**Supplementary Figures** 



# Figure S1. Retrograde tracing of input to the functionally mapped AAF.

# **Related to Figure 1**

(A) DAPI staining of a brain slice. MGd, dorsal part of the medial geniculate body; MGv, ventral part of the medial geniculate body; Po, posterior complex of the thalamus. (B) Cell bodies labeled with CTB-488 were injected into the AAF. (C) Image of a neighboring section showing the landmarks of the hippocampus (Hip). Scale bar, 500 µm.





(A) An example image of the auditory cortex surface under stereoscope (4X amplification). (B) Tungsten electrode (coated with DiI) and glass pipette (coated with DiO) penetration tracks in the same paired recording.  $\theta$  represents the difference in penetration angle. (C) Distribution of  $\Delta$ Angle ( $\theta$ ) for 15 pairs of successfully reconstructed tracks. (D) Calculation of horizontal and depth error at an 800-µm travel distance. (E) Relationship between  $\Delta$ Angle and location error at an 800-µm travel distance.



Figure S3. Comparison of TRFs averaged from adjacent neurons and those of MUA at the same location. Related to Figure 1

(A) TRFs of adjacent neurons in AAF in 5 experiments. Color represents average spike rate; white dashed curves outline the TRF of MUA at the same location. (B) Left panel, TRF averaged from adjacent neurons shown in (A). Right panel, TRF of MUA at the same location.



# Figure S4. Linearity of current-voltage relationship.

#### **Related to Experimental Procedures**

(A) White noise evoked synaptic current at different holding potentials (-100 mV, -70 mV, -30 mV and 0 mV).(B) I-V curve. Currents were averaged within a time window of 1-2 ms (pink) and 20-22 ms (black) after

synaptic onset. Bar, SD.



Figure S5. The excitatory and inhibitory TRFs largely overlapped. Related to Discussion

(A, B) TRFs of excitatory (EPSC) and inhibitory (IPSC) inputs with dashed lines outlining the TRF boundary.(C) Comparison of excitatory and inhibitory TRF boundaries for L4 and L5 cells. (D) Fraction of the non-overlapping area compared with the total TRF area. Bar, SD, t test, p = 0.22.



#### Figure S6. Criteria for defining responsive neurons.

#### **Related to Experimental Procedures**

(A) Spike TRFs of 5 sequential repetitions for the cell shown in Figure 1B. (B, C) Spike responses to a preferred tone in 5 repetitions for example L4 and L5 cells. Bar represents sound duration. (D) Average percentage of responsive trials to preferred tones for responsive L4 (n = 83) and L5 (n = 111) populations, p = 0.54, t test. Error bar, SD. (E) Spontaneous firing rate in L4 and L5 populations, p = 0.63, t test. Error bar, SD. (F) Distribution of z-scores for the total population of recorded cells (n = 346). Z-score > 3 is used as a criterion to define responsive neurons.



# Figure S7. Accuracy of depth determination.

# **Related to Experimental Procedures**

(A) Image showing a biocytin labeled cell in L5. Vertical line indicates the actual depth of this cell, which is 28  $\mu$ m shorter than the travel distance. (B) Depth based on reconstruction plotted against depth expected from the travel distance (n = 22). Blue line represents the unity line.