

1033

$$Y = R$$

1034

1035 Where L is the vertical reading from the left sensor, R is the vertical reading from the right sensor, and  
1036  $\theta$  is the angle between the sensors (78 degrees). X or Y values exceeding 100 cm/s were excluded.  
1037 Then, X and Y values were interpolated at 21.3Hz as fluorescence signals, and linear velocity D was  
1038 computed as:

1039

1040

$$D = (X^2 + Y^2)^{\frac{1}{2}}$$

1041

#### 1042 Rotational velocity calculation and directional preference:

1043 To compute rotational velocity, lateral readings from both computer sensors were first converted to  
1044 radians/sec, and then averaged. Readings corresponding to angular velocities exceeding a magnitude

1045 of 15 radians/s were excluded. Readings were then converted to angular displacement, interpolated at  
1046 21.3Hz, and then converted to rotational velocity.

1047  
1048 Identification of sustained periods of movement with high and low linear velocity:

1049 We identified periods of high versus low linear velocity similarly to Barbera et al.<sup>12</sup>. Briefly, we first  
1050 smoothed linear velocity traces using a 1.5Hz low-pass filter (MATLAB commands “butter” and then  
1051 “filtfilt” for zero-phase filtering). Periods of high speed movement were identified as the time periods  
1052 with linear velocity exceeding 5 cm/s for more than 2 seconds (43 data points). Similarly, periods of  
1053 low speed movement were those where linear velocity remained below 1 cm/s for more than 2  
1054 seconds. Periods overlapping with the beginning or end of the session were excluded.

1055  
1056 Movement onset, offset and peak velocity identification:

1057 To identify movement onset, offset, and peak velocity, we used the unsmoothed linear velocity trace.  
1058 To identify movement onsets, we first identified acceleration transition points as those when linear  
1059 velocity rose to above 5cm/s. A movement onset was then defined as the second data point prior to  
1060 the transition points where velocity remained  $\leq 5\text{cm/s}$  for at least  $\sim 500\text{ms}$  (10 data points) before, and  
1061 exceeded 40 cm/s at some point within  $\sim 1000\text{ms}$  (20 data points) after. To identify movement offsets,  
1062 we first identified deceleration transition points as those when linear velocity decreased to below  
1063 5cm/s. Movement offset was defined as the point before the deceleration transition point where  
1064 velocity exceeded 15 cm/s within  $\sim 500\text{ms}$  before, and also remained  $\leq 5\text{cm/s}$  for at least  $\sim 500\text{ms}$  after  
1065 the transition point. Peak velocity was identified as local maximum using the MATLAB function  
1066 “findpeaks”, which were then thresholded at 55 cm/s. To identify rotational onsets, we identified the  
1067 acceleration transition point when absolute rotational velocity increased to above 0.5 radians/s. A  
1068 rotation onset was then identified as the second data point prior to the transition point where velocity  
1069 remained  $\leq 0.5$  radians/s for at least  $\sim 500\text{ms}$  before, and also exceeded 2 radians/s within  $\sim 1000\text{ms}$   
1070 after. Movement onsets and offsets that extended beyond the length of the session were truncated to  
1071 the beginning and end of the session.

1072  
1073 **Relationship between calcium activity and movement**

1074  
1075 Movement triggered calcium fluorescence:

1076 Population movement-triggered fluorescence was computed on  $\Delta F/F$  values from each neuron over  
1077 100 time points (Figures S4 and S7), or 200 time points (Figures 2D and 2F), on either side of each  
1078 movement event (*onset, offset and peak velocity*). All recording sessions from all mice were used.  
1079 Error bar plots were plotted neuron-wise, and the value for each neuron is the mean of the 40 data  
1080 points before or after each movement event. These neuron-wise averages and statistics were  
1081 computed after first identifying and averaging windows of 50 time points on either side of each event  
1082 for each neuron.

1083  
1084 To compare the timing of maxima in fluorescence around velocity peaks, we examined a window of  
1085 100 time points before and after each velocity peak. We constructed a mixed effects model, averaging  
1086 fluorescence values over a 6 second window for each neuron, grouped in 1-second bins, beginning 2  
1087 seconds before each velocity peak. Models were fitted using maximum likelihood, MATLAB function  
1088 “fitlme”, and p-values were determined using the residual degrees of freedom. The full mixed-effects  
1089 model appeared as, in Wilkinson notation:

1090  
1091 Fluorescence  $\sim 1 + \text{Time} * \text{Cell\_type} + (1 | \text{Event\_ID}) + (1 | \text{Cell\_ID}) + (1 | \text{Mouse})$

1092  
1093 Where, “Time” refers to the time bin, “Cell\_type” to the genotype of the respective cell. Random  
1094 intercepts were included for each mouse (“Mouse”), for each individual cell (“Cell\_ID”), and for each  
1095 peak-velocity event (“Event\_ID”). In these models, we identified the time period of maximum  
1096 fluorescence by finding the bin with the highest expected value. After identifying a significant  
1097 interaction term between Time and Cell\_type, we broke up this model by cell type. P-values were

1098 corrected using the Benjamini-Hochberg procedure after pooling all p-values across all time bin  
1099 comparisons to baseline in all models (15 total comparisons) <sup>56</sup>.

1100  
1101 To determine whether or not a neuron was significantly positively, or negatively, modulated by speed  
1102 or rotation, we used one-tailed rank-sum tests to compare the fluorescence values of the 40 data  
1103 points before versus after an identified movement onset, using a p-value threshold of 0.025. Neurons  
1104 were then assigned to one of the four categories: speed-only, rotation-only, conjunctive, and neither.

1105  
1106 To assess the proportion of neurons positively modulated in the 500ms before movement onset  
1107 (Figure S2H), we analyzed each neuron's average fluorescence during baseline time points (-950 to -  
1108 500 ms before movement onset) to the pre-movement period (-450ms to movement onset) using one-  
1109 sided rank-sum tests with p-value cutoffs of 0.025. To quantify the time difference between PV and  
1110 MSN fluorescence increases at movement onset, we identified the first time point in the movement  
1111 onset window when averaged  $\Delta F/F$  trace of all PV cells or MSNs of that session exceeded a  $\Delta F/F$  of  
1112 0.02. This represents a moderate increase in  $\Delta F/F$  from baseline. The latency between PV cells and  
1113 MSNs responses were then calculated across all sessions where this criterion was met (n=13  
1114 sessions in total).

1115  
1116 For proximity tests (Figure S9B-C), we first computed the proportion of neurons within 100um of a  
1117 neuron that belongs to a certain category. We then compared the proportions surrounding neurons of  
1118 a given category to proportions surrounding all other neurons. These two sets of proportions were  
1119 compared neuron-wise. Neurons without any neighbors were excluded from analysis, and only MSNs  
1120 were used for this analysis.

1121  
1122 Neuron pair-wise asymmetric correlation analysis:

1123 An asymmetric correlation coefficient from neuron A to B was calculated as described previously using  
1124 deconvolved calcium traces<sup>12,33</sup> using the equation:

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1126 
$$r_{A \rightarrow B} = \frac{n_{A \cap B}}{n_A}$$

1127  
1128 That is, the number of events in the deconvolved traces in neuron A that overlapped with events in  
1129 neuron B were divided by the total number of events in neuron A. Pair-wise correlation between  
1130 neurons A and B was then calculated as the averaged correlation coefficients in both directions, A to B  
1131 and B to A.

1132  
1133 To compute baseline correlation coefficients, the deconvolved traces were randomly shifted circularly  
1134 and uniformly over the entire length as discussed by Barbera et al.<sup>12</sup>. This process was repeated 5000  
1135 times for each neuron pair, and the correlation coefficients of these shifted traces were used to  
1136 determine significance using one-sided p-values of <0.05 (Figure 4). Correlations with deconvolved  
1137 traces with no events were labeled as NaN, and they were considered not significant. The random  
1138 values in the plots (Figure 4D-E) are the mean shuffled correlation coefficients for each neuron pair.

1139  
1140 To compare the proportions of neuron pairs with significant correlations that were spatially close (<100  
1141 um) or spatially far ( $\geq 750$  and <1500 um), we constructed a generalized linear model:

1142  
1143  $Is\_significant \sim 1 + Cell\_pair\_type * Distance$

1144  
1145 where "Is\_significant" is a random variable corresponding to whether or not the particular pair is  
1146 significantly correlated, "Cell\_pair\_type" is a categorical variable corresponding to "MSN-CHI", "MSN-  
1147 MSN", or "MSN-PV", and "Distance" is an indicator variable that equals 1 if the pair is spatially far. We  
1148 used a binomial distribution with an identity link function. To compare differences between the time  
1149 periods, we compared the interaction terms for all three pair-types.

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For Figure 4A, we used the complete-linkage clustering function of MATLAB (linkage(\*,'complete'), followed by cluster(\*, 'cutoff', [distance cutoff], 'criterion', 'distance'), where [distance cutoff] was equivalent to 100 um). We statistically compared within-cluster and outside of cluster groups by treating each directional measure of asymmetric correlation as independent, and computed the rank-sum between the directional within-cluster and out-of-cluster groups. In this case, a correlation coefficient from A→B, where B has no events, has a correlation coefficient of 0, and in the opposite case (from B to A), of NaN.

For Figure 5A, the number of neuron pairs with significant correlations divided by the average number of neuron pairs with significant correlations in all bins, were plotted over their anatomical distance. The distance between pairs of neurons centroid(A) – centroid(B) or centroid(B) – centroid(A), was assigned randomly.

Neuron pair-wise Pearson's correlation analysis:

Because of the sparseness of calcium events during periods of low speed movement, we used Pearson's correlation (Figure 4B and 4C). During each motor bout, the correlation coefficients were determined between each MSN pairs, and then averaged across all motor bouts for each session. Shuffled traces (1000 times) were created by concatenating ΔF/F values from high or low velocity time periods, for each ROI, and circularly shifting each concatenated trace by a random, uniformly-distributed value, over the length of the trace. Then, the shuffled traces were broken into segments equal to the length of the original high speed or low speed segments. Correlation values for each pair during high and low time periods were averaged over all 1000 repetitions.

Correlation of MSNs subsets with distinct CHI populations:

To examine whether the same set of MSNs was correlated with CHIs that were positively or negatively modulated by movement (Figure S7G), we analyzed sessions with at least 1 positively movement-modulated CHI and 1 negatively movement-modulated CHI (n=8 sessions). To test whether the MSNs correlated with the positively modulated CHIs and the MSNs correlated with negatively modulated CHIs belong to different communities, we calculated the Jaccard index of the two sets of MSNs as follows:

$$Jaccard\ Index = \frac{MSN_+ \cap MSN_-}{MSN_+ \cup MSN_-}$$

In this equation, "MSN<sub>+</sub>" represents the set of MSNs correlated with at least one positively modulated CHI using the pair-wise asymmetric correlation analysis, and "MSN<sub>-</sub>" represents the set of MSNs correlated with at least one negatively modulated CHI. To test whether the MSNs correlated with these two types of CHIs were non-overlapping, we performed a permutation test (Figure S7G). Briefly, we first identified MSNs that exhibit significant correlation with any CHI. We then performed a bootstrapping procedure 5000 times without replacement. For each CHI, we randomly assigned from this pool of MSNs a number of MSNs equal to the number correlated with the given CHI. Then, a Jaccard Index was computed for each iteration.

Neuron-triggered fluorescence analysis:

Neuron-triggered fluorescence was determined using deconvolved calcium traces (Figure 7A, B). In these analyses, triggering events are the time point immediate before calcium event onset. Unless otherwise noted, triggering events within 50 time points of the beginning or 51 time points of the end of the trace were not included for analysis. We centered fluorescence values for each MSN around each triggering event, and compared changes in ΔF/F values during the 10 data points (~500ms) after each event using a rank-sum test (ROI-event-wise). Standard error of the mean across the entire ~5 second interval was calculated with Welford's algorithm<sup>57</sup>.



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Neuron-triggered speed and rotation:

To analyze changes in velocity following an event in a given cell type, we constructed a mixed-effects model using the MATLAB function “fitlme” on the normalized data (data was normalized for each cell type to the data point coincident with event onset, by subtracting and then dividing). In Wilkinson-notation the model was:

$$\text{Velocity} \sim 1 + \text{Time} * \text{Cell\_type} + (1 | \text{Mouse}) + (1 | \text{Cell\_ID})$$

“Time” is a categorical variable corresponding to time bin. “Cell\_type” corresponds to PV, MSN, or CHI in Figure 2H, or positively or negatively modulated cells in Figures S4H and S7H. We accounted for variance in intercept introduced by each mouse (“Mouse”) and each individual cell (“Cell\_ID”). We broke up the model by cell type in order to test the effect of time by removing all terms relating to cell type, and created smaller models. P-values were corrected for the collections of p-values obtained from each of the smaller models using the Benjamini-Hochberg procedure.

Neuron-triggered rotation (Figure S8C) was analyzed in a similar way, using the absolute value of the rotational velocity in place of speed.

To determine the rate at which speed changed following a calcium event (Figure S2I), we first computed the normalized event triggered speed for each neuron. We then used the normalized speed values for each neuron between 0 and 1.5 seconds after each calcium event to construct a series of simple linear models. These models had predictors including only an intercept and the instantaneous time values, from which we obtained a slope value for each neuron. We compared slope values between the three neuron types using the following linear mixed-effects model:

$$\text{Coefficients} \sim 1 + \text{Cell\_type} + (1 | \text{Mouse})$$

Here, “Coefficients” represent the slopes of the simple linear models, “Mouse” and “Cell\_type” are as described previously.

Neuron co-activation analysis:

Co-activation was calculated using deconvolved calcium traces. We calculated the total number of co-active MSN pairs around each triggering event, defined as the data point before calcium event onset, and the total number of possible MSN pairs across all triggering events, for all neurons across all recording sessions. We then calculated the proportion of co-active pairs over all events out of total pairs over all events.

To compare across neuron types (Figure 7C), we averaged the two data points following each triggering event subtracted by the value at the triggering point, and the errors of the two data points were propagated. We then used the z-tests to compare between cell types. Shaded error bar plots

(Figure 7C) are standard error estimates computed from:  $\sqrt{\frac{p(p-1)}{n}}$ , where p is the probability of the neuron pair being coactive at that time point, and n is the number of total pairs over all events. Error bar plots were centered at triggering onset.

To detect changes in movement speed around MSN-MSN peak co-activation (Figure 7F), we summed the deconvolved traces for all MSNs recorded in each session, and identified MSN-MSN co-activation as when the summed activity exceeded 2 standard deviations above the mean. Peak co-activation within each co-activation period is identified as the local maximum. If two local maxima of the same amplitude occurred in the same co-activation period, the first peak was used. Co-activations within 100 time points of the beginning or end of a recording session were excluded.

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Calculation of predictor performance using regression analysis:

To estimate how each cell type predicted movement or population MSN activity (Figure 6), we built linear models, and assessed the model performance using correlations similar to the time-series forecasting cross-validation method described by Hyndman and Athanasopoulos<sup>58</sup>, and that used by Parker et al.<sup>11</sup>. We used the framework of partitioning a time series into equally-sized, temporally continuous sets for training and testing models. Data of the entire session was separated into 10 consecutive and equally sized segments. Linear model parameters were determined using 9 segments, and then the values for the last segment were predicted using these parameters. The Pearson correlation coefficient between the predicted values and the actual values of this last segment was used to assess predictive ability. This process was repeated 10 times, and the Pearson correlation coefficients were averaged for a given predictor. To plot predicted values for both summed MSN fluorescence and speed, predictors were determined using the entire session.

To predict linear velocity using MSN, PV, CHI cells (Figures 6D, 6H), we first binned  $\Delta F/F$  traces and linear speed to 0.1 second bins.  $\Delta F/F$  traces were then combined, and their average Z-scored  $\Delta F/F$  value was used as the predictor variable. Then, this predictor along with an intercept was tested using our modified cross-validation. To estimate the predictor performance of MSN subpopulations, a random subsample of MSNs with equal number of simultaneously recorded interneurons in the session was used. This process was repeated 5000 times, and the average value was used as the predictor performance for each recording session. For single interneurons and single MSNs, the average value across all single neurons was used as the predictor performance for each session. For sessions in which there was only 1 interneuron, the mean value over all single MSNs was used as the value of the MSN subpopulation.

To predict MSN population fluorescence (Figures 6B, 6F), we used summed population MSN fluorescence as the response variable. Each predictor was binned as above. To estimate the predictor performance of MSN subpopulations, random subsample of MSNs with equal number of simultaneously recorded interneurons in the session was used, and the MSN population response was calculated without the sub-sampled MSNs. This process was repeated 5000 times and the average value was used as the predictor performance. An intercept term was also included in each model. These analyses were also performed using only positively- or negatively-modulated interneurons (Figures S4I and S7I), for sessions that had at least 1 positively and 1 negatively modulated interneuron.

To compare predictor performance (Figure S12), we incorporated cell type into a simple, multivariate mixed-effects model:

$$\text{Velocity} \sim 1 + \text{Fluorescence} + \text{Cell\_type:Fluorescence} + \text{Velocity\_lag1} + \text{Velocity\_lag2} + (1|\text{Session}) + (1+\text{Fluorescence}|\text{Cell\_ID})$$

“Cell\_type” is the genotype of the predicting neuron, “Fluorescence” is the z-scored average fluorescence over 0.1 second bins, “Session” is a session ID corresponding to the fluorescence trace, and Cell\_ID corresponds to each cell. Velocity is the z-scored, binned velocity, and Velocity\_lag1 and Velocity\_lag2 are analogous terms accounting for the autocorrelation of Velocity values. For the complete analysis, data from all 28 sessions were used, and MSNs were used as the reference cell type variable. In order to plot this analysis, the above mixed effects model was also run separately for each session with at least 1 interneuron (10 CHI sessions and 17 PV sessions), and the coefficient of the interaction term that corresponded to Cell\_type:Fluorescence was plotted. Because each session was modeled separately, the (1|Session) term was excluded from these models. Each dot in this plot corresponds to a coefficient from each of these smaller models, and the lines correspond to the coefficient values from the larger model.

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## Optogenetic activation induced changes in calcium activity and movement

Laser-triggered Changes in calcium fluorescence, calcium event probability, and neuron co-activation:  
ROI identification, trace extraction, and movement data processing were largely the same as described above for nonoptogenetic sessions. One session displayed two brief (~2 and ~14 seconds) large motion artifacts during part of the recording, and we replaced the affected data points with interpolated values. Another session displayed a baseline shift toward the beginning (~50 seconds), these data were excluded from further analysis.

To examine laser induced fluorescence change in MSNs, PV cells, and CHIs (Figure S11), we aligned the  $\Delta F/F$  of each cell to laser illumination period. The difference in fluorescence following laser stimulation onset (20 time points) versus the 20 time points immediately prior to laser onset was compared cell-wise.

To examine the change in calcium event probability, we used deconvolved calcium traces (Fig. S11E-F). Event probability was aligned to laser onset for each neuron, and we normalized the event probability by averaging the across all sessions the respective neuron type in the corresponding mice.

To examine changes in neuron co-activation (Figure 7D-E), we first computed MSN-MSN co-activation at the onset of MSN triggering events during non-laser time period by averaging the two time points immediately following each event onset. Events within 50 time points of the beginning of the session or the end of the session were not included. To compute the mean co-activation of MSN-MSN pairs during each laser period, all neuron pairs were combined after normalizing co-activation values by dividing by the mean co-activation during non-laser epochs. This was repeated for non-laser epochs as well, in Figure 7E. To plot MSN-triggered co-activation during non-laser period (7D), we analyzed the 50 time points on either side of the triggering event after discarding events within 100 time points of the beginning or end of a recording session. To plot the laser-triggered MSN-MSN co-activation time series, we plotted the mean and the standard error of the mean across all time points after normalizing via the previously mentioned Welford's algorithm<sup>57</sup>.

For MSN-triggered and laser-triggered co-activation for both Chat-Chrimson and PV-Chrimson mice, we used pairwise z-tests and the sample means and sample standard errors of the means as estimates to test significance. Figure 7E was plotted event-pair wise with event-pairs included from all sessions.

### Laser-triggered changes in movement velocity:

We calculated normalized movement velocity to the movement speed immediately before each laser onset, and then subtracted 1 to represent changes. To test whether or not there was a significant change in speed during laser activation, we used a linear mixed-effects model on the normalized velocity. Our models appear as follows:

$$\text{Velocity} \sim 1 + \text{Time} + (1|\text{Mouse}) + (1|\text{Event\_ID}) + (1+\text{TimeBin}|\text{Event\_ID}) + (1+\text{Mouse}|\text{Event\_ID})$$

"Event\_ID" here corresponds to unique laser epochs. We tested for a significant main effect of time using an ANOVA on this model, and computed post-hoc t-tests for each time bin versus the time bin at time t=0. P-values were corrected within each model using the Benjamini-Hochberg procedure. We then separated data into those events occurring only in sustained low-speed time periods, and then repeated the analysis on these smaller data sets. Both Figures 3B and 3C are plotted event-wise.

### Laser-triggered changes in direction:

To distinguish between vigorous directional change from small rotational velocity changes associated with balancing behavior, we set a threshold where directional changes exceeding 1 radian/second

1358 were counted as vigorous directional changes. To quantify whether there were more vigorous  
1359 directional changes during laser-on time periods versus laser-off time periods, we identified all  
1360 directional changes for each session, and compared the probability of such changes during each  
1361 laser-on versus laser-off time period across all sessions. We compared the difference in the  
1362 probabilities of directional changes during the rising and falling phases of PV or CHI calcium events  
1363 (including events continuous with the beginning of the session), versus other time periods (Figure  
1364 S8A,C), neuron wise.

1365  
1366 *Correlations of MSN activity with laser-induced PV activation*

1367 To examine the uniformity of PVs influence on MSNs, while the population of PV interneurons was  
1368 activated optogenetically, we calculated the number of MSNs that had significant asymmetric  
1369 correlation coefficients during optogenetic stimulation in PV-Chrimson-TDT mice. This analysis was  
1370 identically to the neuron pair-wise asymmetric correlations described above in the non-optogenetic  
1371 mice. To compute significance, we randomly and circularly shifted the deconvolved MSN traces 5000  
1372 times to generate a distribution of randomly shifted MSN traces. We then computed the asymmetric  
1373 correlation coefficients between the randomly shifted MSN traces and laser-on time periods. To test  
1374 whether or not PV optogenetic activation homogeneously affected the MSN population (Figure S4G),  
1375 we randomly sampled with replacement 10000 times the binary values as to whether or not an MSN  
1376 was significantly correlated with laser-on time periods. We compared the proportion of MSNs  
1377 significantly correlated to zero by taking the total number of random samples with no significantly  
1378 correlated values and dividing by the total number of samples (10000). We compared the proportions  
1379 of MSNs to a value of one analogously.

1380  
1381 **Histology and quantification:**

1382 At the end of the experiments, all mice were transcardially perfused and tissue was processed to  
1383 confirm GCaMP6f as well as tdTomato expression, and cannula placement. Mice were perfused with  
1384 30 mL 0.01M phosphate buffered saline (Fisher Scientific, BP2944-100, Pittsburgh, PA), followed by  
1385 30 mL 4% paraformaldehyde (Sigma Aldrich, 158127, St. Louis, MO). Brains were carefully removed  
1386 and post-fixed overnight in 4% paraformaldehyde before being transferred to a 30% sucrose solution.  
1387 Brains were sectioned horizontally at a thickness of 40 $\mu$ m with a freezing microtome (CM 2000R;  
1388 Leica). Tissue sections were collected throughout the striatum. A subset of sections (4-6 sections per  
1389 animal) were stained with antibodies against PV (rabbit anti-PV, SWANT PV25 1:1000) or Chat (goat  
1390 anti-Chat antibody, Millipore AB144P 1:500), followed by Alexa Fluor 633 goat anti-rabbit secondary  
1391 antibody for PV staining (Invitrogen A11011, 1:1000) or Alexa Fluor 633 donkey anti-goat secondary  
1392 antibody for Chat staining (Life Technologies, A21082 1:200). Antibodies and dilution concentrations  
1393 were previously reported<sup>59,60</sup>. Briefly, sections were rinsed with 0.05 M Tris-HCL-buffer (Tris, pH=7.6),  
1394 followed by 60 mins rinse in blocking buffer containing 5% serum and 0.2% Triton. Sections were  
1395 incubated for 24 hours with primary antibody. Sections were then rinsed 3X at 10 min each in Tris-  
1396 HCL and then incubated with the secondary antibody for 2 hours. Sections were rinsed again and then  
1397 mounted on gelatin-coated slides using anti-fade mounting media (Vectashield H-1400).

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1399 Confocal images were taken on an Olympus FV1000 scanning confocal microscope using a 60X  
1400 water immersion lens. All images were comprised of Z-stacks taken at 5  $\mu$ m intervals throughout the  
1401 40 $\mu$ m slices. Stacks were taken from horizontal sections as near as possible to the bottom of the  
1402 imaging window and all imaging stacks were taken within 1 mm of the imaging plane. Areas were  
1403 chosen to include similarly dense tdTomato cell counts across animals by an experimenter blinded to  
1404 the animal strain. To confirm targeting specificity, each tdTomato+ cell was categorized as immune-  
1405 positive or immune-negative for the antibody stain. We also quantified the number of immune-positive  
1406 cells from each stack that were tdTomato+ to estimate the infection efficiency near the imaging  
1407 window. We analyzed 2-4 non-overlapping confocal stacks from 4-6 slices per animal from a large  
1408 subset of animals that made up our GCaMP imaging dataset (n=7 PV-Cre) and (n=7 Chat-Cre mice).  
1409 Cell counts were pooled across slices stained for the same marker for each animal, and averaged to  
1410 produce a single data point for quantification.

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## Statistical Tests

Sample sizes were estimated based on neuron yields from pilot studies and published anatomical descriptions of PV and CHI population densities using G\*Power 3.1.9.2. We applied a two tailed Wilcoxon-Mann-Whitney test utilizing a  $\beta/\alpha$  ratio=1.0, an effect size of 0.5. Additional assumptions included the expectations of 300 neurons/recording and PV and CHI density of 2.5% of all cells. Our sample sizes are also consistent with previous publications of calcium imaging in the striatum<sup>14,38,51</sup>.

Researchers were aware of the animal strain at the time of recording but analysis was done off-line by a researcher that was unaware of cell identity. For histology experiments, areas were chosen to include similarly dense tdTomato cell counts across animals by an experimenter blinded to the animal strain.

Data recording sessions were randomized between PV and Chat animals over the course of the study. Optogenetic stimulations sessions were randomized in Chrimson and tdT control animals with several non-stimulation days occurring between the two sessions. For all calcium imaging sessions, no direct replication was performed although each animal was subjected to calcium imaging 1-3 times and data was collapsed together for analysis. For confocal images, we analyzed 2-4 non-overlapping confocal stacks from 4-6 slices which were pooled together per animal and averaged for quantification.

The detailed statistical tests were described above in related analysis. Briefly, the following statistical tests were used in this study: Chi-square tests, Wilcoxon rank-sum tests, sign-tests, Kruskal-Wallis tests, Friedman tests, Fisher tests, binomial tests, z-tests, and Pearson's correlation. When conducting non-parametric tests we did not make any assumptions of data, including normality. For z-tests, the sample sizes of the distributions were large enough that by the central limit theorem, their means were expected to follow normal distributions. If present in a sign-test, ties were removed from consideration automatically and their presence was noted in the manuscript. All values that evaluated to "NaN" were treated as missing data.

All tests were two-tailed unless noted otherwise. Post-hoc tests were conducted using the MATLAB function "multcompare" following a Kruskal-Wallis or a Friedman test. Use of this function was noted in the manuscript as "Tukey's HSD", as it utilizes for both Kruskal-Wallis and Friedman Tukey-like procedures for non-parametric data.

Data were plotted using neuron-weighted averages unless otherwise noted. Standard error of the mean was computed using the standard formula, based on the assumption of normality of the mean regardless of the shape of the underlying distribution whose mean we describe. Because non-parametric statistics are used throughout the manuscript, estimated standard errors of the mean were shown primarily as a tool for visualization. Unless otherwise noted, box plots were constructed via the MATLAB function "boxplot", and outliers were removed for visualization. Outliers were determined automatically by the "boxplot" function. They were defined as data points that were greater than the third quartile value plus 1.5 times the distance between quartile three and quartile one, or data points that were less than the first quartile value minus 1.5 times the distance between quartile three and quartile one.

Please refer to the Life Sciences Reporting Summary for a detailed description of all statistical tests used and Study Design.

### Data availability statement

All raw data will be available by request.

### Software availability statement:

1464 All source code and custom scripts for analysis will be made available at request.  
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