**Data and code used for making Extended Data Fig. 3**

**Folder: ExtDataFig3a-mpq**

Analysis

* ExtDataFig03\_AveTimeCourse\_OptStim\_Neuron.m: Code for plotting the time course of ΔF/F recorded from dPR1 and TN1A neurite in Extended Data Fig. 3a,h.
* ExtDataFig03\_AveTimeCourse\_OptStim\_Song.m: Code for plotting the time course of and the proportions of pulse and sine song in Extended Data Fig. 3a,h.
* TS\_Img.mat: Time stamps of calcium imaging.
* TS\_OptStimImg.mat: Timings of optogenetic stimulation during calcium imaging.
* ExtDataFig03\_Example\_TimeCourseRaw\_dPR1\_all.m: Code for plotting example traces for dPR1 ΔF/F in Extended Data Fig. 3b.
* ExtDataFig03\_Correlations.m: Code for plotting the histogram of the Pearson’s correlation coefficients between pairs of neurons recorded simultaneously in Extended Data Fig. 3c,j.
* ExtDataFig03\_AveTimeCourseSongTrans.m: Code for plotting calcium signals and song probabilities during song type transitions in Extended Data Fig. 3d,k.
* ExtDataFig03\_BidirRespMod\_Normalized.m: Code for plotting the mean change in ΔF/F after song-type transitions relative to ΔF/F before the transitions in Extended Data Fig. 3e,l.
* ExtDataFig03\_AveTimeCourseSongTransPrePostDur.m: Code for plotting calcium signals and song probabilities during song type transitions separately for short and long song bouts in Extended Data Fig. 3f,m.
* ExtDataFig03\_DecayRateComparison\_PtoS\_OptOffset.m: Code for comparing half decay time of the dPR1 neurite GCaMP signals during pulse-to-sine transitions and after the offset of the song driver stimulation in Extended Data Fig. 3g.
* ExtDataFig03\_Example\_TimeCourseRaw\_TN1SG\_all.m: Code for plotting example traces for TN1A ΔF/F in Extended Data Fig. 3i.
* ExtDataFig03\_Example\_TimeCourseRaw\_dPR1\_IsolatedSong.m: Code for plotting the example trace in Extended Data Fig. 3p.
* ExtDataFig03\_Example\_TimeCourseRaw\_TN1SG\_IsolatedSong.m: Code for plotting the example trace in Extended Data Fig. 3q.

Data/Summary\_GENOTYPE

* EthogramComb.mat: File containing the time course of pulse/sine songs. Row: fly ID; Column: time bins at the resolution of microphone recording (1 kHz).
* EthogramCombImg.mat: Same as EthogramComb.mat but the time resolution of calcium imaging.
* EthogramCombPulseTrain.mat: Same as EthogramComb.mat but the inter-pulse intervals are designated as “pulse.”
* FtimeCourseComb.mat: Mean calcium signals (F) in each ROI and the optogenetic stimulation strength in each trial. F\_comb: Time course of F for each ROI (ROI x Time bins x Blocks). Stim\_comb: Stimulation strength (from 1 to 6) in each trial (Column: block; Row: trial).
* Dataset.csv: A spreadsheet summarizing the imaged ROIs and singing behavior in each experiment.
* SongExplorer: A folder containing audio data and song segmentation results for each recording.
* Transitions.mat: Variables for running ExtDataFig03\_AveTimeCourseSongTrans.m.
* SongTypePrefIndex.mat: Variables for running ExtDataFig03\_BidirRespMod\_Normalized.m.

**Folder: ExtDataFig3no**

Analysis

* ExtDataFig03\_AveTimeCourse\_OptStim\_SelfOpt\_TTX\_Neuron.m: Code for plotting the time course of ΔF/F recorded from dPR1 and TN1A neurons in Extended Data Fig. 3n,o.
* TS\_Img.mat: Time stamps of calcium imaging.
* TS\_OptStimImg.mat: Timings of optogenetic stimulation during calcium imaging.

Data/Summary\_GENOTYPE

* FtimeCourseComb.mat: Mean calcium signals (F) in each ROI and the optogenetic stimulation strength in each trial. F\_comb: Time course of F for each ROI (ROI x Time bins x Blocks). Stim\_comb: Stimulation strength (from 1 to 6) in each trial (Column: block; Row: trial).

**Folder: ExtDataFig1ad**

* IHC\_dPR1: Expression pattern of dPR1 split-Gal4.
* IHC\_TN1A Expression pattern of TN1A split-Gal4.

**Folder: ExtDataFig1b**

* MCFO\_dPR1: Multi-color flip-out image for dPR1 split-Gal4.

**Folder: ExtDataFig1c**

* IHC\_dPR1\_dsx: Images showing the coexpression of Gal4-driven mVenus and dsx. Green: mVenus. Magenta: dsx.

**Folder: ExtDataFig1e**

* MCFO\_TN1A: Multi-color flip-out image for TN1A split-Gal4.

**Folder: ExtDataFig1f-j**

Analysis

* ExtDataFig01\_Example\_TimeCourse\_OptStim\_Song\_dPR1.m: Code for plotting the example song trace during optogenetic stimulation in Extended Data Fig. 1f.
* ExtDataFig01\_TuningCurve\_OptStim\_Song.m: Code for plotting the tuning curves of pulse and sine songs induced by optogenetics in Extended Data Fig. 1g,i.
* ExtDataFig01\_Example\_TimeCourse\_OptStim\_Song\_dPR1.m: Code for plotting the example song trace during optogenetic stimulation in Extended Data Fig. 1h.

Data-Summary

* dataset.csv: A spreadsheet describing the genotype for each microphone recording channel in each experiment.
* EthogramComb\_\*: Files containing the time course of pulse/sine songs. Row: fly ID; Column: time bins.
* EthogramCombPulseTrain\_\*: Files containing the time course of pulse/sine songs. Row: fly ID; Column: time bins.
* Mic\_\*: Flies containing audio data for each experiment.
* OptStim\_\*: Files containing the timings of optogenetic stimulation in each experiment.

**Folder: Fig1k**

* IHC\_22D03LexA\_LexAopCsChrtdT: Expression pattern of 22D03-LexA in a male fly.

**Folder: Fig1l**

* IHC: Expression pattern of 22D03-LexA in a male and a female fly.

**Folder: Fig1m-q**

Analysis

* ExtDataFig01\_GrandAverTimeCourse\_OptStim\_Song\_SongDriver.m: Code for plotting the time course of pulse and sine song during optogenetic stimulation of the song driver in Extended Data Fig. 1m.
* ExtDataFig01\_TuningCurve\_OptStim\_Song\_SongDriver.m: Code for plotting the tuning curves of pulse and sine songs induced by optogenetics in Extended Data Fig. 1m,n.
* BoutLength\_WT.mat: File containing the song bout lengths of a control condition (w1118 crossed to UAS-CsChrimson).
* ExtDataFig01\_BoutLength.m: Code for plotting histograms of pulse and sine song bouts in Extended Data Fig. 1o.
* ExtDataFig01\_GrandAveTimeCourse\_OptStim\_Song\_pIP10.m: Code for plotting the time course of pulse and sine song during optogenetic stimulation of the pIP10 split-Gal4 in Extended Data Fig. 1p.
* ExtDataFig01\_TuningCurve\_OptStim\_Song\_pIP10.m: Code for plotting the tuning curves of pulse and sine songs induced by optogenetics in Extended Data Fig. 1r.

Data-Summary, Data-Summary\_pIP10

* Mic\_\*: Flies containing audio data for each experiment.
* EthogramCombPulseTrain.mat: Files containing the time course of pulse/sine songs. Row: fly ID; Column: time bins.
* StimComb.mat: Stimulation strength (from 1 to 6) in each trial (Column: block; Row: trial).