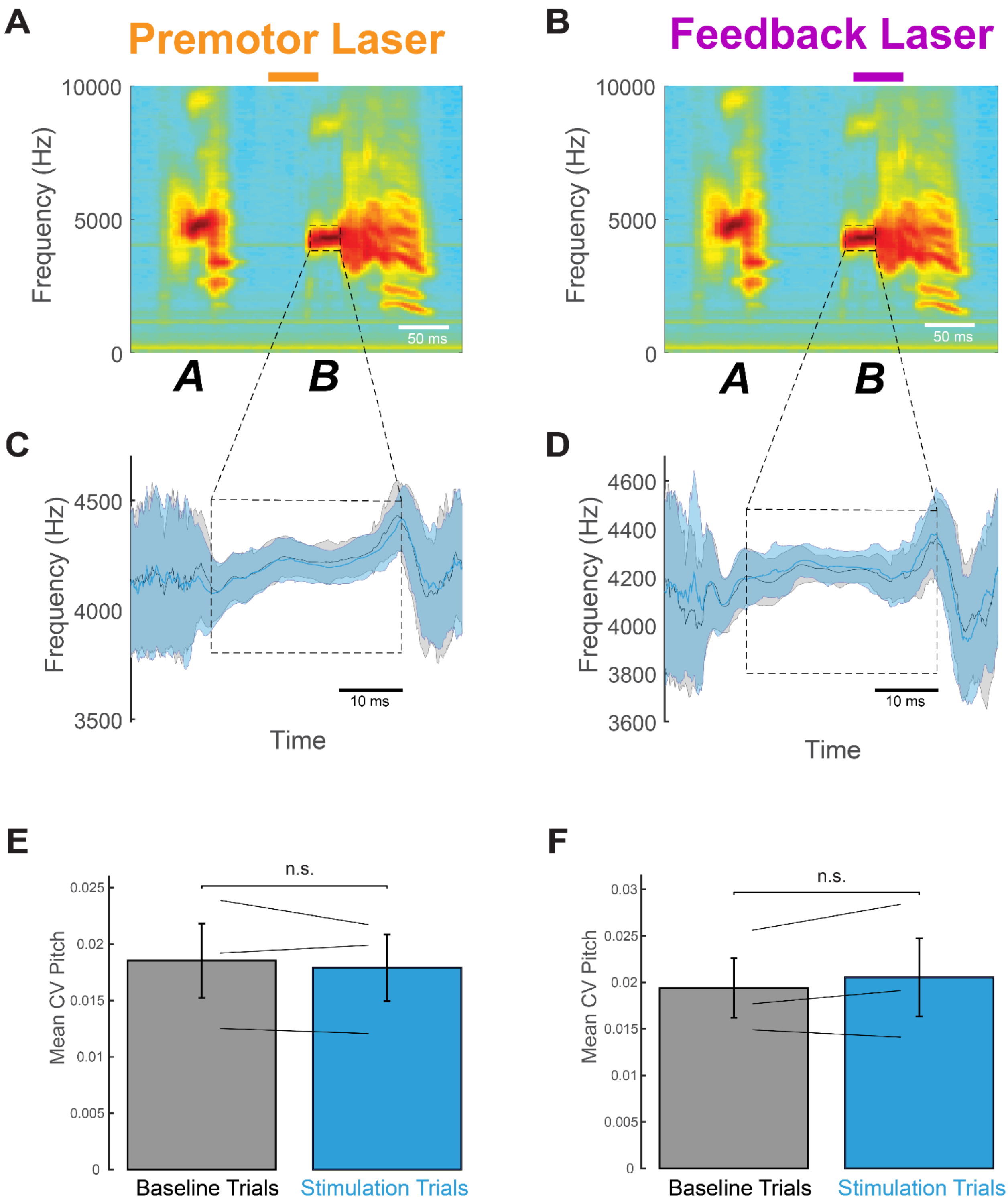
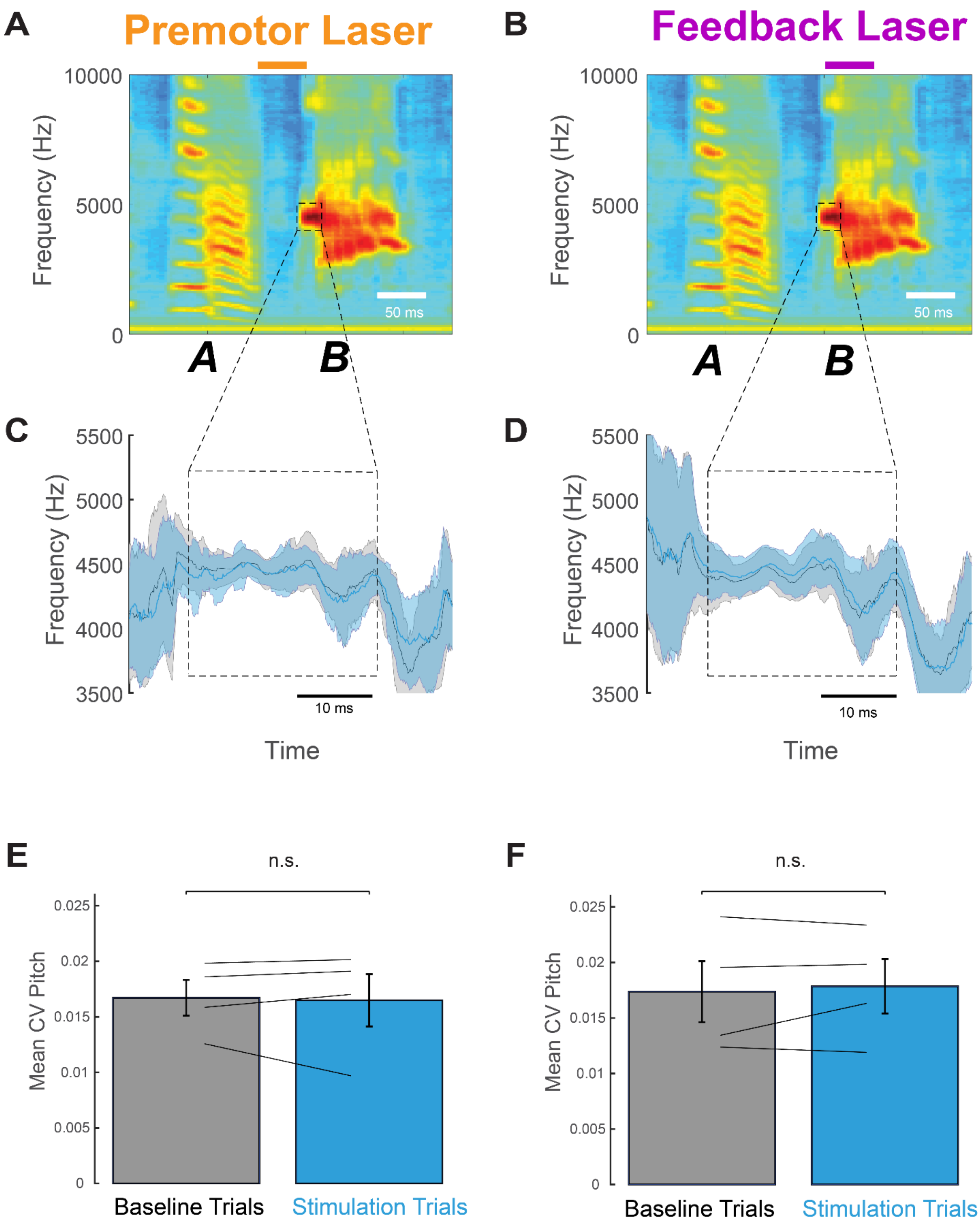
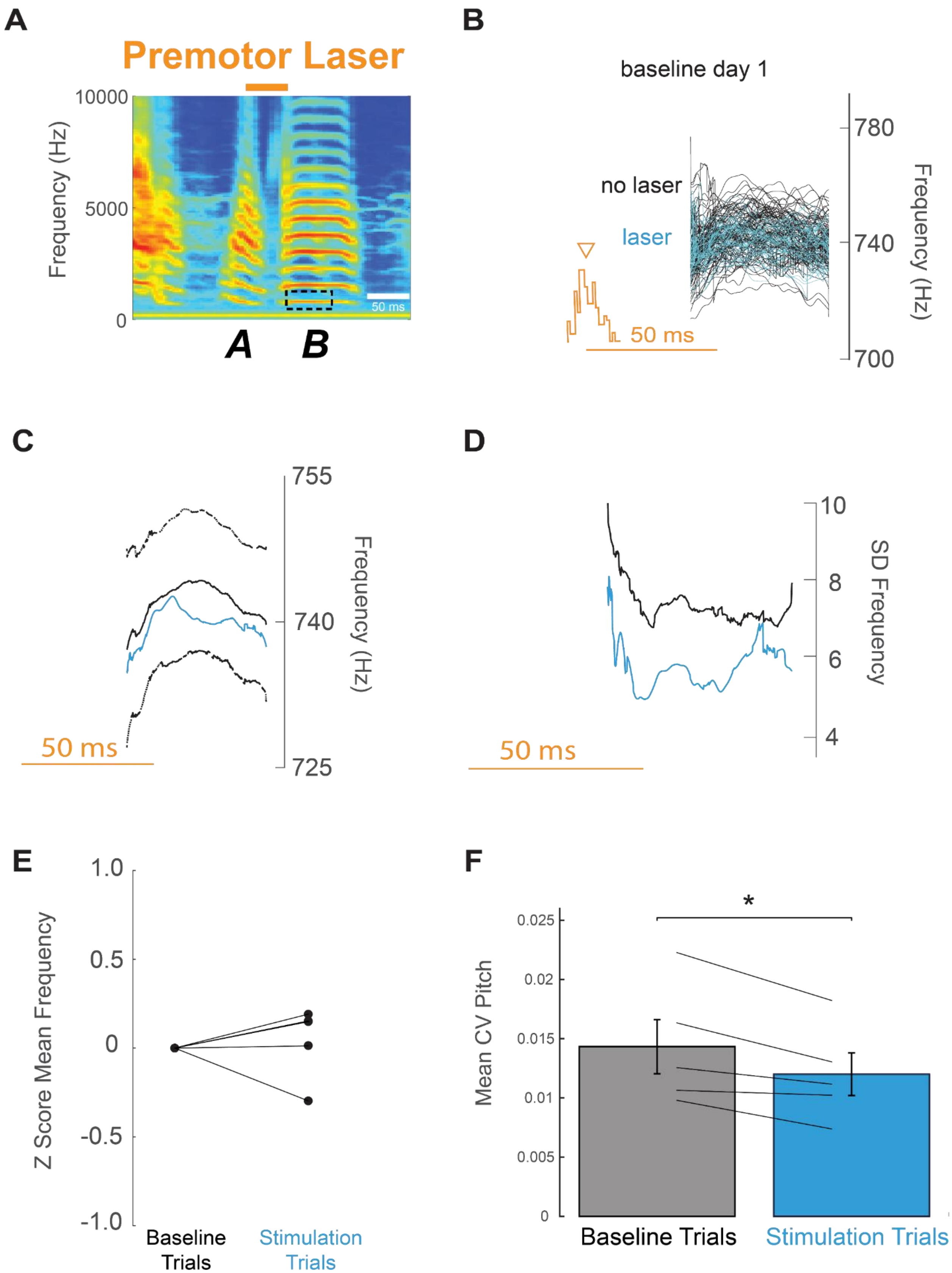

A
Premotor Laser
B
Feedback Laser
Frequency (Hz)
10000
5000
0
50 ms
A
B
C
D
4500
4000
3500
4600
4400
4200
3800
3600
10 ms
Time
E
F
n.s.
Mean CV Pitch
0.025
0.02
0.015
0.01
0.005
0
0.03
Baseline Trials
Stimulation Trials

Supplementary Figure 1: Stimulation of Aiv_{VTA} terminals does not have acute effects on song motor production., Related to Figure 2 (A) Spectrogram showing two syllables of song, Syllable *B* is targeted to receive premotor Aiv_{VTA} laser stimulation on a random 50% of renditions throughout the day. (B) Same as in A but for feedback stimulation delivered 50 ms later. (C) Contour plot demonstrating the mean and standard deviation of pitch of syllable *B* on 50% of trials that receive laser stimulation (blue) and 50% of trials that do not receive laser stimulation (catch trials, gray). (D) Same as in C except for feedback stimulation. (E) Bar graph of mean CV pitch showing no significant changes with premotor Aiv_{VTA} stimulation ($n = 3$ syllables, $n = 3$ birds, $p = 0.5268$, paired t test). (F) Bar graph of mean CV pitch showing no significant changes with auditory feedback Aiv_{VTA} stimulation ($n = 3$ syllables, $n = 3$ birds, $p = 0.3873$, paired t test).



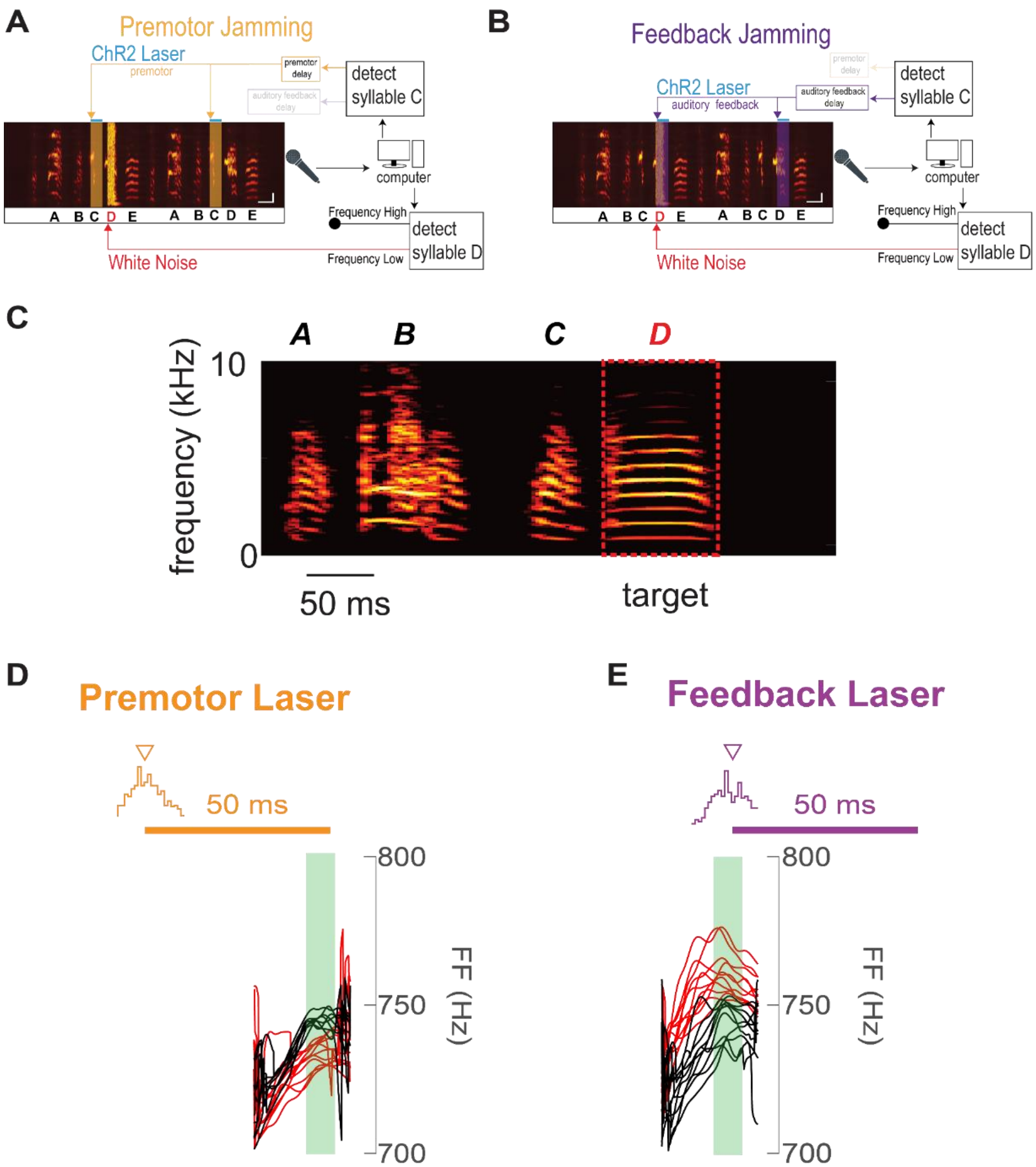
A
Premotor Laser
10000
5000
0
Frequency (Hz)
50 ms
A
B
B
Feedback Laser
10000
5000
0
Frequency (Hz)
50 ms
A
B
C
5500
5000
4500
4000
3500
Frequency (Hz)
10 ms
Time
D
5500
5000
4500
4000
3500
Frequency (Hz)
10 ms
Time
E
n.s.
0.025
0.02
0.015
0.01
0.005
0
Mean CV Pitch
Baseline Trials
Stimulation Trials
F
n.s.
0.025
0.02
0.015
0.01
0.005
0
Mean CV Pitch
Baseline Trials
Stimulation Trials

Supplementary Figure 2: Stimulation of VP_{VTA} terminals does not have acute effects on song motor production., Related to Figure 2 (A) Spectrogram showing two syllables of song, Syllable B is targeted to receive premotor VP_{VTA} laser stimulation on a random 50% of renditions throughout the day. (B) Same as in A but for feedback stimulation delivered 50 ms later. (C) Contour plot demonstrating the mean and standard deviation of pitch of syllable *B* on 50% of trials that receive laser stimulation (blue) and 50% of trials that do not receive laser stimulation (catch trials, gray). (D) Same as in C except for feedback stimulation. (E) Bar graph of mean CV pitch showing no significant changes with premotor VP_{VTA} stimulation (n = 4 syllables, n = 4 birds, p = 0.8247, paired t test). (F) Bar graph of mean CV pitch showing no significant changes with auditory feedback VP_{VTA} stimulation (n = 4 syllables, n = 4 birds, p = 0.5996, paired t test).



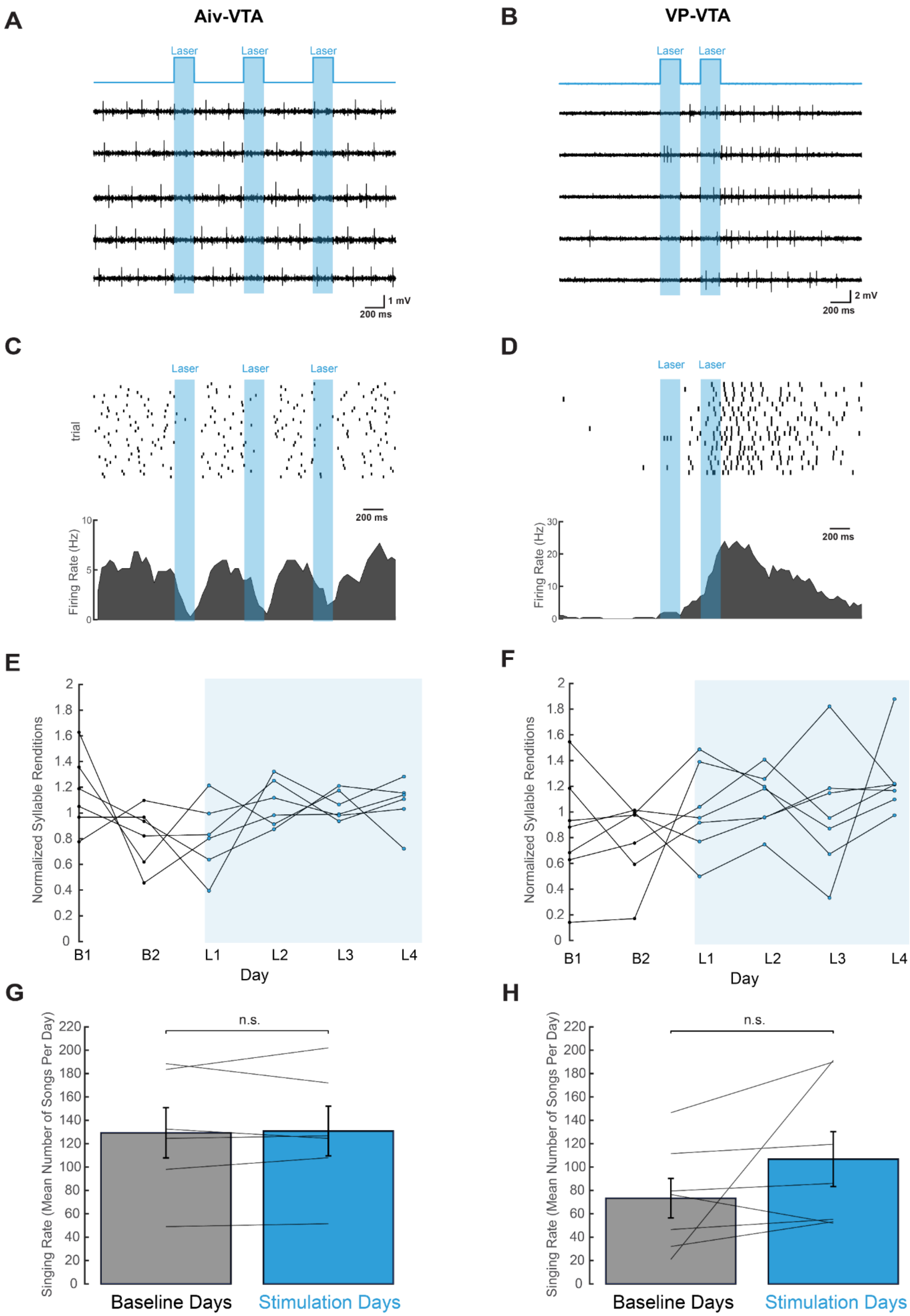
A
Premotor Laser
10000
5000
0
Frequency (Hz)
50 ms
A
B
B
baseline day 1
no laser
laser
50 ms
780
740
700
Frequency (Hz)
C
755
740
725
Frequency (Hz)
50 ms
D
10
8
6
4
SD Frequency
50 ms
E
1.0
0.5
0
-0.5
-1.0
Z Score Mean Frequency
Baseline Trials
Stimulation Trials
F
*
0.025
0.02
0.015
0.01
0.005
0
Mean CV Pitch
Baseline Trials
Stimulation Trials

Supplementary Figure 3: Stimulation of LMAN can acutely effect song motor production., Related to Figure 3 (A) Spectrogram showing two syllables of song, Syllable *B* is targeted to receive premotor LMAN laser stimulation randomly on a subset of renditions throughout the day. (B) Contour plot demonstrating raw contours of syllable *B* on 50% of trials that receive laser (blue) and 50% of trials that do not receive laser (catch trials, gray) (orange histogram shows jitter in onset of laser with mean shown with triangle.) (C) Mean pitch and one standard deviation above and below on non-stimulation “catch” trials. Mean pitch on laser trials (blue) (D) Plot of standard deviation of pitch for no laser (black) and laser stimulation (blue) trials. (E) Z score change in mean frequency with laser stimulation (blue). (F) Bar graph of mean CV pitch showing small but significant decreases with laser stimulation ($n = 5$ syllables, $n = 5$ birds, $p = 0.0234$, paired t test).



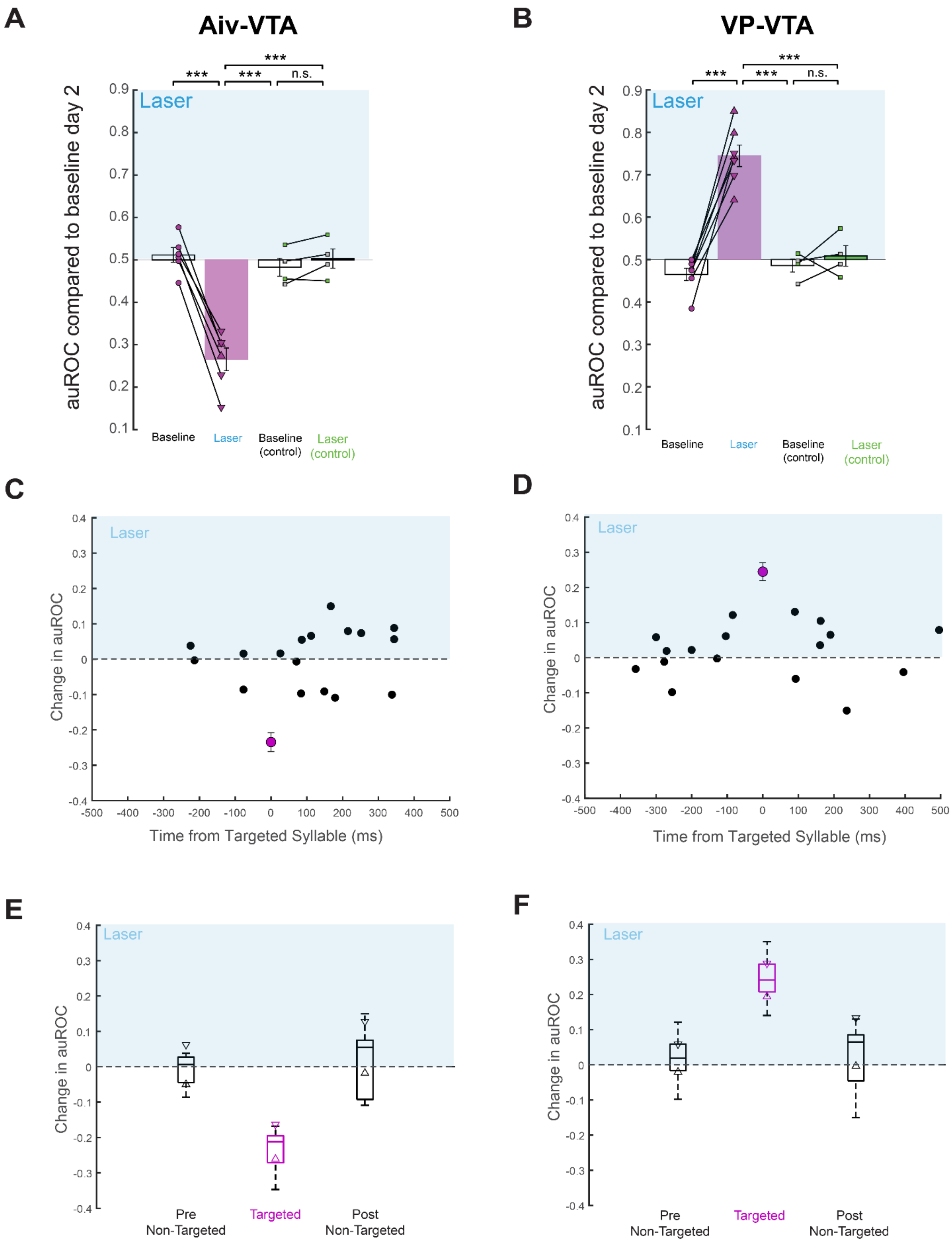
A
Premotor Jamming
ChR2 Laser
premotor
premotor delay
auditory feedback delay
detect syllable C
computer
A B C D E A B C D E
Frequency High
detect syllable D
Frequency Low
White Noise
B
Feedback Jamming
premotor delay
ChR2 Laser
auditory feedback
auditory feedback delay
detect syllable C
computer
A B C D E A B C D E
Frequency High
detect syllable D
Frequency Low
White Noise
C
A B C D
frequency (kHz)
10
0
50 ms
target
D
Premotor Laser
50 ms
800
750
700
FF (Hz)
E
Feedback Laser
50 ms
800
750
700
FF (Hz)

Supplementary Figure 4: Sensitivity and specificity of online dual syllable detection for independent optical and auditory stimulus delivery., Related to Figure 3 (A) Schematic of dual syllable targeting optogenetic interference experiment, auditory feedback white noise is delivered in a pitch contingent fashion to syllable D while syllable C is used to trigger a laser on every trial to disrupt neural activity during a premotor production period relative to the production syllable D. (B) Schematic of dual syllable targeting optogenetic interference experiment: auditory feedback white noise is delivered in a pitch contingent fashion to syllable D while syllable C is used to trigger a laser on every trial to disrupt neural activity during a auditory feedback evaluation period relative to the production syllable D. (C) Spectrogram of song of bird used in LMAN (D) Contours of early trials (black) and late trials (red) on premotor jamming day, green box shows where pitch is measured. Histogram of premotor laser stimulation timing mean onset is 49.3 ms prior to pitch measurement (green box), standard deviation is 9.3 ms. (E) Contours of first early trials (black) and late trials (red) on auditory feedback jamming day, green box shows where pitch is measured. Histogram of premotor laser stimulation timing mean onset is 5.6 ms after pitch measurement (green box), standard deviation is 10.5 ms.



A
Aiv-VTA
Laser
Laser
Laser
1 mV
200 ms
B
VP-VTA
Laser
Laser
2 mV
200 ms
C
Laser
Laser
Laser
trial
200 ms
Firing Rate (Hz)
D
Laser
Laser
200 ms
Firing Rate (Hz)
E
Normalized Syllable Renditions
B1
B2
L1
L2
L3
L4
Day
F
Normalized Syllable Renditions
B1
B2
L1
L2
L3
L4
Day
G
n.s.
Singing Rate (Mean Number of Songs Per Day)
Baseline Days
Stimulation Days
H
n.s.
Singing Rate (Mean Number of Songs Per Day)
Baseline Days
Stimulation Days

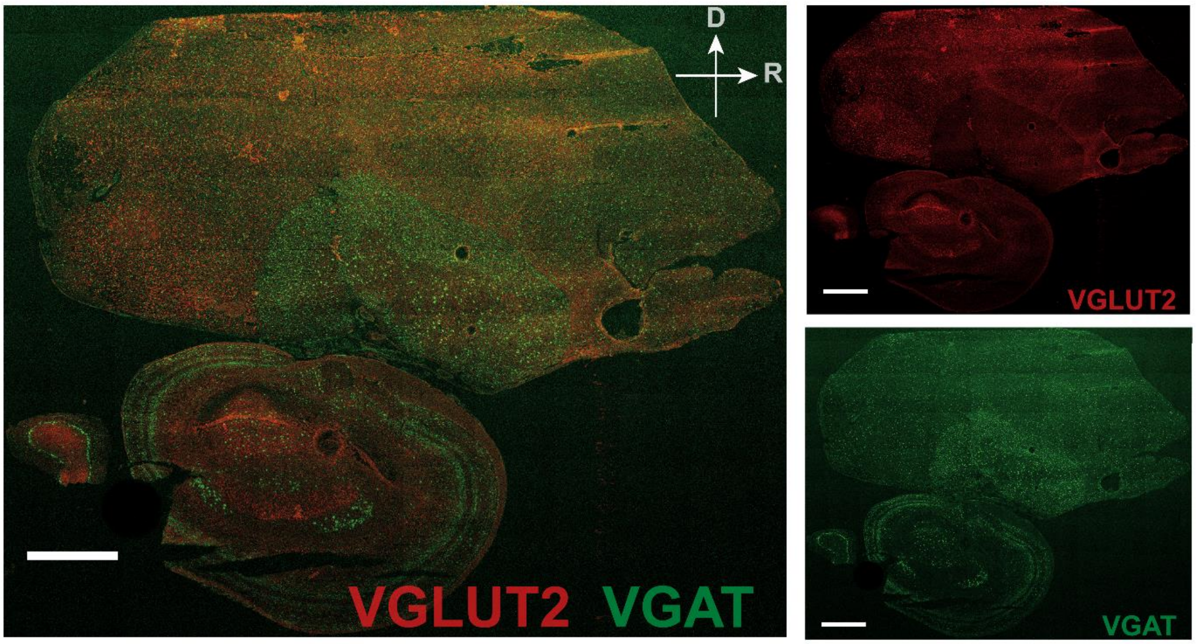
Supplementary Figure 5: Functional expression of ChR2 in Aiv and VP terminals in VTA and Stimulation of Aiv_{VTA}, VP_{VTA} does not significantly alter singing rate. Related to Figure 4,5 (A) Top, Laser Pulse delivery (blue). Bottom, 5 trials of recording showing raw activity of recorded VTA unit. (B) Top, Laser Pulse delivery (blue). Bottom, 5 trials of recording showing raw activity of recorded VTA unit. (C) Top, raster plot of sorted spikes on each trial represented with a tick mark. Bottom, Peristimulus Time Histogram (PSTH) showing smoothed firing rate averaged across all trials demonstrating modulations in the firing rate of this VTA unit linked to Aiv terminal stimulation. (D) Top, raster plot of sorted spikes on each trial represented with a tick mark. Bottom, PSTH showing smoothed firing rate averaged across all trials demonstrating modulations in firing rate of this unit linked to VP terminal stimulation. (E) Plot of normalized number of “catch” syllable renditions (set at 5% of total trials) during each of 6 days of the experiment, 2 baseline days (B1, B2, black) and 4 stimulation days (L1, L2, L3, L4, blue), shaded regions indicates days where laser is delivered normalized by the average number of renditions sang by that bird over the six experimental days. (F) Same as in E, except for VP_{VTA} Stimulation. (G) Bar graph of singing rate the average number of syllable renditions on two baseline days (gray) and four Aiv_{VTA} laser stimulation days (blue) ($n = 6$ syllables, $p = 0.786$ paired t test) (H) Same as in E except for VP_{VTA} laser stimulation ($n = 7$ syllables, $p = 0.2145$ paired t test).


A
Aiv-VTA
B
VP-VTA
Laser
auROC compared to baseline day 2
***
n.s.
Baseline
Laser
Baseline (control)
Laser (control)
C
D
Change in auROC
Time from Targeted Syllable (ms)
E
F
Pre Non-Targeted
Targeted
Post Non-Targeted

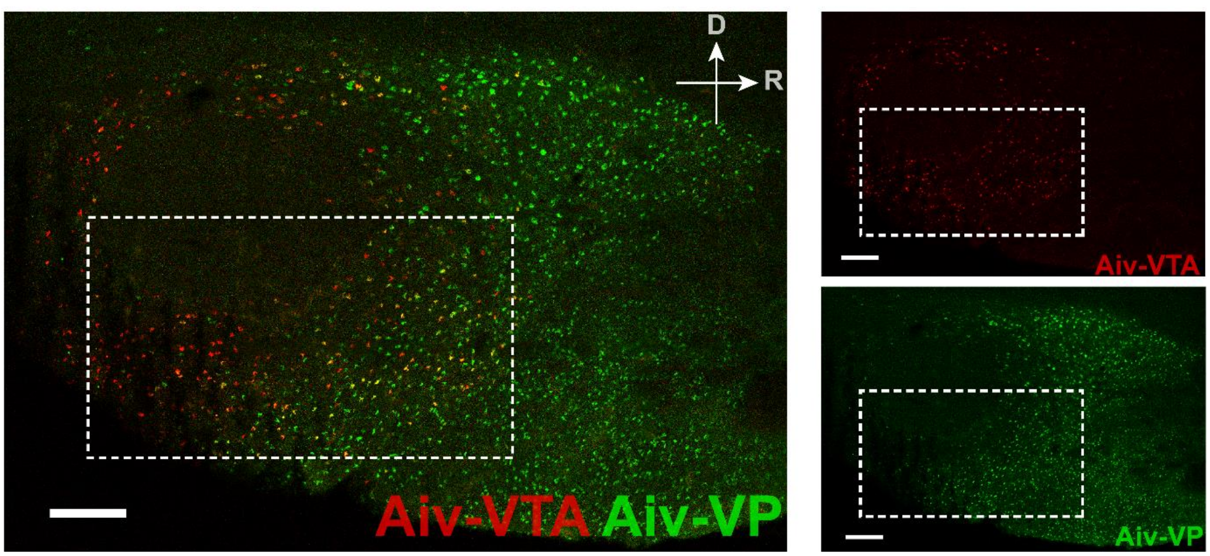
Supplementary Figure 6: Pitch-contingent optogenetic stimulation of Aiv and VP terminals in VTA drives syllable-specific changes in pitch., Related to Figure 4,5

(A) Mean change in auROC of target syllable pitch, relative to B2, on B1 (baseline) and L4 (laser) (n = 6 syllables, n =4 birds, p= 0.000032, paired t test). Right, same but in control birds (Laser(control)) (green, GFP; gray, no injection, n = 4 syllables, 4 birds; p = 0.1476, paired t test). (B) Mean change in auROC of target syllable pitch between B1 and B2 (Baseline), and B2 and L4 of VP_{VTA} terminal stimulation in experimental birds (Laser) (n = 7 syllables, n = 6 birds, p= 0.000044, paired t test). Right, same but in control birds (Laser(control)) (green, GFP; gray, no injection, n = 4 syllables, 4 birds; p = 0.4993, paired t test). (C) Absolute change in auROC of non-targeted syllables for Aiv_{VTA} stimulation as a function of milliseconds in song in distance from the target syllable (n = 17 non-targeted syllables from n = 4 birds). Negative numbers indicate that the syllable preceded the target syllable. Purple circle indicates the mean and S.E.M. of the absolute change in auROC of all Aiv_{VTA} targeted syllables. All values shown as mean ± standard error of the mean (S.E.M.). (D) Absolute change in auROC of non-targeted syllables for VP_{VTA} stimulation as a function of milliseconds in song from the targeted syllable (n = 18 non-targeted syllables from n = 4 birds). Negative numbers indicate that the syllable preceded the target syllable. Purple circle indicates the mean and S.E.M. of the absolute change in auROC of all VP_{VTA} targeted syllables. All values shown as mean ± standard error of the mean (S.E.M.). (E) Boxplot of change in auROC of non-targeted syllables before the targeted syllable (Pre Non-Targeted), targeted syllables (Targeted, magenta), and non-targeted syllables after the targeted syllable (Post Non-Targeted) with Aiv_{VTA} stimulation. (F) Same as E except for VP_{VTA} stimulation.

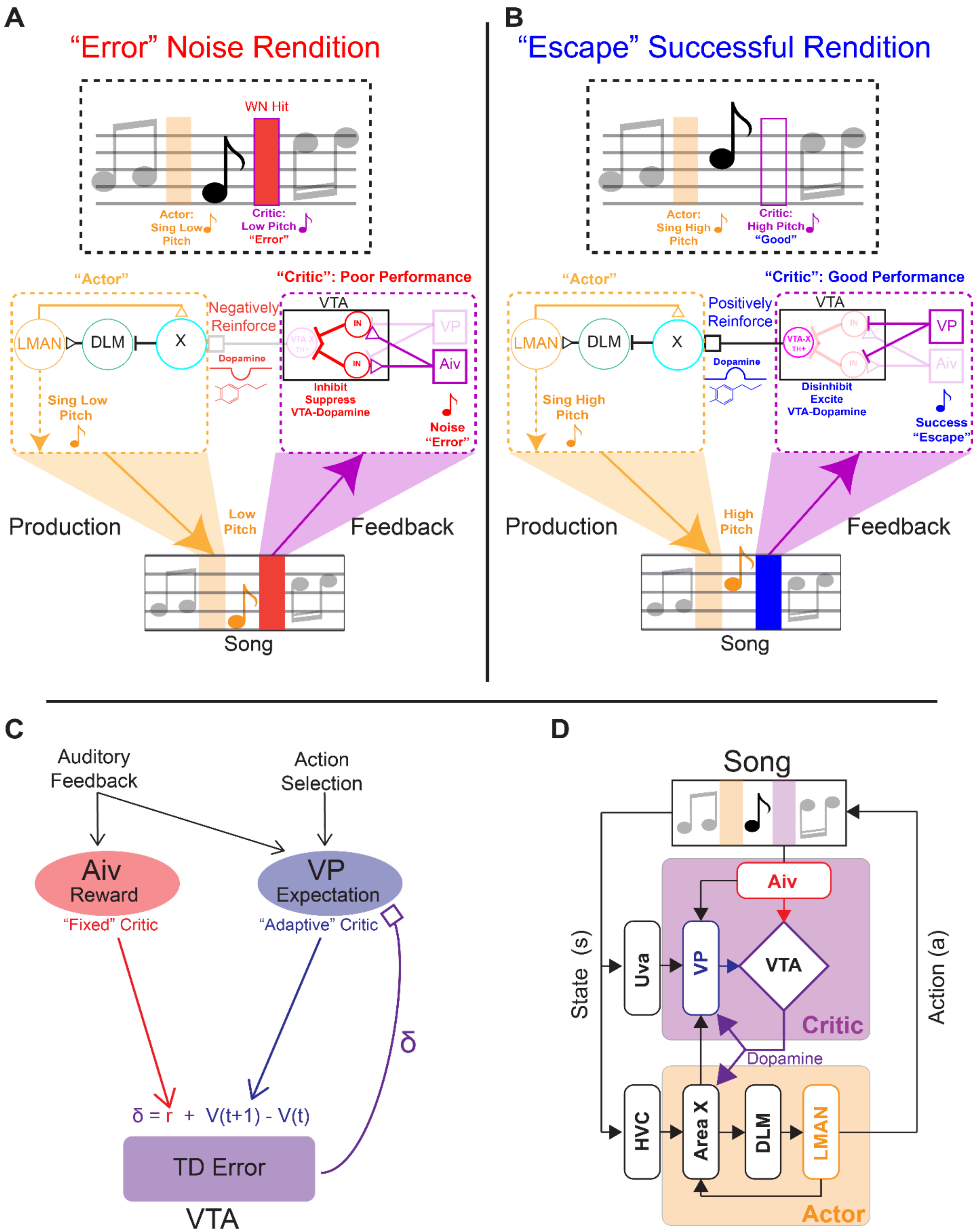
A



B



Supplementary Figure 7: Anatomy and diagram of actor-critic circuit in the songbird., Related to Figure 7 (A) Left, Overlay section of zebra finch brain with fluorescence in situ probe against VGLUT2 (red) and VGAT (green), scale bar 1 mm. Top right, same section with only VGLUT2 (red) channel. Bottom right, same section with only VGAT (green) channel. (B) Left, overlay of arcopallial section of zebra finch brain with CTB injection into VTA (red) and VP (green), scale bar 200 µm. Top right, same section with only Aiv_{VTA} (red) channel. Bottom right, same section with only Aiv_{VP} (green) channel.


A
"Error" Noise Rendition
WN Hit
Actor: Sing Low Pitch
Critic: Low Pitch "Error"
"Actor"
"Critic": Poor Performance
LMAN
DLM
X
Negatively Reinforce
Dopamine
VTA
IN
VP
Aiv
Inhibit Suppress VTA-Dopamine
Sing Low Pitch
Noise "Error"
Production
Low Pitch
Feedback
Song
B
"Escape" Successful Rendition
Actor: Sing High Pitch
Critic: High Pitch "Good"
"Actor"
"Critic": Good Performance
Positively Reinforce
Dopamine
VTA
Disinhibit Excite VTA-Dopamine
Sing High Pitch
Success "Escape"
Production
High Pitch
Feedback
Song
C
Auditory Feedback
Action Selection
Aiv Reward
"Fixed" Critic
VP Expectation
"Adaptive" Critic
δ
δ = r + V(t+1) - V(t)
TD Error
VTA
D
Song
State (s)
Action (a)
Aiv
Uva
VP
VTA
Critic
Dopamine
HVC
Area X
DLM
LMAN
Actor

Supplementary Figure 8: Circuit Models of Pitch Learning., Related to Figure 8 (A) Schematic of activity in songbird vocal learning circuit on “error” renditions that received noise. (E) Schematic of activity in songbird vocal learning circuit on successful renditions that escape noise. (C) TD error model wherein Aiv is a “fixed” critic conveying negative reward (vocal error) and VP is an “adaptive” critic conveying an expectation. These two signals are combined within the local VTA network to compute a TD error. (D) A hypothetical “actor-critic” circuit architecture that may support vocal learning (with inspiration from (Takahashi et al., 2008, Chen et al., 2019).

Supplementary Table 1: VGLUT2 and VGAT Probe Sequence., Related to Figure 7

VGLUT2	gctgggattc tggtgcaata tactgggtgg tcttctgtgt tctatgtata tgggagcttt ggtatagtct ggtacatgtt ttggcttttg gtttcatatg agagtcctgc aaagcatcct acaattacag atgaagaaag gagatacata gaagaaagca ttggagagag tgccaacctc ctaggtgcaa tggaaaaata caaaacacca tggagaaaat tttttacatc tatgccagtc tatgcaataa tagttgcaaa cttctgtaga agctggactt tttacttgct gctaattagt cagcctgctt actttgagga agtgtttgga tttgaaataa gcaaggtggg tattttatct gctgtgccac atttagtgat gacaattatt gttcctattg ggggacaaat tgccgacttt ttaagaagca ggcagattgt ttcaacgact aatgtgagaa agataatgaa ctgtggaggt tttggtatgg aagcaactct tcttctggtg gtgggatatt cacacagcaa aggagtggct atttcattct tagtgcttgc tgtaggcttt agtggatttg ccatatctgg attcaatgtc aaccatttag acattgcccc aaggtatgct agcattttaa tggggatttc taatggagtc gggaccttgt ctggaatggt ttgccctata attgttggag caatgacaaa gaacaagaca cgtgaagaat ggcagtatgt cttcctcatt gctgctttag tccattatgg aggtgtgatc ttctatggca tatttgcttc a***ccctatagt gagtcgtatt acgcgcg***
VGAT	ccaacgccat ccaggggatg tttgttctgg gcctgcccta tgccatcctt cacggtggat acctaggact ctttttaata attttcgctg cagtggtttg ctgctacact gggaaaatcc ttattgcctg tctttacgaa gagaatgagg atggggagat agtcagggtg agagactcct acgtggacat tgcgaacgcg tgctgcgcgc cccgcttccc caccctcggg ggcagaattg tcaacgtggc tcagatcatt gaactggtca tgacctgcat cctctatgtg gtggtcagtg ggaacctgat gtacaacagc ttccccaacc tgcccgtctc ccagaagtcg tggtccatca ttgccacggc agtgctcctg ccttgtgcgt tcttgaagaa cctcaaggca gtctccaagt tcagcttgct ctgcacatta gcccactttg tcatcaacat cctggtgatc gcctactgcc tctccagggc gcgcgactgg gcctgggaca aagtcaagtt ttacattgat gtcaagaagt ttcccatctc cattggcatc attgtcttca gctacacctc ccagatcttt ctgccttcct tggaggggaa catgcagaac cccaaggagt ttcactgcat gatgaactgg actcacatag cagcttgcat ccttaaggga ctctttgcct tggtcgccta cctgacctgg gctgatgaga ccaaggaggt cattacagac aacttgccat ccaccattag ggcagtagtc aatattttct tggtggccaa agccttgctc tcgtacccct tgccgttctt tgcagctgta gaagtcctgg agcgatccct tttccaagat ggaaacaggg ctttcttccc caactgctat gggggtgacg ggcggctcaa atcctgggga ctcaccctca gatgtgccct ggtagttttc accctgctca ***ccctatagtg agtcgtatta cgcgcg***

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