nature neuroscience

Temporal plasticity in the primary auditory cortex induced by operant perceptual learning

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Processing of rapidly successive acoustic stimuli can be markedly improved by sensory training. To investigate the cortical mechanisms underlying such temporal plasticity, we trained rats in a 'sound maze' in which navigation using only auditory cues led to a target location paired with food reward. In this task, the repetition rate of noise pulses increased as the distance between the rat and target location decreased. After training in the sound maze, neurons in the primary auditory cortex (A1) showed greater responses to high-rate noise pulses and stronger phase-locking of responses to the stimuli; they also showed shorter post-stimulation suppression and stronger rebound activation. These improved temporal dynamics transferred to trains of pure-tone pips. Control animals that received identical sound stimulation but were given free access to food showed the same results as naive rats. We conclude that this auditory perceptual learning results in improvements in temporal processing, which may be mediated by enhanced cortical response dynamics.

Natural, biologically relevant sounds are temporally modulated, and can be recognized largely on the basis of their temporal modulation properties[1–6]. The responses of auditory neurons to temporally modulated sounds differ systematically along the auditory pathway, with more centrally located neurons responding to more slowly modulated sounds. At the eighth nerve, for example, sound-evoked action potentials may be phase-locked to the sound waveform at frequencies up to several kilohertz[7,8] or to the envelope of amplitude-modulated tones at rates above 1 kHz[9]. Central auditory neurons respond to sounds with less temporal fidelity[10]. Auditory thalamic neurons can follow stimuli repeated at rates up to 50 Hz, and cortical neurons rarely follow those with temporal modulation rates higher than 20 Hz[11–14]. When stimulated with sounds repeated at 100 Hz or higher, many cortical neurons show sustained responses[11,15]. It has been hypothesized that in the auditory cortex, high-rate temporal modulations are represented by the firing rate of these sustained responses, whereas low-rate modulations are represented by the spike timing of phase-locked responses[11]. Animal vocalizations are primarily modulated in the range of 2–50 Hz[1], and are likely coded in the auditory cortex by responses that are phase-locked to important stimulus features.

In humans, speech comprehension is correlated with the cortical responses that are phase-locked to the envelopes of the stimulus[16]. Impaired cortical processing of rapidly successive stimuli, as assessed by sensory perception tasks and cortical responses, is commonly associated with auditory and language disabilities[17–20]. It has been proposed that improvements in cortical response dynamics underlie training-induced amelioration of language impairments[21,22].

Cortical representations of temporally modulated sounds are influenced by early experience. Rats reared in continuous noise or an acoustic environment dominated by sounds repeated at low temporal rates show reduced cortical representations of high-rate sounds[23]. Congenitally deaf people who receive a cochlear implant at a young age often develop cortical responses to rapidly successive stimuli, whereas those who receive an implant at later ages do not[24–26]. In adulthood, simple, passive sensory exposure is ineffective, and intensive perceptual training is required to improve temporal acoustic information processing[21].

Cortical mechanisms of training-induced improvement in temporal information processing are not well understood. Pairing electrical stimulation of cholinergic basal forebrain with tone pips repeated at a high temporal rate has been shown to enhance A1 responses to high-rate stimuli[27]. However, similar plasticity effects have not been demonstrated under more physiological conditions such as behavioral training. In a recent study, adult owl monkeys were trained in a go/no-go task to detect an increase in the envelope modulation rate of a sinusoidally modulated tone[28]. The neuronal responses recorded in the primary auditory cortex of trained monkeys were globally suppressed, with weaker suppression at target rates than at the standard rate[28].

Here, we designed a sound maze to train rats for temporal rate discrimination. Animals were trained to find a randomly chosen target location using the temporal rate of continuously presented repetitive noise pulses as the only cue. The temporal rate varied with the distance between the animal and the target location: shorter distances corresponded with faster repetition rates. There is a fundamental difference between our sound maze task and the go/no-go task used in a previous study[28]; temporal rates can be grouped into two categories (standard and target) and represented accordingly in the go/no-go task, whereas all relevant rates must be differentially represented for the rat to navigate the sound maze. Also, the go/no-go paradigm potentially requires detection of only a difference between two

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Published online 1 August 2004; doi:10.1038/nn1293

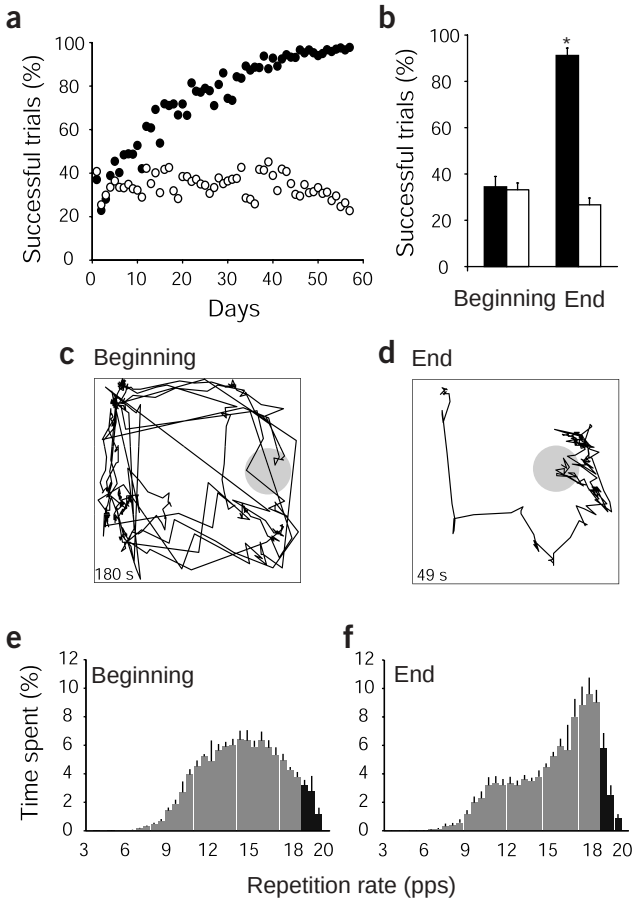

Figure 1 Learning a temporal rate discrimination task. (**a**) Performance of an individual subject across the entire training period. Filled circles, percentage of successful trials; open circles, chance-level performance. (**b**) Mean performance levels of all animals at the beginning and the end of the training period. Solid bars, percentage of successful trials; open bar, chance-level performance (*$P < 0.001$, comparing the final performance with chance performance or performance at the beginning). Error bars depict s.e.m. (**c**,**d**) Navigation traces of an animal at the beginning (**c**) and end (**d**) of the training period. Duration of the trial shown at bottom. The gray circles indicate the positions and the size of the targets. Well-trained animals were able to approach the target quickly, guided by the temporal cue. (**e**,**f**) Percentage of time each animal spent in locations corresponding to various stimulus repetition rates. Shown here are means of all animals. Darker bars indicate time spent in the target regions. At the end of the training, animals spent significantly more time at temporal rates that were close to the target rates, indicating that they were using the temporal cue to find the targets.

sounds, whereas sound maze performance is contingent upon the discrimination of continuous temporal sound properties and not just difference alone. Our sound maze task may thus be closer to sensory training for language-impaired individuals because speech information is coded in a broad range of temporal modulations[2,6]. In the present study, we examined the effects of sound maze training on cortical representations of sounds presented in rapid succession.

RESULTS

Behavioral learning

The behavioral performance of experimental animals reached a plateau after approximately 2 months of training (**Fig. 1a**). At the start of training, animals seemed to move randomly in the training chamber (**Fig. 1c**). The performance of the animals (35 ± 4% of trials were completed successfully, on average) did not differ significantly from chance (34 ± 3%; ANOVA, *post-hoc* Bonferroni's test, $P > 0.5$, **Fig. 1b**). By the end of the training, average performance had significantly improved to 91 ± 3% ($P < 0.001$) and was much higher than the chance level of 27 ± 3% ($P < 0.001$). There was a slight reduction of chance-level performance after the training ($P > 0.05$), which was likely due to changes in the animals' movement patterns in the training chamber. Well-trained animals were able to approach the target quickly, which resulted in a smaller area covered by the animals' movement and, thereby, lower probability that the animals hit targets by chance.

To confirm that experimental animals used repetition rate as the cue for finding the target, we analyzed their time spent at each repetition rate (**Fig. 1e**,**f**). At the beginning of the training period, animals did not show a preference for any particular repetition rate. Animals spent the least time at the high rates because these rates represented the concentric areas that get progressively smaller as one nears the target. They also spent relatively little time at the low rates because such low rates occurred only when the animals were very far away from the targets, which was rare. For example, the lowest rate of 2 pulses per second (pps) occurred only when the animal and the target were in diagonally opposite corners. By the end of training, animals spent significantly more time in areas corresponding to repetition rates from 17 to 18.5 pps (paired *t*-test, $P < 0.05$). This is consistent with the observation that the animals stayed longer in areas near the target (**Fig. 1d**), because the task was more difficult at high rates and required more refined temporal discrimination. In a successful trial, an animal was required to stay in the target only for 2 s before it was allowed to retrieve the food reward, which explains the limited time spent at the target rates. Two animals that were trained to locate a 10-pps target reached maximum performance in 2–3 d (data not shown), indicating that animals learned the task quickly, and that the progressive improvements in performance observed in our experimental animals mainly reflected enhancement in perceptual ability, but not in learning of the behavioral task.

Temporal rate representations

Representations of temporal modulation rate in A1 were examined by recording cortical responses to noise pulses of various temporal repetition rates (from 198 sites in five experimental animals, 185 sites in five auditory control animals and 135 sites in five naive animals; all subsequent quantitative analyses were based on these samples). In naive and auditory control animals, most A1 neurons could follow stimuli at or below 12.5 pps and missed responses to some noise pulses at 15–20 pps (**Fig. 2a**). A small number of neurons in experimental animals responded to every noise pulse in the entire temporal rate range tested (**Fig. 2a**). Quantitative analysis indicated that A1 neuron responses to single noise pulses, as measured by number of spikes in response to the first noise pulse, were not different across the three groups (experimental, 1.76 ± 0.08 spikes per noise pulse; auditory control, 1.69 ± 0.10 spikes/n.p.; naive, 1.62 ± 0.10 spikes/n.p.; ANOVA, $P > 0.1$). The latency of responses to the first noise pulse was also comparable among the groups (experimental, 11.0 ± 0.3 ms; auditory control, 11.6 ± 0.4 ms; naive, 11.0 ± 0.3 ms; Kruskal-Wallis test, $P > 0.5$). The spontaneous discharge rate was not different between naive and experimental groups, but was significantly lower for the auditory control groups (experimental, 29.39 ± 1.06; auditory control, 21.05 ± 1.27; naive, 29.21 ± 2.16;

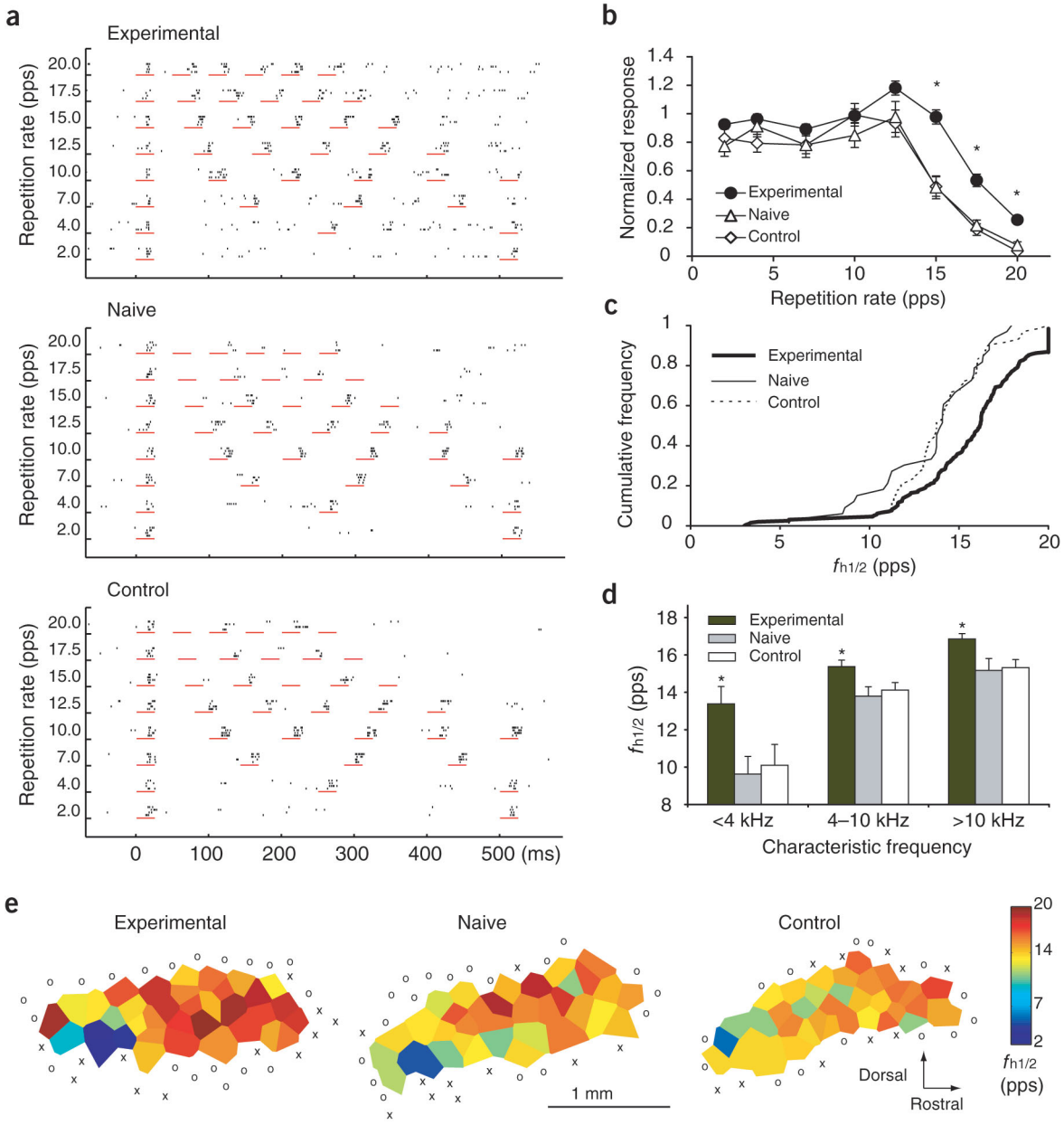

Figure 2 Training enhances cortical responses to high-rate noise pulses. (**a**) Raster plot examples of the cortical responses to pulsed noises. The repetition rates of the noise pulses are indicated on the vertical axis. Red lines indicate pulse durations. The experimental case (top plot) was from a neuron that responded well to 20 pps noise pulses, which does not represent mean properties of this group. (**b**) Repetition rate transfer functions of cortical responses to noise pulses (*$P < 0.001$, experimental versus naive and control groups). Error bars depict s.e.m. (**c**) Highest temporal rate at which cortical responses were half of the maximum ($f_{h1/2}$). There is a significant rightward shift of the $f_{h1/2}$ distributions for experimental animals compared to naive and control animals ($P < 0.05$), manifesting enhanced responses to higher-rate noise pulses. (**d**) $f_{h1/2}$ of neurons with different characteristic frequencies. Enhanced temporal response dynamics were seen in neurons of different CFs (*$P < 0.05$). Error bars depict s.e.m. (**e**) Representative A1 $f_{h1/2}$ maps.

ANOVA, experimental versus naive, $P > 0.5$; auditory control versus experimental or naive, $P < 0.001$).

We quantified repetition-rate transfer functions (RRTFs), in which the magnitude of normalized cortical responses is defined as a function of the stimulus repetition rate (**Fig. 2b**). A1 neurons in experimental animals responded more strongly to noise pulses at rates from 15 to 20 pps than did those in the auditory control and naive animals (ANOVA, $P < 0.001$). In naive and auditory control animals, most neurons had either low-pass or band-pass RRTF (naive, 73% low-pass and 27% band-pass; auditory, 69% low-pass, 29% band-pass and 2% band-reject). By contrast, 13% of neurons in experimental animals were high-pass, band-reject or nonselective (60% low-pass, 27% band-pass, 2% high-pass, 8% band-reject, 3% nonselective; χ^2-test on total percentage of high-pass, band-reject and nonselective neurons across three groups, $P < 0.05$), all of which responded well to high-rate stimuli. Cortical processing of high temporal rate stimuli, as measured by the estimated highest rate at which RRTF was at half of its maximum ($f_{h1/2}$), was significantly improved in the experimental animals

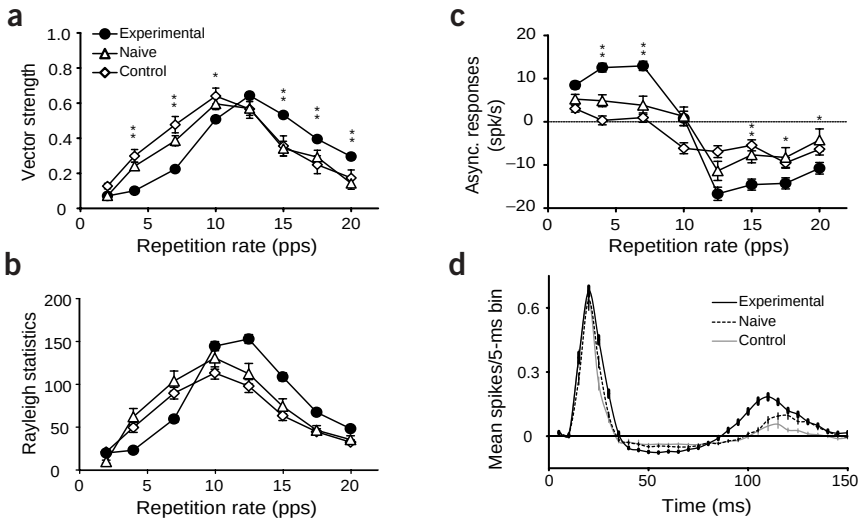

Figure 3 Training enhances cortical phase-locked responses. (**a**) Vector strength of cortical responses as a measure of phase-locking of responses to repetitive noise pulses. (**b**) Rayleigh statistic measuring the significance of response phase-locking. A Rayleigh statistic value above 5.991 corresponds to $P < 0.05$ and 13.816 indicates $P < 0.001$. (**c**) Asynchronous responses to repetitive noise pulses, defined as the mean firing rate in time windows from 40 ms after the onset of noise pulses to the onset of the next pulses, minus mean spontaneous firing rate. (**d**) Recovery dynamics of cortical neurons estimated with the firing rates during and after the neurons were activated by a noise pulse minus mean spontaneous firing rates. Data in **a**–**d** are derived from all 518 recordings and error bars depict s.e.m. $*P < 0.05$, $**P < 0.01$ comparing experimental versus naive and control groups.

(**Fig. 2c**, Kruskal-Wallis, *post-hoc* Bonferroni's test, $P < 0.05$). As indicated in cortical maps (**Fig. 2e**), the temporal processing capacity (that is, $f_{h1/2}$) has a coarsely topographic organization: neurons with a high characteristic frequency (CF) in rostral A1 generally responded better to high-rate stimuli than did low-CF neurons in caudal A1 (linear regression of $f_{h1/2}$ and CF of all neurons, $R = 0.328$, $P < 0.0001$). However, the improved cortical responses to high-rate sounds in experimental animals were not accompanied by altered CF distributions (Kolmogorov-Smirnoff test between CF distributions of any two groups, $P > 0.05$, data not shown). Furthermore, mean $f_{h1/2}$ was significantly higher across all CF ranges for the experimental group than for the naive and auditory control groups ($P < 0.05$; **Fig. 2d**,**e**).

In all three groups of animals, we observed significant correlations between response latency and CF, and latency and $f_{h1/2}$ (linear regression for latency versus CF and latency versus $f_{h1/2}$, $P < 0.001$, for all groups). Thus, high-CF neurons tended to have shorter response latencies and responded better to high-rate noise pulses. However, as we have shown, 'sound maze' training significantly increased $f_{h1/2}$ without changing response latency.

Cortical response dynamics

To further characterize spike timing relative to stimulus phases, we calculated vector strength, a measure of phase-locking of the responses to periodic stimuli, and the Rayleigh statistic, which estimates the significance level of the vector strength, taking into account the total number of spikes[29]. The responses of a neuron were considered phase-locked to the repetitive stimuli if the Rayleigh statistic was greater than 5.991, corresponding to $P < 0.05$. Training significantly reduced the percentage of neurons that showed no phase-locked responses at high repetition rate (at 20 pps: experimental, 7.1%; auditory control, 19.4%; naive, 22.3%; χ^2 test, $P < 0.0005$). Mean vector strength and Rayleigh statistics followed similar trends: neurons generally showed a high degree of phase-locking at 10–12.5 pps. Moreover, training significantly enhanced phase-locked responses at higher temporal rates, and significantly reduced it at lower temporal rates (**Fig. 3a**,**b**).

Phase-locked responses typically occurred in a window 7–40 ms after the onset of each noise pulse. To examine how repetitive noise pulses influenced cortical firing between the phase-locking windows, we quantified cortical responses that were asynchronous with respect to the noise pulses. The magnitude of asynchronous responses was defined as the mean firing rate outside the phase-locking windows minus spontaneous firing rate (see Methods). At low repetition rates, asynchronous responses were generally stronger in experimental animals than in naive and auditory controls (**Fig. 3c**, repeated-measures ANOVA, $P < 0.001$). At high temporal rates, asynchronous responses were suppressed, as indicated by below-spontaneous firing rates, because the sampling windows were in periods of suppressed cortical excitability (see below and **Fig. 3d**). The suppression was stronger in experimental than in naive and auditory control animals (repeated-measures ANOVA for 12.5–20 pps, $P < 0.001$).

The fact that cortical responses to repetitive noise pulses were enhanced by training, whereas those to single noise pulses were unchanged suggests that training altered the dynamics of cortical excitability following sound stimulation. We estimated neuronal excitability by measuring the deviation of neuronal firing rate from spontaneous firing rate. After a burst of spikes activated by pulsed noise, there was a brief period of suppression as indicated by a below-spontaneous discharge rate. This was followed by a period of

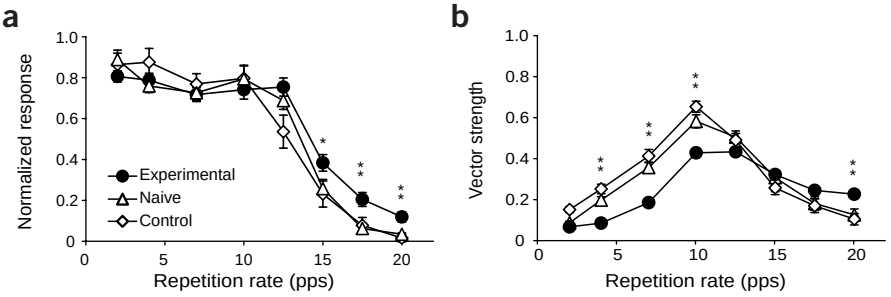

Figure 4 Training effects on cortical responses to repetitive tone pips. (**a**) Repetition rate transfer function of cortical response to tone pips. Training enhanced cortical responses to tone pips at 15–20 pps. The characteristic frequency of the recorded neuron was chosen to be the frequency of the testing tone pips. (**b**) Vector strength of cortical responses to repetitive tone pips. Significantly stronger phase-locking was observed in responses to 20-pps tone pips. $*P < 0.05$, $**P < 0.01$, experimental versus naive and control groups. Error bars depict s.e.m.

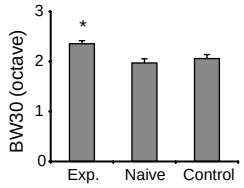

Figure 5 Spectral tuning bandwidth measured at 30 dB above the threshold. Training with broadband noise stimuli resulted in broadened spectral tuning in A1 neurons. *$P < 0.001$, experimental versus naive and control groups. Error bars depict s.e.m.

enhanced or 'rebound' excitability manifested by an above-spontaneous discharge rate (**Fig. 3d**). The rebound excitation activated by the pulsed noise was longer-lasting for the neurons of experimental animals than for those of naive and auditory control animals (ANOVA comparing firing rates 25–35 ms after the noise onset, $P < 0.05$). The rebound excitation also occurred earlier in neurons of experimental animals than in those of naive and control animals (experimental, 90 ms after noise onset; naive, 105 ms; auditory control, 105 ms). Overall, neurons in experimental animals were suppressed for approximately 50 ms following noise stimulation, whereas neurons of naive and auditory control animals were suppressed for approximately 65 ms. The post-depression facilitation was also greater and peaked earlier for neurons in experimental animals than in naive and auditory control animals (Kruskal-Willis test comparing excitability at the maxima, $P < 0.001$; comparing time to the maxima, $P < 0.001$). The shortened suppression in neurons of the experimental animals likely improved cortical response dynamics; the durations from the excitation peak to the rebound excitation peak of individual units were significantly correlated with their $f_{h1/2}$ measures (correlation analysis, $r = -0.31$, $P < 0.001$) and with the half-height upper limits of their vector strength-rate functions ($r = -0.26$, $P < 0.001$).

Transfer of improved cortical dynamics

To determine whether the plasticity effects were specific to the broadband noise stimuli used in the training, we recorded cortical responses to repeated tone pips at various temporal rates. The cortical CF was chosen as the carrier frequency of the tone pips. Both normalized responses and the vector strength were significantly higher at high temporal rates for neurons of the experimental group than for naive or auditory control groups (**Fig. 4**). Vector strength was lower at low rates for neurons of the experimental group.

Spectral plasticity

Examination of frequency representations indicated that all three groups of animals (experimental, auditory control and the naive) had a tonotopically organized A1. The size of A1 was similar among the three groups (experimental, 1.20 ± 0.08 mm^2; auditory control, 1.19 ± 0.07 mm^2; naive, 1.16 ± 0.07 mm^2; ANOVA, $P > 0.5$). There were no significant differences between the three groups in the basic response properties of A1 neurons, such as response latency (experimental, 11.4 ± 0.2 ms; auditory control, 11.1 ± 0.3 ms; naive, 11.0 ± 0.2 ms; ANOVA, $P > 0.1$), response magnitude (experimental, 2.12 ± 0.06 spikes per noise pulse; auditory control, 2.03 ± 0.07 spikes/n.p.; naive 2.03 ± 0.08 spikes/n.p.; ANOVA, $P > 0.5$) or response threshold (experimental, 30.3 ± 1.0 dB; auditory control, 28.1 ± 1.2 dB; naive, 31.6 ± 1.4 dB, MANOVA with CF as an independent variable, $P > 0.5$). Analysis of the frequency-intensity receptive field indicated that training with noise broadened the tuning curves of A1 neurons. Response bandwidth was significantly greater for the experimental animals than the auditory control or naive animals, when measured at 30 dB above threshold (**Fig. 5**; ANOVA, $P < 0.001$) or at 60 dB sound pressure level (experimental, 2.61 ± 0.08 octaves; auditory control, 2.36 ± 0.08 octaves; naive 2.16 ± 0.09 octaves; ANOVA, $P < 0.01$).

DISCUSSION

Our results indicate that cortical temporal processing capacity can be markedly improved through perceptual learning. This is achieved through altered cortical response dynamics: after training, a briefer post-excitatory suppression period is followed by a quicker and more robust rebound of neuronal excitability. Plasticity effects were transferred to stimuli with different but overlapping spectral composition (from broadband noise pulses to tone pips). The type of temporal plasticity observed in the present study may underlie training-dependent improvements in sensory perception of rapidly successive stimuli in auditory and language-impaired persons.

Previous studies have shown that cortical responses to high-rate repetitive sounds are correlated with the response latency to single sound stimuli, with short-latency neurons responding better to high-rate stimuli[12,30]. This finding was replicated in our study. However, temporal discrimination training increased cortical temporal filter frequencies without changing response latencies. These results suggest that intrinsic temporal response properties and temporal plasticity effects might be mediated by separate mechanisms. Modeling studies have suggested that both thalamocortical synapses and intracortical inhibitory circuits are involved in regulating cortical temporal filter properties[31–33]. Altering thalamocortical input will likely alter both temporal tuning and response latency, whereas changing intracortical circuits could specifically affect temporal tuning. Thus, our results suggest that temporal discrimination training improves cortical response dynamics through intracortical mechanisms.

The vector strength and RRTF quantify different and complementary aspects of cortical temporal processing—the degree of phase-locking and the magnitude of phase-locked responses in defined response windows, respectively. They each have certain limitations, and therefore should be considered together. For instance, response vector strength was greater for 20-pps stimuli than for 2-pps stimuli, even for naive animals (**Fig. 3a**,**b**), which seems to suggest that animals process high-rate stimuli better than low-rate stimuli. This paradox arose because vector strength measurements missed an important difference: low vector strength at low temporal rates was due to relatively high asynchronous responses (**Fig. 3c**), whereas the number of spikes activated by each noise pulse was not low (**Fig. 2b**); low vector strength at high repetition rate resulted from a lack of phase-locked responses to the trailing sounds (the second to sixth noise pulses; see **Figs. 2a** and **3a**). At low rates, phase-locked responses, which were synchronized in a population of neurons, were more computationally powerful than asynchronous responses. Thus, although vector strength of individual neurons was weak at low temporal rates, the population activity may selectively amplify the synchronized, phase-locked responses and improve temporal coding and perception.

In experimental animals we observed reduced vector strength at temporal rates below 10 pps. This may be accounted for by the fact that, because the experimental design emphasized training for high-rate perception (see Methods), stimuli of these low rates were rarely presented during training (**Fig. 1e**,**f**). The lack of training with these rates may have resulted in competitive loss of their effective representations. Alternatively, there may be a limit for the bandwidth of cortical phase-locking characteristics (that is, vector strength functions): as it

shifts towards higher rates, phase-locking at lower rates declines. This issue could be resolved in further experiments using a logarithmic rate-distance scale (instead of the linear scale used in our experiment) in which both high-rate and low-rate stimuli are equally emphasized.

The cortical response dynamics recorded in the present study were likely influenced by anesthesia. Nevertheless, temporal response characteristics recorded under anesthesia have been well correlated with some perceptual behaviors, therefore proving to be informative[28,34]. The results reported here probably did not fully document how the training had reshaped A1. Anesthesia may have also isolated A1 from influences of other brain areas, which could be subject to training-induced plasticity. Further studies in awake, behaving animals would elucidate how these factors contribute to improved sensory perception.

In the present study, experimental animals were trained to find a target location using only temporal rate as a cue. Although the target location was correlated with high rates, all temporal rates presented were important in guiding the animals to targets in the sound maze. Consequently, cortical responses to high-rate stimuli were improved and those to low-rate stimuli, which were already robust in naive animals, were not altered. In contrast, monkeys trained in a go/no-go task had reduced cortical responses to the low-rate standard stimuli, thereby increasing the contrast of high-rate target stimuli[28]. Studies of spectral plasticity also demonstrated multiple forms of plasticity. In animals in which a sound was consistently paired with a foot-shock, cortical responses to the sound became enhanced[35,36]. When both reinforced and non-reinforced tones were presented in a differential conditioning paradigm, representations of frequencies near the reinforced tone were enhanced, enhancing sensitivity to spectral contrast[37]. When animals were trained to discriminate tones of target frequencies from standard frequencies, both enhanced responses to target frequencies and reduced responses to standard frequencies were observed[38]. It seems that cortical spectral and temporal feature representations can be increased or decreased depending on the behavioral task. These diverse effects are likely mediated by multiple neuromodulator systems. For instance, cholinergic activation is sufficient to increase representations of coincidental stimuli, whereas dopaminergic activation may enhance representations of preceding stimuli and reduce those of trailing stimuli[27,39–41]. The similarities between the effects of our behavioral training and those of cholinergic stimulation pairing with high-rate repetitive sounds suggest that the cholinergic system is importantly involved in this form of plasticity[27].

METHODS

Behavioral training. All procedures were approved under University of California at San Francisco Animal Care Facility protocols. Ten female Sprague-Dawley rats (weighing 290–310 g) were arbitrarily assigned to the experimental ($n = 5$) or auditory control ($n = 5$) group. Animals were food-deprived to reduce their body weight to 80–85% of their normal weight. During 2-h daily training sessions, each animal was placed in a wire cage (0.6 × 0.6 × 0.2 m) that was located in a continuously ventilated sound isolation chamber. Water was freely available during training. Four strain gauges were mounted at the corners of a plastic floor plate inside the wire cage, and used to monitor the distribution of the body weight of the animal, through which the position of the animal on the pre-calibrated cage floor was determined approximately every 0.2 s with a computer. In each trial, a small circular area (0.14 m in diameter) on the cage floor was randomly selected as the target location. Computer-generated white noise bursts (60 dB SPL, 25 ms duration, 5 ms on/off ramps) were continuously delivered through a speaker placed in the sound isolation chamber. The repetition rate of the noise bursts ranged from 2 to 20 pps and varied linearly with the distance between the animal and the target location: the shorter the distance, the faster the repetition rate (rates greater than 18.5 pps indicated that the target location had been reached). This task is more difficult when the animal gets closer to the target and the rate becomes higher, because rate differences are proportional to the magnitude of the rate as expressed in Weber's law (for example, it is more difficult to distinguish 20 from 19 pps than it is to distinguish 3 from 2 pps). Thus the task emphasizes the processing of high-rate sounds, which are poorly represented in the cortex. A trial was considered successful if the animal found the target within 3 min and stayed in the target location for at least 2 s. Each successful trial was followed by a 5-s timeout, during which the animal received a food pellet delivered to a receptacle at a corner of the wire cage. After the timeout, a new trial began with a new random target location. If the animal failed a trial, a new trial began immediately. Our pilot studies indicated that these task parameters (3-min trial length, 2-s stay in target, 0.14 m target size) allowed robust learning and low chance-level performance. When an experimental animal was being trained, an auditory control animal (also food deprived) was placed in a similar cage in a separate sound isolation chamber for the same period of 2 h where food was freely available. Noise pulses that were played to the experimental animal were also simultaneously played to the auditory control. However, the repetition rate of the noise pulses signaled the target location for the experimental animal but was irrelevant for the control animal. The performance of experimental animals was quantified using the success rate (that is, the percentage of successful trials). Chance-level performance was estimated for individual animals and for each training session. For each trial, we moved the real target to a newly chosen random location, which is called a dummy target, and determined whether the animal would have hit the dummy target based on its actual movement traces. For each training session, the percentage of trials in which an animal would have hit the dummy target was considered its chance-level performance. The rationale is that if the animal did not use the cue, the real target and the dummy target would have no meaningful difference (both were at random locations and were of the same size), and the chance of hitting either of them would be equal. In the contrary, if an animal's real performance was better than its chance-level performance (the animal hit the real target more often that the dummy target), it could only be because the animal used the temporal rate cue, which was related to the location of the target but not the dummy target. This method effectively accounted for potential changes in an animal's movement patterns, which could affect the estimated chance-level performance. Consequently, the chance-level performance could change during training if animals change their movement patterns.

Electrophysiological recording. Behavioral training ended once the performance of the animals leveled off (that is, did not significantly increase over a 10-d period). Within 24 h after the last training session, animals were anesthetized with sodium pentobarbital (50 mg per kg body weight). Throughout the surgical procedures and during the recording session, a state of areflexia was maintained with supplemental doses of dilute pentobarbital (8 mg/ml, injected intraperitoneally). The trachea was cannulated to ensure adequate ventilation and the cisternae magnum drained of cerebrospinal fluid to minimize cerebral edema. The skull was secured in a head holder leaving the ears unobstructed. After reflecting the right temporalis muscle, the auditory cortex was exposed and the dura was resected. The cortex was maintained under a thin layer of viscous silicone oil to prevent desiccation. Five age-matched naive animals were also mapped as controls.

Cortical responses were recorded with parylene-coated tungsten microelectrodes (1–2 MΩ at 1 kHz; FHC, Inc.). Recording sites were chosen to evenly sample from the auditory cortex while avoiding blood vessels and were marked on a magnified digital image of the cortical surface vasculature. At each recording site the microelectrode was lowered orthogonally into the cortex to a depth of 470–550 μm (layers 4/5), where vigorous stimulus-driven responses were recorded. The signal was amplified (×10,000), filtered (0.3–10 kHz) and monitored online. Acoustic stimuli were generated using TDT System II (Tucker-Davis Technology) and delivered to the left ear through a calibrated earphone (STAX 54) positioned inside the pinnae. A software package (SigCal, SigGen and Brainware, Tucker-Davis Technology)

was used to calibrate the earphone, generate acoustic stimuli, monitor cortical response properties online, and store data for offline analysis. The evoked spikes of a neuron or a small cluster of neurons were collected at each site.

Cortical mapping and data analysis. Frequency-intensity receptive fields were reconstructed by presenting pure tones of 60 frequencies (1–30 kHz, 0.1 octave increments, 25-ms duration, 5-ms ramps) at eight sound intensities (0–70 dB SPL in 10-dB increments) to the contralateral ear in a random, interleaved sequence at a rate of 2 pps. All offline analyses were performed automatically with custom-written Matlab programs (MathWorks). The receptive field was smoothed with a nine-pixel median filter. The outline of the receptive field was defined as the tuning curve. The characteristic frequency (CF) was defined as the frequency at the tip of the tuning curve. When a tuning curve had a broad tip or multiple peaks, the median frequency at the threshold intensity was chosen as the CF. Response bandwidth was calculated at various sound intensities (for example, at 60 dB or at 10–30 dB above the threshold). The response latency was defined as the time from stimulus onset to the earliest response (a response was 4 standard deviations above the mean spontaneous firing rate, corresponding to $P < 0.02$ for Poisson distributions), using post-stimulus time histograms of responses to all tone pips. Response magnitude was defined as the average number of spikes per tone, 7–40 ms after stimulus onset (cortical responses have a minimal latency of 7 ms), for five frequencies nearest the CF at 70 dB SPL.

The area of primary auditory cortex (A1) was defined as previously described[42]. To generate A1 maps, Voronoi tessellation (a Matlab routine) was performed to create tessellated polygons, with the electrode penetration sites at their centers. Each polygon was assigned the characteristics (for instance, the temporal response properties) of the corresponding penetration site. In this way, every point on the surface of an auditory cortex can be linked to the characteristics experimentally derived from the sampled cortical site that was closest to this point.

To analyze modulation rate transfer functions of the A1 neurons, trains of six noise pulses (25 ms duration, 5 ms ramps, 60 dB SPL) were delivered through the speaker four times at each of 10 repetition rates (2, 4, 5, 7, 10, 12.5, 15, 17.5, 20 pps) in a randomly interleaved sequence. Neuronal spikes were collected from 200 ms before the first tone pip to at least 200 ms after the sixth tone pip. The magnitude of cortical responses to each noise pulse was quantified as the average number of spikes activated by the noise pulse (7–40 ms after stimulus onset) minus the estimated number of spontaneous spikes during this period (spontaneous firing rate × 34 ms). The normalized cortical response at each repetition rate was calculated as the average response magnitude to the last five noise pulses divided by the response magnitude to the first noise pulse. The repetition rate transfer function (RRTF) is the normalized cortical response as a function of temporal rate[27]. Based on the shape of the RRTF, a neuron was categorized as one of the following: low-pass if its 2-pps, but not 20-pps, response was greater than half of the maximum response; high-pass if its 20-pps, but not 2-pps, response was greater than half of the maximum response; band-pass if both its 2-pps and 20-pps responses were less than half of the maximum response; band-reject if both its 2-pps and 20-pps responses were greater than half of the maximum response, and response at one or more of the other rates was less that half of the maximum; or non-selective if responses to all temporal rates were greater than half of the maximum response. In addition, the cortical ability for processing high-rate stimuli was estimated with the highest temporal rate at which the RRTF was at least half of its maximum, referred to as $f_{h1/2}$. Vector strength and the Rayleigh statistic were used to quantify how well spikes were time-locked to the noise pulses[29], using the following equation:

$$\text{vector strength} = (1/n)\sqrt{\Sigma\,(\cos(2\pi t_i/T))^2 + \Sigma\,(\sin(2\pi t_i/T))^2}$$

In this formula, t_i ($i = 1, 2 \ldots n$) is the time between the onset of the first noise pulses and the i^{th} spike (for a total of n spikes) and T is the period between two consecutive noise pulses. Spikes that occurred during a $6T$ period after the onset of the first noise pulse were included to compute vector strength. It should be noted that the vector strength measure is influenced by inter-stimulus interval; when the timing relationship between the stimulus and the spikes is the same, longer intervals (that is, those greater T values) tend to result in higher vector strength values. The Rayleigh statistic is $2n(\text{vector strength})^2$, with critical values of 5.991 for $\alpha = 0.05$ and 13.816 for $\alpha = 0.001$. As the circular spike distribution is not strictly a von Mises distribution, the Rayleigh statistic may underestimate the non-uniformity of the distribution. Asynchronous responses to repetitive noise pulses were quantified using mean response firing rate (relative to mean spontaneous firing rate) of the neuron during periods from 40 ms after the onset of a noise pulse to immediately before the next noise pulse.

Statistical analyses were done using StatView (SAS Institute). Data are presented as mean ± s.e.m.

ACKNOWLEDGMENTS

This research was supported by National Institutes of Health grants NS-10414 and NS-34835, the Coleman Fund, the Sandler Fund, the Mental Insight Foundation and the National Organization for Hearing Research Foundation. We thank J. Davis for help with the experiments. We thank D. Polley and C.-L. Teng for comments on this manuscript.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Received 12 April; accepted 29 June 2004
Published online at http://www.nature.com/natureneuroscience/

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