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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). Recent 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 timescale 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.

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