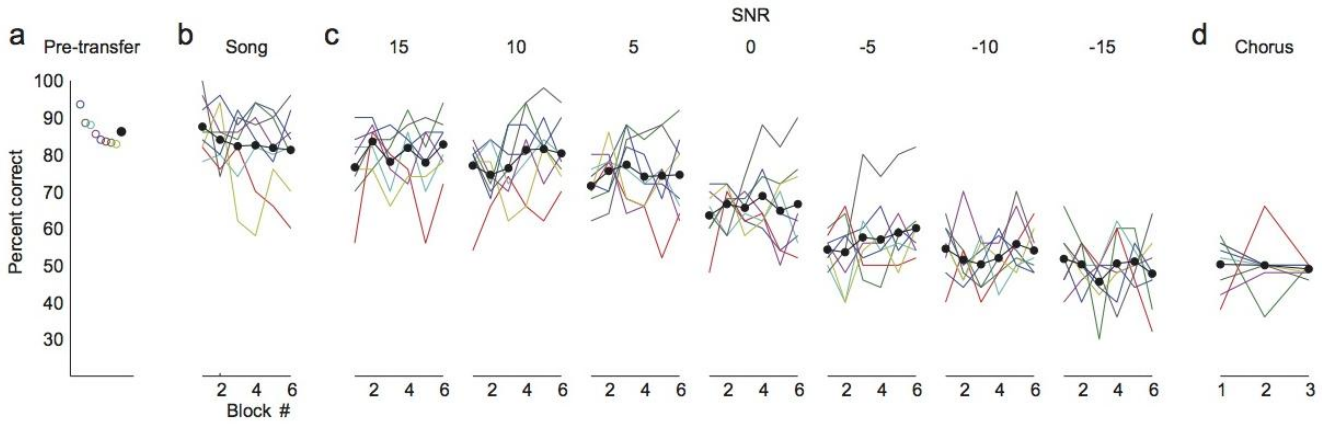
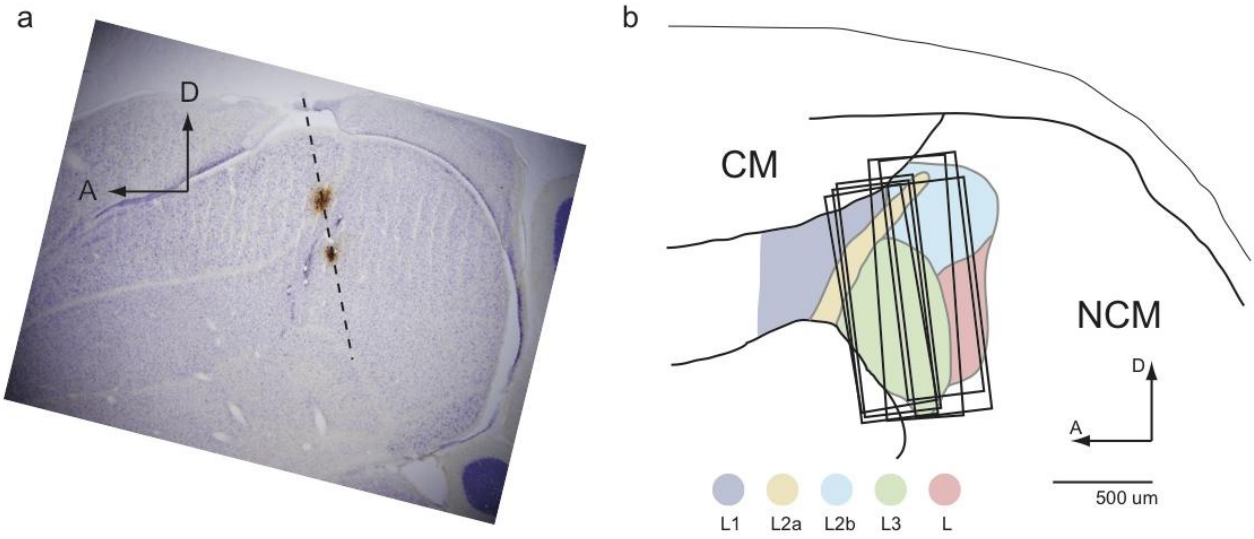
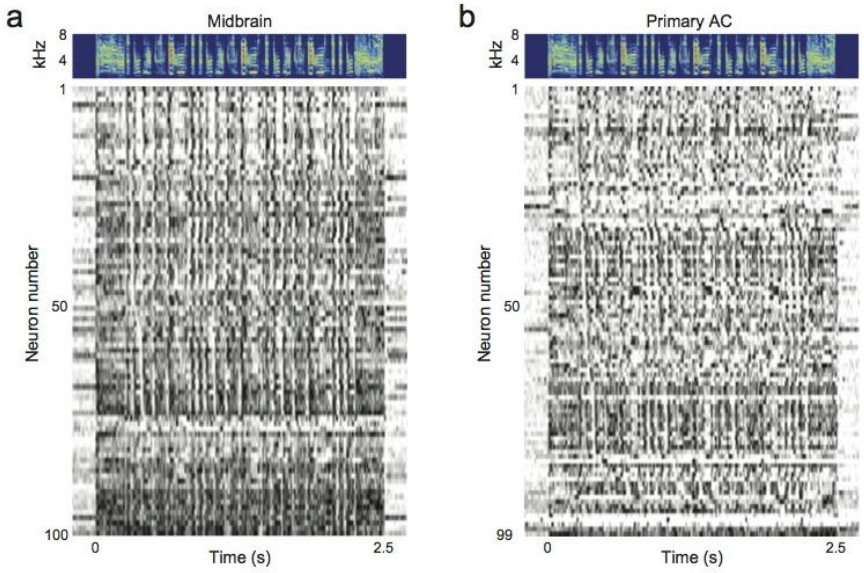
Supplementary Figure 1

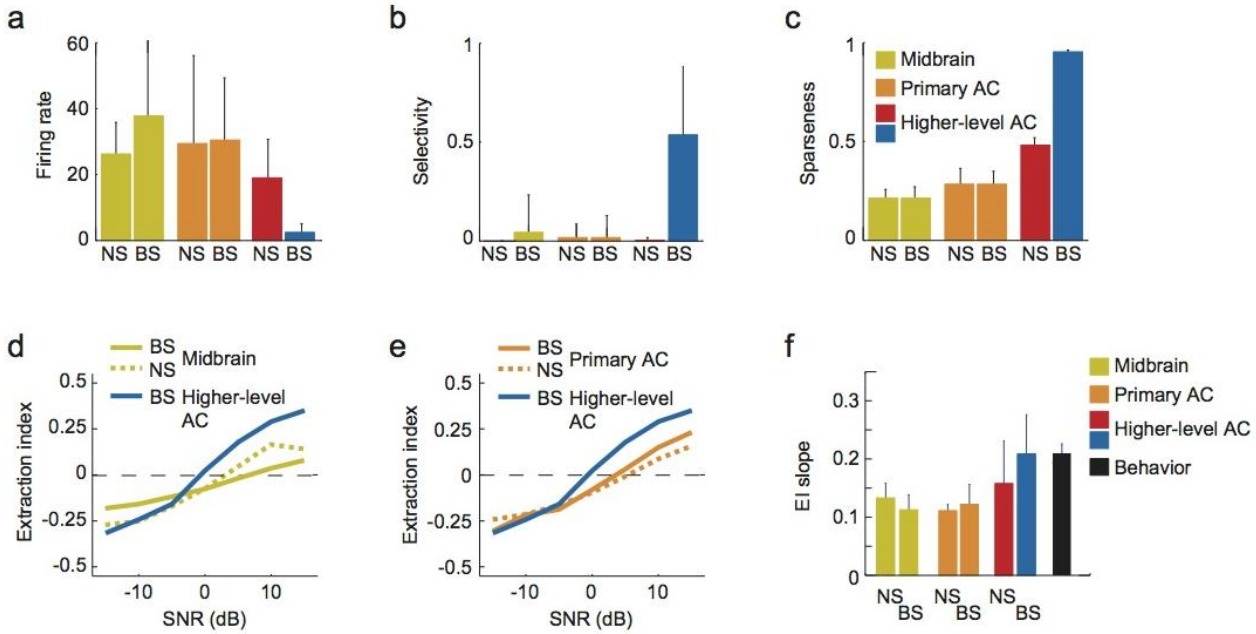



a
D
A
b
CM
NCM
D
A
500 um
L1
L2a
L2b
L3
L

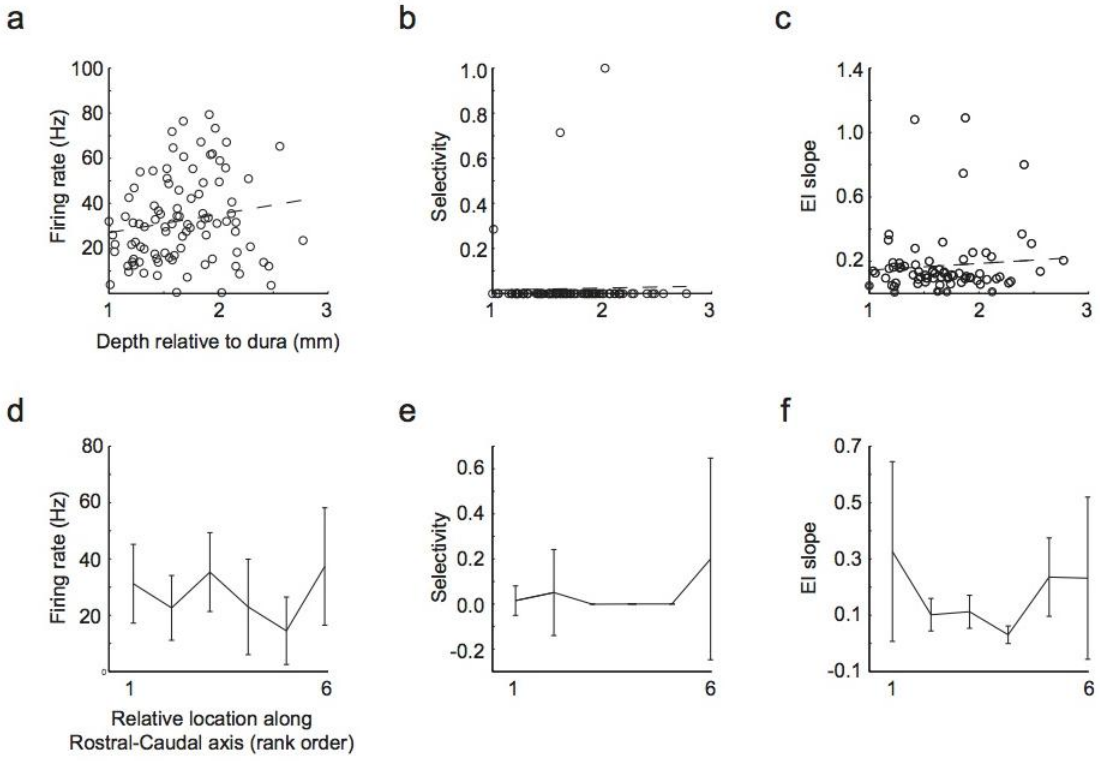
Supplementary Figure 3

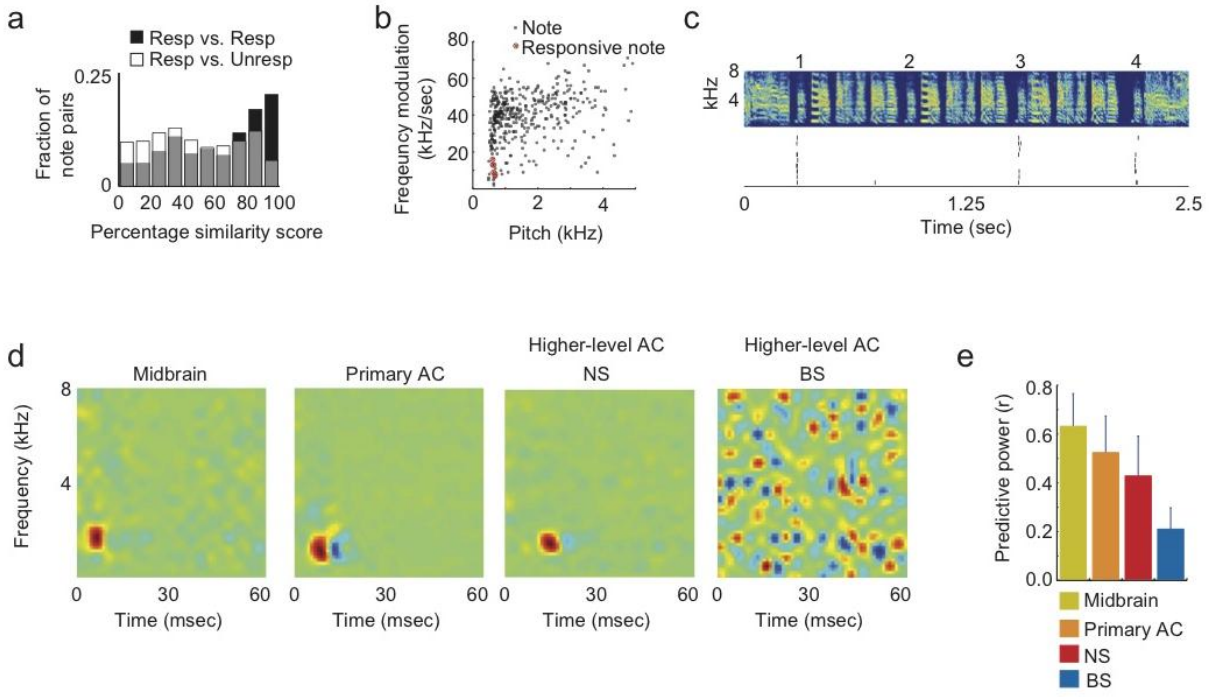


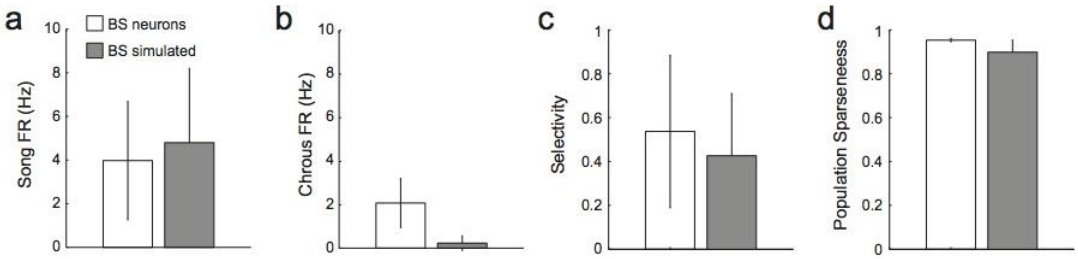
Supplementary Figure 4



Supplementary Figure 5




a
Resp vs. Resp
Resp vs. Unresp
Fraction of note pairs
0.25
0
0 20 40 60 80 100
Percentage similarity score
b
Note
Responsive note
Freqeuncy modulation (kHz/sec)
80
60
40
20
0 2 4
Pitch (kHz)
c
kHz
8
4
1 2 3 4
0 1.25 2.5
Time (sec)
d
Midbrain
Primary AC
Higher-level AC NS
Higher-level AC BS
Frequency (kHz)
8
4
0 30 60
Time (msec)
e
Predictive power (r)
0.8
0.6
0.4
0.2
0.0
Midbrain
Primary AC
NS
BS


a
BS neurons
BS simulated
Song FR (Hz)
10
8
6
4
2
0
b
Chrous FR (Hz)
10
8
6
4
2
0
c
Selectivity
1
0.8
0.6
0.4
0.2
0
d
Population Sparseness
1
0.8
0.6
0.4
0.2
0

Supplemental Figure Legends

Supplemental Figure 1 – Behavioral performance over time, related to Figure 1

(A) Percent correct on the last 200 trials of baseline training on songs alone. Each colored circle shows performance for one bird, filled circle shows mean. Dots are distributed along the x-axis so that the circles do not obscure one another. (B-D) Percent correct performance after transfer to auditory scenes, broken into blocks of 50 trials in each stimulus category. Six blocks of songs and scenes, and three blocks of chorus, comprise 2550 total testing trials. Chorus-alone trials were presented half as frequently as other trial types. (B) Performance on songs alone. Colored lines show individual birds, filled circles show mean. (C) Performance on auditory scenes at varying SNRs. (D) Performance on chorus presented alone.

Supplemental Figure 2 – Histological analysis of primary AC recordings, related to Figure 2

(A) BDA labeling in a parasagittal section located 1 mm lateral of the midline. Injections were made on the final recording day. In each bird, Field L (primary AC) neurons were recorded over a 300 um range of medial-lateral locations, between 1.1 and 1.4 mm lateral to the midline. (B) Schematic of recording locations in Field L, collapsed along the medial-lateral axis. Black rectangles estimate the anatomical region within which single units were recorded in a single hemisphere of an individual bird (6 hemispheres in 4 birds). The location, size and angle of each rectangle was estimated from BDA injections (as in A) and notes made throughout recording. Black lines show three anatomical boundaries that were used to register histological sections to one another and to a previously published map of Field L regions (based on Fortune and Margoliash, 1992).

Supplemental Figure 3 – Midbrain and primary AC neurograms, related to Figure 3

(A-B) Neurograms showing the population representation of a song in the midbrain (A) and primary AC (B). Each row shows the average firing rate over time for an individual neuron. Gray scale is 0 (white) to 100 (black) spikes/sec.

Supplemental Figure 4 – Segregation of NS and BS populations in the midbrain, primary AC and higher-level AC, related to Figures 3 and 4

(A-C) Firing rate (A), selectivity (B) and sparseness (C) of BS and NS neurons in each area (mean + S.D.). (D-E) Extraction index curves for BS and NS neurons in the midbrain (D) and primary AC (E). (F) Slope of extraction index curves for BS and NS neurons in each area,

compared to the slopes of psychometric functions measured from behaving birds.

Supplemental Figure 5 – Responses of primary AC neurons as a function of estimated recording site, related to Figures 3 and 4

(A-C) Firing rate (A), selectivity (B) and extraction index slope (C) of primary AC neurons as a function of recording depth measured relative to the dura. None of these parameters varied significantly as a function of recording depth. (D-F) Firing rate (D), selectivity (E) and extraction index slope (F) as a function of average recording location along the rostral-caudal axis. For each hemisphere for which we had histological data (see Figure S2), we calculated the average rostral-caudal location of the recording region. We then ranked each hemisphere from most rostral (1) to most caudal (6) and averaged the parameters of all neurons recorded in each hemisphere. None of these parameters varied significantly as a function of rostral-caudal location.

Supplemental Figure 6 – Response selectivity of BS neurons in the higher-level AC, related to Figure 6

(A) Acoustic similarity of pairs of notes to which a BS neuron responded (black) and pairs of notes selected at random (white). Pairs of notes that both evoked a response were more acoustically similar to one another compared to pairs of notes selected at random ($p<0.001$, Wilcoxon). Histograms show acoustic similarity scores measured using the responses of all BS neurons that responded to at least 2 notes (n = 117 neurons). (B) The pitch and frequency modulation of every note within the set of 15 vocalizations (n = 403 notes). Red circles indicate the subset of notes to which a single neuron responded (n = 5 notes). These 5 notes had similar pitch and frequency modulation. Other notes with nearly identical pitch and frequency modulation failed to evoke a response. (C) Example BS neuron's response to a vocalization with a note repeated 4 times. The neuron responds reliably on the first, third and fourth iteration, but not the second, indicating that spectrotemporal tuning alone cannot account for this neurons spiking pattern. (D) Example STRFs from individual neurons in the midbrain and primary AC, and from BS and NS neurons in the higher-level AC. Unlike neurons in other populations, STRFs from BS neurons in the higher-level AC lack obvious spectrotemporal tuning. (E) Predictive power of STRFs estimated from each population (mean + S.D.) All groups were significantly different from one another (Kruskal-Wallis, $p<0.05$).

Supplemental Figure 7 – Comparison of recorded and simulated neurons, related to Figure 8

(A-D) Simulated BS neurons respond to song similarly to recorded BS neurons. (A) Song firing rate. (B) Chorus firing rate. (C) Song selectivity. (D) Population sparseness.

Distribution of neurons across subjects and brain areas, related to Figure 2

			NCM	
Bird	**MLd**	**Field L**	**NS**	**BS**
1	11	9	5	14
2	11	7	6	13
3	12	12	5	16
4	19	16	4	16
5	6	19	6	19
6	10	13	3	19
7	20	10	3	15
8	11	13	3	23
Total:	100	99	35	135

Supplemental Experimental Procedures

Histology

During the last physiology session, iontophoretic injections of BDA (5 uA square pulse, 14 ms period) were made at multiple locations along the electrode tracts in each area (MLd, Field L and NCM). One day after the last physiology session, the animal was deeply anesthetized with nembutal and was perfused with 10% formalin. The brain was removed and placed in 30% sucrose solution for approximately one week. Tissue sections were sliced (40 um) on a freezing microtome. BDA injection sites were visualized by processing tissue with DAB and 0.001% hydrogen peroxide in PBS. We estimated the volume within which neurons were recorded over multiple days of physiology based on the reconstructed recording tract from the final day and records from previous days noting the location of the electrode penetration (medial-lateral and rostral-caudal) and the recording depth for each neuron. See Figures S2 and S5.