

# Associative learning shapes the neural code for stimulus magnitude in primary auditory cortex

Daniel B. Polley\*, Marc A. Heiser, David T. Blake, Christoph E. Schreiner, and Michael M. Merzenich

Coleman Memorial Laboratory, W. M. Keck Center for Integrative Neuroscience, and Departments of Otolaryngology and Physiology, University of California, San Francisco, CA 94143

Contributed by Michael M. Merzenich, October 12, 2004

Since the dawn of experimental psychology, researchers have sought an understanding of the fundamental relationship between the amplitude of sensory stimuli and the magnitudes of their perceptual representations. Contemporary theories support the view that magnitude is encoded by a linear increase in firing rate established in the primary afferent pathways. In the present study, we have investigated sound intensity coding in the rat primary auditory cortex (AI) and describe its plasticity by following paired stimulus reinforcement and instrumental conditioning paradigms. In trained animals, population-response strengths in AI became more strongly nonlinear with increasing stimulus intensity. Individual AI responses became selective to more restricted ranges of sound intensities and, as a population, represented a broader range of preferred sound levels. These experiments demonstrate that the representation of stimulus magnitude can be powerfully reshaped by associative learning processes and suggest that the code for sound intensity within AI can be derived from intensity-tuned neurons that change, rather than simply increase, their firing rates in proportion to increases in sound intensity.

intensity | plasticity | Pavlovian | sound | perceptual learning

A complete rendering of the neural code for a stimulus event must portray not only how the stimulus engages the receptor sheet over space and time but also how stimulus magnitude impacts the spatiotemporal patterns of neural responses in central sensory structures. Magnitude is an inherent property of all stimuli, and its subjective perceptual estimation, when assayed psychophysically, conforms to a power function whereby a constant percentage increase in the stimulus magnitude produces a constant percentage increase in the sensed effect (1). Neurophysiological recordings have shown that increasing stimulus magnitude is transduced by peripheral sense organs following the power law then “represented” by a linear increase in response strength across sensory system levels feeding “primary” cortical areas (2–4). In the auditory system, the magnitude and modulation of the sound-pressure envelope provide information that is essential for the perception of the distance separating an animal or human from other sound sources and convey critical information for vocal communication (5). Although the psychophysical thresholds for human loudness discrimination (1, 6) and putative neural codes for loudness based on firing rate or spatial patterns of excitation have been described in the eighth nerve, auditory midbrain and primary auditory cortex (AI) (7–13), very little is known about how, or if, loudness discrimination thresholds can be altered by training or how the effects of such perceptual learning would affect the hypothesized “neural codes” for loudness.

In the present study, we demonstrate that training animals to associate changes in sound intensity with reward induced large-scale plasticity in the neuronal response representation of sound intensity in the AI. Rats were trained in three stimulus-reward conditioning paradigms: (i) instrumental conditioning (IC), in which reward delivery depended on motor actions guided by auditory feedback; (ii) paired stimulus reinforcement (PSR), in which particular sound intensities were reliably paired with

rewards without the requirement for motor responses; and (iii) unpaired stimulus reinforcement (USR), in which exposure to sound stimuli of varying intensity was not reliably paired with reward. We adopted a yoked control experimental design to ensure that IC, PSR, and USR rats received an identical sensory stimulus controlled by the IC rat and differed only in their requirements for reward delivery (Fig. 1*a*). After training, discharge rate versus intensity level functions (RLFs) were derived from neural responses in AI and compared with the responses obtained from naïve control (NC) rats.

## Methods

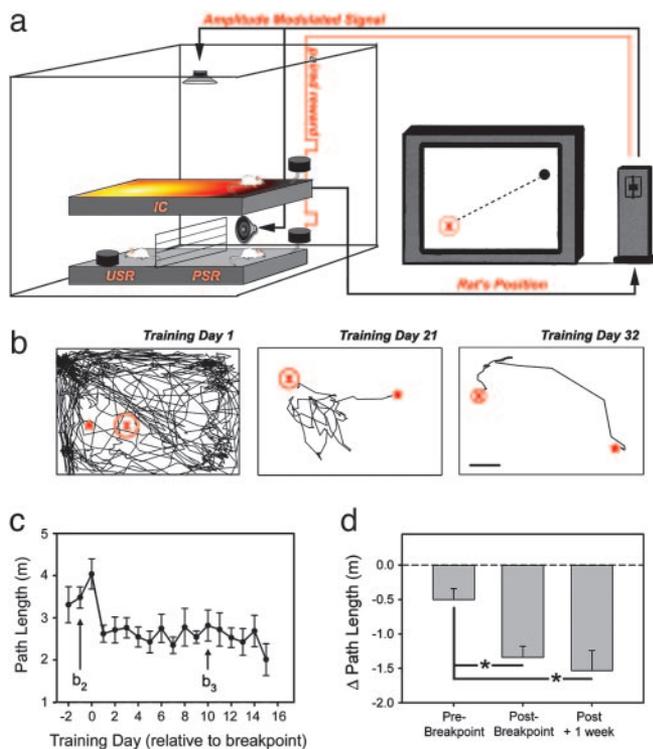
**Behavioral Training.** Twenty-five adult Sprague–Dawley rats (age, 12–16 wk) were used in this study. Rats were divided into four treatment groups: NC ( $n = 5$ ), IC ( $n = 8$ ), PSR ( $n = 5$ ), and USR ( $n = 7$ ). Behavioral training took place over 4–7 wk in a two-tiered behavioral apparatus contained within a sound-attenuated chamber. Each tier was outfitted with a single acoustically transparent wire cage (Fig. 1*a*). The top cage was used for “bull’s-eye” IC, and the bottom cage was partitioned into two separate zones, one dedicated for USR and the other for PSR. The behavior of rats in the PSR and USR groups was not measured. In the bull’s-eye task, rats were allowed to move freely on a rectangular platform (58 × 38 cm) that rested on force transducers. A LABVIEW program (National Instruments, Austin, TX) triangulated the rat’s position in two-dimensional space in real time according to the distribution of weight across the force transducers. A speaker (Vifa, Danish Sound Technology, Videbæk, Denmark) suspended 44 cm above the platform continuously delivered an amplitude-modulated stimulus for the duration of a single trial. The training stimulus was a band-limited noise (0.65-octave bandwidth) with an amplitude envelope modulated by 10% at 8 Hz. For each trial, the center frequency of the band-limited noise stimulus was randomly selected from a 2.2- to 28-kHz range. The intensity of the band-limited noise was proportional to the distance between the rat and bull’s-eye (see supporting information, which is published on the PNAS web site, for details).

For each trial, the computer pseudorandomly selected a circumscribed area on the platform to serve as a virtual bull’s-eye. The rat’s task, on any given trial, was to use the sound intensity cues to locate the position of the bull’s-eye within 3 min to receive a pellet reward. As training progressed, the bull’s-eye diameter was decreased from 10 cm to 4 cm, and the amount of time the rat was required to continuously remain within the perimeter of the bull’s-eye was increased from 0 s to 1.5 s to discourage random search strategies and promote identification

Abbreviations: AI, primary auditory cortex; IC, instrumental conditioning; PSR, paired stimulus reinforcement; USR, unpaired stimulus reinforcement; RLF, discharge rate versus intensity level function; BL, best level; NC, naïve control; SPL, sound pressure level; K-S, Kolmogorov–Smirnov.

\*To whom correspondence should be addressed at: Keck Center for Integrative Neuroscience, 513 Parnassus Avenue, San Francisco, CA 94143-0732. E-mail: dpolley@phy.ucsf.edu.

© 2004 by The National Academy of Sciences of the USA



**Fig. 1.** Design and performance measurements in an auditory learning task. (a) Rats in IC, USR, and PSR training groups were simultaneously exposed to the same amplitude-modulated stimulus. A computer continuously adjusted the sound intensity in real time as a function of the distance between the rat and the virtual bull's-eye, a randomly selected spatial location associated with either a 20- or 70-dB SPL stimulus. IC rats identified and navigated a sound intensity gradient to find the bull's-eye and obtain a food reward. Reinforcement in USR and PSR rats was independent of their behavior. In PSR rats, the reward was paired to reward delivery for the IC rat and, therefore, paired with a particular sound intensity, and, in USR rats, food was available ad libitum. The filled black circle, the X, and the red circle drawn in the computer monitor illustrate the current position of the IC rat, the position of the bull's-eye, and the diameter of the bull's-eye, respectively. The color gradient on the bull's-eye platform represents the gradient of sound levels the rat experienced for a particular trial. (b) Black lines represent a rat's path in a single trial over three stages of training. The starting point for the trial is indicated by the star; the position and diameter of the bull's-eye are represented by an X and red circle, respectively. (Scale bar, 10 cm.) (c) Average path length per day shown relative to the behavioral breakpoint for all rats. Arrows indicate when the single path data from training days 21 (b<sub>2</sub>) and 32 (b<sub>3</sub>) shown in *b* occurred relative to the breakpoint. (d) Change in path length before, immediately after, and 1 wk after the behavioral breakpoint. Asterisks indicate *P* values < 0.0005 from paired *t* tests.

of the bull's-eye position through the use of sound intensity cues. As a final test to ensure that the rat's behavior was specifically controlled by sound level, three rats that had been trained for several weeks to locate bull's-eyes associated with high-intensity stimuli were subjected to a series of "catch trials" in which the sound-level-reward contingency was suddenly reversed so that the bull's-eye was associated with a low-intensity stimulus. In all cases, the path length and trial time exhibited an immediate and substantial increase, demonstrating that behavior was truly guided by sound level.

**Electrophysiological Recording.** Rats were anesthetized with sodium pentobarbital (50 mg/kg followed by  $\approx 15$  mg/kg supplements as needed), the auditory cortex was surgically exposed, and multiunit neural responses were recorded with parylene-insulated tungsten microelectrodes (1–2 M $\Omega$ ; FHC, Bowdoin-

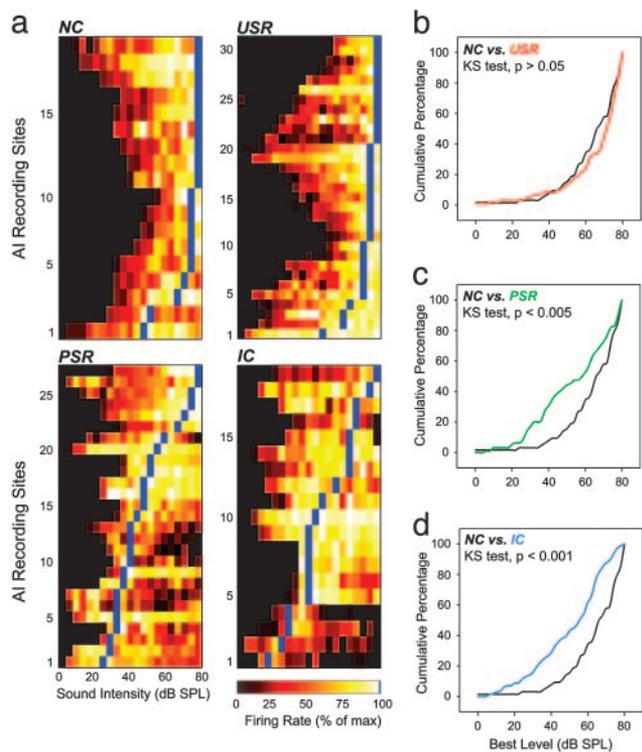
ham, ME). Recording sites ( $\approx 20$  per mm<sup>2</sup>) were evenly distributed across the AI tonotopic map to sample neural responses with characteristic frequencies ranging from 1 to 30 kHz. At each site, the electrode was lowered 450–600  $\mu$ m orthogonal to the pial surface, roughly corresponding to layer IV. Frequency/intensity response areas were reconstructed by presenting 50 pure-tone frequencies (20-ms duration, 5-ms raised cosine ramps) at each of eight sound intensities to the contralateral ear. The ipsilateral ear was plugged with sound-attenuating putty to ensure that sound stimuli were presented monaurally. AI was defined based on short latency (8–20 ms) evoked onset responses in the most medial auditory field containing a complete tonotopic gradient running at  $\approx 15^\circ$  relative to the horizontal plane. Recordings made outside of this frequency gradient that had long latency responses, unusually high thresholds, or very broad tuning were considered to be non-AI sites and were not included in this analysis.

Once we were certain that AI had been identified, we began a more thorough analysis of intensity tuning in AI neurons. Spike RLFs were derived from a subset of recording sites by presenting tone pips (100-ms duration with 500-ms interstimulus intervals) at the characteristic frequencies defined for a particular site at sound levels ranging from 0- to 80-dB sound pressure level (SPL) in 2-dB SPL increments. Sound levels were presented in a random order and repeated 10 times each. The neural response strength at each sound level was obtained by calculating the average spike rate within a 30-ms window beginning at the onset response and subtracting the average baseline spike rate collected during the 50 ms preceding stimulus onset. The following measures were derived from each RLF: (i) minimum response threshold, (ii) transition point, (iii) best level (BL), and (iv) monotonicity. If the spike rate remained at zero for two consecutive sound levels, all sound levels less or equal to the greater of the two levels were considered subthreshold. Minimum response threshold was defined as the first sound level in the suprathreshold region of the RLF (see Fig. 3, arrow). In many cases, the RLF consisted of a fast-growing low-level portion and a saturated or decreasing response function at higher sound levels. The transition point was defined as the highest sound level within the fast-growing region (see Fig. 3, star). BL was defined as the sound level that evoked the greatest magnitude response (see Fig. 3, diamond). Monotonicity was defined as the slope of the RLF between the transition point and the highest sound level estimated by a linear regression analysis. In the event that the RLF increased linearly above the minimum response threshold (e.g., see Fig. 3*a*) and a transition point could not be determined, a linear regression analysis was performed on all sound levels above threshold. In both cases, the slope of the regression function was used as a quantitative measure of monotonicity, whereby a negative slope corresponded to a nonmonotonic response and a slope of zero or greater corresponded to a monotonic response function. All analyses were performed blind to experimental condition.

## Results

We created a loudness discrimination task in which the IC rat had to remain motionless over a small region on a two-dimensional platform (the bull's-eye) to obtain a food reward. The virtual bull's-eye target was consistently associated with either the high (70-dB SPL) or low (20-dB SPL) end of a 50-dB intensive spectrum. The spatial position of the bull's-eye varied randomly for each trial. In an adaptive procedure, the loudness of band-limited noise stimuli were proportional to the distances between the rat and bull's-eye, which required that rats use sound intensity as the cue to locate the bull's-eye location.

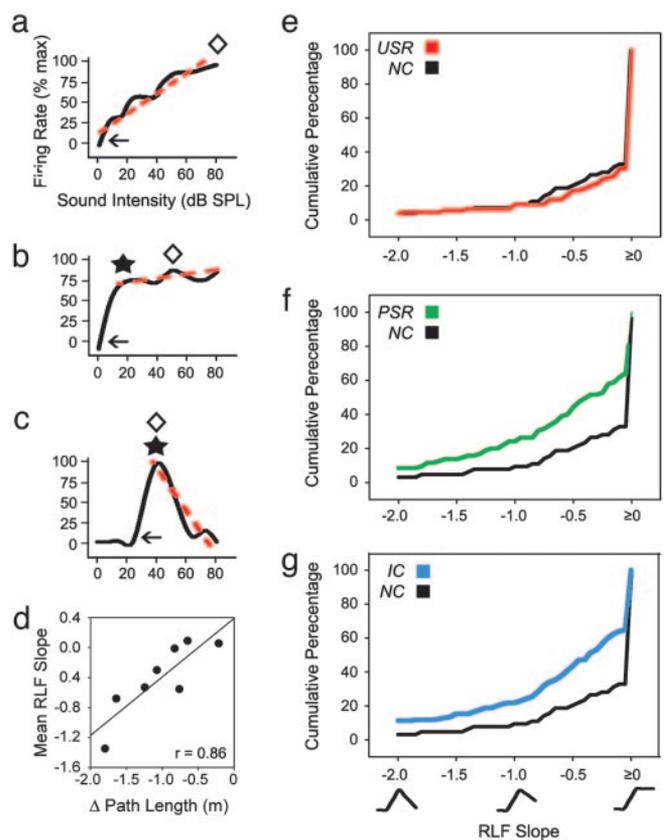
All IC rats learned to discriminate fine changes in loudness to approach the target intensity bull's-eye then remain motionless over the bull's-eye until rewarded. In the initial days of training,



**Fig. 2.** Associative learning changes the encoding of sound intensity in AI neurons. (a) Neurograms depict all RLFs recorded within AI from a single animal in each group. Each row represents a RLF from a single recording site, with the firing rate represented by a pseudocolor scale. Rows are sorted by BL such that BLs that correspond to lower sound intensities are presented at the bottom of each neurogram. Response at each intensity are normalized to the response evoked by the BL (100%/blue). (b–d) Cumulative percentage histograms compare the distributions of BL values for NC rats (black line for all graphs) with USR (b), PSR (c), and IC (d) rats. Significant differences in BL distributions obtained with a two-sample K–S test are indicated for each comparison.

rats remained along the perimeter of the bull’s-ey platform (Fig. 1*b Left*). Over a period of several weeks, the path length to the bull’s-eye decreased (Fig. 1*b Center and Right*). The average path length initially fluctuated around a relatively high level over several days and then increased slightly for 1 d, operationally defined as the “behavioral breakpoint,” before dropping to consistently lower values for the remainder of training (Fig. 1*c*). The decrease in path length between the breakpoint and the following day was significant ( $t = 4.93$ ,  $P < 0.005$ ), but the increase between the breakpoint and the prior day was not ( $t = 1.89$ ,  $P > 0.05$ ). Furthermore, the average path length after the behavioral breakpoint was significantly shorter than before the behavioral breakpoint and continued to remain at a lower value 1 wk later (paired  $t$  test;  $P < 0.0005$  for both comparisons) (Fig. 1*d*). The time required to find the bull’s-eye did not change significantly over the course of training [ $F(16,64) = 1.36$ ,  $P > 0.05$ ], which, when considered in conjunction with the decrease in path length, indicates that the rats moved more slowly as training progressed.

Substantial changes in the cortical encoding of sound intensity were recorded in IC ( $n = 8$ , 170 sampled sites) and PSR ( $n = 5$ , 95 sites) rats compared with NC ( $n = 5$ , 64 sites) and USR ( $n = 7$ , 107 sites) rats after 4–7 wk of intensity discrimination training. AI was distinguished from other auditory cortical fields based on tonotopic maps reconstructed from high-density ( $\approx 20$  penetrations per  $\text{mm}^2$ ) sampling of characteristic frequency tuning. AI was reliably identified by a continuous and complete low–high



**Fig. 3.** Associative learning increases the proportion of nonmonotonic RLFs. (a–c) Example RLFs illustrate the minimum response threshold, transition point, and BL with an arrow, star, and diamond, respectively. (a) Note that in linearly increasing RLFs, the transition point cannot be defined. The red dotted line in each example depicts the slope of the linear regression line used to calculate monotonicity. (d) Decrease in path length (after breakpoint–before breakpoint) is significantly correlated with the average RLF slope. The relationship is fit with a linear regression. (e–g) Cumulative percentage functions depict the distribution of monotonicity values in USR (e), PSR (f), and IC (g) rats. The distribution of monotonicity values from NC rats is shown as a black line in each graph for comparison.

tonotopic gradient running caudal to rostral at an  $\approx 15^\circ$  angle relative to the horizontal plane. RLFs were derived from a subset of recording sites judged to be within AI. Neural firing rate recorded in NC and USR rats typically increased continuously (monotonically) with increases in sound intensity, and the sound intensity that evoked the greatest response, the BL, was clustered around the highest sound intensities (Fig. 2*a Upper*). After either PSR or IC training, neural responses were more frequently tuned to a restricted range of sound intensities, and BLs were more evenly distributed across the intensive range (Fig. 2*a Lower*). Quantitative comparison of the BL distributions from NC rats revealed a significant skewness toward high intensities compared with either PSR or IC rat BL distributions [two-sample Kolmogorov–Smirnov (K–S) tests: PSR,  $P < 0.005$ ; IC,  $P < 0.0001$ ] (Fig. 2*c and d*). BL distributions from NC rats were not different from the distributions recorded in USR rats (two-sample K–S test;  $P > 0.05$ ) (Fig. 2*b*).

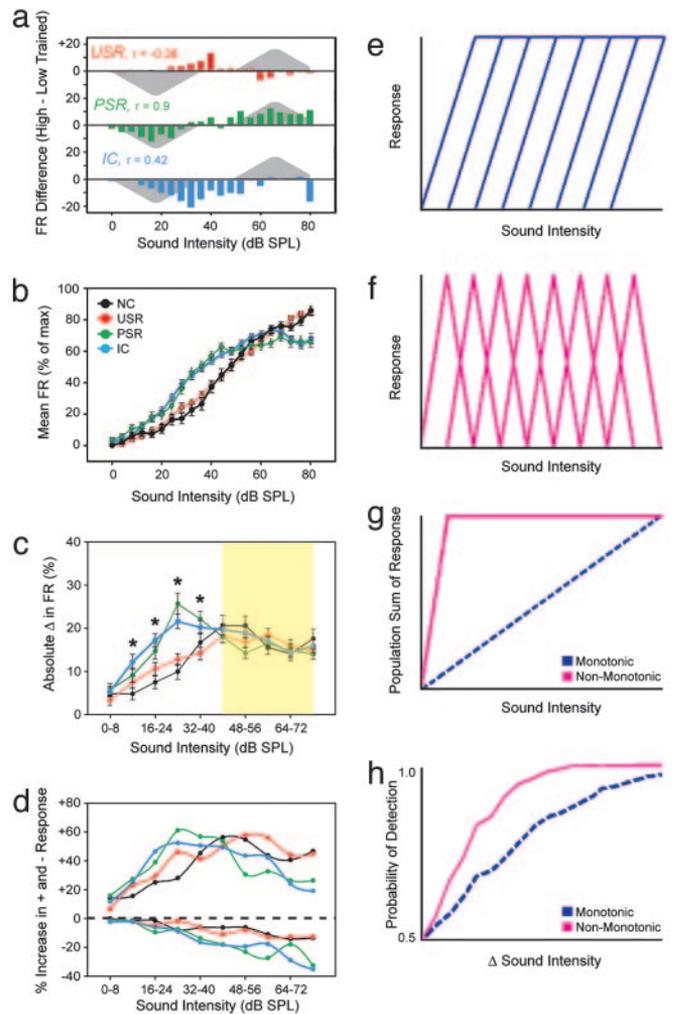
By using a 50% decrease in firing rate at the highest sound intensity relative to the firing rate at the BL as a rough index of a nonmonotonic response, we observed that 14% and 18% of recording sites were nonmonotonic in NC and USR rats, respectively. By contrast, 38% and 32% met that criterion in PSR and IC rats, respectively. A quantitative analysis of monotonicity distributions between NC and USR recordings confirmed that

RLF slopes were predominantly monotonic in both groups and that their distributions did not differ (two-sample K-S test,  $P > 0.05$ ) (Fig. 3e). In PSR and IC rats, by contrast, the distributions of slope functions were significantly shifted toward nonmonotonic values compared with NC distributions (two sample K-S tests: PSR,  $P < 0.005$ ; IC,  $P < 0.0005$ ) (Fig. 3f and g). As a result, a significantly greater proportion of neural responses were tuned to restricted ranges of moderate or low sound intensities in trained animals.

The increased proportion of nonmonotonic RLFs in trained animals was not simply the byproduct of an overall greater level of cortical inhibition after training. Spontaneous ( $8.47 \pm 0.6$  versus  $7.04 \pm 0.41$  spikes per s;  $t = 1.91$ ,  $P > 0.05$ ) and BL-evoked ( $121.51 \pm 3.93$  versus  $119.37 \pm 4.09$  spikes per s;  $t = 0.36$ ,  $P > 0.05$ ) firing rates measured in trained (average of IC and PSR groups) versus untrained (NC rats) animals respectively were not different after training. Spectral tuning curve bandwidths measured 20 dB above threshold were significantly broader in trained (average of IC and PSR groups) animals than in NC ( $1.25 \pm 0.03$  versus  $1.09 \pm 0.03$  octaves;  $t = 3.66$ ,  $P < 0.0005$ ). This difference is likely attributable to the spectral bandwidth (0.65 octave) used in the training stimulus, given that continuous exposure to wide band noise has been shown to increase receptive field size in younger rats (14). Thus, cortical response features unrelated to sound intensity were either unchanged or slightly more excitable in PSR and IC rats, whereas cortical responses to higher intensity stimuli became selectively suppressed.

Importantly, nonmonotonicity was significantly correlated with the extent of behavioral improvement in the bull's-eye task. Upon screening all measured physiological variables as a correlate of the instrumental learning (see supporting information), only the mean slope value was significantly correlated with the behavioral improvement ( $r^2 = 0.73$ ,  $P < 0.01$ ), whereby rats with more negative (nonmonotonic) RLF slopes also showed the greatest decrease in path length after the behavioral breakpoint compared with that before the behavioral breakpoint (Fig. 3d).

We observed several important differences between the plasticity expressed in IC versus PSR rats that directly pertain to the different behavioral requirements in the two tasks. In the instrumental learning task, the IC rat located the bull's-eye by following a sound intensity gradient. In IC rats, therefore, all intensities, including the target intensity, provided information crucial for obtaining rewards. For PSR rats, in contrast, only intensities at or near the target intensity predicted the onset of reward. Based on this difference, we formulated two hypotheses: (i) The specific conditioning strategy (high- versus low-intensity sounds paired with reward) would potentiate the neural response to stimuli presented at the target intensities in PSR, but not IC, rats, and (ii) BL values in IC rats should be better contained within the range of intensities encountered in the bull's-eye task (20- to 70-dB SPL) than in PSR rats. Subsequent analysis upheld both of these predictions. We found that neural response strengths evoked by tones played at or near the intensity that immediately preceded rewards were selectively potentiated in PSR rats but not in IC rats (Fig. 4a). The difference in neural responses at each intensity (mean firing rate in high-intensity-trained rats minus mean firing rate in low-intensity-trained rats) was strongly correlated with an idealized difference function in which the responses were selectively potentiated at or near the target intensity (Fig. 4a, gray shading) in PSR rats ( $r = 0.9$ ,  $P < 0.001$ ) but not in IC ( $r = 0.42$ ) or USR ( $r = -0.31$ ) rats. The correlation strength was stable across a broad range of idealized difference function models (see supporting information). Furthermore, we found that a significantly greater proportion of recordings sites had BL values distributed within the range of sound intensities encountered in the bull's-eye task (20- to 70-dB SPL) in IC rats (81%) compared with PSR rats (69%;  $\chi^2 = 4.2$ ;  $P < 0.05$ ). Therefore, the different behavioral requirements



**Fig. 4.** Plasticity of the cortical population intensity code. (a–d) Data from NC, USR, PSR, and IC rats are shown in black, red, green, and blue, respectively. (a) Plot of the difference between neural response magnitude at each sound intensity in low-target-trained rats (20 dB) and responses to each sound intensity in high-target-trained rats (70 dB). Gray shading represents a hypothetical difference function in which responses were potentiated near the target intensity. The correlation between the actual response and the idealized response was calculated, and the Pearson correlation coefficient is shown. (b) Population growth of magnitude functions created by averaging all individual RLFs in NC, USR, PSR, and IC rats. (c) Absolute change in response strength between consecutive 8-dB intensity bins. Yellow shading indicates a 40-dB range over which the absolute change in response remained fairly constant for all groups. Asterisks indicate significant differences in absolute change by using pair-wise comparisons between trained (average of PSR and IC) and untrained (average of USR and NC) rats. (d) Percentage of recording sites in which response strength either increased (values above the dotted zero line) or decreased (values below the dotted zero line) by  $\geq 10\%$ . (e and h) Theoretical output from an array of monotonic (e) and nonmonotonic (f) neurons. (g and h) Population responses created from the sum of monotonic or nonmonotonic response functions (g) can be used to predict the probability of detecting changes in sound intensity (h).

between IC and PSR learning correspond to predictable differences in the expression of plasticity. The distribution of monotonicity and minimum response threshold did not differ between high-intensity-target-trained versus low-intensity-target-trained animals in the USR, PSR, or IC groups (two-sample K-S tests,  $P > 0.05$  for all tests).

Changes in sound intensity representation described at individual recording sites translate into distinct cortical population

codes used to represent sound intensity between trained and untrained animals. The neuronal population growth of response functions, created by averaging all normalized RLFs for each group, showed how a population-rate code might serve as a code for sound intensity in control and conditioned animals. Because minimum response thresholds varied and RLFs were mostly monotonic in NC and USR rats, it is not surprising that the population growth of magnitude functions increased linearly with sound intensity in both groups (linear regression analysis,  $P < 0.01$  for both groups) (Fig. 4*b*). By contrast, the population growth of magnitude functions in PSR and IC rats were significantly different from NC rats [PSR,  $F(1,157) = 7.77$ ,  $P < 0.01$ ; IC,  $F(1,232) = 14.86$ ,  $P < 0.001$ ]. Population growth of magnitude functions in trained animals increased rapidly and then flattened out across the upper half of the intensive range (Fig. 4*b*). This nonlinear appearance was attributable to steep initial RLF slopes and a nearly equal mixture of monotonic RLFs with flat or positive slopes and nonmonotonic RLFs with negative slopes that collectively produced a flat slope across the range of higher intensities.

At first glance, the population-rate code for sound intensity in USR and NC rats appeared superior because each intensity evoked a unique response level, whereas, in IC and PSR rats, responses changed by less than a standard error across a 100-fold increase in sound intensity. However, a more detailed analysis of the population firing-rate code in trained animals revealed the emergence of a combined rate and spatial code for sound intensity. Comparison of the absolute changes in firing rate across the entire range of intensities, independent of whether responses increased or decreased, demonstrated a significantly greater proportional change in response strength over the lower half of the intensive range in trained animals compared with untrained animals, but, importantly, the absolute change in response strengths remained surprisingly flat across the upper half of the intensive range and was similar between all four groups [ $F(3,434) = 0.99$ ,  $P > 0.05$ ] (Fig. 4*c*). Therefore, the change in firing rate over a 100-fold change in sound pressure level (Fig. 4*c*, yellow shading) was linear in IC and PSR rats, yet the increase in firing rate was nonlinear. The bidirectional coding scheme that emerged after training confers several advantages. First, bidirectional coding permits a marked increase in firing rate across the lower intensities while still allowing for a proportional change in response strength across higher intensities without reaching saturation; second, it suggests that a change in the spatial code for sound intensity emerges with training. In USR and NC rats, an increase in sound intensity is disproportionately represented by a population of neurons that increase firing rate. In PSR- and IC-trained rats, there was an approximately equal contribution from two distinct neural populations: one that decreased firing rate and another that increased their firing rate across a range of higher intensities (Fig. 4*d*). Therefore, both place- and rate-based cortical population codes provide for a greater overall dynamic range in trained animals.

A model was created that illustrated the theoretical advantages conferred by nonmonotonic RLFs for a firing-rate-based code representing changes in sound intensity (see supporting information). The model featured separate neuronal populations comprised exclusively of either monotonic or nonmonotonic response types to represent the two theoretical extremes. As was roughly observed in our trained rats, each extreme had identical distributions of response thresholds and each covered the same range of sound intensities. In the monotonic population (Fig. 4*e*), each neuron monotonically increased its firing rate from the same minimum to the same maximum. Each nonmonotonic neuron increased to an identical firing rate achieved at a variable sound intensity then monotonically decreased back to the minimum firing rate at higher stimulus levels (Fig. 4*f*). Population growth of magnitude functions were generated for

each population. Comparison of the experimental (Fig. 4*b*) and simulated (Fig. 4*g*) data revealed a close similarity between the monotonic response function and the NC and USR growth of response functions. The PSR and IC rat response functions appeared intermediate to the monotonic and nonmonotonic extremes, reflecting the mixture of monotonic and nonmonotonic response functions described in these animals. Because nonmonotonic neurons encoded stimulus-intensity changes with both increasing and decreasing firing-rate changes, the nonmonotonic population had twice the firing-rate change for a given change in sound intensity compared with the monotonic population, translating to enhanced receiver-operator characteristics. In the IC and PSR groups, therefore, the larger fraction of the population with nonmonotonic RLFs directly implies an increased detectability of changes in sound intensity (Fig. 4*h*).

## Discussion

Several aspects of the animal model and recording techniques are worth mentioning. First, unlike other species, such as the mustached bat or cat, that have a significant proportion of nonmonotonic RLFs in AI (9, 15), recordings strictly limited to the middle cortical layers of AI in the rat have shown that the vast majority of RLFs are monotonic (16, 17), in agreement with our findings. Second, monotonicity estimation is also influenced by single versus multiunit recording techniques (11). However, in the present study, the emphasis is placed on plasticity, or difference, in the proportion of nonmonotonic neurons in trained versus untrained rats. Because identical recording methods were used in all groups, multiunit recordings could influence absolute estimation of nonmonotonic neurons recorded but would not create a bias in estimations made in trained versus untrained animals.

It is well known that spectral and temporal selectivity can be modified by associative learning in the adult auditory system. Learning-based plasticity in the spectral and temporal domains is typically expressed as a change in receptive field bandwidth or preferential tuning (18–22) and/or selective modulation of firing rate to reinforced and nonreinforced stimuli (23–25). In the present study, we have demonstrated that the proportion of nonmonotonic RLFs is significantly increased in PSR- and IC-trained animals, tantamount to a sharpening of the receptive field in the intensive domain. Furthermore, we have shown that response strength can be selectively potentiated at or near the target intensity in PSR animals. Therefore, both the response selectivity and response strength of AI neurons can be reshaped in the intensive domain, similar to training-induced changes in spectral or temporal domains. It is clear, however, that exposure to the same stimuli, when not reliably coupled to reward, had no measurable impact on monotonicity, BL distributions, or population response functions compared with NC.

After loudness listening training, we have shown that a linear population growth of magnitude function became more strongly nonlinear, in clear contradistinction to the linearly increasing functions that have been traditionally reported (in nonloudness-conditioned animals) by others (2–4, 7). The absolute change in firing rate, however, remains constant across the upper half of the intensive range, suggesting that a neural “change code” can effectively represent loudness. This finding can be interpreted as upholding the general form of Weber’s law, which states that changes in response must bear a constant ratio to changes in stimulus intensity (26, 27). At the same time, this finding refutes the specific, widespread neurological interpretation that an increase in stimulus magnitude is represented by a monotonically increasing growth of response strength. This marked plasticity in the neuronal response representation of stimulus intensity that emerges after associational learning clearly requires a reconsideration of our classical and contemporary views about neural magnitude coding explaining perceptual magnitude estimation.

We thank Drs. Shaowen Bao and Alisa Woods for their involvement in the design, construction, and software programming for the bull's-eye behavior task; Emily Stryker, Amy Deipolyi, and Jo Jefferis for help with behavioral training and data analysis; and Drs. Ralph Beitel and Loren

Frank for thorough reading of the manuscript. This work was supported by the Coleman Fund; the Sooy Fund; National Institutes of Health Grants NS 010414-32, NS 34835, and DC 2260; and National Research Service Award Fellowship F32 DC05711.

1. Stevens, S. S. (1975) *Psychophysics: Introduction to Its Perceptual, Neural, and Social Prospects* (Wiley, New York).
2. Werner, G. & Mountcastle, V. B. (1965) *J. Neurophysiol.* **28**, 359–397.
3. Mountcastle, V. B., Poggio, G. F. & Werner, G. (1963) *J. Neurophysiol.* **26**, 807–834.
4. Johnson, K. O., Hsiao, S. S. & Blake, D. T. (1996) in *Somesthesia and the Neurobiology of the Somatosensory Cortex*, eds. Franzen, O., Johansson, R. & Terenius, L. (Birkhäuser, Basel), pp. 213–228.
5. Moore, B. J. (1995) *Hearing* (Academic, New York).
6. Buus, S. & Florentine, M. (1991) *J. Acoust. Soc. Am.* **90**, 1371–1380.
7. Stevens, S. S. (1970) *Science* **170**, 1043–1050.
8. Suga, N. (1977) *Science* **196**, 64–67.
9. Phillips, D. P. & Irvine, D. R. (1981) *J. Neurophysiol.* **45**, 48–58.
10. Heil, P., Rajan, R. & Irvine, D. R. (1994) *Hear. Res.* **76**, 188–202.
11. Sutter, M. L. & Schreiner, C. E. (1995) *J. Neurophysiol.* **73**, 190–204.
12. Heil, P. & Neubauer, H. (2003) *Proc. Natl. Acad. Sci. USA* **100**, 6151–6156.
13. Ehret, G. E. & Schreiner, C. E. (2005) in *The Inferior Colliculus*, eds. Winer, J. A. & Schreiner, C. E. (Springer, New York), pp. 312–345.
14. Chang, E. F. & Merzenich, M. M. (2003) *Science* **300**, 498–502.
15. Suga, N. & Manabe, T. (1982) *J. Neurophysiol.* **47**, 225–255.
16. Phillips, D. P. & Kelly, J. B. (1989) *Hear. Res.* **37**, 269–279.
17. Doron, N. N., Ledoux, J. E. & Semple, M. N. (2002) *J. Comp. Neurol.* **453**, 345–360.
18. Bakin, J. S. & Weinberger, N. M. (1990) *Brain Res.* **536**, 271–286.
19. Recanzone, G. H., Schreiner, C. E. & Merzenich, M. M. (1993) *J. Neurosci.* **13**, 87–103.
20. Edeline, J. M., Pham, P. & Weinberger, N. M. (1993) *Behav. Neurosci.* **107**, 539–551.
21. Linkenhoker, B. A. & Knudsen, E. I. (2002) *Nature* **419**, 293–296.
22. Fritz, J., Shamma, S., Elhilali, M. & Klein, D. (2003) *Nat. Neurosci.* **6**, 1216–1223.
23. Blake, D. T., Strata, F., Churchland, A. K. & Merzenich, M. M. (2002) *Proc. Natl. Acad. Sci. USA* **99**, 10114–10119.
24. Beitel, R. E., Schreiner, C. E., Cheung, S. W., Wang, X. & Merzenich, M. M. (2003) *Proc. Natl. Acad. Sci. USA* **100**, 11070–11075.
25. Bao, S., Chang, E. F., Woods, J. & Merzenich, M. M. (2004) *Nat. Neurosci.* **7**, 974–981.
26. Weber, E. H. (1846) in *Handwörterbuch der Physiologie*, ed. Wagner, R. (Braunschweig, Vieweg), Vol. III, pp. 481–588.
27. Fechner, G. T. (1860) *Elemente der Psychophysik* (Breitkopf and Hartel, Leipzig).