**Data and code used for making Fig. 1**

**Folder: Fig1a**

Analysis

* Fig01\_Example\_RawTraces\_WT.m: Code for plotting the example song trace in Fig. 1a.
* Mic\_20210923\_02\_1.mat: Audio recording data.

**Folder: Fig1b**

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

**Folder: Fig1cd**

Analysis

* Fig01\_AveTimeCourse\_OptStim\_Song.m: Code for plotting the average time course of pulse and sine song during optogenetic activation of dPR1 and TN1A neurons in isolated male flies in Fig. 1c,d.

Data/Summary

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

**Folder: Fig1f**

* Fig01\_Example\_TimeCourse\_OptStim\_Song\_Intact.m: Code for plotting the example song trace during optogenetic stimulation of the song driver in Fig. 1f.
* Mic\_20210318\_01.mat: Audio recording data.
* EthogramComb.mat: File containing the time course of pulse/sine songs. Row: fly ID; Column: time bins.

**Folder: Fig1g-k**

Analysis

* Fig01\_Example\_dPR1\_Frames.m: Code for plotting example frames of a recording from dPR1 in Fig. 1g.
* file\_00021.tif: Raw imaging data.
* F2d\_20210604\_02.mat: Time course of mean F value in each ROI.
* TS\_Img.mat: Time stamps of calcium imaging.
* TS\_OptStimImg.mat: Timings of optogenetic stimulation during calcium imaging.
* Fig01\_Example\_dPR1\_Frames.m: Code for plotting the time courses of delta F/F for the neuropil of dPR1 (Fig. 1h) and TN1A (Fig. 1k) neurons.
* Fig01\_AveTimeCourseSongTrans.m: Code for plotting calcium signals and song probabilities during song type transitions in Fig. 1i,l.
* Fig01\_BidirRespMod\_Normalized.m: Code for plotting the mean change in ΔF/F after song-type transitions relative to ΔF/F before the transitions in Fig. 1j,m.

Data/Summary\_GENOTYPE

* Dataset.csv: A spreadsheet summarizing the imaged ROIs and singing behavior in each experiment.
* 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.
* 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).
* SongExplorer: A folder containing audio data and song segmentation results for each recording.
* Transitions.mat: Variables for running Fig01\_AveTimeCourseSongTrans.m.
* SongTypePrefIndex.mat: Variables for running Fig01\_BidirRespMod\_Normalized.m.