UNBALANCED SYNAPTIC INHIBITION CAN CREATE INTENSITY-TUNED AUDITORY CORTEX NEURONS


Abstract—Intensity-tuned auditory cortex neurons have spike rates that are nonmonotonic functions of sound intensity: their spike rate initially increases and peaks as sound intensity is increased, then decreases as sound intensity is further increased. They are either “unbalanced,” receiving disproportionately large synaptic inhibition at high sound intensities; or “balanced,” receiving intensity-tuned synaptic excitation and identically tuned synaptic inhibition which neither creates enhances nor creates intensity-tuning. It has remained unknown if the synaptic inhibition received by unbalanced neurons enhances intensity-tuning already present in the synaptic excitation, or if it creates intensity-tuning that is not present in the synaptic excitation. Here we show, using in vivo whole cell recordings in pentobarbital-anesthetized rats, that in some unbalanced intensity-tuned auditory cortex neurons synaptic inhibition enhances the intensity-tuning; while in others it actually creates the intensity-tuning. The lack of balance between synaptic excitation and inhibition was not always apparent in their peak amplitudes, but could sometimes be revealed only by considering their relative timing. Since synaptic inhibition is essentially cortical in origin, the unbalanced neurons in which inhibition creates intensity-tuning provide examples of auditory feature-selectivity arising de novo at the auditory cortex. © 2007 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: nonmonotonic, whole-cell, timing, feature selectivity.

The intensity of a sound is often behaviorally important. For example, it can convey the distance of a sound source, or prosodic information in speech and music. Most auditory cortex neurons have monotonic spike rate versus sound intensity functions; they encode sound intensity by increasing spike rate as sound intensity is increased. A different encoding strategy, however, is used by the intensity-tuned neurons whose spike rates are nonmonotonic functions of sound intensity: their spike rate initially increases and peaks as sound intensity is increased, then decreases as sound intensity is further increased (Phillips et al., 1985; Phillips and Kelly, 1989; Heil, 1997a,b). The number of intensity-tuned auditory cortex neurons increases in rats trained to perform a task requiring fine intensity discrimination, which suggests that intensity-tuned neurons are required for sound intensity to be precisely encoded (Polley et al., 2004, 2006).

There are no intensity-tuned neurons at the auditory periphery, so central inhibition is required for the formation of intensity-tuned neurons. As there are intensity-tuned neurons at subcortical auditory stations, that central inhibition is not necessarily cortical (Rhode and Smith, 1986; Ding and Voigt, 1997; Ding et al., 1999; Davis and Young, 2000; Pollak et al., 1978; Ryan and Miller, 1978; Palombi and Caspary, 1996a,b; Sivaramakrishnan et al., 2004; Wang et al., 2005).

However, Ojima and Murakami’s (2002) intracellular study of the cat auditory cortex described 12 “unbalanced” intensity-tuned neurons which received disproportionately large synaptic inhibition at high intensities. As Ojima and Murakami (2002) did not measure synaptic excitation and inhibition separately, they could not determine whether inhibition enhanced intensity-tuning that was already present in the excitation, or whether the interaction of inhibition with excitation created intensity-tuning that was not present in the excitation. In Wehr and Zador’s (2003) intracellular study of the rat auditory cortex synaptic excitation and inhibition were separately measured. They described seven “balanced” intensity-tuned neurons which received intensity-tuned synaptic excitation and identically tuned synaptic inhibition which neither enhanced nor created intensity-tuning. The sample of Wehr and Zador (2003) would not be inconsistent with unbalanced intensity-tuned neurons being present in the auditory cortex of the rat, as they are in the cat.

We therefore performed in vivo whole cell measurements of the synaptic excitation and inhibition received by intensity-tuned auditory cortex neurons of pentobarbital-anesthetized rats. As approximately 85% of neurons in core regions of the rat auditory cortex are not intensity-tuned (Phillips and Kelly 1989; Doron et al., 2002), we trained some of the rats on a variant of the abovementioned intensity discrimination task to increase the number of intensity-tuned neurons in their auditory cortices, and thereby improve our chances of obtaining in vivo whole cell recordings from such neurons (Polley et al., 2004, 2006).

This work has been previously available as an abstract and an e-print (Tan et al., 2005, 2006).
Behavioral training

All experiments in this study were approved by the University of California, San Francisco Institutional Animal Care and Use Committee Protocols, and were in accordance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996). All efforts were made to minimize the number of animals used and their suffering. Adult female Sprague-Dawley rats were used. Some of the rats were trained as described by Polley et al. (2006). Rats were trained to identify a target auditory stimulus from a set of distracter auditory stimuli. The auditory stimuli consisted of 150 ms tone pips (10 ms linear ramps) of variable frequency and intensity. Rats were rewarded for making a Go response shortly after the presentation of a 35 dB sound pressure level (SPL) tone at any frequency. Training for making a Go response shortly after the presentation of a 35 dB SPL tone pips was performed in an acoustically transparent operant training chamber (20×20×18 cm, length×width×height) contained within a sound-attenuated chamber. Input and output devices (photobeam detector, food dispenser, sound card, house light) and software were manufactured by Med Associates (Georgia, VT, USA).

A single behavioral trial was defined as the length of time between the onsets of two successive tones. The intertrial interval was selected at random from a range of 3–5 s. A rat’s behavioral state at any point in time could be classified as either “Go” or “NoGo.” Rats were in the Go state when the photobeam was interrupted. All other states were considered NoGo. For a given trial, the rat could elicit one of five reinforcement states. The first four states were given by the combinations of responses (Go or NoGo) and stimulus properties (target or non-target). Go responses within 3 s of a target were scored as a hit, and a failure to respond within this time window was scored as a miss. A Go response within 3 s of a non-target stimulus was scored as a false positive, and the absence of a response was scored as a withhold. The fifth state, false alarm, was defined as a Go response that occurs >3 s after stimulus presentation. A hit triggered delivery of a food pellet (45 mg; BioServe, Frenchtown, NJ, USA). A miss, false positive, and a false alarm initiated a 5 s “time-out” period during which time the house lights were turned off and no stimuli were presented. A withhold did not produce a reward or a time out. Trials were grouped into blocks of 50. At the conclusion of each block, a target response ratio (number of hits/number of target trials) and a non-target response ratio (number of false positives/number of non-target trials) were calculated. The target and non-target response ratio criteria were set to 80% and 30%, respectively.

Rats were shaped in three phases. During phase A, rats were trained to make a nose poke response to obtain a food reward. During phase B, rats were trained to make a nose poke only after presentation of an auditory stimulus (5 kHz at 35 dB SPL). During phase C, rats were conditioned to make discriminative responses to the target stimulus and not to a limited set of non-target stimuli. We implemented an adaptive tracking paradigm whereby the task difficulty increased as rats became increasingly proficient at identifying the target sound intensity. This was achieved by creating six levels of the task in which the sound intensity of the distractor stimuli became increasingly similar to the 35 dB SPL target intensity (mean distractor tone sound intensities: 64, 59, 53, 47, 41, 38 dB SPL for levels 1–6, respectively). If, at the conclusion of a block of trials, the behavior satisfied the target and non-target response ratio criteria, rats were advanced to the next level (unless they were working on level 6). If the behavior did not meet the criteria, the task difficulty was decreased by one level (unless they were working on level 1). Rats began each day of training at level 1. Rats received 47 ± 14 (mean ± standard deviation) days of training.

Surgery

Experiments were carried out in a sound-attenuating chamber. Each rat was anesthetized by i.p. injection of sodium pentobarbital (50–80 mg/kg), with the dose adjusted to make the rat areflexic. The rat was maintained in an areflexic state for the rest of the experiment by further i.p. injections of sodium pentobarbital (20–60 mg/kg) when necessary. The rat was placed on a heating pad, and its temperature maintained at ~37 °C. Prior to any skin incision, bupivacaine was injected s.c. at the incision site. A tracheotomy was performed to secure the airway. The head was held fixed by a custom-made device that clamped it at both orbits and the palate, leaving the ears unobstructed. A cisternal drain was performed. The right auditory cortex was exposed by retracting the skin and muscle overlying it, followed by a craniotomy and a durotomy. The cortical surface was kept moist with normal saline. The auditory cortex was coarsely mapped at 500–800 μm below the pial surface with a parylene-coated tungsten electrode to determine the location of intensity-tuned multiunit sites.

Whole cell recordings

A silver wire, one end of which was coated with silver chloride, served as the reference electrode against which potentials were measured; its chlorided end was inserted between the skull and the dura. The reference electrode was assigned a potential of 0 mV. The potential of the cerebrospinal fluid was assumed to be uniform and equal to that of the reference electrode.

Patch pipettes with resistances of 7 MΩ were made. Patch pipettes contained a cesium-based solution that consisted of (in mM) 130 Cs-glucuronate, 5 TEA-Cl, 4 MgATP, 0.3 GTP, 10 phosphocreatine, 10 Hepes, 0.2 EGTA, 5 QX-314, pH 7.3. The pia was broken by slowly lowering and raising the jagged tip of a broken pipette in and out of the cortex. An unbroken pipette was lowered into the cortex, with the pressure in the pipette greater than atmospheric. Dimpling of the cortical surface was not visually detectable. The cortical depth of the pipette tip was estimated according to the distance it had traveled. The cortex was covered with 4% agarose in normal saline. An oscillating potential was set up at the pipette tip; the oscillating potential had a time average of ~50 mV; its period was much faster than the breathing rate. The resulting current oscillation was measured. When the amplitude of the current oscillation decreased and an even slower oscillation whose period was the breathing rate of the animal became superimposed on the current oscillation, the pipette tip might be touching the cell membrane of a neuron. At this point, the pipette was slowly advanced to further reduce the amplitude of the current oscillation. The pressure in the pipette was suddenly reduced to less than atmospheric and then returned to atmospheric. Often a giga-ohm seal would spontaneously form within 1 min; if not, additional gentle suction sometimes helped. The pipette capacitance was compensated. A pulse of reduced pressure in the pipette would often break the cell membrane and bring the recording into whole cell mode. The whole cell capacitance was compensated and the initial series resistance (25–60 MΩ) was compensated to achieve an effective series resistance of 15–30 MΩ. The input resistance was 100–400 MΩ. Signals were filtered at 5 kHz and sampled at 10 kHz. A Multiclamp 700B amplifier (Molecular Devices Corporation, Sunnyvale, CA, USA) was used.

Stimuli

Noise bursts were delivered by a calibrated free field speaker directed toward the left ear. The intensities of the noise bursts measured at the position of the ear ranged from 0 dB SPL to 80 dB SPL, and were evenly spaced over that range. Noise bursts were white, 50 ms in duration with 5 ms linear rising and falling phases. The intensities were pseudorandomly interleaved.
Predicted membrane potential

The predicted membrane potential $V_p$ was calculated using

$$C_m \frac{dV_p(t)}{dt} = G_r(t)(V_p(t) - E_r) + G_e(t)(V_p(t) - E_e) + G_i(t)(V_p(t) - E_i),$$

where $C_m$ is the membrane capacitance, $G_r(t)$ the resting or leak conductance, $E_r$ the resting membrane potential, $G_e(t)$ the excitatory synaptic conductance, $G_i(t)$ the inhibitory synaptic conductance, and $E_e$ and $E_i$ are the reversal potentials of the excitatory and inhibitory synaptic conductance, respectively. Eq. 1 is a good model of the membrane voltage of cortical neurons in the absence of spiking (McCormick et al., 1987; Troyer and Miller 1997). $C_m$ was derived from the whole cell capacitance compensation procedure. $G_r$ was derived using

$$I(V) = G_r(V - E_r),$$

where $V$ is the clamping voltage, and $I(V)$ the resting or leak current. Measurement of $I(V)$ at two different values of $V$ yielded a system of two equations which could then be solved for $G_r$ and $E_r$ was measured in current clamp. $G_e(t)$ and $G_i(t)$ were derived using

$$\Delta I(t, V) = G_e(t)(V - E_e) + G_i(t)(V - E_i),$$

where $\Delta I(t, V)$ is the amplitude of the synaptic current, relative to the resting current at $V$. The values of $E_e$, $E_i$ were set by the ionic composition of the pipette solution and the cerebrospinal fluid (Johnston and Wu, 1995; Davson and Segal, 1996); the value of $E_r$ was based on the permeability of GABA-A conductances to Cl$^-$, but it should be noted that they also pass HCO$_3^-$ (Bormann et al., 1987). $G_e(t)$ and $G_i(t)$ were the two unknowns in Eq. 3 at any particular $t$. Measurement of $\Delta I(t, V)$ at two different values of $V$ yielded a system of two equations which could then be solved for $G_e(t)$ and $G_i(t)$ at any particular $t$ (Borg-Graham et al., 1998; Hirsch et al., 1998; Anderson et al., 2000a). Currents into the neuron were assigned a negative value. $E_e$ and $E_i$ were 0 mV and $-70$ mV respectively. We did not correct the measured membrane potential for the series resistance or the junction potential; these errors are approximately 10 mV in magnitude; in a previous study, errors of this magnitude did not significantly affect the initial phases of excitatory and inhibitory conductances, which are the phases important for intensity-tuning (Tan et al., 2004).

RESULTS

Rats were either untrained, or trained on an intensity discrimination task which increases the number of intensity-tuned neurons in the auditory cortex, to improve our chances of obtaining in vivo whole cell recordings from such neurons (Polley et al., 2004, 2006). The auditory cortices of anesthetized rats were coarsely mapped to locate intensity-tuned multiunit sites, to which whole cell recording attempts were then directed. Noise bursts ranging from 0 dB to 80 dB in 5 dB steps, with at least 10 repetitions per intensity, were used to determine intensity-tuning. The patch pipette contained cesium, TEA, and QX-314, which blocked spiking and most intrinsic conductances, including voltage-dependent sodium channels, and prevented them from contaminating measurements of the synaptic conductances. Because spiking was blocked, we will use peak membrane potential as a gauge of spike rate. We will also use peak values to describe synaptic currents. We use peak values for simplicity; this is occasionally problematic, and will receive further comment at the appropriate junctures. Synaptic excitation was seen as inward currents when the neuron was voltage-clamped at $-70$ mV, near the inhibitory reversal potential; synaptic inhibition was seen as outward currents when the neuron was clamped more positively, nearer the excitatory reversal potential. Whole cell recordings were obtained from 33 neurons at intensity-tuned multiunit sites in seven untrained rats and seven trained rats. Complete synaptic current records were obtained for most of these neurons, but the membrane potential records were incomplete for most of them. As will become apparent, complete synaptic current and membrane potential records are often necessary to reach our conclusions. We have therefore refrained from drawing conclusions about most of these neurons. Fortunately, in six neurons, sufficient data were obtained to determine both the neuron’s intensity-tuning, and the balance of synaptic excitation and inhibition underlying that tuning. Of the six neurons, one was monotonic and balanced, while five were intensity-tuned and unbalanced. The five intensity-tuned neurons are each described in detail below. Our conclusion is that in some neurons, synaptic inhibition enhances intensity-tuning that is already present in the excitation; while in others, synaptic inhibition creates intensity-tuning that is not present in the excitation.

Unbalanced synaptic inhibition can enhance intensity-tuning

Responses from an unbalanced neuron, from a trained rat, are shown in Fig. 1. Fig. 1A shows the average synaptic currents obtained at 45 dB and 70 dB. The inward currents representing synaptic excitation (blue traces, downward deflections) were obtained when the neuron was clamped at $-70$ mV, and the outward currents representing synaptic inhibition (red traces, upward deflections) obtained when the neuron was clamped at 0 mV. The synaptic excitation has the same peak amplitude at both intensities, but no synaptic inhibition is observed at 45 dB, while there is substantial synaptic inhibition at 70 dB. This suggests that synaptic inhibition would cause there to be less depolarization of the membrane potential at 70 dB than at 45 dB, and thus contribute to intensity tuning in this neuron. Fig. 1B graphs the peak average synaptic excitation and inhibition (blue and red lines respectively) versus intensity (error bars indicate standard error of the mean). The synaptic excitation is itself a nonmonotonic function of intensity, showing a distinct peak around 40 dB to 50 dB. From 40 dB to 70 dB, inhibition increases faster than excitation, suggesting that inhibition enhances the intensity-tuning of this neuron. However, from 75 dB to 80 dB, inhibition decreases as excitation increases. Thus this neuron might show two peaks in its intensity-tuning profile, but it is also possible that the timing of the inhibition at 75 dB and 80 dB is shifted so as to decrease the membrane depolarization at those intensities. It might be possible to distinguish between these possibilities by using the average synaptic excitation and inhibition to predict the average membrane potential; however, it would not be possible to ascertain the correctness of the prediction, as membrane potential records were not obtained for this neuron.
For this neuron, a problem with examining the peak average synaptic current is that the average excitatory currents at different intensities have different time courses (Fig. 1A). Accordingly, another function of the average synaptic current, such as the time-integrated magnitude, might behave quite differently as intensity is varied. This problem is resolved by examining the synaptic currents evoked by each presentation of a noise burst (Fig. 1C). At intensities ranging from 40 dB to 50 dB, there is great variability in the excitatory currents, with most of the noise bursts evoking only a small excitatory current, but a few evoking much larger excitatory currents with peak magnitudes greater than 100 pA. In contrast, at intensities ranging from 65 dB to 75 dB, no similarly large excitatory currents are evoked by any noise burst, indicating that the synaptic excitation itself is intensity-tuned. The inhibitory currents are also variable, but large inhibitory currents are seen both in the 40 dB to 60 dB range, where there are large excitatory currents, as well as the 65 dB to 75 dB range, where there are no large excitatory currents, indicating that synaptic excitation and inhibition are unbalanced in this neuron. The intensity-tuning of the synaptic excitation, and the unbalanced synaptic excitation and inhibition, which were suggested by examining the peak average synaptic current, are thus confirmed by examining the individual synaptic currents. They also confirm that membrane potential records are required to ascertain if the membrane potential has been correctly predicted from the synaptic currents, as several rather different predicted membrane potentials are possible, depending on how the variable individual excitatory and inhibitory currents are paired.

Responses from a second, unbalanced intensity-tuned neuron, from a trained rat, are shown in Fig. 2. Fig. 2A shows the average membrane potential responses at 15 dB, 25 dB and 75 dB. The noise-evoked depolarization at 25 dB is greater than at 15 dB, but that at 75 dB is less than at 25 dB, indicating a nonmonotonic intensity response function. Fig. 2B graphs the peak average membrane potential versus sound intensity. The peak average membrane potential increases to a maximum at 25 dB, then decreases above that, showing that this neuron is intensity-tuned. (Fig. 2C and 2D shows the same data as Fig. 2A and 2B respectively, but with the membrane potential immediately preceding sound onset subtracted from each trace before averaging.) Fig. 2E shows the average syn-
Fig. 2. Unbalanced neuron in which inhibition enhances intensity-tuning, from a trained animal. (A) Average membrane potential responses at three intensities. (B) Peak average membrane potential versus sound intensity. (C) Same as A, but with the membrane potential immediately preceding sound onset subtracted from each trace before averaging. (D) Same as B, but with the membrane potential immediately preceding sound onset subtracted from each trace before averaging. (E) Average synaptic currents at two intensities, at −70 mV (blue) or −30 mV (red). (F) Peak average inward (blue) and outward (red) currents versus sound intensity. (G) Predicted membrane potential responses at three intensities. (H) Peak predicted membrane potential versus sound intensity.
aptic currents obtained at 25 dB and 80 dB. When the neuron is clamped at −70 mV the inward currents (blue traces, downward deflections) representing synaptic excitation are equal for 25 dB and 80 dB. In contrast, at −30 mV the outward current (red traces, upward deflections) representing synaptic inhibition is much greater at 80 dB than at 25 dB. There is thus disproportionately large inhibition at the higher intensity which will either enhance or create intensity-tuning.

The synaptic current at −30 mV at 25 dB displays an initial inward portion that represents synaptic excitation. This inward current at −30 mV is as large as the inward current at −70 mV, indicating that the excitatory conductance has a nonlinear dependence on the membrane potential, perhaps due to NMDA receptor activation. This nonlinearity might cause synaptic inhibition to be underestimated. It will not, however, affect the conclusion that synaptic excitation and inhibition are unbalanced in this neuron. This is because the inward current at −70 mV is the same at both 25 dB and 80 dB. Unlike the synaptic current at −30 mV at 25 dB, there appears to be no indication of the nonlinearity in the synaptic current at −30 mV at 80 dB; however, it may be present but not apparent as an inward current, because the synaptic inhibition is disproportionately so much larger that the net current remains outward. Furthermore, the outward current at 80 dB remains disproportionally larger at 60 ms after tone onset, when the nonlinearity is no longer present, confirming that synaptic excitation and inhibition are unbalanced in this neuron.

Fig. 2F graphs the peak average inward and outward currents (blue and red lines respectively) versus intensity. The magnitude of the peak average inward current reaches a maximum at 25 dB, then decreases at higher intensities. The maximum of the peak average inward current occurs at the same intensity as that of the peak average membrane potential, showing that the intensity-tuning of the membrane potential is partially present in the excitation. Yet the peak average inward current increases above 65 dB, while the peak average membrane potential decreases, showing that excitation alone cannot account for the membrane potential at intensities above 65 dB. In that intensity range, the increase with intensity of the peak average outward current is much sharper than that of the peak average inward current. This increasing ratio of inhibition to excitation can explain the continued decrease in membrane potential above 65 dB. To confirm these points, we used the excitatory and inhibitory currents to predict the membrane potential. Fig. 2G shows example predicted membrane potential traces at 15 dB, 25 dB and 75 dB; these resemble the actual membrane potential traces at those intensities in Fig. 2A. The graph of peak predicted membrane potential versus intensity shown in Fig. 2H matches the actual curve in Fig. 2B. This is thus an example of an unbalanced intensity-tuned neuron in which synaptic inhibition enhances, but does not create intensity-tuning.

Unbalanced synaptic inhibition can create intensity-tuning

Responses from a third, unbalanced intensity-tuned neuron, from a trained rat, are shown in Fig. 3. Fig. 3A shows the average membrane potential responses at three intensities. The noise-evoked depolarization at 25 dB is greater than at 55 dB, but that at 80 dB is less than at 55 dB, indicating a nonmonotonic intensity response function. Fig. 3B graphs the peak average membrane potential versus sound intensity. The peak average membrane potential increases to a maximum at 55 dB, then decreases above that, showing that this neuron is intensity-tuned. Fig. 3E shows the average synaptic currents obtained at 55 dB and 80 dB. At −70 mV, the inward currents representing synaptic excitation are equal for 55 dB and 80 dB. In contrast, at −20 mV the outward current representing synaptic inhibition is much greater at 80 dB than at 25 dB. Like the neuron of Fig. 2, there is thus disproportionately large inhibition at the higher intensity which will either enhance or create intensity-tuning. Fig. 3F graphs the peak average inward and outward currents versus intensity. Unlike the neuron of Fig. 2, the peak average inward current is not intensity-tuned, but increases monotonically with intensity. Therefore the intensity-tuning must be created by synaptic inhibition. The sharper increase with intensity of the peak average outward current over that of the peak average inward current for intensities above 55 dB, produces an increasing ratio of inhibition to excitation that can explain the continued decrease in membrane potential above 55 dB. Fig. 3G shows example predicted membrane potential traces at 25 dB, 55 dB and 80 dB; these resemble the actual membrane potential traces at those intensities in Fig. 3A. The graph of peak predicted membrane potential versus intensity shown in Fig. 3H matches the actual curve in Fig. 3B. This is thus an example of an unbalanced intensity-tuned neuron in which synaptic inhibition actually creates intensity-tuning.

The responses from a fourth, unbalanced intensity-tuned neuron, from an untrained rat, are shown in Fig. 4. (This neuron was incorrectly noted to be from a trained rat in Tan et al., 2006.) Again, Fig. 4A and 4B demonstrates that the membrane potential is intensity-tuned. (Fig. 4C and 4D shows the same data as Fig. 4A and 4B respectively, but with the membrane potential immediately preceding sound onset subtracted from each trace before averaging.) In this case, examining the peak membrane potential suggests, but does not confirm intensity-tuning: the curves of Fig. 4B and 4D have maxima at different intensities, indicating that the intensity at which the maximum occurs is uncertain, and suggesting that the intensity at which the minimum occurs is also uncertain. However, Fig. 4A and 4C shows that the membrane potential at 80 dB, compared with that at 60 dB, clearly exhibits only the shortest depolarization, but a sustained and much greater hyperpolarization, confirming that the membrane potential is intensity-tuned. Fig. 4E shows examples of the excita-
Fig. 3. Unbalanced neuron in which inhibition creates intensity-tuning, from a trained animal. (A) Average membrane potential responses at three intensities. (B) Peak average membrane potential versus sound intensity. (C) Same as A, but with the membrane potential immediately preceding sound onset subtracted from each trace before averaging. (D) Same as B, but with the membrane potential immediately preceding sound onset subtracted from each trace before averaging. (E) Average synaptic currents at two intensities, at −70 mV (blue) or −20 mV (red). (F) Peak average inward (blue) and outward (red) currents versus sound intensity. (G) Predicted membrane potential responses at three intensities. (H) Peak predicted membrane potential versus sound intensity.
Fig. 4. Unbalanced neuron in which inhibition creates intensity-tuning, from an untrained animal. (A) Average membrane potential responses at three intensities. (B) Peak average membrane potential versus sound intensity. (C) Same as A, but with the membrane potential immediately preceding sound onset subtracted from each trace before averaging. (D) Same as B, but with the membrane potential immediately preceding sound onset subtracted from each trace before averaging. (E) Average synaptic currents at two intensities, at −70 mV (blue) or −30 mV (red). (F) Peak average inward (blue) and outward (red) currents versus sound intensity. (G) Predicted membrane potential responses at two intensities. (H) Peak predicted membrane potential versus sound intensity.
tory and inhibitory synaptic currents, showing disproportionately large inhibition at the higher intensities. Fig. 4F shows that the peak average excitatory currents are monotonic, and display no intensity-tuning. Therefore the intensity-tuning must be created by synaptic inhibition. The peak average inhibitory current does indeed increase more quickly from 65 dB to 70 dB. However, it increases just as quickly as the excitation from 70 dB to 80 dB, even though the membrane potential continues to decrease in that range (Fig. 4B). A linear least squares fit of the peak of inhibition versus the peak of excitation yields a correlation coefficient whose square is 0.63, within the range of a balanced neuron (Wehr and Zador, 2003; Zhang et al., 2003). This suggests that changes in the timing of the inhibition relative to the excitation are responsible for the further decrease of the membrane potential from 70 dB to 80 dB. To test this, we used the excitatory and inhibitory currents to predict the membrane potential. Fig. 4G shows example predicted membrane potential traces at 60 dB and 80 dB; these resemble the actual membrane potential traces at those intensities in Fig. 4A. The graph of peak predicted membrane potential versus intensity is shown in Fig. 4H, and resembles the actual curve of Fig. 4B. The final fall-off in the peak predicted membrane potential is too small to indicate intensity-tuning. As was the case with the peak membrane potential, examining the peak predicted membrane underestimates intensity-tuning. Fig. 4G shows that the predicted membrane potential at 80 dB, compared with that at 60 dB, clearly exhibits only the shortest depolarization, but a sustained and much greater hyperpolarization, confirming that unbalanced inhibition also creates intensity-tuning in this neuron, in part through changes in its timing relative to excitation.

Fig. 5 shows a fifth, unbalanced intensity-tuned neuron, from a trained rat. Once again, Fig. 5A and 5B demonstrates that the membrane potential is intensity-tuned. Fig. 5E shows examples of the excitatory and inhibitory synaptic currents. Fig. 5F shows that the synaptic excitation is a monotonic function of intensity. Thus synaptic inhibition must create the intensity tuning. However, a linear least squares fit of the peak of inhibition versus the peak of excitation yields a correlation coefficient whose square is 0.90, well within the range of a balanced neuron (Wehr and Zador, 2003; Zhang et al., 2003). It appears that changes in the relative timing of excitation and inhibition account for the intensity-tuning of the membrane potential. To test this, we used the excitatory and inhibitory currents to predict the membrane potential. Fig. 5G shows example predicted membrane potential traces at three intensities; these resemble the actual membrane potential traces at those intensities in Fig. 5A. The graph of peak predicted membrane potential versus intensity shown in Fig. 5H matches the actual curve in Fig. 5B, confirming that unbalanced inhibition creates intensity-tuning in this neuron through changes in its timing relative to excitation.

DISCUSSION
We have described five unbalanced intensity-tuned neurons in the auditory cortex. In some of these synaptic inhibition enhanced the intensity-tuning, while in others it actually created the intensity-tuning. There are thus at least three different patterns of synaptic input which underlie intensity-tuning: balanced intensity-tuned excitation and inhibition (Wehr and Zador, 2003), unbalanced inhibition which enhances intensity-tuning (Figs. 1, 2; also recently reported by Wu et al., 2006), and unbalanced inhibition which creates intensity tuning (Figs. 3, 4, 5). As previously suggested (Ojima and Murakami, 2002), the lack of balance between synaptic excitation and inhibition was not always apparent in their peak amplitudes, but could sometimes be revealed only by considering their relative timing (Figs. 4, 5). These diverse patterns are schematically summarized in Fig. 6. Since synaptic inhibition is essentially cortical in origin, the unbalanced neurons in which inhibition creates intensity tuning provide examples of auditory feature-selectivity arising de novo at the auditory cortex.

The schematic in Fig. 6 does not distinguish between the synaptic mechanisms underlying intensity-tuning to noise bursts (this paper) and to tone pips (Ojima and Murakami, 2002; Wehr and Zador, 2003; Wu et al., 2006). Both noise bursts and tone pips can be used to observe the increase in the number of intensity-tuned neurons produced by behavioral training (Polley et al., 2004, 2006). However, the relationship between spike rate versus sound intensity functions obtained with different stimuli is not necessarily straightforward (Phillips, 1988; Calford and Semple, 1995; Sutter and Loftus, 2003); intensity-tuning to noise bursts, for example, does not imply intensity-tuning to tone pips (Phillips and Cynader, 1985). Additional studies of the synaptic mechanisms underlying intensity-tuning to various stimuli are required to determine how the schematized patterns of synaptic input should be further divided.

We used pentobarbital anesthesia to reproduce the conditions under which the observation was made that the behavioral training increases the number of intensity-tuned neurons in the rat auditory cortex (Polley et al., 2004, 2006). Pentobarbital increases the duration of synaptic inhibition (Nicoll et al., 1975). Under pentobarbital anesthesia, tone-evoked inhibitory input outlasts the excitatory input; whereas under ketamine anesthesia, tone-evoked inhibitory and excitatory inputs have similar durations (Wehr and Zador, 2005). If the duration of the inhibitory input were the only difference between pentobarbital and ketamine anesthesia, we would expect our conclusions about the synaptic inputs underlying intensity-tuning to also hold in ketamine-anesthetized rats, because it is the initial phase of the inhibitory conductance, when there is also excitatory input with which it interacts, that is important for intensity-tuning. This expectation is consistent with the similar prevalence of intensity-tuned neurons in barbiturate- and ketamine-anesthetized cats (Calford and Semple, 1995). However, as the tone-evoked synaptic inputs of pentobarbital- and ketamine-anesthetized rats also differ in the initial phases (Wehr and Zador, 2005), we cannot be certain about whether the synaptic mechanisms underlying intensity-tuning in pentobarbital-anesthetized
Fig. 5. Unbalanced neuron in which inhibition creates intensity-tuning, from a trained animal. (A) Average membrane potential responses at three intensities. (B) Peak average membrane potential versus sound intensity. (C) Same as A, but with the membrane potential immediately preceding sound onset subtracted from each trace before averaging. (D) Same as B, but with the membrane potential immediately preceding sound onset subtracted from each trace before averaging. (E) Average synaptic currents at two intensities, at −70 mV (blue) or 0 mV (red). (F) Peak average inward (blue) and outward (red) currents versus sound intensity. (G) Predicted membrane potential responses at two intensities. (H) Peak predicted membrane potential versus sound intensity.
We have used the peak membrane potential as a gauge of spike rate. This is reasonable, because the spike rate is often modeled as a monotonic function of the membrane potential in cortical neurons (Miller and Troyer, 2002; Priebe et al., 2004). The spike rate is also often modeled as a threshold-monotonic function of the membrane potential (Anderson et al., 2000b; Carandini and Ferster, 2000). The spike threshold sharpens frequency tuning (De Ribaupierre et al., 1972; Volkov and Galazjuk, 1991; Ojima and Murakami, 2002; Tan et al., 2004), and should sharpen intensity-tuning similarly. The spike rate may also be modeled as a function of the net synaptic current at spike threshold, although it is uncertain if this holds for transient synaptic inputs like those observed here (Koch et al., 1995; Wilent and Contreras, 2005). In such a model, since spike rate is a function of net synaptic current at spike threshold, the value of the spike threshold, relative to the excitatory and inhibitory reversal potentials, will affect the intensity at which spike rate is maximum, if the neuron is unbalanced. In particular, the maximum may be shifted beyond the range of intensities used in the experiment such that an intensity-tuned neuron appears monotonic, or vice versa. We note that changing the resting conductance can have a similar effect. Interestingly, nucleus basalis stimulation affects the resting conductance of auditory cortex neurons (Metherate and Ashe, 1993). As behavioral training probably activates the nucleus basalis (Weinberger, 2004), a change in resting conductance during behavioral training may permit the conversion of imperfectly balanced, monotonic neurons into unbalanced, intensity-tuned neurons. The appropriateness of the various models relating synaptic inputs, membrane potential and spike rate thus requires further investigation. The issue may be experimentally addressed by recording the

![Fig. 6](image-url)
spikes in cell-attached mode before whole-cell mode is established (Margrie et al., 2002), or by removing pharmacological blockers of spiking from the intracellular solution (Borg-Graham et al., 1998; Wehr and Zador, 2003).

A previous study addressed the role of synaptic inhibition in creating intensity-tuning by using a pharmacological blocker of synaptic inhibition (Wang et al., 2002). They found that only 2 of 31 intensity-tuned neurons became monotonic when inhibition was blocked. However, they also found that 12 of 38 monotonic neurons became intensity-tuned when inhibition was blocked. It is not straightforward to fit their data and ours into a single picture, as disrupting inhibition pharmacologically raises the baseline spike rate of many neurons, which may result in the engagement of additional voltage-gated intrinsic conductances that can produce intensity-tuning (Sivaramakrishnan et al., 2004), and affects the inhibition at all neurons in a network in which there may be complex feedback connections (Oswald et al., 2006a,b).

The receptive fields of neurons vary with layer and area of the auditory cortex (Phillips and Orman, 1984; Volkov and Galazjuk, 1991; Schreiner et al., 1992; Phillips et al., 1994; Ojima and Murakami, 2002; Kalatsky et al., 2005; Ahmed et al., 2006), as does the distribution of inhibitory interneurons (Hendry and Jones, 1991; McMullen et al., 1994; Prieto et al., 1994a,b; Cruikshank et al., 2001). This suggests that the diverse patterns of synaptic input underlying intensity-tuning may be spatially organized. In the primary visual cortex, diverse patterns of synaptic input are also present, with some of the patterns known to be organized by layer or area (Borg-Graham et al., 1998; Hirsch et al., 1998; Anderson et al., 2000a, 2001; Martinez et al., 2002; Monier et al., 2003; Marino et al., 2005).

As the behavioral training converts balanced, monotonic neurons into intensity-tuned neurons (Polley et al., 2004, 2006), the question is raised as to which patterns of synaptic input the new intensity-tuned neurons receive. If the majority of intensity-tuned neurons in trained rats are unbalanced, then balanced, monotonic neurons are probably being converted to unbalanced, intensity-tuned neurons. This would constrain models of cortical circuitry and plasticity, which would have to ensure the balance of synaptic excitation and inhibition in monotonic neurons in an untrained rat, but not so robustly that training cannot undo it (Froemke et al., 2005). The existence in trained rats of a substantial proportion of unbalanced, intensity-tuned neurons in which inhibition creates intensity-tuning would further suggest that the conversion of monotonic neurons to intensity-tuned neurons can be accomplished through the potentiation of synaptic inhibition, rather than the depression of synaptic excitation, at high sound intensities. Since balanced intensity-tuned neurons (Wehr and Zador, 2003), unbalanced neurons in which synaptic inhibition enhances intensity-tuning (Wu et al., 2006), and unbalanced neurons in which synaptic inhibition creates intensity-tuning (Fig. 4) have been observed in untrained rats; but only the latter two classes in trained rats (Figs. 1, 2, 3, 5), it cannot be ruled out that training also converts balanced intensity-tuned neurons to unbalanced, intensity-tuned neurons. However, all these possibilities remain hypotheses, because an accurate estimate of the relative number of neurons with each of the patterns of synaptic input in either trained or untrained rats cannot be obtained from the small number of neurons in our sample. A more extensive comparison of intensity-tuned neurons in trained versus untrained rats is required to determine if training recapitulates existing synaptic mechanisms or invokes ones qualitatively different to achieve an increased incidence of intensity-tuning.

Acknowledgments—We thank Tom Babcock for his help with the behavioral training, and Robert C. Froemke, Julie L. Schnapf and Michael P. Stryker for their comments on drafts of this paper. This work was supported by the National Institutes of Health (DC002260; NS34835). A.Y.Y.T. is a recipient of the Howard Hughes Predoctoral Fellowship.

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(Accepted 5 January 2007)
(Available online 22 February 2007)