

Nonoverlapping Sets of Synapses Drive On Responses and Off Responses in Auditory Cortex

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SUMMARY

Neurons in visual, somatosensory, and auditory cortex can respond to the termination as well as the onset of a sensory stimulus. In auditory cortex, these off responses may underlie the ability of the auditory system to use sound offsets as cues for perceptual grouping. Off responses have been widely proposed to arise from postinhibitory rebound, but this hypothesis has never been directly tested. We used *in vivo* whole-cell recordings to measure the synaptic inhibition evoked by sound onset. We find that inhibition is invariably transient, indicating that off responses are not caused by postinhibitory rebound in auditory cortical neurons. Instead, on and off responses appear to be driven by distinct sets of synapses, because they have distinct frequency tuning and different excitatory-inhibitory balance. Furthermore, an on-on sequence causes complete forward suppression, whereas an off-on sequence causes no suppression at all. We conclude that on and off responses are driven by largely nonoverlapping sets of synaptic inputs.

INTRODUCTION

The sudden disappearance of an object or sound is often just as striking as its sudden appearance. The neural correlate of this percept is likely to be the robust responses evoked by stimulus offset in many visual, somatosensory, and auditory cortical neurons. In the visual cortex, these off responses are thought to arise from push-pull synaptic interactions between opponent bright- and dark-sensitive (ON and OFF) pathways that originate in retinal bipolar cells and remain anatomically segregated until they converge onto visual cortical neurons (Ferster, 1988; Jin et al., 2008; Liang et al., 2008). In somatosensory and auditory cortex, which do not show opponent processing, the mechanisms underlying off responses are unknown. Until recently, it has been controversial whether off responses can even be observed in primary auditory cortex (A1). Early studies reported the absence of off responses in A1, leading to the argument that sound offsets are perceptually less important than sound onsets (Phillips et al., 2002). However, sound offsets are important cues for perceptual grouping (Bregman, 1990; Bregman et al., 1994;

Plack and White, 2000). Moreover, the failure to observe off responses in A1 was likely due to the use of barbiturate anesthesia, because more recent reports of prominent off responses in A1 have been from animals that were either awake (Fishman and Steinschneider, 2009; Qin et al., 2007; Recanzone, 2000) or were anesthetized with ketamine or halothane (Moshitch et al., 2006; Volkov and Galazjuk, 1991). What are the cellular and synaptic mechanisms underlying these off responses in auditory cortex?

At least three types of mechanisms have been proposed to underlie off responses in auditory cortex (Figure 1). One proposal is that off responses are generated by the same neural mechanisms that generate on responses or responses to other rapid changes of sound intensity (Qin et al., 2007). For example, on and off responses could both be driven by presynaptic neurons that respond both to sound onset and offset (Figure 1A). A second proposal is that off responses are generated at the cellular level, by a rebound from sustained hyperpolarization—usually assumed to arise from long-lasting synaptic inhibition (Figure 1B) (Calford and Webster, 1981; He et al., 1997; Heil et al., 1992; Takahashi et al., 2004; Volkov and Galazjuk, 1991). This implies that information about sound offset is conveyed by a separate (inhibitory) channel from sound onset; thus, neurons could exhibit on responses, off responses, or both, depending on which channels they receive. A third proposal is that on and off responses in cortical neurons are driven by two separate, excitatory channels (Figure 1C). This is consistent with reports of off responses at multiple subcortical levels of the auditory system, including the auditory brainstem response (Henry, 1985a), dorsal cochlear nucleus (Young and Brownell, 1976), inferior colliculus (Pérez-González et al., 2006), and auditory thalamus (He, 2001). In this view, off responses could either originate at the cochlea (e.g., from a mechanical transient in the basilar membrane caused by sound offset [Suga et al., 1975]) or could be generated by postinhibitory rebound at some point along the auditory hierarchy (Kuwada and Batra, 1999), but in either case would be conveyed to the auditory cortex by a set of synapses distinct from those activated by sound onset.

Here we demonstrate that on and off responses have distinct frequency tuning, at both the spiking and subthreshold level. Moreover, the balance of synaptic excitation and inhibition is typically different for on and off responses. We show that this different excitatory-inhibitory balance is a consequence of the different frequency tuning of on and off responses, because responses can be purely excitatory at receptive field edges, which differ for on and off responses. Taken together, these results suggest that on and off responses in A1 are driven by

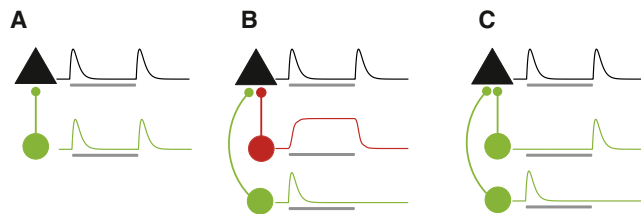


Figure 1. Three Hypotheses for the Synaptic Mechanisms underlying On and Off Responses in Auditory Cortical Neurons

In each case, spiking on and off responses (black traces) are produced in the black neuron by inputs from excitatory (green) or inhibitory (red) presynaptic neurons. The gray horizontal bars indicate sound stimuli.

(A) On and off responses are driven by the same sets of synapses. Only excitatory synaptic inputs are shown (inhibition would be identical to excitation).

(B) Off responses are generated by a rebound from sustained synaptic inhibition. Only excitatory synaptic inputs are shown (inhibition would be identical to excitation).

two distinct sets of synaptic inputs. To further test this idea, we used forward suppression, which likely acts via synaptic depression (Chung et al., 2002; Wehr and Zador, 2005). We demonstrate that an on-on sequence causes complete forward suppression but that an off-on sequence causes no suppression at all, consistent with largely nonoverlapping sets of synaptic inputs that drive on and off responses.

RESULTS

Distinct Frequency Tuning

We first set out to characterize off responses in A1 using single-unit extracellular recordings (using the cell-attached patch technique). An example of a neuron with robust off responses is shown in Figure 2A. To verify that off responses were locked to stimulus offset, we varied stimulus duration for this and all extracellular and intracellular recordings included in this report. Off responses (blue regions in Figure 2A) were time-locked to stimulus offset and became stronger with increasing tone duration (Figure 2A, inset). To compare the frequency tuning of on and off responses, we presented an array of tones (1–40 kHz, 0–80 dB). Responses of a different neuron to this array (Figure 2B) showed the characteristic V-shaped receptive field for the on responses (orange). The region of off responses (blue) was shifted to higher frequencies compared to the on responses. A few weak off responses can also be seen along the low-frequency flank of the on receptive field (e.g., 1.0 kHz, 80 dB). This difference in frequency tuning was typical of our sample of neurons that exhibited off responses. The on response receptive fields for 18 neurons in primary auditory cortex (Figure 2C) show the characteristic V-shape, centered at the characteristic frequency (CF). These neurons were distributed throughout A1 (CFs ranged from 2.9–28.2 kHz; mean, 7.6 kHz; SD, 5.7 kHz). The off responses of these neurons were tuned 1–2 octaves above the on response CF (Figures 2D–2F). A much less robust region of off responses can also be seen 1–2 octaves below CF (Figure 2D). A direct comparison of the receptive fields

(Figure 2E) for on responses (orange) and off responses (blue) reveals that they are largely nonoverlapping. Thus, on and off responses show distinct frequency tuning, consistent with the possibility that they are driven by distinct sets of synaptic inputs.

Off Responses Are Not a Postinhibitory Rebound

To test the hypothesis that off responses are produced by rebound from synaptic inhibition that is sustained throughout the duration of a tone, we used *in vivo* whole-cell methods to record tone-evoked synaptic currents. We selected neurons that showed clear off responses to at least one tone frequency. By voltage clamping neurons to three different holding potentials, we could disentangle the excitatory and inhibitory contributions to each response. We blocked intrinsic voltage-dependent conductances by including QX-314, Cs⁺, and TEA in the patch pipettes. At hyperpolarized holding potentials (−88 mV in Figure 3A, dark blue trace), transient inward synaptic currents were evoked at tone onset. Because this holding potential is near the inhibitory reversal potential, these currents were predominantly excitatory. At depolarized potentials (+12 mV in Figure 3A, magenta trace), transient outward synaptic currents were evoked at tone onset; because this holding potential is near the excitatory reversal potential, these currents were predominantly inhibitory. Both the excitatory and inhibitory currents evoked by tone onset were transient, lasting only about 100 ms, despite the 400 ms tone duration. This indicates that inhibitory synaptic currents are not sustained throughout the duration of the tone. We used these synaptic currents to estimate the underlying excitatory and inhibitory synaptic conductances (Figure 3B). In agreement with inspection of the synaptic currents, tone onset evoked transient excitatory (green) and inhibitory (red) synaptic conductances. Across a wide range of tone durations (100–1600 ms), inhibition lasted only about 100 ms after tone onset (Figure 3C). In many cells, off responses could occur without any onset-evoked inhibition (Figures 3D and 3E). For 27% of the tones that evoked off responses (total offset-evoked synaptic conductance >1 nS), there was no onset-evoked inhibition (inhibitory synaptic conductance was <1 nS; $n = 75/278$ off responses in 16/25 cells). For tones that did evoke inhibition at onset, it was transient, with a duration of 85 ± 64 ms (mean $\pm$ SD, $n = 203$ on responses in 25 neurons, tone durations 400–1600 ms). The duration of inhibition was uncorrelated with tone duration ($r = 0.08$, n.s.), further demonstrating that inhibition was not sustained throughout tone duration. Nevertheless, each of these tones produced off responses, indicating that off responses were not produced by rebound from sustained synaptic inhibition. Likewise, off responses were not produced by rebound from hyperpolarization due to intrinsic membrane properties, which were blocked in these neurons. We cannot rule out the possibility that in intact neurons (for which we haven't blocked voltage-gated channels), an intrinsic rebound mechanism could partially contribute to off responses to the subset of short (~100 ms) tones that evoke onset inhibition. However, the fact that we observed off responses across a wide range of tone durations, with voltage-gated channels blocked, indicates that off responses do not critically depend on rebound from inhibition, suggesting the operation of a more general—i.e., synaptic—mechanism.

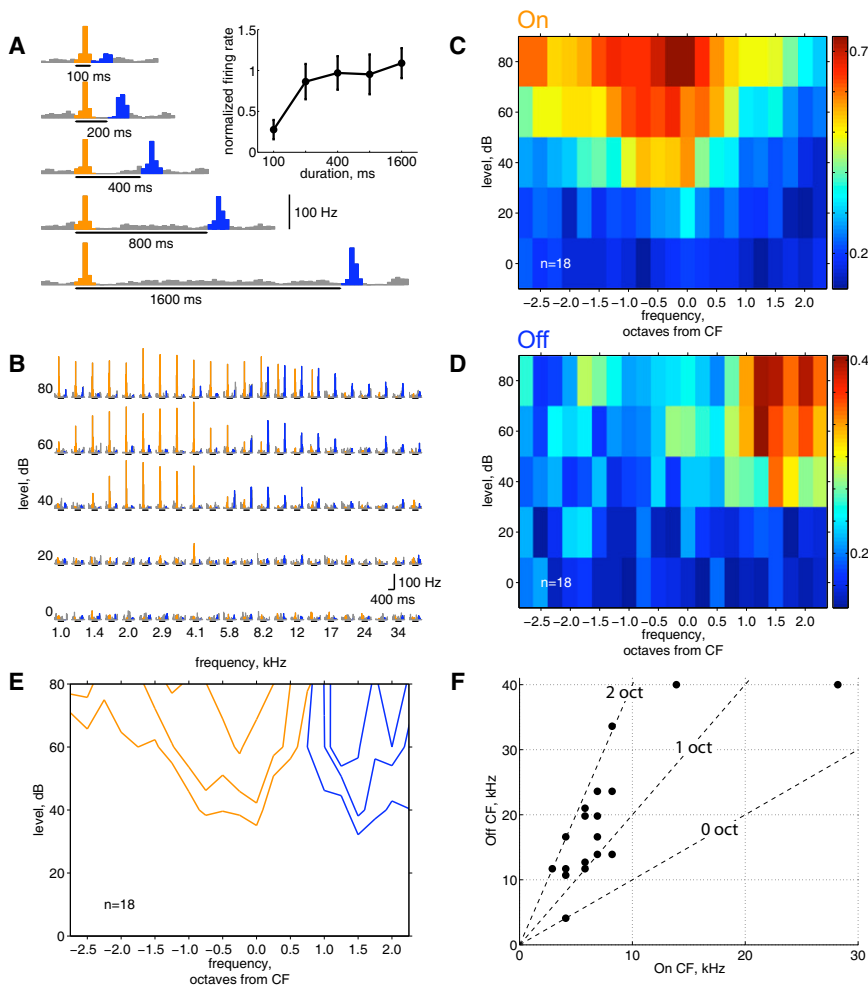


Figure 2. Off Responses Are Tuned One to Two Octaves above On Responses

(A) Example of single-unit responses to a 40 kHz, 80 dB SPL tone at five different durations. Colors indicate 100 ms windows following tone onset (orange) and offset (blue). Bar underneath each histogram indicates tone presentation. (Inset) Off response magnitude as a function of tone duration, averaged across five neurons. Responses are normalized to the mean on response for each tone.

(B) Responses of a different neuron to a wide range of tone frequencies (1–40 kHz) and levels (0–80 dB), all with duration 400 ms. CF for this site was 4.1 kHz. Note that off responses (blue) were evoked at higher frequencies than on responses (orange).

(C) On responses to the same tone array, averaged across 18 neurons. Responses are spike counts in a 100 ms window following tone onset (i.e., orange regions in A and B), aligned to CF and normalized to the maximal on response for each neuron. Note that CF is defined by on responses here and in all figures except Figure 2F.

(D) Off responses (spike counts in a 100 ms window following tone offset, i.e., blue regions in A and B) for the same neurons, aligned to CF and normalized to the maximal on response for each neuron.

(E) Contour plots for the population on responses in (C) (orange) and the population off responses in (D) (blue). Contours indicate 85th, 90th, and 95th percentiles.

(F) Comparison of CF for on responses (On CF) to that for off responses (Off CF) for each neuron ($n = 18$).

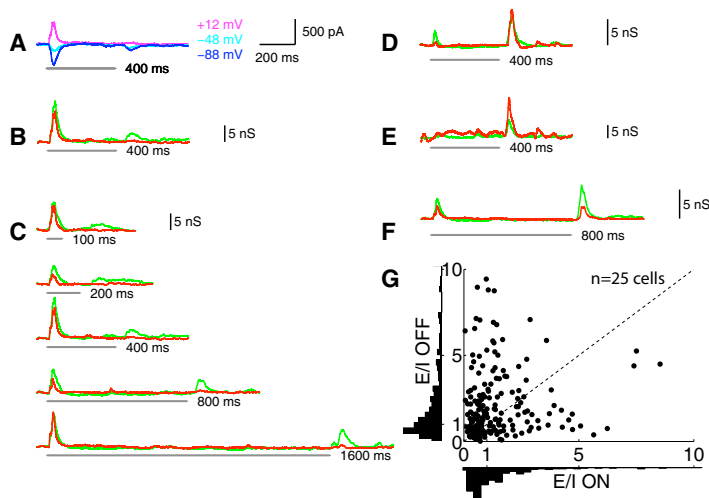
Distinct Excitatory-Inhibitory Balance

If on and off responses are driven by the same sets of synapses, they should exhibit an identical balance of excitation to inhibition. In contrast, if on and off responses are driven by distinct sets of synapses, they could in principle exhibit a different balance of excitation and inhibition. The on responses in Figures 3A–3C consist of a roughly balanced mixture of excitation and inhibition, whereas the off responses are purely excitatory. This can even be seen directly from the synaptic currents at +12 mV (Figure 3A, magenta line), which show outward currents for the on responses but not for the off responses. This off response was purely excitatory across a wide range of tone durations (Figure 3C); although the off response increased in amplitude with longer tones, it remained purely excitatory. This indicates that the sets of synapses underlying the on and off responses cannot be identical, since one set includes inhibition whereas the other does not.

This different excitatory-inhibitory balance for on and off responses was typical of all of our recorded neurons. Many neurons (for some tone frequencies) showed off responses that were roughly balanced in amplitude, but showed on responses consisting of nearly pure excitation (an example is

shown in Figure 3D). Approximately balanced off responses could also occur in the absence of an on response (an example is shown in Figure 3E) or together with a balanced on response (Figure 3F). In general, the ratio of excitation to inhibition for the on response showed no correlation with the ratio for the off response to the same tone, across our sample of 25 off-responsive neurons (Figure 3G, $r = 0.04$, n.s.; this correlation was also n.s. for each duration analyzed separately). The fact that the synaptic balance of on responses was independent from that of off responses provides further evidence that distinct sets of synapses underlie on and off responses.

What accounts for this different balance of excitation to inhibition? The simplest interpretation is that the sets of synapses underlying on and off responses are largely nonoverlapping and therefore can have independent excitatory-inhibitory composition. An alternative possibility is that the sets of synapses underlying on and off responses are largely overlapping anatomically but can show functionally different excitatory-inhibitory composition because excitatory and inhibitory synapses are subject to differential synaptic depression. Inhibitory inputs depress more strongly in auditory cortex *in vitro* (Metherate and Ashe, 1994) and can be more strongly affected



(E) Example (from a different cell) of a tone that evoked no on response but an off response with roughly equal magnitudes of excitation and inhibition. Tone was 400 ms, 33.6 kHz, 80 dB SPL.

(F) Example from a different cell showing a tone that evoked both an on response and an off response with roughly equal magnitudes of excitation and inhibition. Tone was 400 ms, 25.5 kHz, 80 dB SPL.

(G) E/I ratio for on and off responses across the population ($n = 25$ cells). Each dot ($n = 217$) represents the trial-averaged response to a tone 400 ms or longer. Only responses with both strong on and off responses were included (total synaptic conductance > 1 nS for both). The ratio of peak excitatory conductance to peak inhibitory conductance for the off response (E/I OFF) is plotted against that for the on response for that tone (E/I ON). The E/I ratios for on and off responses were uncorrelated ($r = 0.04$, $p = 0.53$). Subpanels show E/I distributions separately for on and off responses.

by forward suppression in vivo (Wehr and Zador, 2005). In vivo, when two sounds are presented in rapid succession, most cells ($\sim 80\%$) show identical excitatory-inhibitory balance for both responses. In a subset of cells ($\sim 20\%$), inhibition recovers more slowly from forward suppression, so that the second sound evokes purely excitatory responses when delivered within about 100 ms. An example of such a purely excitatory response to the second in a pair of clicks is shown in Figures 4A and 4B. If an on response causes forward suppression of the inhibitory component of the subsequent off response, this mechanism could account for a purely excitatory off response, even if both responses were produced by the anatomically identical set of synapses.

To test whether this mechanism might account for the different excitatory-inhibitory balance we observed in on and off responses, we presented click pairs to a subset of cells. In 2/5 (40%) of these cells, we observed a purely excitatory response to the second in a pair of clicks (P2), for click separations of 100 ms (the shortest interval tested). Assuming that clicks and tones produce similar synaptic depression, this suggests that differential depression of inhibition could in some cases account for purely excitatory off responses. However, the relatively short time course of this effect (< 200 ms, Figure 4B) suggests that it cannot explain purely excitatory off responses for tones longer than 200 ms. Moreover, the occurrence of a purely excitatory P2 was independent of the occurrence of purely excitatory off responses. For example, the cell in Figures 4C and 4D showed balanced excitatory-inhibitory responses to both clicks in a pair, but purely excitatory off responses to tones with the same durations as the click separations (Figure 4D, arrow). Interestingly, the cell in Figures 4E and 4F showed a purely excitatory

Figure 3. Balance of Excitation to Inhibition Typically Differs for On and Off Responses

(A) Example of tone-evoked synaptic currents recorded at three different holding potentials. The gray bar indicates the tone (400 ms, 19.8 kHz, 80 dB SPL). Holding potential is indicated by trace color. Note that at +12 mV (magenta) outward currents are transient and not sustained throughout the tone. Note also that at -88 mV (dark blue) inward currents are evoked for both the on response and off response, whereas at +12 mV (magenta) outward currents are evoked only for the on response but not for the off response.

(B) Excitatory (green) and inhibitory (red) synaptic conductances calculated from the currents in (A). Note that the on response is transient and consists of both excitation and inhibition, but the off response consists of pure excitation.

(C) Excitatory and inhibitory conductances for the same cell, tone frequency, and level as in (A) and (B), for five different tone durations. Note the purely excitatory off responses.

(D) Example (from a different cell) of a nearly pure excitatory on response, but an off response with equal magnitudes of excitation and inhibition. Note that this is the reverse of the example in (A)–(C). Tone was 400 ms, 23.6 kHz, 80 dB SPL.

P2 response for only a 100 ms click separation (Figure 4E) and a purely excitatory off response for only a 100 ms tone (Figure 4F), suggesting that these purely excitatory responses may share an underlying mechanism. However, the on responses to these tones were purely excitatory even when the off responses consisted of balanced excitation and inhibition, which strongly suggests that the on and off responses are driven by distinct sets of synaptic inputs. We conclude that differential forward suppression of inhibitory inputs can occur, but in most cases cannot account for the differing excitatory-inhibitory composition of on and off responses. This further supports the conclusion that on and off responses are likely driven by distinct sets of synaptic inputs.

Pure Excitation at Receptive Field Edges

Although excitation and inhibition are typically balanced throughout the receptive field, pure excitation can often be observed at the edges of the receptive field (Wehr and Zador, 2003). This observation, combined with our finding that off responses are tuned 1–2 octaves higher than on responses, led us to the following hypothesis: purely excitatory on and off responses are both evoked by tones at the edges of their respective receptive fields. Thus, a tone within the core of the on receptive field might lie at the edge of the off receptive field and thereby evoke a balanced on response and a purely excitatory off response. Conversely, a tone at the edge of the on receptive field might lie within the core of the off receptive field, and thereby evoke a purely excitatory on response and a balanced off response. In this view, the excitatory-inhibitory balance of both on and off responses is governed by a common principle—that tones at receptive field edges can evoke pure

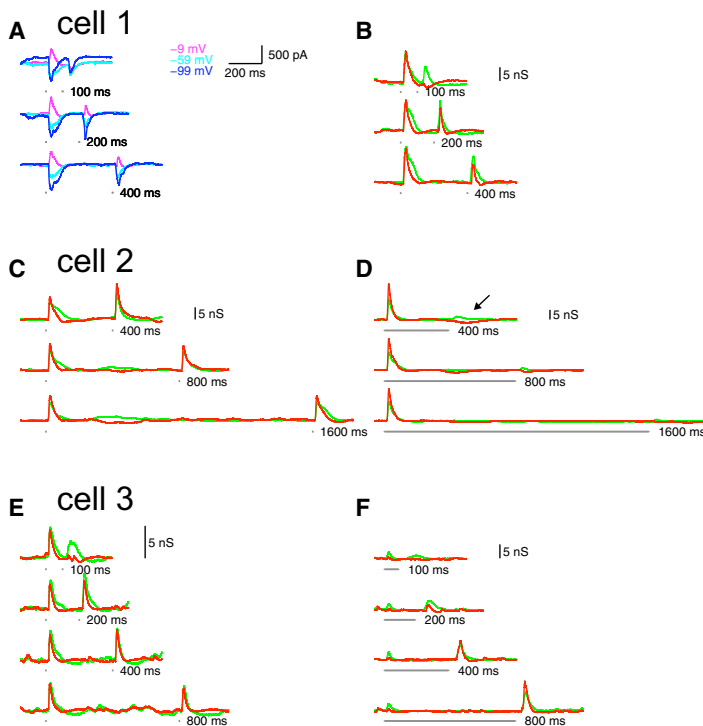


Figure 4. Forward Suppression Does Not Account for the Different E/I Balance of Off Responses

(A) Example of synaptic currents from a neuron that showed pure excitation for the second of a pair of clicks, for short click separation. Holding potential is indicated by trace color, the gray bars indicate the clicks (10 ms, 80 dB SPL white noise bursts). Note that for a click separation of 100 ms inward currents were evoked by both clicks at -99 mV (dark blue), whereas at -9 mV (magenta) outward currents were evoked only for the first but not for the second click. At longer click separations outward currents were evoked by both clicks.

(B) Excitatory (green) and inhibitory (red) synaptic conductances calculated from the currents in (A). Note the purely excitatory response to the second click for a separation of 100 ms.

(C and D) Example of a neuron that showed balanced excitation and inhibition for both clicks in a pair, for any click separation (C), but unbalanced off responses for any tone duration (D).

(E and F) Example of a neuron that showed pure excitation for the second of a pair of clicks, only for short click separation (E), but purely excitatory on responses for any tone duration (F). The off response is very small and purely excitatory for a 100 ms tone, perhaps due to forward suppression, but off responses show balanced excitation and inhibition at longer durations.

excitation—and the different excitatory-inhibitory balance of on and off responses evoked by individual tones is a consequence of the fact that on and off responses are driven by distinct sets of synaptic inputs with largely nonoverlapping frequency tuning.

To test this hypothesis, we examined the excitatory-inhibitory composition of on and off responses throughout the tonal receptive field. Figure 5 shows the excitatory and inhibitory receptive fields for both on responses (solid lines) and off responses (dashed lines) of an example neuron. Within the core of the on receptive field (e.g., at 4.1 kHz, see inset), excitation and inhibition were approximately balanced in amplitude. At the edges of the on receptive field (e.g., at 1.7 kHz, or at 13.9 kHz), on responses consisted of nearly pure excitation. At 33.6 kHz, within the core of the off receptive field, the off response was approximately balanced, but because this frequency is at the far edge of the on receptive field, the on response was purely excitatory. At the edge of the off receptive field (13.9 kHz and 28.2 kHz), off responses consisted of nearly pure excitation. Because 13.9 kHz lies at the edges of both receptive fields, both on and off responses were purely excitatory. Most of the off receptive field probably exceeded 33.6 kHz, the highest frequency tested for this cell. This pattern can also be seen at the population level, in the sample of 13 cells for which we measured the complete frequency tuning of synaptic inputs. Figure 6A shows the excitatory and inhibitory tuning curves for on responses averaged across the population. Excitation and inhibition were approximately balanced within the receptive field core (<1 octave from CF), but were predominantly excitatory at the edges (e.g., at -2 or $+2$ octaves from CF). Because not all neurons show pure excitation at the edges of their receptive fields, this effect is less prominent in the population average

than in some individual neurons (such as the one in Figure 5). For off responses (Figure 6B), similarly, excitation dominated at the receptive field edges (across a wide range, from -2 to 2 octaves from CF) but was more balanced with inhibition closer to the core (i.e., ≥ 2 octaves above CF). Consistent with this, the ratios of excitation to inhibition for individual on responses were clustered around 1 within an octave of CF (Figure 6C), but could range much higher at the receptive field edges (1–2 octaves from CF) for a subset of cells. Likewise for off responses, the ratios of excitation to inhibition in the core of the off receptive field (≥ 2 octaves above CF) were clustered around 1 (Figure 6D) but could range much higher at the edge of the off receptive field (<1 octave from CF). It is likely that we undersampled the core of the off receptive fields in Figure 6B. Because off responses were typically tuned 1–2 octaves above on responses, the core of the off receptive field was likely to be higher than 40 kHz (the highest frequency we could deliver with our system) for the two high-CF (>10 kHz) neurons in our sample.

Lack of Forward Suppression

These results suggest that on and off responses are driven by largely nonoverlapping sets of synaptic inputs. This predicts that forward suppression, which is thought to be mediated by synaptic depression, should act between on responses but not between on and off responses. In other words, an on response should cause forward suppression of a subsequent on response, because they are mediated by the same set of synapses. In contrast, an off response should *not* cause forward suppression of a subsequent on response, because they are mediated by different sets of synapses. To test this prediction, we recorded extracellularly from single neurons using the cell-attached patch

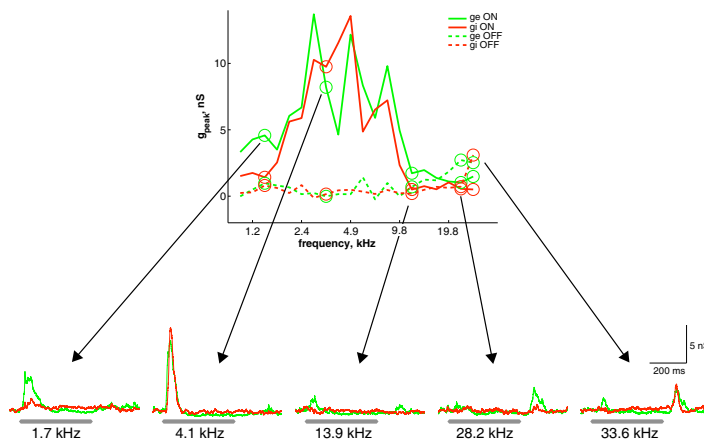


Figure 5. Both Unbalanced On and Off Responses Can Be Explained by Pure Excitation at the Edges of the Receptive Field

Synaptic tuning curve for an example neuron, with peak excitatory (green) and inhibitory (red) conductances plotted as a function of tone frequency. On responses are shown by solid lines, off responses are shown by dashed lines. (Insets) Synaptic conductance traces at five representative frequencies within the receptive field, indicated by open circles in the synaptic tuning curve. On responses show roughly balanced excitation and inhibition at 4.1 kHz, within the core of the on receptive field, but show nearly pure excitation at 1.7 and 13.9 kHz, at the edges of the on receptive field. Off responses show roughly balanced excitation and inhibition at 33.6 kHz, within the core of the off receptive field, but show nearly pure excitation at 13.9 and 28.2 kHz, at the edges of the off receptive field. Tones were 80 dB SPL.

technique and identified 52 neurons that exhibited both on and off responses to the same tone frequency. We restricted our analysis to 33 of these neurons for which the on and off responses were equivalent (i.e., spike counts were not significantly different at the $p < 0.001$ level, two-sample two-tailed t test). **Figure 7A** shows an example of spiking on responses evoked by a pair of 25 ms tones separated by 100 ms (onset-to-onset, magenta lines). Although the first tone evoked a brisk on response, the on response to the second tone was completely abolished by forward suppression (arrow). The responses of this site to a pair of 400 ms tones of the same frequency are shown in black. The magenta and black traces are aligned such that the onset of the first 25 ms tone lines up with the offset of the first 400 ms tone, and the onsets of the

second tones line up with each other. The 400 ms tone evoked a brisk off response (black) that is equal in magnitude to the 25 ms tone on response (magenta). However, unlike the forward suppression seen for the on responses, the off response caused no forward suppression of the subsequent on response (arrow), even though the two responses had the same 100 ms separation as the 25 ms on responses. This was true for all extracellularly recorded neurons in our sample. **Figure 7B** shows the spiking responses averaged across the population ($n = 33$ neurons). Whereas on responses caused complete suppression of a second on response 100 ms later (magenta), off responses caused no suppression at all for on responses occurring 100 ms later (black). This confirms our prediction and supports our hypothesis that the set of synapses activated by on

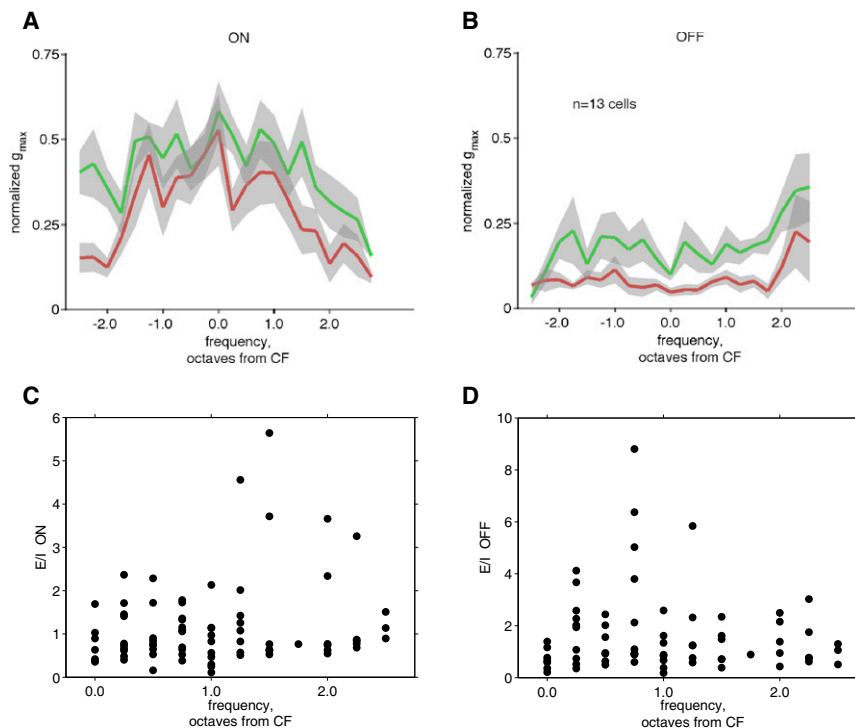


Figure 6. Both Unbalanced On and Off Responses Can Be Explained by Pure Excitation at the Edges of the Receptive Field (Group Data)

(A and B) Synaptic tuning curves for on responses (A) and off responses (B) averaged across 13 neurons, with normalized peak excitatory (green) and inhibitory (red) conductances plotted as a function of tone frequency (aligned to CF for each neuron, where CF is defined by on responses). Shaded regions indicate SEM. Note the nearly pure excitation at the edges of the receptive fields for both on and off responses.

(C and D) E/I ratio for on responses (C) and off responses (D) across the population ($n = 13$ cells). Each dot represents the trial-averaged response to a tone 400 ms or longer. Only responses with both strong on and off responses were included (total synaptic conductance > 1 nS for both). The ratio of peak excitatory conductance to peak inhibitory conductance is plotted against frequency (the absolute value of the distance from CF).

Tones were 80 dB SPL for (A)–(D).

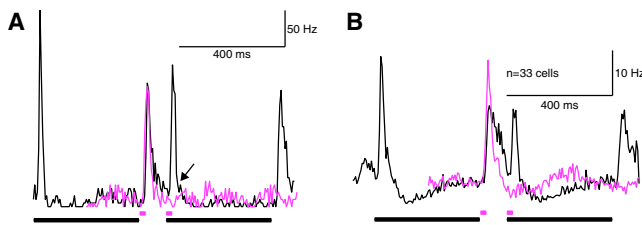


Figure 7. Off Responses Do Not Cause Forward Suppression of On Responses

(A) Example of single-unit spiking responses to tone pairs (19.8 kHz, 80 dB). This tone frequency was chosen such that the on response to a 25 ms tone (magenta) was equivalent to the off response to a 400 ms tone (black). Note that the on response to the first 25 ms tone (magenta) caused complete forward suppression of the response to the second 25 ms tone (arrow). In contrast, the off response to the 400 ms tone (black) caused no suppression of the on response to the second 400 ms tone (arrow). Separation was 100 ms between onsets for 25 ms tones (magenta) and between offset and onset for 400 ms tones (black). Means of 40 trials.

(B) Single-unit responses as in (A) averaged across 33 neurons for which on responses to the 25 ms tone were equivalent to the off responses to the 400 ms tone. On responses invariably suppressed on responses to tones at a separation of 100 ms, but off responses caused no suppression of on responses to tones at a separation of 100 ms.

responses is mostly distinct from the set of synapses activated by off responses.

Because voltage-gated conductances were not blocked in our extracellular recordings, it is possible that a rebound from transient inhibition (if present) could partially contribute to off responses. This nonsynaptic component would not be expected to contribute to forward suppression. However, firing rates during the 400 ms tone completely returned to baseline after the on response (Figure 7B), indicating that rebound does not contribute to the off responses. We verified this by comparing the firing rates during the last 100 ms of the tone to a 100 ms prestimulus baseline period. These firing rates were not significantly different (5.8 ± 4.0 Hz versus 5.4 ± 4.0 Hz, n.s., $n = 33$ neurons), suggesting that off responses were primarily synaptic and therefore were driven by mostly different synapses than on responses. Note also that our conclusions based on forward suppression assume that the spiking responses of neurons presynaptic to those we recorded from had transient on and/or off responses, like those in Figure 7. This is very likely to be the case, since on and off responses were transient in all auditory cortical neurons in our sample and the vast majority of thalamic (MGBv) neurons in anesthetized animals (Calford, 1983; He, 2001, 2002).

DISCUSSION

Here we have demonstrated that auditory cortical neurons can respond both to the onsets and offsets of sounds but that these responses are driven by distinct sets of synapses with different properties. The frequency tuning of on and off responses was largely nonoverlapping at both the spiking and subthreshold level, with off responses typically tuned 1–2 octaves above on responses. Off responses were not driven by a rebound from

sustained synaptic inhibition, which we never observed; rather, both on and off responses were driven by transient synaptic inputs. The balance of synaptic excitation and inhibition in these transient on and off responses was typically different. In most cases, this was not due to differential forward suppression of inhibition. Rather, the different excitatory-inhibitory composition of on and off responses can be understood as a straightforward consequence of their different frequency tuning. Excitation and inhibition are roughly balanced in amplitude throughout most of the frequency receptive field, but tones at the edges of the receptive field often evoke pure excitation (Wehr and Zador, 2003). Because the on and off receptive fields are largely nonoverlapping, tones within one receptive field are at the edge of the other, thus producing different excitatory-inhibitory composition. The different frequency tuning and excitatory-inhibitory composition underlying on and off responses strongly suggest that they are driven by largely nonoverlapping sets of synapses. This predicts that on responses should cause forward suppression of subsequent on responses, since they activate the same set of synapses, and forward suppression is likely to act via synaptic depression (Wehr and Zador, 2005). In contrast, off responses should cause no forward suppression of subsequent on responses, since they activate different sets of synapses. Our findings confirmed these predictions, leading us to conclude that on and off responses are driven by largely nonoverlapping sets of synaptic inputs.

Although many studies have reported the observation of off responses in auditory cortex (Brugge and Merzenich, 1973; Chimoto et al., 2002; Evans and Whitfield, 1964; He et al., 1997; Heil et al., 1992; Moshitch et al., 2006; Pelleg-Toiba and Wollberg, 1989; Pfingst and O'Connor, 1981; Takahashi et al., 2004; Volkov and Galazjuk, 1991), only a few have systematically compared on and off response properties (Fishman and Steinschneider, 2009; Qin et al., 2007; Recanzone, 2000). In agreement with their results, we found that off responses had different frequency tuning, longer duration, and smaller amplitude than on responses. We found that off responses were consistently tuned 1–2 octaves above on responses (with a rare second peak below on responses), which is consistent with reports that cortical on and off responses show complementary tuning in awake primates (Fishman and Steinschneider, 2009; Pelleg-Toiba and Wollberg, 1989), but contrasts with the finding by Qin et al. that the tuning of on and off responses showed no consistent relationship in the awake cat (Qin et al., 2007). This discrepancy could be a species difference or could be a result of anesthesia. Because our sample in this study was heavily biased toward off-responsive neurons, we have not reported the percentages of neurons showing off responses, nor of those showing only on responses (indeed, only off-responsive neurons are included in our sample). Previous reports of the prevalence of off-responsive neurons are typically about 30% (30% in awake monkey [Recanzone, 2000], 31% in ketamine-anesthetized cat [Volkov and Galazjuk, 1991], 28% in halothane-anesthetized cat [Moshitch et al., 2006], but 59% in awake cat [Qin et al., 2007]). Responses in awake animals show a greater diversity of temporal dynamics than those in anesthetized animals, including sustained responses to long tones (Fishman and Steinschneider, 2009; Recanzone, 2000; Wang et al.,

2005). Which set or sets of synapses are activated during sustained responses in awake animals remains an open question.

Our results are also consistent with the segregation of on and off pathways in the auditory thalamus, in which on-responding neurons are found mainly in the core of the ventral division (MGBv), whereas off-responding neurons form a surrounding sheet involving the edges of MGBv as well as numerous neighboring nuclei (He, 2001). Because A1 receives projections from both MGBv and neighboring nuclei (Lee and Winer, 2008), these distinct populations of thalamic neurons may be the sources of the distinct sets of synapses activated in the on and off responses of cortical neurons. This scenario is reminiscent of the anatomical segregation of ON and OFF pathways in the retina and lateral geniculate nucleus, which are progressively combined in simple and complex cells in the visual cortex (Jin et al., 2008).

In turn, thalamic off-responsive neurons likely get their input from off-responsive neurons in inferior colliculus. Because off responses have also been reported in the cochlear nuclei, auditory nerve, and basilar membrane mechanics (Henry, 1985a, 1985b; Suga et al., 1975; Young and Brownell, 1976), it seems likely that information about sound offsets is processed along the auditory hierarchy in a fashion similar to information about sound onsets. The fact that both the auditory brainstem response and cochlear microphonic on and off responses show different frequency tuning suggests that the distinct frequency tuning we observed in cortical on and off responses may have its origin at the basilar membrane. In addition, off responses may be enhanced or even generated *de novo* at any subcortical station—for example, by postinhibitory rebound, as seen in the superior olivary complex (Kuwada and Batra, 1999). Nevertheless, we have demonstrated here that such a postinhibitory rebound mechanism does not underlie off responses in primary auditory cortex. Taken together, these findings are consistent with a model in which on and off responses are each generated at distinct locations on the basilar membrane and are processed in parallel by independent pathways all along the auditory hierarchy.

Sound offsets are important for perceptual grouping and auditory scene analysis. One view is that on and off responses are symmetrical representations of amplitude transients and as such constitute the neural correlates of the perception of temporal edges, which can then serve as perceptual grouping cues. For example, asynchronous sound offsets serve as cues for the segmentation of a complex tones into its components (Bregman et al., 1994; Plack and White, 2000). This suggests that the auditory system may use off responses to reset itself at points of sudden amplitude change, in order to carry out a fresh perceptual grouping analysis (Bregman et al., 1994). Similarly, the success of the continuity illusion (in which a tone is perceived as continuing through a noise-filled gap) depends on the ability of the noise to mask the offset of the tone. Without the off response, according to this view, the auditory system has no reason to infer that the tone has stopped, leading to illusory continuity of the tone (Bregman, 1990). Thus, although in principle the cessation of a neuronal response could implicitly provide complete information about tone offset, off responses are an example of the explicit neural representation of computed features that are relevant to perceptual analysis.

An alternative view is that there is a perceptual asymmetry, such that information about sound onsets is more heavily weighted than information about sound offsets (Phillips et al., 2002). This perceptual asymmetry has been argued to parallel the more elaborate neurophysiological representation of onsets compared to offsets, although it is unclear how strongly this conclusion depends on studies that used barbiturate anesthesia, in which off responses are not seen. Indeed, in awake animals, off responses have been shown to be superior for the precise temporal coding of sound envelopes of amplitude-modulated sounds (Kuwada and Batra, 1999). Off responses have also been suggested to play a role in temporal integration, for example in the construction of duration selectivity seen in neurons in the dorsal zone of cat auditory cortex (He, 2001). However, the different frequency tuning that we observed for on and off responses suggests that the onset and offset of a given sound are represented by largely nonoverlapping populations of A1 neurons. Thus, temporal integration of these signals would need to be carried out by combining information from these separate populations, perhaps in downstream cortical areas.

EXPERIMENTAL PROCEDURES

Physiology

We recorded from the left primary auditory cortex of anesthetized (30 mg/kg ketamine, 0.24 mg/kg medetomidine) rats aged 18–35 days postnatal. All procedures were in strict accordance with the National Institutes of Health guidelines as approved by the University of Oregon Animal Care and Use Committee. Recordings were made from primary auditory cortex (A1) as determined by the frequency-amplitude tuning properties of cells and local field potentials, based on the criteria of Polley et al. (2007). For whole-cell recordings (26 cells), we used standard blind patch-clamp methods (Wehr and Zador, 2003). Subpial depth of whole-cell recordings ranged from 238 to 770 μm , as determined from micromanipulator travel. Internal solution contained, in mM, Cs-gluconate 140, HEPES 10, MgCl_2 2, CaCl_2 0.05, MgATP 4, NaGTP 0.4, Na₂Phosphocreatine 10, BAPTA 10, TEA 4, QX-314 6, pH 7.25, diluted to 290 mOsm, producing a calculated reversal potential of -85 mV for Cl^- conductances. Most voltage-dependent channels implicated in rebound from hyperpolarization should be blocked, because QX-314 blocks calcium as well as sodium currents, and intracellular Cs⁺ and TEA block potassium currents (Hille, 1992; Lüthi and McCormick, 1998; Talbot and Sayer, 1996). Across the population, input resistance was 68 ± 49 M Ω , and series resistance was 31 ± 27 M Ω (median $\pm$ interquartile range, $n = 13$ cells for which series and input resistance were measured). Holding potentials were stepped (using a 1 s ramp) to a pseudorandom sequence of three values using an Axopatch 200b amplifier. At each potential, after a 1 s equilibration period, ten 10 mV voltage pulses were delivered to monitor series and input resistance, followed by acoustic stimuli. For single-unit recordings (52 cells) we used the loose cell-attached patch recording method (DeWeese et al., 2003) with a pipette internal solution of 0.9% saline. Subpial depth of cell-attached recordings ranged from 160 to 825 μm , as determined from micromanipulator travel. Whole-cell and cell-attached recordings were only included in this report if they showed reliable responses that were time-locked to tone offset at multiple tone durations.

Stimuli

To characterize the frequency tuning of single-unit on and off responses, we used a pseudorandomly interleaved tone array consisting of 22 frequencies (logarithmically spaced from 1 to 40 kHz) and five amplitudes (from 0 to 80 dB), with 400 ms duration, 5 ms 10%–90% cosine-squared ramps, and a 500 ms interstimulus interval (ISI). We defined characteristic frequency (CF) as the frequency at which on responses (spikes or synaptic currents) could be reliably evoked at the lowest intensity; if on responses were evoked over a range of frequencies, CF was defined as the center of this range. “dB”

indicates dB SPL throughout. For whole-cell recordings, we used a similar tone array (but with 25 ms tones and 300 ms ISI) to identify CF, and then used an iso-intensity tone array to separately characterize on and off responses (same frequencies as above, at either 70 or 80 dB, 400 ms duration, 10 ms ramps, 500 ms ISI). To verify that off responses were locked to tone offset, we identified a tone frequency and amplitude that evoked robust off responses and then presented that tone at five durations (100, 200, 400, 800, and 1600 ms), with 10 ms ramps and 500 ms ISI. To characterize forward suppression evoked by on and off responses, we identified a tone frequency and amplitude that evoked both on and off responses of comparable magnitude and then presented that tone at two durations (25 ms and 400 ms), both in isolation (with 1000 ms ISI) or in pairs (first pair: durations 25 ms and 25 ms, at 100 ms stimulus onset asynchrony, such that onset followed onset by 100 ms; second pair: durations 400 ms and 400 ms, at 500 ms stimulus onset asynchrony, such that onset followed offset by 100 ms; see Figure 7). All stimuli were generated at a sampling rate of 200 kHz using a 24 bit Lynx22 soundcard and delivered with a Stax SRM-717 driver and SR-303 speaker in free-field configuration (speaker located 15 cm lateral to, and facing, the contralateral ear) in a sound isolation chamber with anechoic surface treatment.

Analysis

To extract spikes, we thresholded cell-attached recordings at seven standard deviations of the extracellular voltage. We quantified spiking responses by counting spikes in a fixed 100 ms window (Figure 2) or 50 ms window (Figure 7) following either tone onset or tone offset; using 50, 75, or 100 ms windows did not change the results (data not shown). Peristimulus time histograms were averaged over 9 to 11 repetitions and binned at 25 ms (Figure 2) or averaged over 39 to 100 repetitions and binned at 5 ms (Figure 7). To average single-unit frequency response areas across neurons, we normalized both on and off responses to the maximal on response for each neuron. Correlation values indicate pairwise linear correlation coefficients.

We computed series resistance from the peak current transients by taking the average across each group of ten pulses and taking the median of those averages over an entire stimulus protocol. We did not use online series resistance compensation. We corrected holding potentials for a calculated liquid junction potential of 12 mV. We computed total synaptic conductance, corrected offline for series resistance, assuming an isopotential neuron as described previously (Wehr and Zador, 2003). Briefly, total synaptic conductance was estimated from the linear regression slope between the trial-averaged synaptic currents and the holding potential. Synaptic currents were averaged over 2 to 28 (mean: 6) repetitions. We then decomposed total synaptic conductance into excitatory and inhibitory components, assuming linearity, as described previously (Wehr and Zador, 2003). Conceptually, this decomposition takes advantage of the fact that at holding potentials near 0 mV (the excitatory synaptic reversal potential), the synaptic current is mainly inhibitory, whereas near -85 mV the synaptic current is mainly excitatory (see Wehr and Zador, 2003, for more details).

We quantified synaptic tuning curves by measuring peak conductance change for each tone in a 200 ms window following tone onset or offset. To average synaptic tuning curves across cells, we normalized each tuning curve separately for excitation and for inhibition, with on and off responses both normalized to the maximal on response. We quantified the duration of onset-evoked synaptic conductances as the amount of time that the conductance continuously exceeded 10% of its maximal value during sound presentation. For some analyses (clearly indicated in Results) we excluded responses that did not exhibit a clear on or an off response—for example, duration and E/I ratio are ill-defined without a clear response. For this purpose we used an inclusion criterion of 1 nS, which is close to the SD of total synaptic conductance averaged across all cells and stimuli (0.94 nS).

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