## **Supporting Information**

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## **SI Materials and Methods**

Optical Intrinsic Signal Stimulus Set and Image Preparation. Our acoustic imaging stimulus was designed to highlight regions of A1 selective for 5- and 13-kHz sounds. Phase-triggered images were recorded over 150 repetitions of a 4-s stimulus. The stimulus used was divided into four 1-s blocks, each consisting of various types of tone pips lasting for 25 ms and having cos<sup>2</sup> ramps. The first second consisted of 5-kHz tone pips with 200-ms spacing. The next second was a randomly timed bracket of 16 1- to 2.5-kHz tones and 6 7.071- to 9.330-kHz tones. The third second was identical to the first second except that 13-kHz tones were used. The fourth second was again a random bracket of tones, but all were over 26 kHz. This procedure ensured that the 5- and 13kHz responsive regions of A1 were activated 180° out of phase with each other. All tones were intensity-adjusted to the rat audiogram relative to 8 kHz at 60 dB (1). Data analysis was performed with VK Imaging software and identified regions of A1 in which hemodynamic changes altered red light absorbance in a manner phase-locked to the 5- and 13-kHz tones. Phase was pseudocolored, and shifting allowed the LF and HF regions to be displayed in green and red, respectively. The LF and HF regions of A1 were then overlaid onto the cortical surface image using

1. Heffner HE, Heffner RS, Contos C, Ott T (1994) Audiogram of the hooded Norway rat. Hear Res 73:244–247. GIMP image software (Free Software Foundation), and any necessary adjustments were made in images aligned using vasculature matching between the two images.

Nose Poke Shaping. On arrival, animals were pair-housed, allowed a minimum of 3 d to adjust to their colony room, and given free access to standard laboratory chow and water. After this rest period, animals were desensitized to handling for 5 min/d for 3-5 d. Free food access was also removed, and each animal received a minimum of 10 g of food immediately following handling. On the last day of handling, food was restricted to 80%. The following day, animals began shaping for the nose poke task in the operant chamber they would later perform tone detection in. All subsequent food would be gained by working in this operant chamber. Initial hold times were 100 ms, and animals were able to earn a minimum of 220 45-mg food pellets each day. Once the rate of successful holds exceeded unrewarded early withdrawals, the hold time was increased by 100 ms until animals reliably held a poke for 800 ms, which took between 8 and 10 sessions. Animals were then kept at this time, and following the third day of holding for 800 ms, they were introduced to the tone detection task.



**Fig. S1.** Imaging and microelectrode recording. (*A*) Optical intrinsic imaging overlaid onto a photograph of the craniotomy and cortical surface. (*B*) Subsequent penetration map showing distribution and characteristic frequency of sites in the HF and LF regions. Penetrations lacking an auditory response are marked with the letter x. (*C*) Two-panel plot shows the sound stimulus (above) and spike-filtered voltage response from one A1 site. (*D*) Action potential onset responses to tone sound stimului of different intensities and frequencies are shown.



Fig. S2. Response strength vs. frequency in control A1 regions. (A) Response strength vs. frequency from control LF region in response to 50-dB SPL tone sounds. (B) Frequency-response relation from control HF region. Shaded area on either side of black line indicates SE measurements.



**Fig. S3.** Spatial reorganization of target representation in A1 at different learning time points. (A–C) Individual tessellation maps of microelectrode recordings in A1. (A) Representative tessellation map of a control animal with a frequency histogram of impulse rates from penetrations in all control animals divided into LF regions (solid line) and HF regions (dashed line). (B) Representative tessellation maps of a 1-day animal with a population frequency histogram. (C) Representative tessellation maps of a 1-day animal. Tessellation maps are set to a rainbow scale representing the number of action potentials per second. (D–F) Population frequency histograms of A1 firing rates for target. A population frequency histogram of control animals (D), 1-day animals (E), and 14-day animals (F) is shown. All histograms are normalized for numbers of total penetrations made in control LF (n = 54) and HF (n = 56) regions, 1-day LF (n = 61) regions. Bins are set to one impulse per second. Histograms were truncated at 20 impulses per second.



Fig. S4. Arc expression in A1 regions of controls. Arc mRNA expression in LF and HF regions of control animals. DAPI staining of cell nuclei is shown in blue. Baseline Arc mRNA expression is very low in A1 but is observable as red foci in a small number of neuronal nuclei (1–2%).