

Synaptic Mechanisms of Forward Suppression in Rat Auditory Cortex

Michael Wehr and Anthony M. Zador*
Cold Spring Harbor Laboratory
1 Bungtown Road
Cold Spring Harbor, New York 11724

Summary

In the auditory cortex, brief sounds elicit a powerful suppression of responsiveness that can persist for hundreds of milliseconds. This forward suppression (sometimes also called forward masking) has usually been attributed to synaptic (GABAergic) inhibition. Here we have used whole-cell recordings *in vivo* to assess the role of synaptic inhibition in forward suppression in auditory cortex. We measured the excitatory and inhibitory synaptic conductances elicited by pairs of brief sounds presented at intervals from tens to hundreds of milliseconds. We find that inhibitory conductances rarely last longer than 50–100 ms, whereas spike responses and synaptic inputs remain suppressed for hundreds of milliseconds. We conclude that postsynaptic inhibition contributes to forward suppression for only the first 50–100 ms after a stimulus and that intracortical contributions to long-lasting suppression must involve other mechanisms, such as synaptic depression.

Introduction

Sounds in a natural environment, such as speech, footsteps, or rustling leaves, are almost always temporally complex. Temporal context can profoundly affect how sounds are perceived and how they are processed by the brain. Temporal separation affects whether sounds are perceptually grouped together into a single object or not, a process known as auditory stream segregation (Bregman, 1990). Temporal separation even determines whether the second in a pair of sounds is perceived at all, a phenomenon known as forward masking (Moore, 1995).

Many of these perceptual phenomena have correlates in the response properties of neurons in auditory cortex. In particular, they may be related to a rapid form of sensory adaptation in cortical neurons, which we refer to as forward suppression (also known as forward masking; Brosch and Schreiner, 1997; Calford and Semple, 1995), although adaptation can also occur over several time scales in auditory cortex (Ulanovsky et al., 2004). Similarly, cortical neurons cannot follow trains of sounds with repetition rates higher than about 5–15 Hz (Creutzfeldt et al., 1980), which corresponds to the perceptual boundary between rhythm (in which each click is heard individually) and pitch (in which click trains create a continuous, tonal percept). Rates in this range also strongly affect the perceptual grouping of similar stimuli, which gives rise to auditory stream seg-

regation (Bregman, 1990). The precise relationships between cortical forward suppression, other cortical physiological phenomena, and perceptual phenomena such as forward masking and stream segregation remain unclear.

Because most thalamic neurons can follow click trains at several tens of hertz or higher, forward suppression is thought to be mainly cortical in origin (Creutzfeldt et al., 1980; Miller et al., 2002). What are the mechanisms by which these response properties are created in the auditory cortex? Intrinsic intracellular mechanisms (such as postdischarge adaptation) do not appear to be responsible (Calford and Semple, 1995). This suggests that forward suppression arises at the circuit level, from the action of the synaptic inputs driving a neuron's response.

Postsynaptic GABAergic inhibition has been widely considered the most likely mechanism for cortical forward suppression (Brosch and Schreiner, 1997; Calford and Semple, 1995; Tan et al., 2004), so much so that the term “inhibition” has sometimes been used interchangeably to refer both to the phenomenology of forward suppression and to the underlying mechanism. However, the hypothesis that long-lasting GABAergic inhibition is responsible for forward suppression has not been tested directly; and indeed there have been several suggestions that synaptic depression, acting at thalamocortical or intracortical synapses (Castro-Alamancos, 1997; Chung et al., 2002; Varela et al., 1997), could account for the phenomenon of forward suppression (Denham, 2001; Eggermont, 1999).

Here we have studied the contribution of GABAergic inhibition to forward suppression by directly measuring the synaptic conductances evoked by pairs of clicks. We find that inhibitory conductances rarely last longer than 50–100 ms, whereas spike responses and synaptic inputs remain suppressed for several hundred milliseconds. Thus, synaptic inhibition is unlikely to play a role in forward suppression beyond the first 100 ms after a stimulus. We conclude that intracortical contributions to long-lasting suppression must involve other mechanisms, such as synaptic depression.

Results

Suppression of Firing

To characterize forward suppression of spiking responses, we used the loose cell-attached patch method to record extracellularly from well-isolated single units in rat primary auditory cortex (A1). Figure 1A shows an example of single-unit responses to pairs of clicks, presented at intervals of 32, 64, 128, 256, and 512 ms. For intervals shorter than 128 ms, the response to the second click was completely suppressed. For longer intervals, the evoked response progressively recovered, although by 512 ms it still had recovered to only 80% of the response to the first click (hence the P2/P1 ratio was 0.8), as shown in Figure 1B (black line). All cells showed suppression at short intervals. At longer in-

*Correspondence: zador@cshl.edu

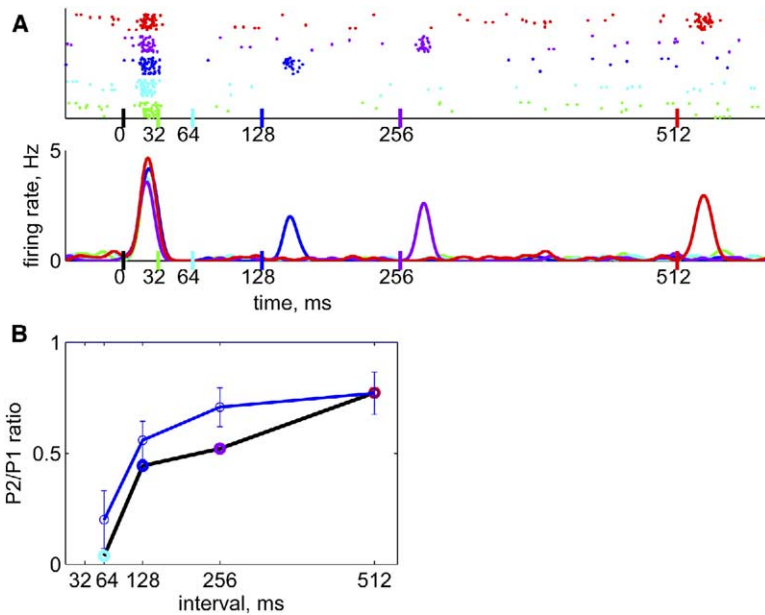


Figure 1. Forward Suppression of Spiking Responses Lasts Hundreds of Milliseconds

(A) Example of single-unit activity recorded in cell-attached mode showing responses to randomly interleaved click pairs (top: rasters, bottom: firing rates, stimuli indicated by ticks on abscissa, colors indicate different intervals). The response to the second click was completely suppressed for intervals shorter than 128 ms and progressively recovered for longer intervals.

(B) The ratio of the second response to the first (P2/P1) as a function of interval for this cell (black line, colored circles indicate different intervals), and for the population of suppressed cells (blue line, $n = 7$ cells, error bars are SEM). Cells with a P2/P1 < 1 at all intervals were classified as suppressed. In this and following figures, P2/P1 is not shown for the shortest interval (32 ms) because the P1 and P2 responses overlap in time.

Intervals, about half of the cells (7/12) showed long-lasting suppression (i.e., $P2/P1 < 1$) at an interval of 256 ms, whereas the other half (5/12) showed transient facilitation at longer intervals (i.e., $P2/P1 > 1$ at 256 ms, see [Experimental Procedures](#)). Because we were interested in the mechanisms underlying forward suppression, we restricted our analysis to cells showing suppression at all intervals. The population mean for these suppressed cells is shown in [Figure 1B](#) (blue line). For all suppressed cells, suppression lasted longer than 512 ms, the longest interval presented.

These dynamics, and the prevalence of suppression in the population, are consistent with previous reports ([Brosch and Schreiner, 1997](#); [Brosch et al., 1999](#); [Creutzfeldt et al., 1980](#); [Eggermont, 1999](#); [Kilgard and Merzenich, 1999](#)). Spontaneous firing rates were generally low (4.5 ± 5.1 Hz, population median \pm interquartile range), but in a few cases we could observe a clear suppression of spontaneous activity that had the same time course as that seen in [Figure 1B](#).

In what follows, we distinguish among several related terms. We will use *forward masking* to refer to the psychoacoustic perceptual phenomenon. We will use *suppression* to refer to phenomenology: the observed reduction in an auditory response. We will use *inhibition* to refer to one candidate mechanism for this suppression: GABAergic inhibition, arising from postsynaptic activation of either GABA_A or GABA_B receptor-gated channels. Finally, we will use the term *synaptic depression* to refer to the reduction in synaptic drive resulting from a decrease in synaptic release probability during fast trains of inputs ([Koch, 1999](#)). We note that synaptic depression may arise in part from activation of presynaptic GABA_B receptors, which acts to decrease synaptic release probability.

Suppression of Synaptic Conductances

What are the mechanisms responsible for the suppression of firing observed after the first click? To address

this question, we recorded intracellularly in vivo from 122 cells in A1 using the whole-cell patch-clamp technique. Specifically, we wanted to test the hypothesis that long-lasting inhibition is responsible for the suppression of firing seen in [Figure 1](#).

To measure directly the inhibitory synaptic conductances elicited by pairs of clicks, we decomposed click-evoked responses into their underlying excitatory and inhibitory components, using methods described previously ([Anderson et al., 2000](#); [Borg-Graham et al., 1998](#); [Las et al., 2005](#); [Monier et al., 2003](#); [Wehr and Zador, 2003](#); [Zhang et al., 2003](#)). The synaptic currents used for the decomposition were obtained by voltage clamping neurons to three different holding potentials. An example of these synaptic currents is shown in [Figure 2a](#). Evoked synaptic currents were inward at hyperpolarized holding potentials and outward at depolarized holding potentials, consistent with a mixture of excitatory and inhibitory conductances ([Anderson et al., 2000](#); [Ferster, 1986](#); [Monier et al., 2003](#); [Wehr and Zador, 2003](#)).

For any holding potential, synaptic currents were suppressed with a time course similar to that of spiking activity. This suggests that a lack of synaptic input, rather than a persistent inhibitory current, is responsible for the suppression of firing. This can be seen directly from the synaptic conductance waveforms for this cell ([Figure 2C](#)), which were extracted from the synaptic currents ([Figure 2B](#), see [Experimental Procedures](#) for details). Inhibitory conductances elicited in this cell by clicks were brief (<100 ms; [Figure 2C](#), red curves), yet the total evoked synaptic conductance (i.e., both excitation and inhibition) remained suppressed for hundreds of milliseconds. In this example, synaptic inputs had only recovered about halfway (57%) by 512 ms, the longest interval presented ([Figure 2D](#)). This long-lasting suppression, therefore, cannot be due to a long-lasting inhibitory conductance in this cell.

This example was typical. Across the population, half

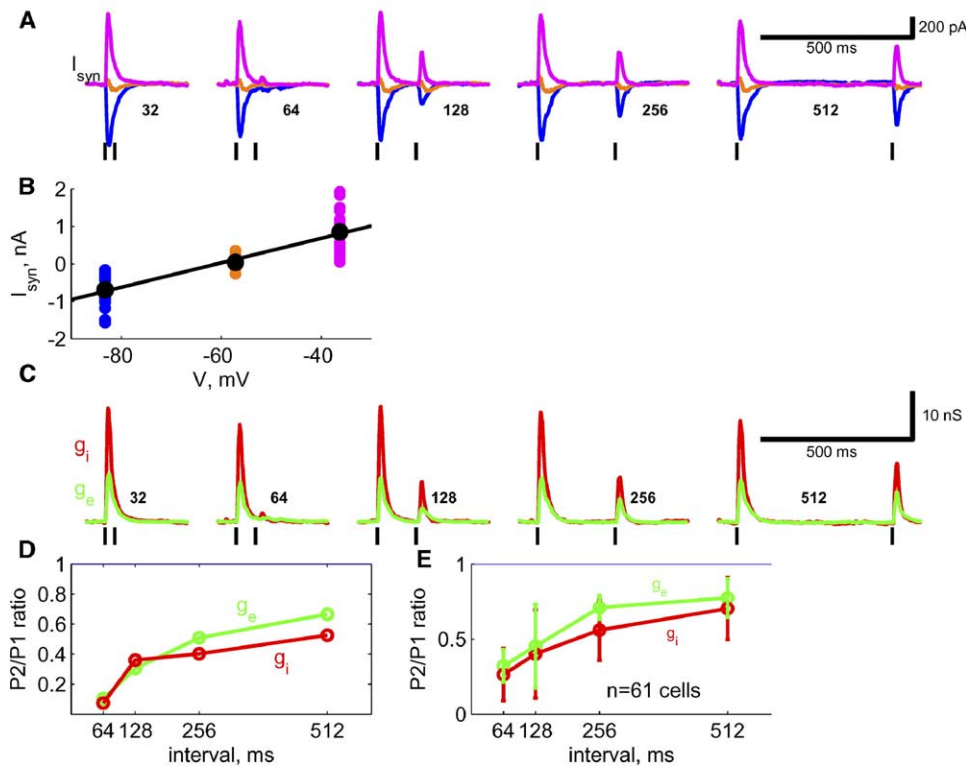


Figure 2. Inhibitory Conductances Are Much Shorter than Forward Suppression

(A) Example of synaptic currents evoked by click pairs at three different holding potentials (pink, -28 mV; orange, -57 mV; blue, -90 mV). Spikes were blocked pharmacologically. Stimuli indicated by ticks along bottom.

(B) Peak synaptic currents (colored dots) are plotted against holding potential (colors as in [A]) for each of 30 repetitions (means indicated by black dots). The regression slope (line) and x-intercept give, respectively, the instantaneous synaptic conductance (33 nS) and net synaptic reversal potential (-61 mV).

(C) Excitatory (green) and inhibitory (red) conductances evoked by pairs of clicks at different intervals. Inhibitory conductances decayed completely within 50–100 ms, yet synaptic conductances remained suppressed long after that, recovering only to 50% of control amplitude even after 512 ms.

(D) P2/P1 ratio for excitatory (green) and inhibitory (red) conductances for this cell.

(E) P2/P1 ratio for the population average of 61 suppressed cells. In all cells, as in the population average, synaptic inputs were suppressed long after inhibition had decayed.

of the cells (61/122) showed suppression at an interval of 256 ms. The other half of the population (61/122) showed facilitation and was excluded from analysis (see [Experimental Procedures](#)). For all suppressed cells ([Figure 2E](#)), synaptic currents and conductances elicited by the second stimulus were suppressed long after inhibitory currents elicited by the first stimulus had decayed. Inhibitory conductance (population mean) decayed to 10% of peak amplitude within 105 ms after stimulus onset and decayed from 90% to 10% of peak amplitude with a time constant of 82 ms ($n = 122$ cells), whereas synaptic conductances typically remained suppressed for hundreds of milliseconds, and this suppression lasted longer than our longest interval of 512 ms in 41/61 cases.

We therefore conclude that the long-lasting suppression of firing we and others have observed ([Figure 1](#)) is not due to prolonged inhibition acting directly on the recorded cell, but is due instead to a suppression, or withdrawal, of all synaptic inputs to the cell (see also [Las et al., 2005](#)). This suggests that either the suppres-

sion is due to long-lasting inhibition in some other population of cells presynaptic to those we recorded from (cortical or subcortical), or else it is due to synaptic depression. The fact that we never observed long-lasting inhibition in any cortical cell indicates that long-lasting suppression is not caused by long-lasting synaptic inhibition at the cortical level. We conclude that long-lasting suppression in the auditory cortex is either inherited from thalamic inputs or generated by synaptic depression at thalamocortical or intracortical synapses.

Prolonged Inhibition and Barbiturates

Although the decomposition shown in [Figure 2](#) represents one of the first systematic measurements of synaptic inhibition during forward suppression (i.e., during pairs of closely separated stimuli), inhibitory synaptic conductances evoked by *isolated* pure tones in A1 neurons have been measured previously ([Tan et al., 2004](#); [Wehr and Zador, 2003](#); [Zhang et al., 2003](#)). Surprisingly, the duration of inhibition reported by these studies has been strikingly different. We previously reported ([Wehr](#)

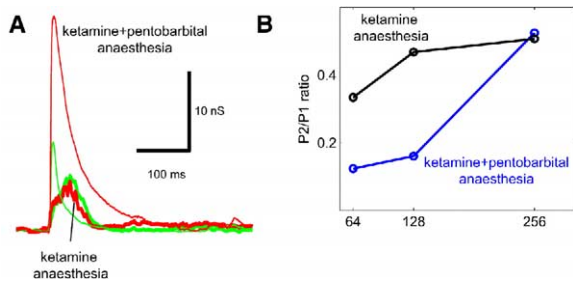


Figure 3. Pentobarbital Anesthesia Prolonged Inhibitory Conductances and Increased Suppression Compared to Ketamine

(A) Excitatory (green) and inhibitory (red) synaptic conductances evoked by a click under ketamine (heavy lines). After pentobarbital administration (50 mg/kg i.p.), the inhibitory conductance in the same cell was greatly increased and prolonged (thin lines). Duration of inhibition increased from <100 to >200 ms.

(B) P2/P1 ratio before (black) and after (blue) pentobarbital for this cell. Forward suppression was greatly enhanced by pentobarbital (i.e., the P2/P1 ratio was greatly decreased) for intervals up to 128 ms.

and Zador, 2003) that tone-evoked inhibitory conductances had a duration of 50–100 ms, similar to the duration of tone-evoked excitatory conductances. In contrast, Tan and colleagues reported large inhibitory conductances with much longer durations (several hundred milliseconds), which far outlasted the excitatory conductances they measured (by 450%). They suggested that this long-lasting inhibition could be responsible for forward suppression of cortical spiking responses and possibly even the perceptual phenomenon of forward masking.

Although the methods we used for measuring synaptic conductances are similar to those used by Tan and colleagues, one important difference is the anesthesia used. In both our previous work and in the present study, we used ketamine anesthesia, whereas Tan and colleagues used pentobarbital; as noted by Tan and colleagues, the mechanism of action of pentobarbital is to prolong the open time of GABA-activated chloride channels (MacDonald et al., 1989; Nicoll et al., 1975). We therefore wondered whether the longer inhibitory durations reported by Tan and colleagues might have been due to the use of pentobarbital anesthesia.

To address this question directly, we measured synaptic conductances evoked by click pairs in single neurons in ketamine-anesthetized animals before and after systemic administration of pentobarbital. In this way, we could directly monitor the effect of pentobarbital on inhibitory conductances. In four neurons, we completed the protocol for measuring conductance before and after the addition of pentobarbital.

Pentobarbital dramatically prolonged evoked inhibitory conductances within 4–5 min after injection (Figure 3), leading to inhibitory conductances with a time course comparable to that reported by Tan and colleagues. For the cell shown in Figure 3A, the time constant of inhibitory decay increased 242%, from 56 ms to 135 ms. The prolonged inhibition caused by pentobarbital led to a profound enhancement of the suppression observed with pairs of clicks. At an interval of 128 ms, inhibition had completely decayed under ketamine,

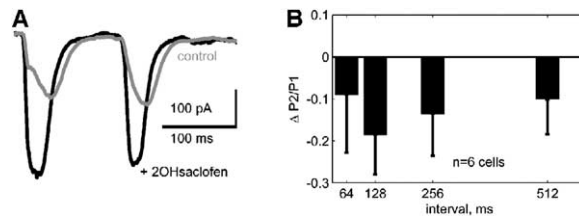


Figure 4. Blocking GABA_B Increased P1 but Decreased P2/P1

(A) Synaptic currents before (gray trace) and after (black trace) application of 25 mM hydroxysaclofen to the cortical surface. Blocking GABA_B therefore increased suppression, which is inconsistent with a postsynaptic suppressive effect of GABA_B activation, but is consistent with a presynaptic GABA_B activation. Holding potential –73 mV.

(B) Hydroxysaclofen-induced change in P2/P1 across six cells tested with hydroxysaclofen. Error bars are SEM.

but was still substantial under pentobarbital (see Figure 3A) and was associated with dramatically increased forward suppression (a 66% reduction in the P2/P1 ratio, Figure 3B). Inhibitory duration was similarly increased in all cells held through intraperitoneal injection. On average, the time constant of inhibitory decay increased by 643% ± 165% (n = 4 cells, mean ± SEM). We conclude that the prolonged inhibitory conductances reported by Tan and colleagues are likely due to pentobarbital anesthesia and are unlikely to account for forward suppression under other conditions.

Contribution of GABA_B

Although we never observed long-lasting inhibitory conductances, it is important to note that QX-314—included in the internal solution to block voltage-dependent conductances and thereby improve isolation of synaptic inputs—has also been reported to block long-lasting inhibitory GABA_B potassium currents (Deisz et al., 1997; Talbot and Sayer, 1996). QX-314 might thereby have masked the contribution of GABA_B to forward suppression under our recording conditions.

We therefore recorded responses to isolated tones at the optimal frequency, either with or without QX-314 in a potassium-based internal solution. Cells recorded *without* QX-314 showed a small, slow inhibitory conductance change, which lasted 200–300 ms after stimulus onset and had a peak amplitude of 1.5 ± 2 nS (mean ± SD), or 12% of the fast evoked synaptic conductance change (n = 17 cells). Cells recorded *with* QX-314 showed no such slow inhibitory conductance change in response to the same stimuli (peak amplitude 0.2 ± 0.4 nS, or 1.7% of the fast evoked conductance change, n = 9 cells).

We wondered whether this small, slow inhibitory conductance could account for the profound forward suppression we observed. To test this directly, we recorded responses to click pairs (without QX-314 in the pipette) before and after application of the selective GABA_B antagonist 2-hydroxysaclofen to the surface of the cortex. If a GABA_B-mediated inhibitory conductance was responsible for suppression, then blocking it would be expected to relieve the suppression and increase the P2/P1 ratio. Instead, we saw the opposite: blocking

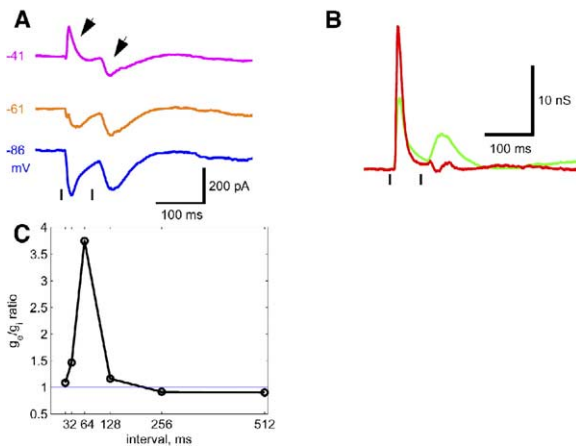


Figure 5. The Ratio of Excitation to Inhibition Was Often Larger for the Second Click Response

(A) Synaptic currents and (B) synaptic conductances for a cell that showed decreased inhibition evoked by the second click at an interval of 64 ms (colors as in Figure 2). The change in reversal potential for the second click response can be seen directly in the synaptic currents (arrows in [A]). (C) For this cell, the ratio of excitation to inhibition (g_e/g_i) increased dramatically during the extent of forward suppression.

GABA_B caused a consistent decrease in the P2/P1 ratio (Figures 4A and 4B). In other words, GABA_B blockade enhanced forward suppression. Hydroxysaclofen also increased P1 amplitude as much as 2-fold (Figure 4A, mean increase was $27\% \pm 21\%$, $n = 6$ cells, mean \pm SEM) and increased input resistance by 21 ± 12 M Ω (or $52\% \pm 27\%$, $n = 4$ cells). Because GABA_B blockade enhanced forward suppression, it seems unlikely that a long-lasting postsynaptic GABA_B inhibitory component makes a substantial contribution to forward suppression.

Preferential Suppression of Inhibition

In the majority of cells, the ratio of excitation to inhibition was the same on both the first and second clicks. That is, both excitatory and inhibitory synaptic inputs were equally suppressed and recovered with a parallel time course. In an intriguing subset ($22/122 = 18\%$) of cells, however, inhibition appeared to be much more strongly suppressed than excitation. This can be seen directly in the synaptic currents (Figure 5A shows an example), where at depolarized holding potentials the first click evokes a net outward current, whereas the second click evokes a net inward current. Thus for this cell, the apparent net synaptic reversal potential changed dramatically on the second click, leading to a strong decrease in the estimated inhibitory conductance (Figure 5B). Thus, our estimate of the ratio of excitation to inhibition (g_e/g_i) increased dramatically during the extent of forward suppression for this cell (Figure 5C). We never observed the opposite, i.e., preferential suppression of excitation. This suggests that, in a subset of cells, forward suppression reduces inhibition preferentially, changing the response properties of the cortical circuitry to the second stimulus.

Subcortical Contribution to Forward Suppression

We have concluded that long-lasting suppression of firing in cortical neurons is caused by a suppression of the synaptic inputs they receive. This suppression could be due to synaptic depression (at thalamocortical or intracortical synapses) or could be inherited (directly or indirectly) from thalamic response properties. The cortical neurons in our sample were presumably a mixed population of cells, some of which did and some of which did not receive direct monosynaptic thalamic input. Thalamic neurons have previously been reported to have temporal response properties that are twice as fast as cortical cells (Miller et al., 2002) and to follow periodic stimuli at much higher rates than cortical neurons (Creutzfeldt et al., 1980). This suggested that forward suppression in cortical cells was unlikely to be inherited from thalamic inputs.

To confirm this in our preparation, we used the cell-attached patch method to record from 11 well-isolated single units in the auditory thalamus (medial geniculate body). Figure 6A shows an example of a thalamic unit that had almost completely recovered (95%) within 64 ms, an interval at which cortical units had only started to recover (35% maximum). Across the population of thalamic units, the distribution of forward suppression at an interval of 64 ms was significantly faster than that for cortical units (Figure 6B, $p < 0.05$, Mann-Whitney). Nevertheless, the two distributions showed some overlap, so that the slowest thalamic neurons were slower than the fastest cortical neurons.

Discussion

In the auditory cortex, brief sounds elicit a powerful suppression of responsiveness that we refer to as forward suppression (also sometimes called forward masking). Forward suppression can last hundreds of milliseconds and can be observed both at the level of spiking output and at the level of the underlying synaptic inputs. Here we have tested the hypothesis that the mechanism responsible for forward suppression is a long-lasting GABAergic inhibition induced by the first sound. We measured the excitatory and inhibitory synaptic conductances elicited by brief sounds presented at intervals from 32 to 512 ms. We found that both the excitatory and inhibitory components of the synaptic drive recovered from suppression in parallel, with a similar time course. Moreover, the inhibitory conductance elicited by the first tone typically lasted 50–100 ms, whereas the suppression of the response to the second tone could last 500 ms or longer. Our main conclusion is that GABA-mediated inhibition does not play a major role in forward suppression beyond about 100 ms.

Our results also reconcile conflicting intracellular evidence about the role of long-lasting inhibition in forward suppression. Several studies have reported that tone-evoked inhibition decays much too rapidly (within 50–100 ms) to account for the long time course of forward suppression (De Ribaupierre et al., 1972; Las et al., 2005; Volkov and Galazjuk, 1991; Wehr and Zador, 2003). Others have reported long-lasting inhibitory conductances and have suggested that this might indeed

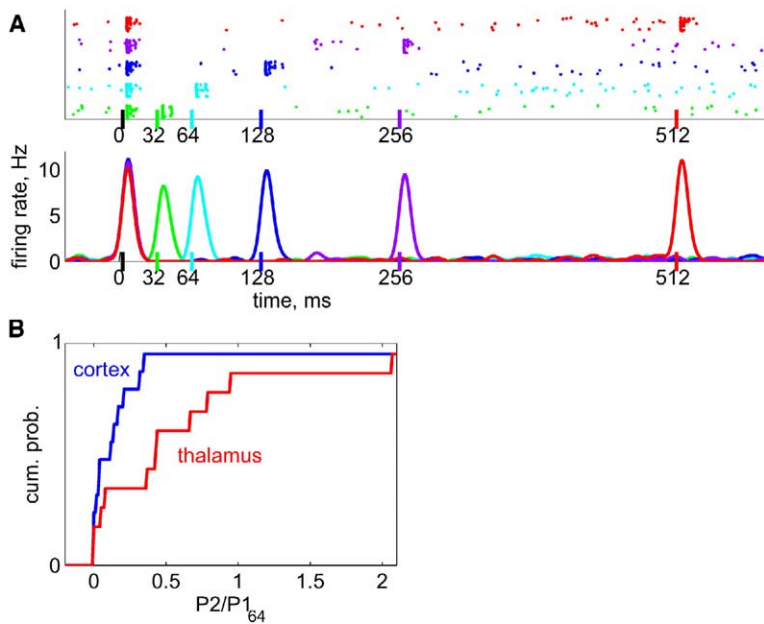


Figure 6. Thalamic Neurons Recover from Forward Suppression More Quickly than Cortical Neurons

(A) Example of single-unit activity recorded in cell-attached mode from a thalamic neuron, which responded strongly at an interval of 32 ms (an interval at which cortical neurons rarely showed any response at all, cf. Figure 1).

(B) Distribution of P2/P1 at an interval of 64 ms for our sample of 11 thalamic neurons and 12 cortical neurons. Thalamic neurons had significantly higher P2/P1 values than cortical neurons at this interval ($p < 0.05$, Mann-Whitney).

account for the long time course of forward suppression (Tan et al., 2004; Volkov and Galazyuk, 1992). Here we have shown that, under barbiturate anesthesia, the GABA_A-mediated chloride conductance is markedly prolonged (643%) and contributes considerably to forward suppression. In the absence of barbiturate anesthesia, however, the GABA_A-mediated conductance is unlikely to contribute to long-lasting forward suppression.

Role of Synaptic Depression

What causes forward suppression for intervals longer than 100 ms? Synaptic depression is the most likely candidate mechanism at these longer intervals. In the somatosensory cortex, synaptic depression is largely responsible for a form of rapid sensory adaptation that is similar (and perhaps analogous) to forward suppression in the auditory cortex (Chung et al., 2002). Suppression in the visual cortex is also more consistent with thalamocortical synaptic depression than with inhibition (Carandini et al., 2002; Freeman et al., 2002). Auditory cortical models have also demonstrated that synaptic depression can account for forward suppression and the low-pass characteristics of cortical responses to repetitive stimuli (Denham, 2001; Eggermont, 1999; Eggermont and Smith, 1995). Finally, our own observations of the effect of GABA_B receptor blockade (Figure 4) are also consistent with a role for synaptic depression in forward suppression (see below). Moreover, although some thalamic units showed forward suppression with a time course similar to that seen in cortex, the population of thalamic units recovered much more quickly than cortical cells, suggesting that inheritance of thalamic response properties is unlikely to fully account for long-lasting forward suppression in the cortex.

At shorter intervals (<100 ms), responses were almost always completely suppressed. Synaptic depression,

which is maximal immediately following the first stimulus (Varela et al., 1997), is likely to make a strong contribution to this suppression. Other mechanisms, however, may also be involved at these short intervals. The synaptic inhibition evoked by the first click typically lasts 50–100 ms and most likely reduces the response to the second click. In addition, at short intervals (<100 ms), thalamic units (Figure 6B) and even auditory nerve fibers (Harris and Dallos, 1979) show partial forward suppression, which would be inherited by cortical cells. Forward suppression at short intervals is therefore likely the result of a complex mixture of several mechanisms.

Role of GABA_B

GABA_B receptors can mediate both postsynaptic and presynaptic effects in cortical neurons. Postsynaptically, they mediate a long-lasting (hundreds of milliseconds) inhibitory potassium conductance. Two lines of evidence suggest that this postsynaptic GABA_B-gated conductance plays little role in forward suppression. First, the QX-314-sensitive inhibitory conductance, to which GABA_B receptors may contribute, was small (12%) compared to fast evoked responses. Second, blocking GABA_B receptors with hydroxysaclofen increased forward suppression, rather than relieving it as would be predicted if forward suppression was due to a postsynaptic GABA_B-mediated inhibitory conductance. Taken together, these observations argue against a major role for postsynaptic GABA_B receptors in forward suppression.

In addition to increasing forward suppression, hydroxysaclofen application also increased P1 (the amplitude of the response to the first click). We speculate that this increase in P1 results from blockade of presynaptic GABA_B receptors; in vitro, GABA_B receptor activation causes a decrease in synaptic release probability (Deisz and Prince, 1989). We further speculate that it is

the increase in P1 that is in turn responsible for the observed increase in forward suppression (decreased P2/P1 ratio). This is consistent with *in vitro* studies showing that P1 and P2 (here interpreted as synaptic release probability on the first and second pulse in a pair, respectively) are inversely correlated (Dobrunz and Stevens, 1997), so that increasing P1 decreases P2 and thereby increases synaptic depression. Thus, if the hydroxysaclofen-induced increase in P1 seen *in vivo* is indeed the result of increased release probability, then the enhancement of forward suppression is the expected result and is due to an enhancement of synaptic depression.

We also note an intriguing difference between the effect of GABA_B receptor antagonists *in vivo*, where they increase P1, and *in vitro*, where in the cortical slice preparation they have no effect on P1 (Gil et al., 1997). One possible explanation is that *in vivo*, GABA_B receptors are tonically activated by residual or background GABA. Persistent activation of *presynaptic* GABA_B receptors would tonically depress release probability at cortical synapses; blockade of *presynaptic* GABA_B receptors by hydroxysaclofen would relieve this depression and increase the response to an isolated stimulus (Figure 4A). Tonic activation of *postsynaptic* GABA_B receptors might also explain the increase in input resistance that we observed following application of hydroxysaclofen. *In vitro*, there is presumably no residual GABA_B receptor activation, so P1 is unaffected by GABA_B antagonists. Residual GABA could thereby explain the different effects of GABA_B receptor blockade on P1 *in vivo* and *in vitro*. Although these interpretations are speculative, we note that our main conclusion—that GABA_B plays at most a small role in forward suppression—does not depend on them.

Change in Circuit Properties

In a subset (18%) of cells, inhibition appeared to be much more strongly suppressed than excitation, evident as a sign change (from outward to inward) of the synaptic current at depolarized holding potentials. This sign change is a direct observation (Figure 5A), but it can be interpreted in two ways: as a change in cellular properties, or as a change in circuit properties. We consider changes in cellular properties (e.g., nonlinearities such as voltage-gated channels or NMDA receptor activation) to be less likely—because we used the voltage-gated channel blocker QX-314, which blocks many voltage-gated channels, and ketamine anesthesia (which is a competitive NMDA receptor antagonist)—but we cannot rule them out.

There are two possible changes in circuit properties that could underlie this sign change phenomenon. One possibility is that forward suppression suppresses inhibition more than excitation (Figure 5B). Indeed, inhibitory synapses are known to exhibit synaptic depression (Deisz and Prince, 1989), and inhibitory inputs depress more strongly than excitatory inputs in auditory cortex (Metherate and Ashe, 1994), although the opposite has been reported in visual cortex (Varela et al., 1999). Alternatively, the sign change could be due to the second input volley arriving with the same ratio of excitation to inhibition, but at a different and more distal set of

synapses. The resulting increase in the electrotonic distance of synaptic inputs would cause an apparent change in the net synaptic reversal potential (Spruston et al., 1993). At present, we cannot distinguish between these two possibilities, but we note that *neither can the soma itself*. Thus, the effect of this phenomenon on spike initiation in the soma will be identical—a decrease in relative inhibition—regardless of the underlying causes in the location and composition of synaptic inputs.

This effective decrease in inhibition for the second click would be expected to increase the spiking response evoked by the second click. This increase could propagate through the cortical circuit and contribute to the facilitation of both spiking outputs and synaptic inputs that we observed in a subset of cells. Thus, response facilitation may be due not just to synaptic facilitation (Castro-Alamancos and Connors, 1996), but to a relative decrease in the effective level of inhibition as well.

Although forward suppression of cortical responses may play a critical role in perceptual forward masking, the precise relationship between these phenomena is not yet clear. Because forward suppression can last much longer than perceptual forward masking, it seems likely that long-lasting suppression also affects related perceptual phenomena such as auditory stream segregation and rhythm perception. The relationships between these perceptual phenomena, and the neural correlates underlying them, remain to be elucidated.

Experimental Procedures

Physiology

We recorded from the left primary auditory cortex of anesthetized (30 mg/kg ketamine, 0.24 mg/kg medetomidine) rats aged 19–26 days postnatal. All procedures were in strict accordance with the National Institutes of Health guidelines as approved by the Cold Spring Harbor Laboratory 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. We recorded from all subpial depths (range: 110–1320 μm , as determined from micromanipulator travel, but 69% were <500 μm). For single-unit recordings, we used the cell-attached patch method (DeWeese et al., 2003), which provides excellent isolation. For whole-cell recordings, we used standard blind patch-clamp methods (Wehr and Zador, 2003). Internal solution contained Cs- or K-gluconate, 140 mM; HEPES, 10 mM; MgCl₂, 2 mM; CaCl₂, 0.05 mM; MgATP, 4 mM; NaGTP, 0.4 mM; Na₂Phosphocreatine, 10 mM; BAPTA, 10 mM; QX-314, 1–10 mM; and MK-801, 25–50 mM (pH 7.25); diluted to 290 mOsm, producing a calculated reversal potential of -85 mV for both K⁺ and Cl⁻ conductances. For cell-attached recordings, we used K-based internal solution and omitted QX-314 and MK-801. For whole-cell recordings, we included QX-314 in the internal solution for 94 cells, Cs⁺ for 77 cells, and MK-801 for 22 cells. Note that in addition to blocking fast sodium channels (and thereby blocking action potentials), QX-314 also blocks many other activity-evoked conductances (Deisz et al., 1997; Talbot and Sayer, 1996). We included MK-801, which has been reported to block NMDA currents intracellularly in this concentration range (Humeau et al., 2003), in an attempt to determine if an NMDA component was responsible for the sign change phenomenon shown in Figure 5. We observed no difference in the prevalence of the sign change phenomenon (17/100 = 17% of cells without MK-801, 5/22 = 23% of cells with MK-801), consistent with the hypothesis that an NMDA component is not responsible for the sign change phenomenon. However, we have no positive evidence that MK-801 was effectively blocking NMDA currents. Because the

two populations showed no differences, we grouped them together for analysis. The subset of cells in which inhibition was preferentially suppressed relative to excitation (i.e., the 22/122 cells that displayed the sign change phenomenon, Figure 5) was classified in this way if the sign of the evoked current (at a depolarized holding potential) changed from outward on P1 to inward on P2. Cell-attached recordings in the thalamus were obtained from the ventral division of the medial geniculate body, as determined by latency (<16 ms) and V-shaped frequency response areas. In addition, we filled a subset of thalamic cells by electroporation (by including 0.5 mM Alexa 594, a fluorescent dye, in the pipette) and recovered morphology and position using standard histological methods. We recovered one cell that showed strong forward suppression and was clearly located in the ventral subdivision. Thus, the distribution we observed in the strength of forward suppression (Figure 6B) cannot be solely accounted for by cells being located in different thalamic subdivisions (Calford and Webster, 1981). In other words, at least some neurons in the ventral division of the medial geniculate body show long-lasting forward suppression.

Because we used a variety of recording technologies, stimuli, and pharmacology, we briefly summarize here the numbers of cells recorded under different conditions. We recorded extracellularly (using cell-attached methods) from 12 neurons in auditory cortex (area A1) and 11 neurons in auditory thalamus (MGB). Of these 12 cortical neurons, 7 showed forward suppression and were therefore included in the analysis of suppression. We recorded intracellularly (using whole-cell methods) from 122 neurons in auditory cortex, presenting click pair stimuli. Of these, 61 neurons showed forward suppression and were therefore included in the analysis of suppression. We reanalyzed an additional 26 neurons (originally recorded as part of a previous study [Wehr and Zador, 2003] using whole-cell methods and isolated pure tone stimuli) to examine the effect of QX-314 on slow inhibitory conductances (9 cells with and 17 cells without QX-314, amplitude measured at 200 ms from stimulus onset). For four additional cells (in four separate ketamine-anesthetized animals), we investigated the effect of barbiturate anesthesia by injecting sodium pentobarbital (25–50 mg/kg) intraperitoneally during the recording (whole-cell recording and click pairs). For six additional cells (in six separate animals), we investigated the effect of GABA_B receptor blockade by applying 25–50 mM 2-hydroxysaclofen to the surface of the cortex (whole-cell recording and click pairs).

Across the population, input resistance was 56 ± 35 M Ω , and series resistance was 20 ± 21 M Ω (median \pm interquartile range, $n = 122$ cells). 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.

Stimuli

We presented 5 ms white noise bursts, which we refer to as clicks, with 1 ms 10%–90% cosine-squared ramps, intensity 102 dB SPL, sampling rate 97.656 kHz, using a System 3 Stimulus Presentation Workstation with an ED1 electrostatic speaker (Tucker-Davis Technologies, Alachua, FL) in free-field configuration (speaker located 8 cm lateral to, and facing, the contralateral ear) in a double-walled sound booth. Click pairs consisted of six pseudorandomly interleaved intervals (16, 32, 64, 128, 256, and 512 ms), with 3–4 s between pairs. Isolated pure tones had duration 25 ms, with 5 ms ramps, and intensity 66 dB SPL.

Analysis

We extracted spike times from single-unit recordings by high-pass filtering and thresholding. For display purposes, we smoothed firing rates by convolving with a Gaussian kernel (standard deviation 5 ms). We quantified single-unit responses using the spike count in a window following stimulus onset (matched to response latency and duration for each cell) and synaptic conductance responses by measuring peak conductance change during a fixed 100 ms window following stimulus onset (relative to a 50 ms baseline period prior to stimulus onset). We calculated P2/P1 ratios by dividing the trial-averaged response to P2 (the second click in a pair) by

the response to the first click (P1, averaged across trials and intervals 128, 256, and 512). Unresponsive cells were not included in this report.

We defined facilitation and suppression as P2/P1 ratios greater than or less than 1, respectively, and partitioned the population into facilitated and suppressed cells based on the P2/P1 ratio at an interval of 256 ms. The P2/P1 ratio was significantly correlated between intervals of 256 and 512 ms ($r^2 = 0.69$, $p < 10^{-4}$), indicating that the distribution of facilitation and suppression reflects true underlying variability in the population. Because facilitation and suppression tended to cancel each other out in the population mean across all cells, however, we excluded facilitated cells from our population analysis of suppression. For longer trains of clicks, we often also observed a transient facilitation at these intervals, which was inevitably overwhelmed by suppression within a few clicks (data not shown), consistent with previous reports (Eggermont and Smith, 1995). Thus, facilitation, while seen for click pairs in half the cells we recorded from, is not present in steady-state responses to repetitive stimuli.

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 (Barry, 1994) of 12 mV. We computed total synaptic conductance, corrected for series resistance, assuming an isopotential neuron as described previously (Wehr and Zador, 2003). Essentially, total synaptic conductance is given by the regression slope between synaptic currents and holding potential (Figure 2B). 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).

Acknowledgments

We gratefully acknowledge support by NIH, the Swartz Foundation, the Mathers Foundation, and the Sloan Foundation.

Received: April 19, 2005

Revised: May 26, 2005

Accepted: June 8, 2005

Published: August 3, 2005

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DOI 10.1016/j.neuron.2005.07.011

One Click, Two Clicks: The Past Shapes the Future in Auditory Cortex

What are the synaptic and cellular mechanisms by which stimulus context shapes cortical responses? In this issue of *Neuron*, Wehr and Zador describe intracellular recordings of responses to click pairs in rat primary auditory cortex (A1) and offer new insights into the successive roles of inhibition and synaptic depression in suppressing responses to the second click in many A1 neurons.

For many years, neurophysiologists studying auditory cortex examined neuronal responses by presenting single, isolated acoustic stimuli. Inspired partly by neuroethological and psychoacoustic studies that revealed the importance of stimulus context in shaping behavioral responses, researchers began to explore the role of stimulus context in auditory cortical processing. The simplest approach was to study responses to two successive sound stimuli and hence the influence of a preceding stimulus on neuronal responses to a subsequent acoustic stimulus. Some of the earliest studies to examine responses to sound pairs observed that some neurons in the bat responded selectively with enhanced responses to pairs of tones or FM chirps at specific interstimulus delays, corresponding to the biologically salient range of echo-delays from biosonar ultrasonic calls (~1–20 ms). In this early work, demonstrating combination-sensitive neural responses, emphasis was on the facilitation of responses to the second stimulus in a pair. Subsequent studies have confirmed the presence of facilitatory responses to pairs of stimuli in HVC in the anesthetized bird (Margoliash and Fortune, 1992), and in auditory cortex in the anesthetized cat (Brosch and Schreiner, 2000), anesthetized macaque (Brosch et al., 1999), and awake marmoset (Bartlett and Wang, 2005). In addition, physiologists observed the inverse phenomena—that some neurons in auditory cortex showed profound inhibition to certain pairs of stimuli in anesthetized cats (Calford and Semple, 1995; Brosch and Schreiner, 1997), awake rabbits (Fitzpatrick et al., 1999), and marmosets (Bartlett and Wang, 2005). Re-

cent studies suggest that the response to the second stimulus in a pair can be enhanced or suppressed for interstimulus intervals ranging from milliseconds to seconds depending on the spectrotemporal properties, the intensity, and the spatial location of the first stimulus. Complex stimulus context and history contribute to shaping responses over an even longer period of time (Ulanovsky et al., 2003, 2004). These results indicate that the cortical responses to sound are highly dynamic and dependent upon stimulus context.

All of the studies mentioned above used extracellular recording techniques to study cortical function. Recently, many investigators have begun to record from auditory cortex using intracellular recording techniques, making it possible to study the synaptic and cellular mechanisms that give rise to auditory responses and stimulus context effects. Earlier studies in A1 (Wehr and Zador, 2003; Tan et al., 2004) using the in vivo whole-cell patch-clamp technique have already led to fundamental new insights, showing that the excitatory and inhibitory inputs to A1 show similar frequency tuning. In such cotuning, unlike earlier lateral inhibition models, tone-evoked inhibition is not required in order to create selective frequency tuning, which hence raises the question as to possible alternate roles for the inhibitory input. Wehr and Zador (2003) observed that tones typically evoke brief excitation in auditory cortex, which was rapidly quenched within a few milliseconds by a long-lasting hyperpolarization. They proposed that the role of this rapid inhibition, right on the heels of stimulus-induced depolarization, was to increase temporal precision by ensuring that neurons spike only at tone onset, rather than during the remainder of tone duration. Although such a mechanism could certainly enhance temporal precision, long-lasting inhibition (100–200 ms) should also presumably reduce the ability of the neuron to spike in response to a second tone and hence limit the ability of cortical neurons to respond to temporal rates greater than about 5–10 Hz, which is too low, even by slow cortical standards.

In their current work, Wehr and Zador (2005 [this issue of *Neuron*]) decided to further explore the role of inhibition in shaping cortical responses by using a two-click stimulus paradigm. In the present paper, they significantly deepen our understanding of the time course and synaptic origin of inhibition and response suppression in auditory cortex by examining the effects of the first click on second click response over a range of different interclick intervals.

Initially, using intracellular techniques, Wehr and Zador measured excitatory and inhibitory conductances elicited by click pairs with interclick intervals ranging from 32–512 ms. They confirmed earlier results from extracellular recording and showed that, for interclick intervals shorter than 128 ms, the response to the second click was almost completely suppressed. Responses slowly recovered but for many neurons were still depressed at 256 and 512 ms. Although inhibition evoked by the first click has hitherto been invoked as the explanation for the decrease in response to the second click, a key finding of their work is that the GABAergic inhibitory conductances elicited by clicks were actually much shorter (<100 ms) than the time course of the

long-lasting suppression. Therefore, they concluded that this suppression was due to other factors, such as synaptic depression (either at thalamocortical [TC] or intracortical [IC] synapses), or was inherited from thalamic inputs. To test the possibility that the long-lasting suppression of firing in cortical neurons was inherited from thalamic inputs, they recorded from neurons in the auditory thalamus. The results demonstrated that the thalamic explanation was unlikely, since forward suppression in the thalamus recovered much more rapidly than in cortex.

Another insight arising from this work highlights the critical importance of anesthesia in shaping cortical responses. The durations of the inhibitory synaptic conductances observed by [Wehr and Zador \(2003, 2005\)](#) in ketamine-anesthetized rat A1 were considerably shorter than those observed by [Tan et al. \(2004\)](#) in pentobarbital-anesthetized rat A1. [Wehr and Zador \(2005\)](#) examined whether the choice of anesthetic could explain this puzzling discrepancy. In an elegant set of experiments, they first measured synaptic conductances evoked by click pairs in neurons in ketamine-anesthetized animals and then measured synaptic conductances evoked by the same stimuli in the same neurons after systemic administration of pentobarbital. They found that pentobarbital, compared to ketamine anesthesia, dramatically prolonged inhibitory conductance, probably because pentobarbital potentiates and increases the duration of GABAergic inhibition. This result illustrates some of the interpretative problems of anesthesia and, in our opinion, emphasizes the value of the awake preparation.

Of course, there are still a number of open and intriguing new questions raised by this study.

- (1) Forward suppression at short intervals (<100 ms) is likely to be a mixture of several cortical and subcortical mechanisms. It will be a challenge for the future to analyze the contributions of these different mechanisms at multiple levels of the auditory system.
- (2) There is good evidence that synaptic depression plays a role in forward suppression. As [Wehr and Zador](#) observe, TC synaptic depression has been demonstrated in the somatosensory cortex and implicated in the visual cortex, and is consistent with many auditory cortical models. But what are the relative contributions of TC and IC synaptic depression in shaping forward suppression at intervals greater than 100 ms in A1? Also, are these effects homogenous for different synaptic connections and throughout all cortical layers? This is unlikely, given earlier observations demonstrating differential sensitivity of TC and IC synapses to neuromodulators, and the recent results of [Ojima and Murakami \(2002\)](#), who found strong hyperpolarization following onset depolarization in layer 3, but not in layer 2 pyramidal neurons in cat primary auditory cortex. It is left for future work to determine the locus, origin, and function of synaptic depression in mediating long-lasting suppression.
- (3) What roles do inhibition and synaptic depression play in awake and freely moving animals in different

behavioral states? There is evidence from the somatosensory system that in behavioral states such as active whisking, there is a dramatic reduction in cortical and thalamic adaptation to repetitive sensory stimulation ([Fanselow and Nicolelis, 1999](#)). Is this also the case in the auditory cortex?

- (4) What is the role, and what are the synaptic mechanisms underlying the cortical cells (half of the recorded cells in their sample, which are not discussed in [Wehr and Zador \[2005\]](#)) that show facilitatory responses to the second click?
- (5) What is the effect of long-lasting suppression on cortical responses to repetitive stimuli such as fast click rates? The literature on rate (or temporal) modulation transfer functions is related to the present results on forward suppression because both show how neuronal spike rate depends upon the temporal intervals between acoustic transients. Using stimulus sequences of tone bursts or clicks, a number of studies have shown that neurons respond in a time-locked fashion to repetition rates up to 5–30 Hz. At higher repetition rates, neurons typically respond to the first element of the sequence, but not at all or weakly to the following elements. Similar results have been obtained with periodic amplitude- or frequency-modulated sounds. As mentioned, the cells in this study that show long-lasting suppression cannot encode click rates higher than around 5–10 Hz. But we know that many cells in A1 can encode responses up to 20–30 Hz, and some cells in auditory cortex can encode click rates that are much higher, using either synchronized or rate coding of high repetition rates up to 200–300 Hz in the awake marmoset ([Lu et al., 2001](#)). Hence, there must be other populations of cortical cells that do not show the strong suppression as described by [Wehr and Zador \(2005\)](#) and must be sensitive to other time intervals. Where are these cells? Do they form a separate network for auditory processing in A1?

In general, how do the physiological results of [Wehr and Zador \(2005\)](#) tie in with known psychoacoustic data? Building a bridge between neurophysiology and psychophysics is a daunting challenge, and it is noteworthy that the authors make a useful contribution to this effort by drawing valuable terminological distinctions in their paper that will be helpful in guiding future studies.

At the perceptual level, many psychoacoustic studies have shown that the sound context influences the detection thresholds of sounds; awareness of sound changes in pitch, timbre, loudness, or location; and the formation of perceptual streams. A simple example of the perceptual value of stimulus context can be demonstrated in forward masking, a phenomenon in which a prior acoustic stimulus (such as a tone or click) reduces the listener's ability to hear the following acoustic stimulus (and elevates behavioral threshold for the second sound). Regardless of the masker, forward masking is usually over within about 100 ms, and since forward masking is also dependent on the duration of the masker, a short click produces the least masking. Human psychophysical studies indicate that we can detect the presence of two clicks separated by a few ms,

although detection thresholds for the second click may become slightly elevated for up to 100 ms. We can even resolve the order of two clicks of different amplitude when the gap is about 2–3 ms. Thus, the psychoacoustic data suggest that the time course of forward masking to clicks is shorter than the time course of the inhibitory conductances described by [Wehr and Zador \(2005\)](#).

Another example of the importance of stimulus context is called the precedence effect. Sound can reach our ears via a number of different paths, which may include multiple reflections of the original source, giving rise to echoes. However, we are still able to easily locate a sound source in a reverberant room. When two brief sounds (such as clicks) are presented in close succession, the perception is of a single fused sound if the interclick interval is sufficiently short (<5–10 ms), and the location of the fused sound is largely determined by the location of the first sound. This precedence effect is an important behavioral phenomenon, and its neural basis has been extensively studied at many levels of auditory processing. Recently, [Fitzpatrick et al. \(1999\)](#) examined responses to click pairs at multiple structures in the ascending auditory system, from auditory nerve to auditory cortex. In agreement with the current results of Wehr and Zador, [Fitzpatrick et al. \(1999\)](#) found cortical suppression to the second click in a click pair lasting up to 200 ms. However, they also observed a population of cells in the auditory cortex of the rabbit that showed recovery functions that were tuned to specific ranges of interclick intervals from 5 to 70 ms, which could encode separations between sound and echo sources from around 2–24 m. Thus, the inhibitory conductances observed in forward suppression are also likely to play a role in sound localization and echo suppression.

In relation to timing, it is intriguing that fast click rates (above 30–40 Hz) or oscillations of amplitude-modulated noise are perceived as one continuous sound, whereas temporal patterns occurring on a slower time-scale are perceptually resolved as individual auditory events (this may be the acoustic counterpart of flicker fusion in the visual system). This perceptual boundary may be related to the time course of the inhibitory synaptic currents described by Wehr and Zador. In general, the existence of a multiple temporal decomposition by the auditory cortex may help explain a variety of auditory perceptual phenomena such as the ability to detect temporal gaps between noises separated by just a few milliseconds as well as the tonal contours of sentences.

Stimulus context may also be important in mediating stream segregation phenomena. Many natural sounds consist of temporal sequences of spectrally complex acoustic events. Depending on their spectral composition, duration, and temporal separation, successive auditory events can be perceived as a single auditory stream or can be segregated into different auditory streams. Forward suppression could play a role in stream tracking for similar stimuli and facilitation of nonmatched stimulus pairs could enhance the contrast for stream segregation ([Bartlett and Wang, 2005](#)). It is very probable that synaptic depression, as well as inhibition, contributes to auditory streaming, since many

perceptual effects occur over a long time course in stream segregation and integration.

Overall, although the perceptual consequences of acoustic stimulus context have been studied intensively, the central neural mechanisms underlying these perceptual phenomena remain mysterious, and the connections with physiological correlates are still highly speculative. There will need to be many more well-designed physiological studies in the behaving animal in order to forge the crucial links with psychophysics and to reveal the neural correlates of auditory perception.

The important contribution of [Wehr and Zador \(2005\)](#) and the discoveries of other cortical physiologists over the past 5 years presage a new era of intracellular *in vivo* recording studies. This approach will lead to a greater understanding of neural responses to acoustic stimuli and complex acoustic scenes and of the effects of different stimulus contexts and distinct behavioral states, and a fuller elucidation of the underlying dynamic synaptic mechanisms in auditory cortex. In conjunction with extracellular recordings and recent studies of ensemble coding in the auditory cortex, a picture begins to emerge of the synaptic, cellular, and network aspects of cortical auditory processing with fascinating implications for the neural basis of auditory perception.

**Jonathan Fritz, Shihab Shamma,
and Mounya Elhilali**
Center for Acoustic and Auditory Research
The Institute for Systems Research
University of Maryland
College Park, Maryland 20742

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