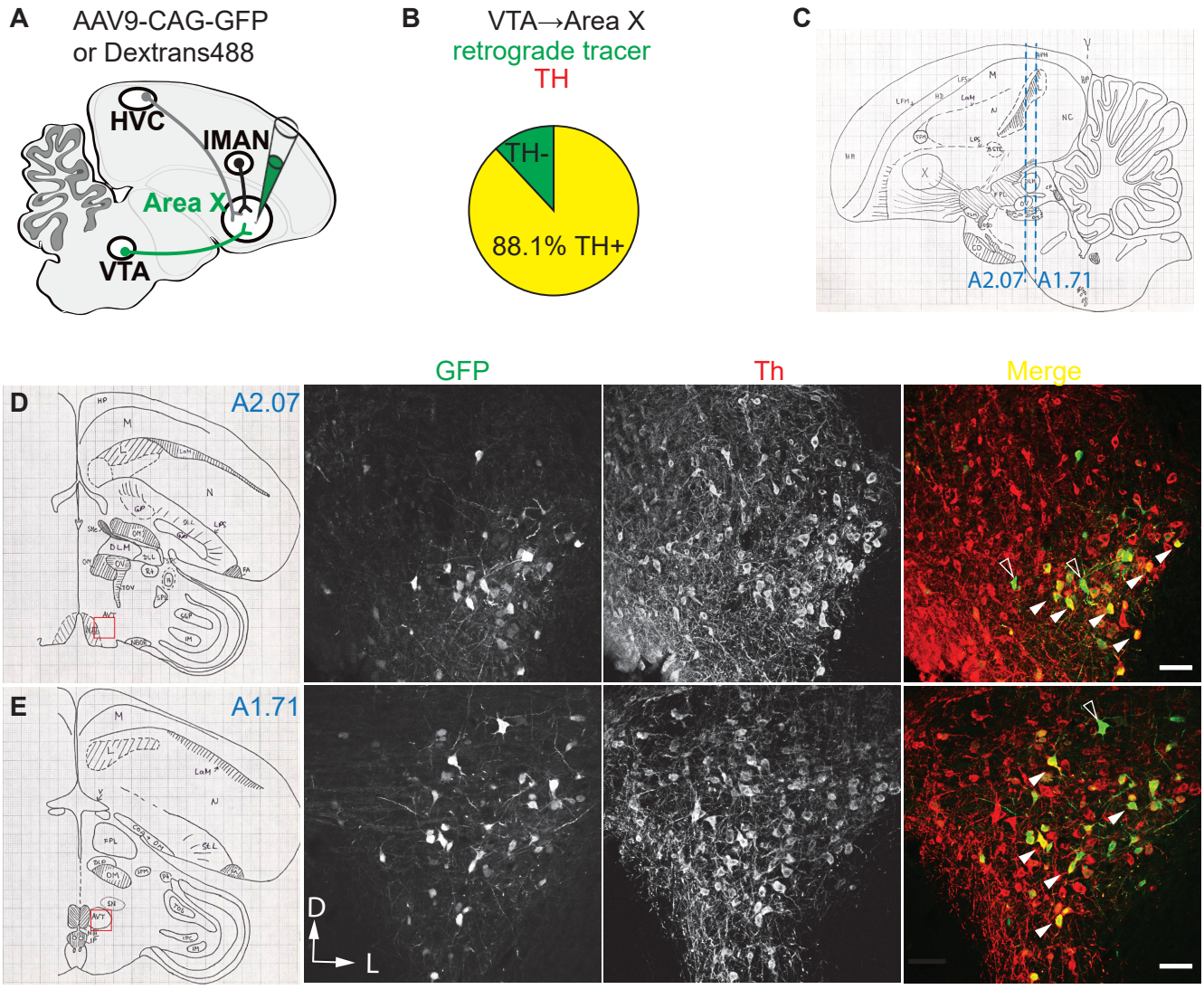
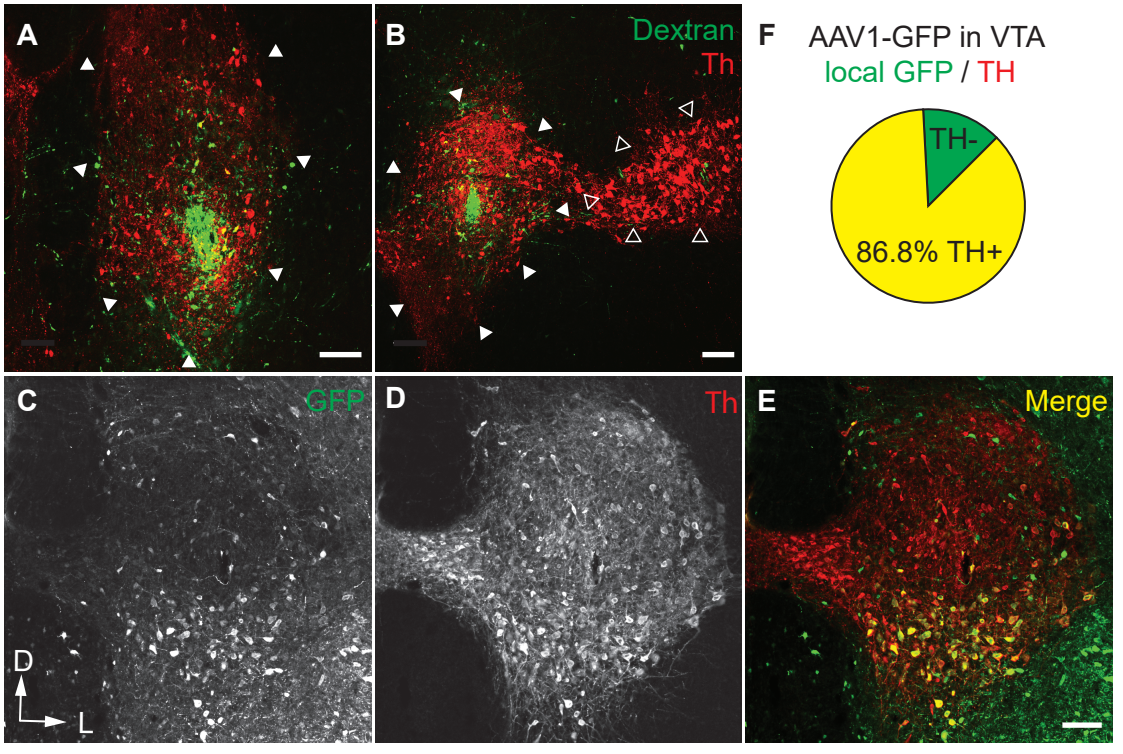
Supplementary Figure S1



Supplemental Figure 1. Anatomical identification of VTA_{AX} neurons. Related to Figure 2.

A. Schematic showing the design of retrograde-labeling experiments. Fluorescent retrograde tracers (dextran, Alexa Fluor, n=4) or AAV9-CAG-GFP(n=3) were injected into Area X.
B. Retrograde-labeling experiments reveal that 88.1% of neurons (1926 out of 2185 in VTA) projecting into Area X area (yellow, filled arrowheads in figures D&E) are TH positive.
C. Schematic representation of the cutting plane in sagittal section (cited from 'a stereotaxic atlas of the brain of the zebra finch'**)**. Blue dashed lines illustrate two transverse sections 2.07mm (A2.07, figure D) and 1.71mm (A1.71, Figure E) rostral of Y-point.
D. and E. Confocal images taken from rostral VTA (figure D, A2.07) and caudal VTA (figure E, A1.71). Most of neurons projecting to Area X, located in the ventrolateral VTA, are TH positive (yellow, filled arrowheads), open arrowheads indicate TH negative Area X projecting neurons(green). Scale bar, 100 µm; D, Dorsal; L, lateral.

Supplementary Figure S2



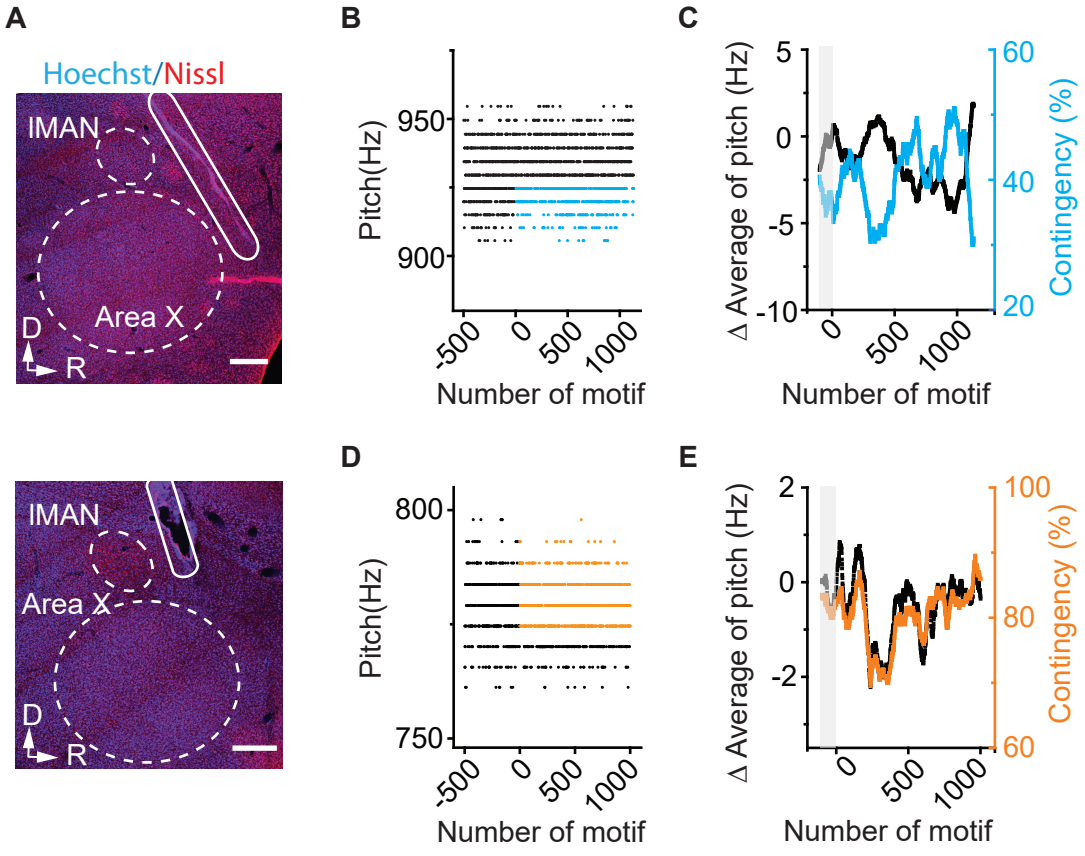
Supplemental Figure 2. Targeting injections to VTA. Related to Figure 2.

A. and B. Representative coronal sections taken from a bird injected with fluorescent tracer (dextran 488) injected into VTA illustrates that our approach provides reliable targeting of VTA (figure A = A2.07 and figure B = A1.71). Filled triangles outline the border of VTA and open triangles outline the border of SNc. Scale bar, 100 µm.

C. –E. Representative coronal sections taken from a bird with AAV1-CAG-GFP directly injected into VTA reveals that most of GFP positive neurons in VTA are TH positive. Scale bar, 100 µm.

F. Injections of AAV1-CAG-GFP into VTA reveal that 86.8% of virally infected neurons (992 out of 1143, 8 hemispheres from 4 birds) in VTA are TH positive.

Supplementary Figure S3



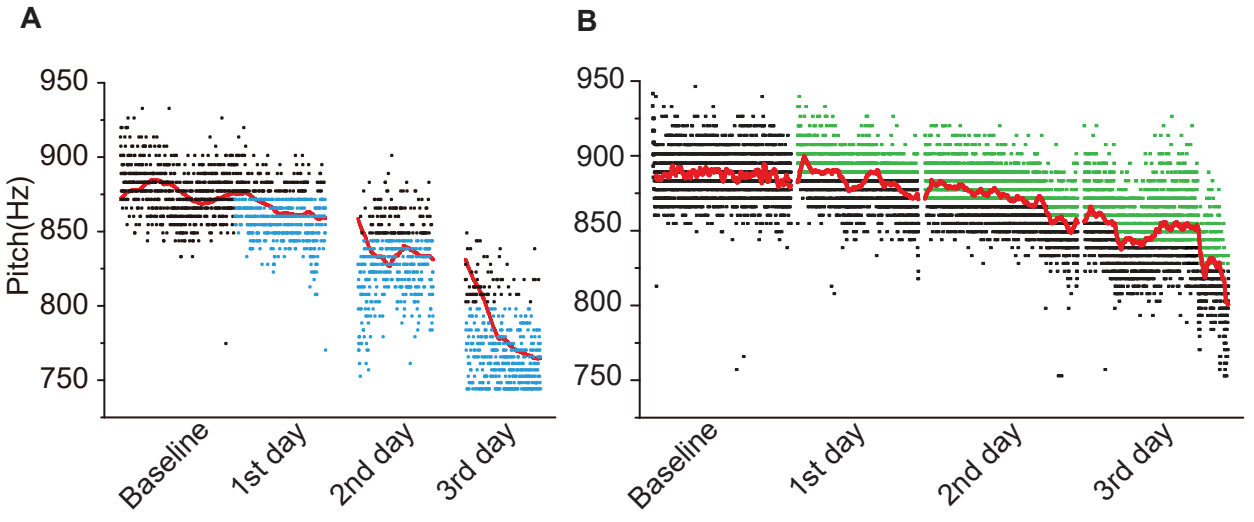
Supplemental Figure 3. Examples of pitch learning with misplacement of optical fiber. (axChR2, n=5 birds, axArchT, n=3 birds). Related to Figure 3 and 4.

A. Parasagittal section shows implantation track of fiber optic misplaced over Area X, either too deep(upper) or shallow(lower). Dashed lines outline both the border of Area X and lMAN. Scale bar, 400 µm.

B. & D. Plot of the pitch of the target syllable before and during optical stimulation (B) or inhibition (D) each point corresponds to one rendition of the syllable. Closed-loop optical manipulation of target syllables to lower pitch variants (blue dots, B) or higher pitch variants (orange dots, D) fail to result in any changes in the number of 'hit' trials in birds with miss-targeted optical fibers.

C. & E Plot of the running average of the pitch and hit rate (contingency) during the day of close-looped optical manipulation illustrates no trend of changes in running average of pitch (black) and concomitant changes in contingency percentage (blue in C, orange in E). Each point corresponds to one rendition of the syllable; gray box indicates the baseline period before optical manipulation.

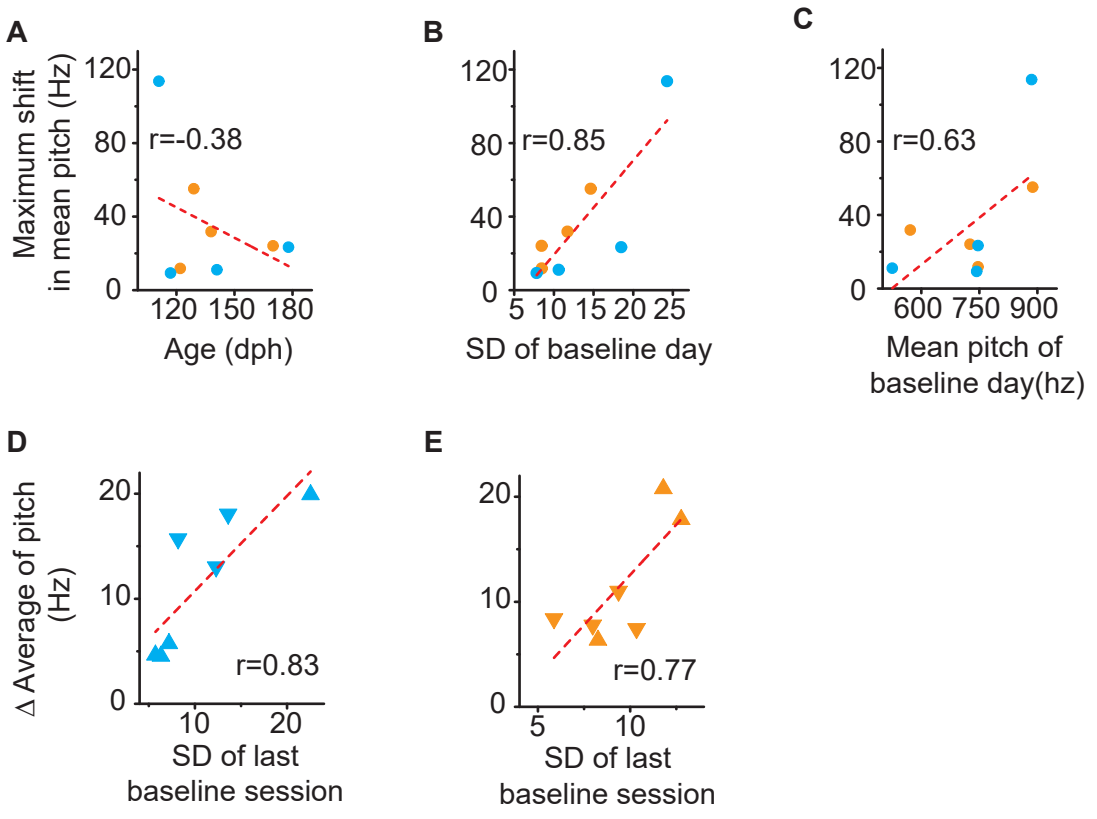
Supplementary Figure S4



Supplemental Figure 4. Raw pitch changes over 3 days for an axChR2 and a axArchT bird. Related to Figure 6.

Representative plot of all pitch variants for one axChR2+bird with illumination to variants with lower pitch (Figure A, blue dot) and one axArchT+ bird with illumination to variants with high pitch (Figure B, green dot). During three consecutive days, both birds exhibited continued decrease in running average of pitch (red line). Black dot, baseline or 'escape' rendition.

Supplementary Figure S5

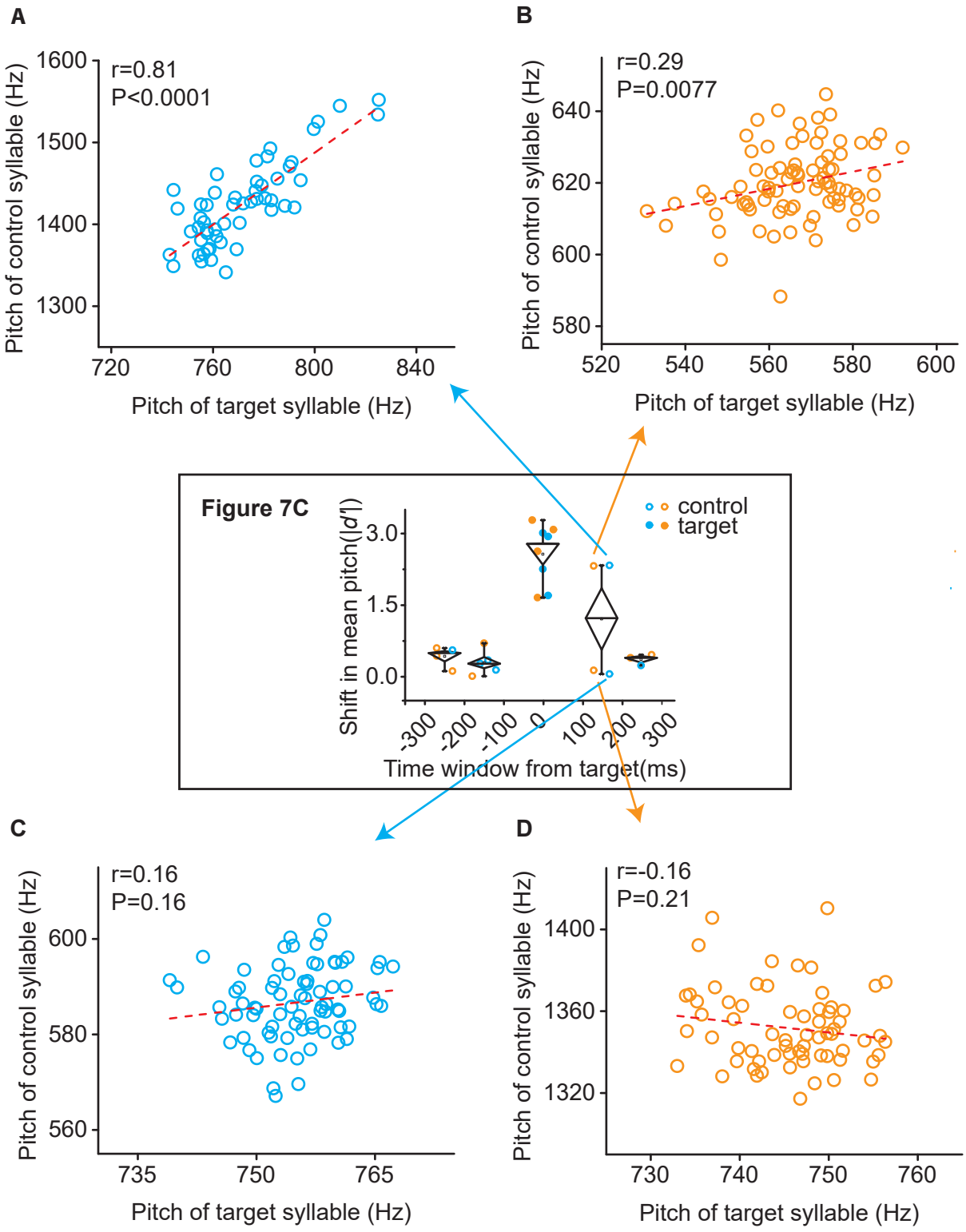


Supplemental Figure 5. Optogenetic changes in pitch correlate with natural variation in syllable pitch. Related to Figure 6.

A. -C Relationship between the magnitude of maximum shift in mean pitch and age of the bird or features of their target syllable. Across excitation and inhibition experiments, there was a positive correlation between the maximum shift in mean pitch and the SD of pitch on baseline day (**B**, r=0.85; p=0.0081, n=8, pearson). In contrast, the magnitude of maximal shift is not related with either age of the bird (**A**, r=-0.38, p=0.35, pearson) or mean pitch of target syllable (**C**, r=0.63, p=0.096, pearson).

D. -E Relationship between the standard deviation(SD) of pitch in last baseline session and the magnitude of shift in running average over the course of the stimulation day(**D**) or inhibition day(**E**). Across experiments, there was a positive correlation between the SD of pitch in last baseline session and the magnitude of shift during both stimulation day (r=0.83, p = 0.020, Pearson) and inhibition day (r=0.77 p = 0.041, Pearson).

Supplementary Figure S6



Supplemental Figure 6. Baseline correlations in pitch between target syllables and control syllables within +100-200 window from 4 individual birds plotted in Figure 7C. Related to Figure 7.

This figure illustrates pitch correlations on baseline day for the four birds plotted in main figure 7C. This analysis reveals that in the two birds in which the pitch of the control syllable shifted following optogenetic manipulations a positive correlation between the pitch of targeted and control syllables existed prior to optogenetic manipulations. For one axChR2+ bird and one axArchT+ bird in which pitch of control syllables shifted (Figure 7C, Blue, axChR2+, d'=2.33; orange, axArchT+, d'=2.32), the pitch of target syllable is positive correlated with the pitch of control syllable on baseline day (**S6 Figure A**, axChR2+, r=0.81, p<0.0001; **S6 Figure B**, axArchT+, r=0.29, p=0.0077). In contrast, the other axChR2+ bird and axArchT+ birds in which pitch of control syllables were not changed (Figure 6C, Blue, axChR2+, d'=0.056; orange, axArchT+, d'=0.13), there is no correlation between the pitch of target syllable and control syllable on baseline day (**S6 Figure C**, axChR2+, r=0.16, p=0.16; **S6 Figure D**, axArchT+, r=-0.16, p=0.21).