Cerebral Cortex, August 2020;30: 4465–4480

doi: 10.1093/cercor/bhaa044
Advance Access Publication Date: 7 March 2020
Original Article

ORIGINAL ARTICLE

Synaptic Recruitment Enhances Gap Termination Responses in Auditory Cortex

Bshara Awwad[1,2], Maciej M Jankowski[1,2] and Israel Nelken[1,2]

[1]Edmond and Lily Safra Center for Brain Sciences, The Hebrew University of Jerusalem, 9190401 Jerusalem, Israel and [2]Department Neurobiology, Silberman Institute of Life Sciences, Hebrew University of Jerusalem, 9190401 Jerusalem, Israel

Address correspondence to Israel Nelken, Edmond and Lily Safra Center for Brain Sciences, Edmond J. Safra Campus, Givat Ram, Jerusalem 91904, Israel. Email: israel.nelken@mail.huji.ac.il

Abstract

The ability to detect short gaps in noise is an important tool for assessing the temporal resolution in the auditory cortex. However, the mere existence of responses to temporal gaps bounded by two short broadband markers is surprising, because of the expected short-term suppression that is prevalent in auditory cortex. Here, we used in-vivo intracellular recordings in anesthetized rats to dissect the synaptic mechanisms that underlie gap-related responses. When a gap is bounded by two short markers, a gap termination response was evoked by the onset of the second marker with minimal contribution from the offset of the first marker. Importantly, we show that the gap termination response was driven by a different (potentially partially overlapping) synaptic population than that underlying the onset response to the first marker. This recruitment of additional synaptic resources is a novel mechanism contributing to the important perceptual task of gap detection.

Key words: auditory cortex, gap detection, rat, short-term plasticity, stimulus-specific adaptation

Introduction

The ability of the auditory system to track rapid changes in sound amplitude is crucial for processing tasks such as speech understanding, and deficits of temporal processing are associated with speech and language disorders, learning disabilities and low quality of life (Katz et al. 1992; Chermak and Musiek 1997; Bellis and Ferre 1999; American Speech-Language-Hearing Association 2005). An important tool for characterizing temporal processing consists of testing the detection of a silent gap in noise. The shortest gaps that can be reliably detected by normal hearing listeners are 1–3 ms long (Hirsh 1959; Green 1971). Gap detection thresholds are affected by age (Snell 1997; Snell and Frisina 2000; Bertoli et al. 2002) and hearing loss (Fitzgibbons and Wightman 1982; Yin et al. 2008). Deficits in gap detection are also present in tinnitus (Turner et al. 2006; Fournier and Hébert 2013), and are sometimes used as behavioral evidence for the presence of tinnitus in animal models (Turner et al. 2006). In humans, temporal gaps evoke cortical responses that include mismatch negativity and P300 (Bertoli et al. 2002; Pratt et al. 2005). In animal models, gaps are associated with a variety of cortical and subcortical responses (Eggermont 2000; Yin et al. 2008; Khouri et al. 2011; Weible et al. 2014; Zhao et al. 2015; Anderson and Linden 2016; Phillips et al. 2017).

Increase in gap thresholds in the absence of hearing loss is observed in subjects suffering from central auditory processing disorders (CAPD). CAPD may also involve difficulty in speech understanding in the presence of background noise, difficulties in following spoken instructions, distraction, and academic difficulties (American Speech-Language-Hearing Association 2005). CAPD prevalence is relatively high among the school-age children (Musiek et al. 1990; Bamiou et al. 2001) and reaches 70% in the older adult population (Stach et al. 1990). Similarly, temporal processing deficits show comorbidity with conditions such as attention deficit hyperactivity disorder, language impairment,

and learning disability, autism, and autoimmune disease (Riccio et al. 1994; Gomez and Condon 1999; Rosen 2003; Bishop and McArthur 2005; Bruner et al. 2009; Bhatara et al. 2013). In consequence, understanding the mechanisms that underlie gap detection may have widespread clinical implications.

In gap stimuli, a silent gap separates two sounds ("markers"), usually consisting of broadband noise (BBN). Each marker defines two auditory events, its onset and offset; the gap itself is bounded by the offset of the first marker and the onset of the second, both of which can evoke a neuronal response. There is a fair amount of information about mechanisms that shape these responses. When the first marker is long enough, offset responses may be driven by sets of synapses that are separate from those driving the onset responses (Scholl et al. 2010), also described as dissociable onset-sensitive and offset-sensitive channels (Anderson and Linden 2016). These responses may sum up with the onset responses due to the second marker, resulting in a marked enhancement of the gap-related responses (Scholl et al. 2010).

In contrast, when the first marker is short, forward suppression often occurs when two successive stimuli are acoustically matched, so that a substantial reduction in the responses to the second marker is expected (Harris and Dallos 1979; Calford and Semple 1995; Brosch and Schreiner 1997; Wehr and Zador 2005; Bleeck et al. 2006; Scholes et al. 2011). Although facilitation of the responses to a subsequent stimulus has been described, it usually occurred when the two stimuli were acoustically different (Brosch and Schreiner 1997; Eggermont 1999; Fitzpatrick et al. 1999; Kilgard and Merzenich 1999; Wehr and Zador 2005).

Here, we show that in rat auditory cortex, onset responses to the first and to the second markers of a gap have distinct synaptic signatures when the first marker is short (~100 ms). These responses differ in their excitation–inhibition balance and are differentially affected by adaptation. This recruitment of new synaptic resources is a novel mechanism that ensures the representation of gaps in cortical activity in the presence of forward masking, when other amplification mechanisms such as the conjunction of on- and off-responses are weak or absent.

Materials and Methods

The study protocol was approved by the joint ethics committee of the Hebrew University and Hadassah Medical Centre for animal welfare. The Hebrew University is an Association for Assessment and Accreditation of Laboratory Animal Care International accredited institution. Sixty-eight adult female sabra rats (Harlan Laboratories) were used for electrophysiological recordings.

Animal Preparation

Surgical procedures have been described in details previously (Taaseh et al. 2011; Yaron et al. 2012; Hershenhoren et al. 2014). Briefly, anesthesia was induced by an initial intramuscular injection of ketamine (~40 mg/kg) and medetomidine (~0.2/kg). Additional smaller doses of ketamine were administered as needed to maintain anesthesia during surgery. After tracheotomy, the animal was ventilated (10–15 mm H_2O peak inlet pressure, 47/min, 15–30 cc per stroke) through the cannula by a mixture of O_2 and halothane (Rhodia Organique Fine Ltd) using a small-animal ventilator (model AWS, Hallowell EMC), and a halothane vaporizer (VIP 3000, Matrx). The halothane level was set around 0.8% as needed. After the end of the surgery, no further ketamine injections were given. The CO_2 level was continuously monitored at the tracheal cannula. The depth of anesthesia was judged by the lack of movement and resistance to the respirator, and halothane level and ventilation pressure were adjusted accordingly. Body temperature was monitored and maintained at 36–38 °C using a rectal thermistor probe and a feedback-controlled heating pad (FHC Inc.). Recordings under halothane anesthesia in auditory cortex have been found to match awake responses more closely than recordings with other anesthetic agents (Moshitch et al. 2006).

Electrophysiology

All electrophysiological recordings were done in the left primary auditory cortex of female sabra rats. These choices were made mostly for convenience. Since gap detection is such an important and basic perceptual task, we do not expect large sex effects, and indeed sex differences have not been reported in any electrophysiological study of gap-related responses. Hemispheric asymmetries have been suggested in rodents (Zatorre et al. 2002) in some high-level auditory tasks, but not in low-level detection tasks such as gap detection. Therefore, we do not expect these choices to have an appreciable effect on the conclusions of the paper.

For intracellular recordings, a small craniotomy (<1 mm) and duratomy were performed above the left auditory cortex. Electrodes were advanced slowly into the brain using a micromanipulator (MP-225, Sutter Instrument Company). Neurons were recorded at depths between 200 and 1200 μm. Most whole-cell recordings were at depths <500 μm.

Extracellular recordings were performed using an array of four glass-coated tungsten electrodes (Alpha-Omega Ltd). Electrode impedance ranged from ~0.5 to 5 MΩ. An additional set of extracellular recordings was performed in two rats using Neuropixels probes (Jun et al. 2017). They were introduced into the cortex vertically at coordinates AP: –5.2 mm posterior to Bregma, at depth between 2.7 and 3.2 mm, corresponding to auditory cortex. Recordings were performed using SpikeGLX (Jun et al. 2017). The data shown here were not further manually sorted, since we report mostly population averages. A1 localization was verified by the spatial extent of the auditory activity and by the recovered location of the electrode.

Intracellular recordings were performed with sharp electrodes and with patch electrodes, which both were prepared from a filamented borosilicate tube (1.5 mm OD, 0.86 mm ID, Sutter Instruments). The resistance of sharp electrodes was 55–100 MΩ and they were filled with 1 M potassium-acetate solution. The bridge was balanced, and capacitance compensation was used in all experiments. The signal was amplified ×10 (NeuroData IR283, Cygnus Technologies, Inc.), sampled at 12.207 kHz (Rx8, TDT, Tucker-Davis Technologies) for online display, and stored for offline analysis. Whole-cell recordings were performed using patch pipettes with impedance of 4–6 MΩ (P-1000, Sutter Instruments). We recorded from seven neurons using internal solution that contained 135 mM Cesium-Met., 4 mM TEA-Cl, 10 mM HEPES, 1 mM MgATP, 0.3 mM NaGTP, 3 mM QX-314, and 10 mM phosphocreatine, for a total osmolality of 300 mOsm. We added MK-801 (1 mM) to this internal solution for another eight neurons. We recorded from another 21 neurons using internal solution that contained 120 mM k-gluconate, 6 mM NaCl, 10 mM HEPES, 1 mM EGTA, 0.1 mM $CaCl_2$, 4 mM Mg-ATP, 0.4 mM Na-GTP, 2 mM KCL, 14 mM Creatine phosphate, and 2 mM $MgCl_2{}^*6H_2O$ (300 mOsm). The recordings were made

with EPC 10 USB amplifier (HEKA). The pipette capacitance and whole cell capacitance were fully compensated. The series resistance was 10–60 MΩ and was partially compensated (40–85%). Currents were measured at a holding potential of −90 mV, near the reversal potential of potassium, and at depolarized holding potential, near the excitatory reversal potential. The data were stored in computer files for offline analysis.

Stimulus and Sound Presentation

All experiments were conducted in a sound-proof chamber (IAC). Sounds were synthesized online using Matlab (The Mathworks Inc.), transduced to voltage signals by a sound card (HDSP9632, RME), attenuated (PA5, TDT), and played through a sealed speaker (EC1, TDT) into the right ear canal of the rat. In A1, the majority of the neurons have their best responses for sounds that are louder in the contralateral ear, and only a few do not respond at all to contralateral stimulation (Higgins et al. 2010).

For pure tones, an attenuation level of 0 dB corresponded approximately to 100 dB SPL with a frequency-dependent variability of ∼10 dB between 1 and 32 kHz. Noise bursts were spectrally generated and had a bandwidth of 0–60 kHz and a spectrum level of -50 dB/$\sqrt{}$Hz.

To determine minimal response thresholds to noise burst, a sequence of 280 noise bursts (200-ms duration, 10-ms linear onset/offset ramps, 500-ms interstimulus intervals [ISI, onset-to-onset]) was presented at seven attenuation levels (−60 to 0 dB, every 10 dB).

In the main experiments, gap stimuli were derived from bursts of BBN with a duration of 200 ms that had 10-ms linear onset/offset ramps. Gaps were always of full depth. Following Eggermont (2000), gap onset was always 100 ms following stimulus onset and gaps had instantaneous onset and offset. The gap durations used in this study were 2–20 ms. Thus, the duration of the first marker was 100 ms, with a 10-ms linear onset ramp and an instantaneous offset. The duration of the second marker varied between 98 and 80 ms, it had an instantaneous onset, and it ended with a 10-ms offset ramp. These parameters ensured that the shortest gaps we used, whose duration was 2 ms, already evoked a significant response. These stimuli were presented at a rate of 2/s. The sound level in these experiments was always 30 dB above noise threshold.

Tests of gaps of all durations consisted of sequences of gap stimuli of 10 different durations ranged from 2 to 20 ms with a resolution of 2 ms as well as a continuous BBN (nominally, a gap duration of 0 ms). Gaps of each duration were repeated 25 or 50 times. These sequences were used in some of the intracellular recordings and in many extracellular recordings.

Two oddball sequences consisted of intermixed gap and noise stimuli. Both sequences had a total of 500 stimulus presentations. In one sequence, the gap stimuli were presented 50 times and continuous noise was presented 450 times, while in the other sequence the proportions were inverted. Sometimes a smaller total number of stimuli were presented, keeping the relative number of stimuli of each type. These sequences were presented with gap durations of 2, 4, and 20 ms.

In addition to these stimuli and stimulus sequences used in the main experiments, additional stimulus conditions were used as controls using extracellular recordings using neuropixels probes. For examining the influence of the duration of the first marker on the gap-related responses, we used 500-ms long noise bursts (with 10-ms linear onset and offset ramps) and placed a 20-ms gap in 10 locations from 50 to 450 ms after stimulus onset. Gap onsets and offsets were instantaneous in these stimuli. ISI varied randomly between 2 and 2.5 s. Each stimulus was repeated 20 times.

As shown later, the gap stimuli evoked responses that were locked to the onsets of the two markers. To control for the difference in the gating of the two markers in the main experiments (10-ms linear ramp for the first marker, instantaneous for the second marker), we also recorded responses to gap stimuli with a gap duration of 20 ms but different gates at stimulus onset and at the gap. We used all four combinations of 10-ms linear ramps and instantaneous (0-ms linear ramps) at onset and at the gap. These stimuli had a duration of 200 ms, with the gap onset at 100 ms. Responses to these stimuli were recorded in a block in which each combination was repeated 20 times in a pseudo-random order, as well as in oddball sequences constructed as described above.

Data Analysis

The data were analyzed using Matlab (The Mathworks Inc.). Spikes were clipped from the intracellular voltage traces. To detect the spikes, the membrane potential was first high-pass filtered with a corner frequency of 30 Hz. The quality of spike detection was verified by visual inspection. The onset of a spike was determined by the maximum acceleration of the rising phase, and its end was determined by the time point when the derivative was closest to zero within a period of 1.5 times the spike width after the peak of the spike. The spikes were then clipped from the unfiltered signal, and were replaced by a straight line from start to end of the spike. The clipped signal thus obtained was considered in this study as the membrane potential signal. Synaptic currents measured in the voltage clamp mode were baseline-corrected using prestimulus values. Extracellularly measured local field potentials (LFP) were baseline-corrected to the 30-ms interval just preceding stimulus onset, and averaged. Response strength was quantified by the depth of the maximal (most negative) trough of the average response in the time window between 0 and 70 ms after stimulus onset. To detect multiunit activity (MUA), the raw extracellular electrode signals were filtered between 200 and 8000 Hz. Large, fast events were marked as spikes. The threshold for spike detection was set to seven times the median of the absolute deviations from the median of the filtered voltage traces (corresponding to more than four standard deviations for Gaussian signals). The resulting spike trains were aligned on stimulus onset and averaged.

The inclusion criterion for LFP, spikes, membrane potential, and membrane currents was the presence of significant responses in the deviant condition for both peaks (P1 and P2) of the gap stimulus. Significance test was performed by a paired t-test between the average of the set of single-trial responses and the corresponding prestimulus activity levels. P1 responses were corrected relative to the prestimulus activity, while P2 responses were corrected relative to the values in the interval between gap onset and 5 ms after gap offset (because the onset of the gap termination responses had a latency greater than 5 ms in all cases).

Results

We measured responses to gap stimuli in auditory cortex of 68 rats using extracellular and intracellular recordings. All the neurons recorded here had a relatively short response latency

(<20 ms), robust responses to tones and to BBN, and a narrow frequency response area, suggesting that the responses are recorded in A1 (most likely given the location of the penetrations) or AAF. The analysis is based on 54 neurons recorded intracellularly using sharp electrodes, 36 neurons recorded intracellularly in a whole-cell configuration, as well as LFP and MUA recorded extracellularly from 156 sites in 10 rats. Control data were recorded extracellularly using neuropixels probes from another two rats.

In the main experiments, the gap stimuli consisted of two BBN bursts (markers) with a short gap (gap duration varied from 2 to 20 ms), occurring 100 ms after stimulus onset (Fig. 1*d*). The onset of the first marker and the offset of the second marker had a linear rise/fall of 10 ms. On the other hand, the onset and offset of the gap (the offset of the first marker and the onset of the second marker) were instantaneous. Broadband markers, rather than pure tones or narrowband markers, were used in order to avoid the effects of "spectral splatter"—the activation of distant frequency channels by abrupt auditory events. Thus, the use of broadband markers ensured the dominance of temporal mechanisms in shaping the responses, without the confounding effects of spectral interactions.

These stimuli are similar to those clinically used to test for gap detection thresholds and have been previously used (Yin et al. 2008; Zhao et al. 2015). Other studies have used either gaps in continuous noise or had first markers whose duration was >200 ms (Eggermont 1999; Weible et al. 2014; Anderson and Linden 2016). The stimuli we adopted for the current study were relatively short but still ensured the presence of responses at the shortest detectable gaps (Eggermont 2000).

Gap-Related Responses Are Onset Responses to the Second Marker (Gap Termination Responses)

The evoked responses consisted of two components (called here P1 and P2), representing the responses evoked by the onset of the stimulus followed by the responses evoked by the onset of the second marker (gap offset; Figs 1*b*,*c* and 2*a*). In the examples shown in Fig. 1*a*–c, the gap-related responses occurred after the onset of the second marker. Furthermore, their latency (relative to the onset of the second marker) was largely independent of gap duration. In consequence, relative to the onset of the first marker, these responses shifted in time by an amount equal to the gap duration. This was true for intracellular recordings (Fig. 1*a*), extracellular recordings of MUA (Fig. 1*b*), and LFPs (Fig. 1*c*).

To quantify these observations, Fig. 1*e* displays the latency of the gap-related responses (relative to gap onset) in Fig. 1*a* as a function of gap duration. The slope of the regression line is 0.9, reflecting the close correspondence between gap duration and the latency of the responses relative to gap onset. Overall, 12 neurons recorded intracellularly with sharp electrodes were tested with 11 conditions consisting of 10 gap durations (2–20 ms) as well as a no-gap stimulus (nominally, gap duration of 0 ms) as in Fig. 1*a*. The estimated slopes are shown in Fig. 1*f* (blue bars). Another five neurons recorded in a whole-cell configuration were tested with three gap durations (10, 20, and 30 ms), and the resulting slopes are displayed in Fig. 1*f* as well (brown bars). Slopes close to unity were found in 16/17 neurons. For one neuron the slope was 0.23, and by inspection, this neuron was the only one in this set that had offset responses to the first marker. We therefore believe that the gap-related response (P2) in most of our recordings is an onset response to the second marker, and that offset responses are largely absent under the conditions we used. Since the P2 responses were onset responses to the second marker, in the following we use the term "gap termination responses" to describe them. This terminology is justified since, although these responses were evoked by the onset of the second marker, they were obviously shaped by the preceding gap—their timing marked the termination of the gap, and their strength was shaped by the duration of the gap.

To determine the shortest gaps that evoked a significant response, Figure 1*g* shows the average membrane potential of the 12 neurons that were tested with 11 gap durations, at the time window of the expected response (11 ms after the onset of the second marker, blue bars) and the average membrane potential of the same neurons in the same time window when responding to a continuous BBN burst (black bars). Similar to previous studies (Eggermont 2000; Yin et al. 2008; Zhao et al. 2015; Anderson and Linden 2016), the membrane potential following gaps as short as 2 ms was significantly higher on average than the membrane potential at the corresponding time window for continuous noise ($t(11) = 2.63$, $P = 0.011$).

Gap Termination Responses Increase Rapidly with Gap Duration

In the following, we used responses to gap stimuli that occurred as the rare stimulus (10% of the stimulus presentations) within an oddball sequence in which the majority of the stimuli (90%) were continuous BBN bursts. As will be shown below, under these conditions, the gap termination responses were maximized.

Our gap stimulus consisted of two BBN markers that were spectrally identical (Fig. 1*d*) with a very short intervening gap. Under these conditions, we expected to observe strong forward suppression (Calford and Semple 1995; Brosch and Schreiner 1997; Fitzpatrick et al. 1999; Wehr and Zador 2005). Figure 2*a* displays the intracellular responses of one neuron to gaps of 2, 4, and 20 ms. As expected, the onset response to the first marker (P1) was similar for the three gap durations. The gap termination response (P2) shifted in time (as expected from the onset response to the second marker), and increased substantially in size following the longer gaps, consistent with previous studies (Zhao et al. 2015). In consequence, the ratio of response magnitudes, P2/P1, increased with gap duration. At a gap duration of 20 ms, the response was already comparable with the onset response (P2/P1∼1). Population results are displayed for these three gap durations in Figure 2*b*. While generally P2 responses were smaller than P1 responses for the shorter gap durations, for gaps duration of 20 ms, 39% of the neurons (21/54) had P2 > P1.

To increase the range of gap durations we tested, we recorded extracellular responses to additional gap durations (2, 4, 10, 15, and 20 ms). Figure 2*c* shows a summary of the LFP responses. These data confirmed that longer gap durations were associated with larger P2/P1 ratio (Fig. 2*c*), and suggested that response saturation occurred for gap durations was longer than 15 ms.

These findings seem incompatible with reports showing substantial, long-duration forward masking for matching pairs of stimuli in auditory cortex (Calford and Semple 1995; Brosch and Schreiner 1997; Fitzpatrick et al. 1999; Wehr and Zador 2005). The time intervals between onsets of the first and second marker in the stimuli used here varied between 102 and 120 ms for gaps of 2–20 ms. As a comparison, Wehr and Zador (2005) studied forward masking using click pairs. In their hands, P2/P1 was on average ∼0.55 for a pair of clicks presented with an interval

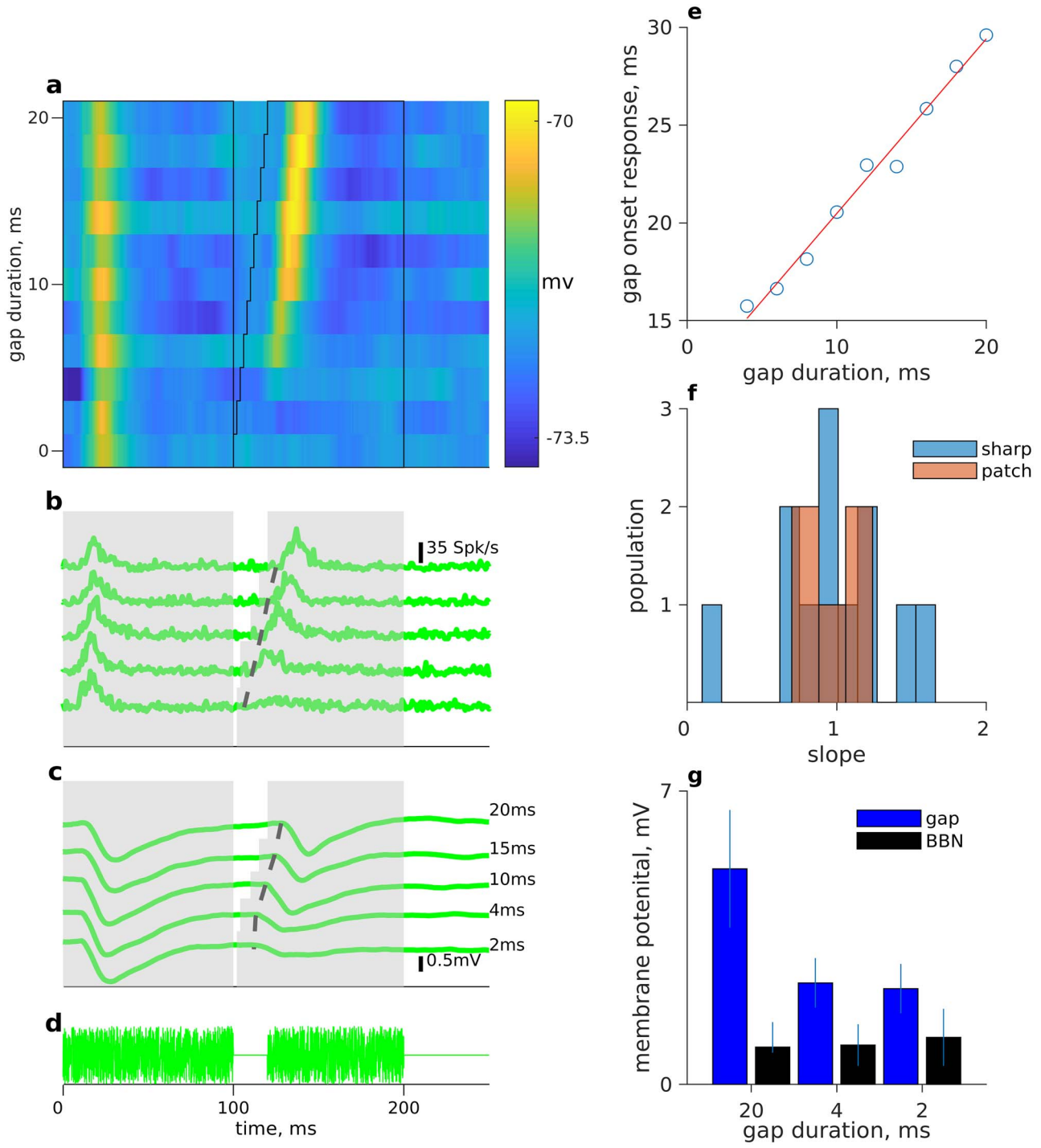

Figure 1. Gap-related responses are onset responses to the second marker. (*a*) Responses to gap stimuli, recorded intracellularly from a neuron in rat auditory cortex. The abscissa is time around the onset of the first marker, the ordinate represents gap duration, and the color represents the membrane potential. The black lines indicate (from left to right) the offset of the first marker (at 100 ms after marker onset), the onset of the second marker, and the offset of the second marker. Each trace represents the average response to 25 repetitions of the corresponding gap stimulus. (*b*) MUA recorded from a site in rat auditory cortex. Each line shows the responses at different gap duration, as in *c*. The gray patches indicate the temporal structure of the stimulus. The dashed line connects the onsets of the responses at the different gap durations. These are the time points in which the response exceeded the pregap activity by 2 SD. (*c*) LFP recorded from one site. Similar conventions as in *b*. Gap durations in *b* and in *c* are the same. (*d*) Waveform of a stimulus with a gap of 20 ms. (*e*) Relationship between gap duration and the onset of the responses, for the neuron in *a*. Note the linear relationship between the two, with a slope close to 1. Regression line is in red. (*f*) Histograms of the slopes of the regression lines for recordings with a sharp electrode ($n = 12$, blue bars) and for recordings in the whole cell configuration with a patch pipette ($n = 5$, brown bars). (*g*) Means of the responses (blue, the maximum of the voltage between 0 and 70 ms after the onset of the second marker) compared with the maximum of the voltage recorded at the same time window, in the same neurons, in response to continuous BBN (black).

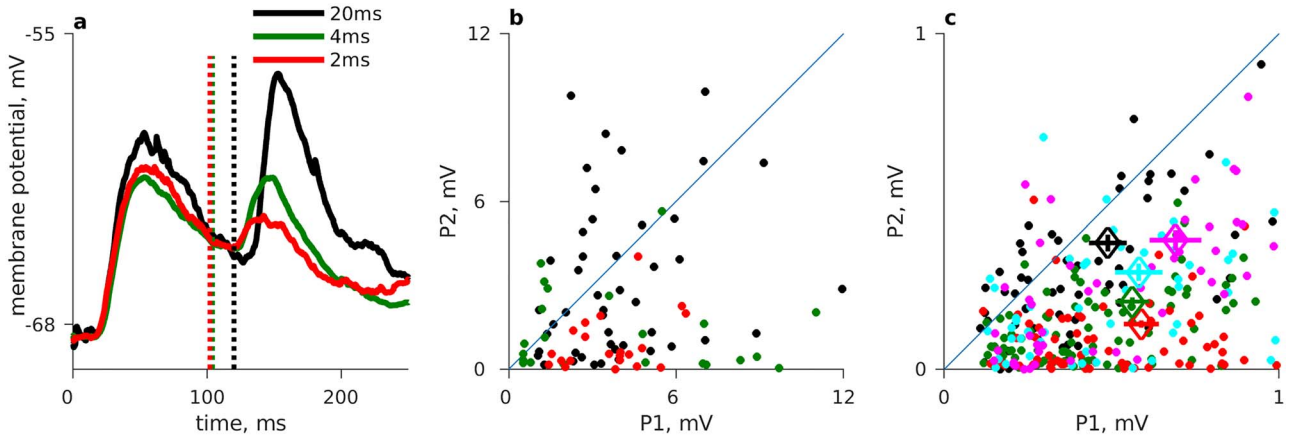

Figure 2. Large responses to short gaps. (*a*) Intracellular responses from one neuron in response to 2-, 4-, and 20-ms gaps. Dashed lines indicate the onset of the second marker (red: 2 ms, green: 4 ms, and black: 20 ms). (b) Population summary for the intracellular recordings. The size of the P1 response is plotted along the abscissa, the P2 response along the ordinate. Colors as in *a*. $n = 21$ for 2-ms gaps, $n = 23$ for 4-ms gaps, $n = 54$ for gaps of 20 ms. (c) Population summary, LFP responses. Similar conventions as in *b*. Gap durations: 2 ms ($n = 82$, red), 4 ms ($n = 100$, green), 10 ms ($n = 60$, cyan), 15 ms ($n = 56$, magenta), and 20 ms ($n = 69$, black). Diamonds indicate the averages for each gap duration and error bars are standard errors of the mean.

of 128 ms between them; for longer stimuli and shorter silent intervals, the forward masking should have been even stronger. In our data, P2/P1 was larger than 0.55 for 61% of the neurons when the gap duration was 20 ms, 41% of the neurons when the gap duration was 4 ms, and 14% of the neurons when the gap duration was 2 ms. Even more remarkably, P2 was larger than P1 ($P2/P1 > 1$) for 39% and 26% of the neurons recorded intracellularly with gaps of 20 and 4 ms, respectively. Thus, gap termination responses were substantially larger than expected from forward masking. We therefore attempted to determine the synaptic mechanisms underlying these remarkably large responses.

Synaptic Fingerprints of Onset and Gap Termination Responses

To estimate the synaptic currents that underlie the gap termination responses, we recorded neuronal responses in the whole-cell configuration using voltage clamp. The currents were typically recorded at holding potentials of −90 and 0 mV, approximately the reversal potentials of the inhibitory and excitatory synaptic currents (Tan et al. 2011).

The excitatory currents were estimated from the recordings at the hyperpolarized holding potential (Fig. 3*a*). In eight of these neurons, the recordings were performed in the presence of MK801, to block NMDA currents (Fig. 3*b*). We compared the currents associated with the P1 and P2 responses (each measured relative to its own baseline) for gap duration of 20 ms ($n = 31$). These currents were highly correlated (Fig. 3c, $r = 0.97$, $df = 29$, $P = 0$). Furthermore, there was no significant difference between the mean current at P1 and P2 ($t(30) = 0.95$, $P = 0.34$). At the level of single neurons, significant differences between the P1 and P2 responses were found in 16/31 cases (nine neurons with $P2 > P1$ and seven neurons with $P2 < P1$, Fig. 3c red and blue, respectively). Unsurprisingly, at these hyperpolarized potentials there were no essential differences between the recordings with (diamond markers in Fig. 3c) and without the NMDA blocker.

The determination of the inhibitory currents turned out to be more complex. The intracellular solution we used included blockers of voltage-sensitive channels (see Methods), but nevertheless, in 23 neurons tested with this solution, the P1 responses (onset of the first marker) failed to reverse in the depolarized holding potential, while the P2 responses (onset of the second marker) reversed more often. One example is displayed in Figure 3*d*, showing the currents measured in the depolarized holding potential. We hypothesized that NMDA currents could underlie the failure to reverse the P1 responses at the depolarized holding potential. Adding the NMDA channel blocker MK-801 to the intracellular solution resulted in the reversal of both P1 and P2 synaptic currents at the depolarized holding potential in all eight tested neurons (Fig. 3*e*). We conclude that NMDA currents could be involved in the excitatory responses, with a larger contribution to the P1 than to the P2 responses.

The peak currents associated with the P1 and the P2 responses at the depolarized holding potential in the presence of NMDA current blockage are plotted in Figure 3*f*. They presumably reflect mostly inhibitory conductances. The magnitudes of the inhibitory currents at P1 and P2 were significantly correlated ($r = 0.32$, $P = 0.44$, $n = 8$), although less strongly than the excitatory currents (Fig. 3c). On average, the currents at P1 and P2 were not significantly different from each other ($t(7) = 1.04$, $P = 0.33$), although three of the eight neurons showed significantly larger inhibitory currents at P1 than at P2.

To further study the relative contributions of excitation and inhibition in the sensory responses, we recorded, from another set of neurons, responses in current clamp while injecting different amounts of current to the neuron (Fig. 4*a*). These recordings were performed using sharp electrodes filled with 1 M potassium acetate. We computed the time-dependent equivalent reversal potential (Fig. 4*b*) by fitting the following equation to the current–voltage curve at each moment in time:

$$I_{inj} = G_{tot}(t) \times (V(t) - E_{eq}(t)),$$

where I_{inj} is the fixed injected current to the neuron, G_{tot} is the total conductance, and $V(t)$ is the membrane potential (Wehr and Zador 2003; Las et al. 2005). Here, $E_{eq} = \frac{G_e * E_e + G_{il} * E_{il}}{G_e + G_{il}}$, where G_e is the excitatory conductance and E_e its reversal potential, and the G_{il} is the sum of the inhibitory and leak conductances, which

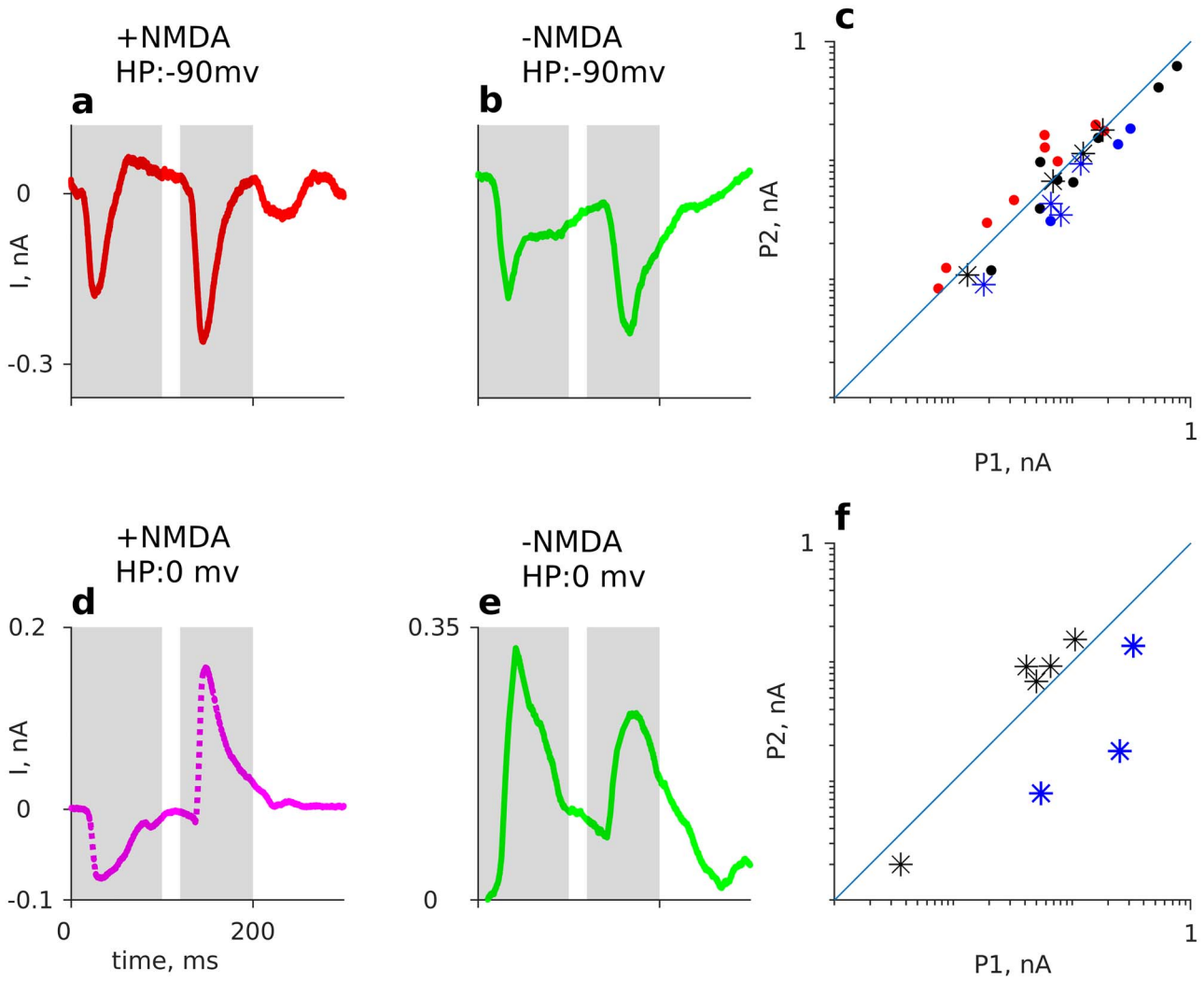

Figure 3. Synaptic currents in response to gap stimuli. (*a*) The excitatory synaptic currents recorded with normal NMDA currents. The gray patches are a schematic representation of the 20-ms gap stimulus. (*b*) The excitatory synaptic current recorded with inactivated NMDA currents. (*c*) Population summary of the magnitudes of the excitatory currents. The peak excitatory current evoked by the first marker (P1) is plotted against the peak excitatory current of evoked by the second marker (P2) for all neurons ($n = 31$). Red (blue) markers represent cases in which the P2 current was significantly larger (smaller) than the P1 current. Black markers represent cases in which the P2 current was not significantly different from the P1 current. Asterisks represent cases recorded with inactivated NMDA currents. (*d*) The current measured from one neuron at a holding potential of 0 mV with normal NMDA currents. Note the failure to invert the current at the onset of the first marker. (*e*) The current measured from one neuron with inactivated NMDA currents. Note the reversal of both response components. (*f*) Population summary of the inhibitory currents recorded inactivated NMDA currents ($n = 8$). Similar conventions as in (*c*).

are assumed to share approximately the same reversal potential E_{il}. Since the conductances change as a function of time during the stimulus, so does E_{eq}. Defining the synaptic balance (also time-dependent)

$$B = \frac{Ge}{Ge + Gil},$$

The equivalent reversal potential E_{eq} can be expressed as the weighted sum of the excitatory and inhibitory equilibrium potentials:

$$E_{eq} = B * E_e + (1\text{-}B) * E_{il}.$$

Thus, changes in E_{eq} indicate changes in B, and therefore changes of the excitatory/inhibitory (E/I) balance.

As expected, in Fig. 4*b*, the equivalent reversal potential was negative (as the weighted mean of E_{il}, which is negative, and E_e, which is ~0). It tended to increase both at onset and at the gap termination response, reflecting the large excitatory input at these times. Generally, E_{eq} was more negative at P2 than at P1 (Fig. 4*c*). These differences were statistically significant ($t(21) = -2.64$, $P = 0.015$). However, there was a large variability between neurons ($r = 0.26$, $df = 19$, $P = 0.24$). At the single neuron level, significant differences between E_{eq} at P1 and P2 were found in 14/22 neurons (five neurons showed P2 > P1 [red dots] and nine neurons showed P2 < P1 [blue dots]). These results imply a change in the E/I balance between the onset and the gap termination responses, with ~1/3 of the neurons showing a relative increase in excitation relative to inhibition. There was a weak tendency of the neurons that showed facilitation in their gap termination responses (P2/P1 > 1 with no current injection) to also have larger E_{eq} during the gap termination response. Indeed, with no current injections, six neurons had P2/P1 > 1, and 16 had P2/P1 < 1. Among the neurons with P2/P1 > 1, the E_{eq}

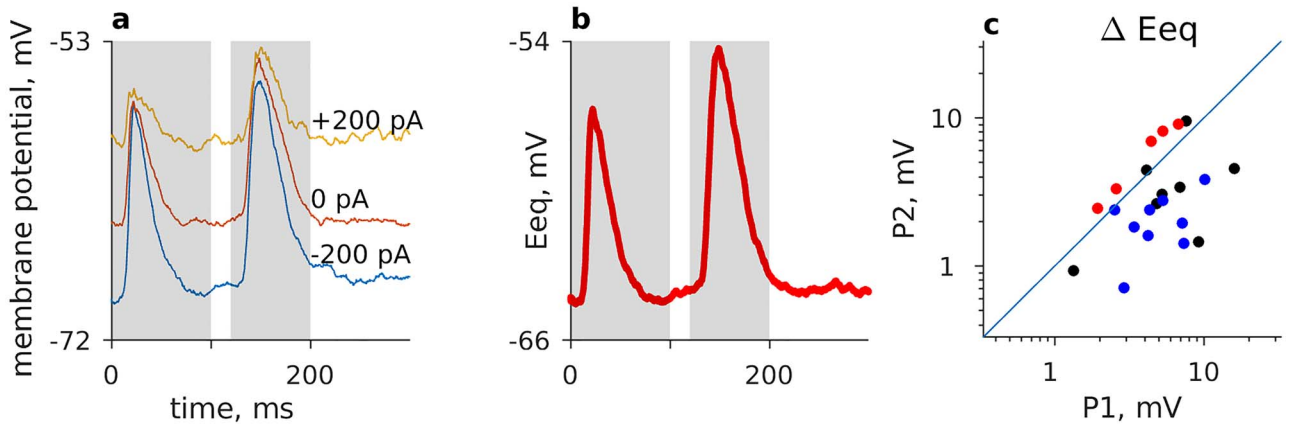

Figure 4. Equivalent synaptic reversal potential in the responses to gap stimuli. (*a*) Example of sensory evoked responses recorded in current clamp mode, with different current injections in one neuron. (*b*) The estimated reversal potential from the same neuron in *a*. (*c*) Population in logarithmic scale ($n = 22$) showing that the reversal potential of P2 and P1 are typically different, indicating changing E/I balance. Colored dots: as in Fig. 3*c*.

of 66% (4/6) was larger at P2 than at P1, showing a shift of the E/I balance towards excitation. In contrast, only 3/16 neurons with P2/P1 < 1 had E_{eq} at P2 larger than at P1. This tendency just failed to reach statistical significance (Fisher's exact test for 2×2 tables, $P = 0.054$).

Differential Adaptation of P1 and P2 Responses

The analysis of the synaptic currents, both in voltage clamp and in current clamp, suggested that P1 and P2 responses had different synaptic profiles. Indeed, P1 responses had a larger contribution of NMDA currents than P2. Furthermore, both excitatory and inhibitory contributions to the P2 responses were larger than expected: synaptic currents are expected to show significant amount of forward masking, with the inhibition being reduced, if anything, more than the excitation (Cohen-Kashi Malina et al. 2013). In fact, the current clamp experiments suggested that inhibition was often larger at P2 than at P1. We therefore hypothesized that the responses at P1 and P2 are generated by different (potentially partially overlapping) sets of synapses. We tested this hypothesis by showing that the P1 and P2 responses could be adapted differentially.

We used stimulus-specific adaptation (SSA) for this purpose. SSA is the larger response evoked by a stimulus that appears rarely compared with the response of the same stimulus when common (Ulanovsky et al. 2003; Taaseh et al. 2011; Yaron et al. 2012). SSA has been mostly studied in the spectral domain, while here we study SSA to a temporal sound feature.

We used BBN bursts and gap stimuli in the oddball configuration (Fig. 5*a*). We compared responses with gap stimuli that were either standard (occurring with a probability of 0.9 in the sequence) or deviant (occurring with a probability of 0.1 in the sequence). The other stimuli in those sequences were always broadband, continuous noise bursts of the same overall duration.

Assume first that the same set of synapses supplies the input at stimulus onset and at the gap. These synapses would show some adaptation simply due to the fact that the stimuli appear at a rate of 2/s. This set of synapses is expected be somewhat more adapted when gaps are standard than when gaps are deviants, because of its higher rate of activation by gaps (which activate the hypothetical set of synapses twice at each stimulus presentation, at onset and again at the gap). On top of that, the gap termination responses themselves would be affected by the presence of an onset response just 100 ms previously, reducing them to a fixed fraction of the onset response. In consequence, while the gap termination responses are expected to be smaller when gaps are standard than when gaps are deviant, after normalization by the onset response, the gap termination responses should be similar when standard and when deviant. In consequence, under these circumstances, SSA is not expected to occur.

On the other hand, if different synaptic populations supply inputs at stimulus onset and at the gap, the excitatory synapses that are active during gap termination responses but not during the onset responses would be activated relatively little when gaps are deviant (10% of the stimuli), and would therefore support strong gap termination responses. When gaps are standard, the same population of synapses would be activated more often, show greater adaptation, and therefore the gap termination responses are expected to be weaker. In consequence, SSA should be present under these conditions.

The responses of a neuron recorded intracellularly to oddball sequences are shown in Figures 5*b*–*d*. Figure 5*b* displays the responses to a gap stimulus (gap duration 20 ms) and to a continuous broadband stimulus, used as the deviant in an oddball sequence in which the gap stimulus was the standard. Figure 5*c* displays the responses to the same two stimuli, for the oddball sequence in which the gap stimulus was deviant. Figure 5*d* compares the average responses with the gap stimuli from the two sequences. Clearly, while for this neuron, the P1 (onset) responses were similar for the gap stimuli when standard and when deviant, the P2 (gap) responses were larger when the gap stimulus was deviant than when it was standard.

Figure 5*e*–*g* compares the gap termination responses for standards and deviants across the population of neurons recorded intracellularly. Each point in the scatter represents the average responses for the gap when deviant (ordinate) versus the average responses for the same gap when standard (abscissa). For statistical analysis, the P2 responses were log transformed in order to ensure approximate normality (the log transformation was approximately the best Box-Cox transformation for these data), and a linear mixed effects model was fitted with gap duration, probability condition (standard/deviant),

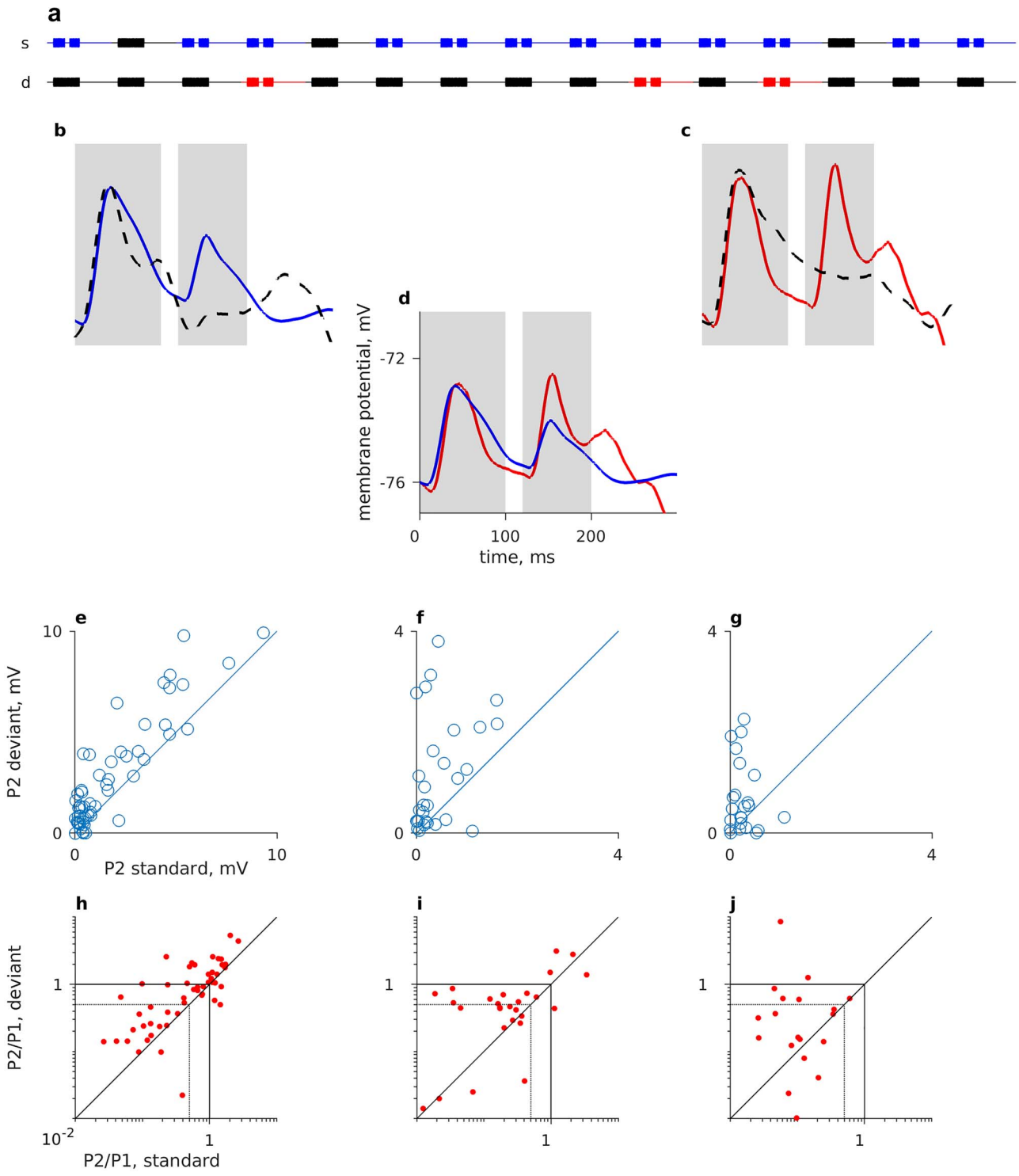

Figure 5. SSA to temporal gaps. (*a*) Schematic examples of an oddball sequence with gaps in the standard condition (top, s, responses to gaps in blue) and of another oddball sequence with gaps in the deviant condition (bottom, d, responses to gaps in red). Black bars indicate continuous noise played as the other stimulus in the sequence (top: as deviant, bottom: as standard). (*b*) Responses of one neuron to the oddball sequence in which the gap stimulus was standard (here and elsewhere, solid blue line) and the continuous noise was deviant (dashed black line). The gray patches are a schematic representation of the 20-ms gap stimulus. (*c*) Responses of the same neuron in (*b*) for the stimuli in the opposite sequence, in which the gap stimulus was deviant (here and elsewhere, solid red line) and the continuous noise was standard (dashed black line). (*d*) Comparison of the responses with the gap stimulus in the two different sequences. (*e–g*) Population summary of the responses recorded intracellularly to gaps of different durations. Each point represents the average responses of P2 in standard sequence (abscissa) versus the average responses of P2 in deviant sequence (ordinate) in the same neuron. (*e*) 20-ms gaps. (*f*) 4-ms gaps. (*g*) 2-ms gaps. (*h–j*) P2/P1 ratio calculated for the responses to the gap stimulus in the standard condition (abscissa) plotted against the same ratio for the responses to the gap stimulus in the deviant condition in the same neuron. The solid lines mark P2/P1 = 1, and dashed lines mark P2/P1 = 0.55.

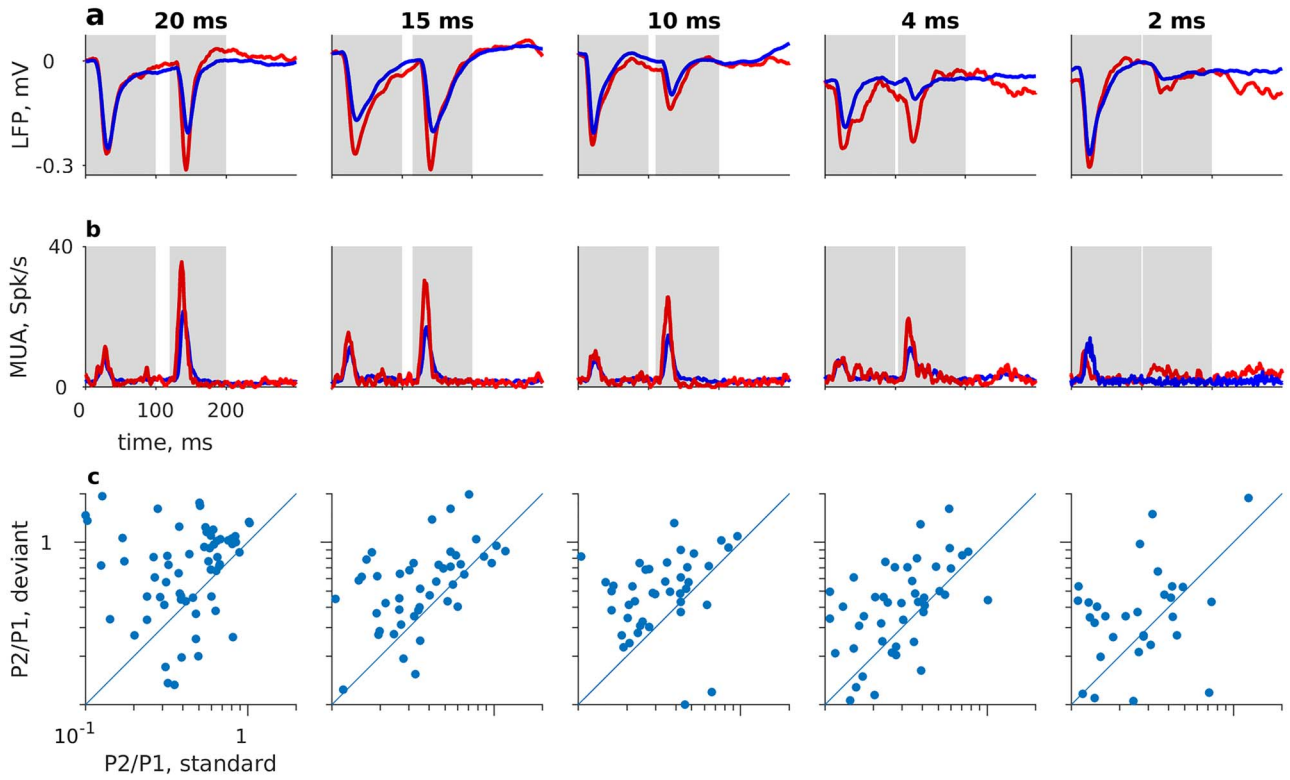

Figure 6. SSA to temporal gaps in extracellular recording. (*a*) Each panel displays the LFP responses recorded from one site to standard (blue) and deviant (red) gaps. Gap duration is marked at the top of each panel. The gray patches are a schematic representation of the gap stimuli. (*b*) Same as (*a*), MUA recordings from one site. (*c*) Population summary of the LFP responses to the gap stimuli. Each panel shows the P2/P1 ratio for gaps in the deviant condition (ordinate) plotted against the P2/P1 ratio in standard condition (abscissa) at the same recording site.

and their interaction as fixed factors, and with neuron and animal as random factors. Gap duration was entered as a numerical variable, so that the model tested a linear dependence of response size on gap duration. There was a significant main effect of probability condition ($F(1,240) = 4.2$, $P = 0.039$; deviant responses were significantly larger than standard responses) as well as gap duration ($F(1,240) = 20.2$, $P = 1.1{*}10^{-5}$; gap termination responses increased with increasing gap duration). However, the interaction was not significant ($F(1,240) = 0.51$, $P = 0.47$), suggesting a multiplicative effect of the probability condition on the P2/P1 ratio, independent of gap duration.

The larger responses at the gap when deviant than when standard could result from activity-dependent fatigue, because (as explained above) the more activity was evoked in oddball sequences with gap standards than in oddball sequences with gap deviants (since gap stimuli evoked a pair of responses, once at onset and a second time at the gap). To show that the larger deviant gap termination responses cannot be accounted for by such fatigue, we used the P1 response as a measure of the overall fatigue induced by the stimulation sequence. We then normalized the gap termination response (P2) by the onset response (P1). Figure 5*h*–*j* compare P2/P1 response ratios for the gaps when standard and when deviant. This ratio would be expected to remain largely constant in the presence of simple activity-dependent fatigue affecting all synaptic inputs. The values P2/P1 = 1 (black lines) and P2/P1 = 0.55 (dashed lines), corresponding to the level of recovery of P2 at an interval of 128 ms reported in Wehr and Zador (2005), are highlighted. These data were analyzed as above, after log transformation, using a linear mixed effects model with probability condition (standard/deviant), gap duration and their interaction as fixed factors, and with neuron and rat as random factors. There was a significant main effect of probability condition ($F(1,214) = 6.96$, $P = 0.0089$) and gap duration ($F(1,214) = 16.0$, $P = 8.9{*}10^{-5}$). As above, the interaction was not significant ($F(1,214) = 1.49$, $P = 0.22$).

Using extracellular recordings, it became possible to test gap termination responses at additional gap durations. We report here the responses of LFP and unsorted MUA. Figure 6*a* shows examples of the average LFP evoked by standard (blue) and deviant (red) gaps. These responses are from different recording sites. In all examples, the deviant response was larger than the standard response. The ratio P2/P1 could be smaller than 1 for standard responses and larger than 1 for the same gap when deviant (e.g., for the gaps of 15 and 20 ms). Figure 6*b* shows examples of MUA responses to gaps, again illustrating the stronger responses evoked by deviant gaps relative to those evoked by standard gaps with the same gap duration.

We compared directly the P2/P1 ratio when gaps were standard and when they were deviant (Fig. 6*c*). Using the same statistical design as for the intracellular data (Fig. 5*h*–*j*) reproduced the same findings—there were significant main effects of probability condition ($F(1,578) = 19.5$, $P = 1.2{*}10^{-5}$) and gap duration ($F(1,578) = 74.7$, $P = 5.4{*}10^{-17}$) but the interaction was not significant ($F(1,578) = 0.13$, $P = 0.71$).

In the neurons recorded intracellularly, it was possible to estimate the excitatory currents and equivalent reversal potentials for the gap termination responses, separately in the standard and deviant conditions. An example of the excitatory

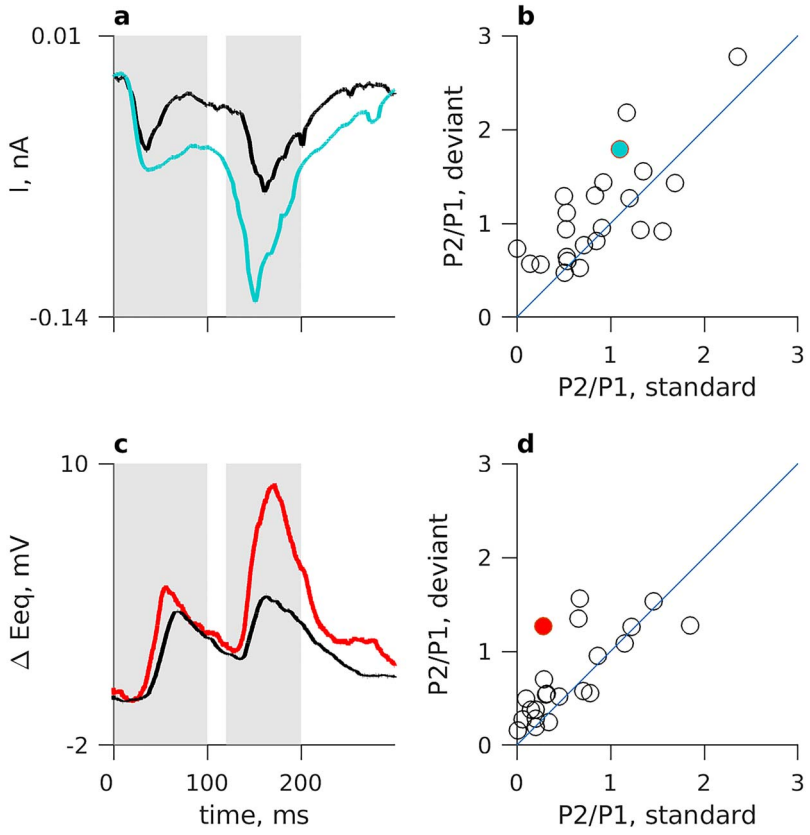

Figure 7. Changing balance between excitation and inhibition in the responses to standard and deviant gap stimuli. (*a*) Excitatory synaptic currents recorded from one neuron. Black: standard; cyan: deviant. (*b*) Population summary of the P2/P1 ratios for the excitatory currents when the gap was standard (abscissa) versus the same ratio when the gap was deviant (ordinate). (*c*) An example of the equivalent synaptic reversal potential estimated in current clamp mode: the blue trace shows the responses for the gap in the standard condition, the red trace in the deviant condition. (*d*) Population summary of the P2/P1 ratios of the equivalent synaptic reversal potentials estimated in current clamp mode for the standard condition (abscissa) versus deviant (ordinate). Points above the diagonal indicate P2/P1 ratios that are larger in the deviant than in the standard condition.

synaptic currents, measured in the voltage clamp mode at a holding potential of −90 mV for standards and deviant gap stimuli, is shown in Fig. 7*a*. The P2/P1 ratios for the excitatory currents tended to be larger when the gaps were deviants (Fig. 7*b*, $t(22) = 2.79$, $P = 0.010$). SSA was not tested in the neurons recorded with MK801 in the intracellular solution, and therefore the inhibitory currents evoked by standards and deviants cannot be compared. However, in current clamp experiments, E_{eq} tended to be larger for gaps when deviants than when standards. One example is shown in Figure 7*c*, and Figure 7*d* displays the population results. The P2/P1 ratio of the E_{eq} was significantly larger on average for deviant than for standard gaps (two tailed paired *t*-test, $t(21) = 2.33$, $P = 0.029$). Thus, when gaps were deviant, not only were the P2 responses larger, but also the E/I balance during the gap termination response shifted to relatively higher levels of excitation even when taking into account the larger P1 responses.

Controls and Extensions

The main experiments used only a limited set of gap stimuli. In order to generalize our conclusions, we tested additional stimulus conditions using extracellular recordings. These experiments were conducted using neuropixels probes, allowing us to record from hundreds of neuronal clusters simultaneously.

The first set of extensions concerned the duration of the first marker, which was fixed at 100 ms in the main experiments. There are two reasons to expect that longer first markers would enhance subsequent gap termination responses. First, longer first markers enhance offset responses, and offset responses to the first marker may sum up with onset responses to the second marker to enhance the gap termination responses (Scholl et al. 2010; Keller et al. 2018). First markers had to be >200-ms long for these effects to become important. Second, with longer first markers, there is more time to recover from synaptic depression caused by the onset response, also potentially enhancing the responses to the onset of the second marker.

The responses to 20-ms gaps inserted at different points along a 500-ms long BBN stimulus are displayed in Figure 8*a*,*b*. Figure 8*a* displays the responses recorded in one penetration, averaged across all units ($n = 176$). The responses are clearly locked to the onset of the second marker (dashed lines) at all conditions. However, contrary to the hypothesis above, instead of a monotonic increase, the responses increased to a maximum when the first marker was 100-ms long, but the responses decreased with longer first markers. The average responses over all recordings (Fig. 8*b*, $n = 1678$ units from $n = 7$ penetrations in two animals) also had a maximum around a first marker duration of 100 ms. At the longest first markers, the gap termination responses decreased to the level of the responses with very short first markers or even lower.

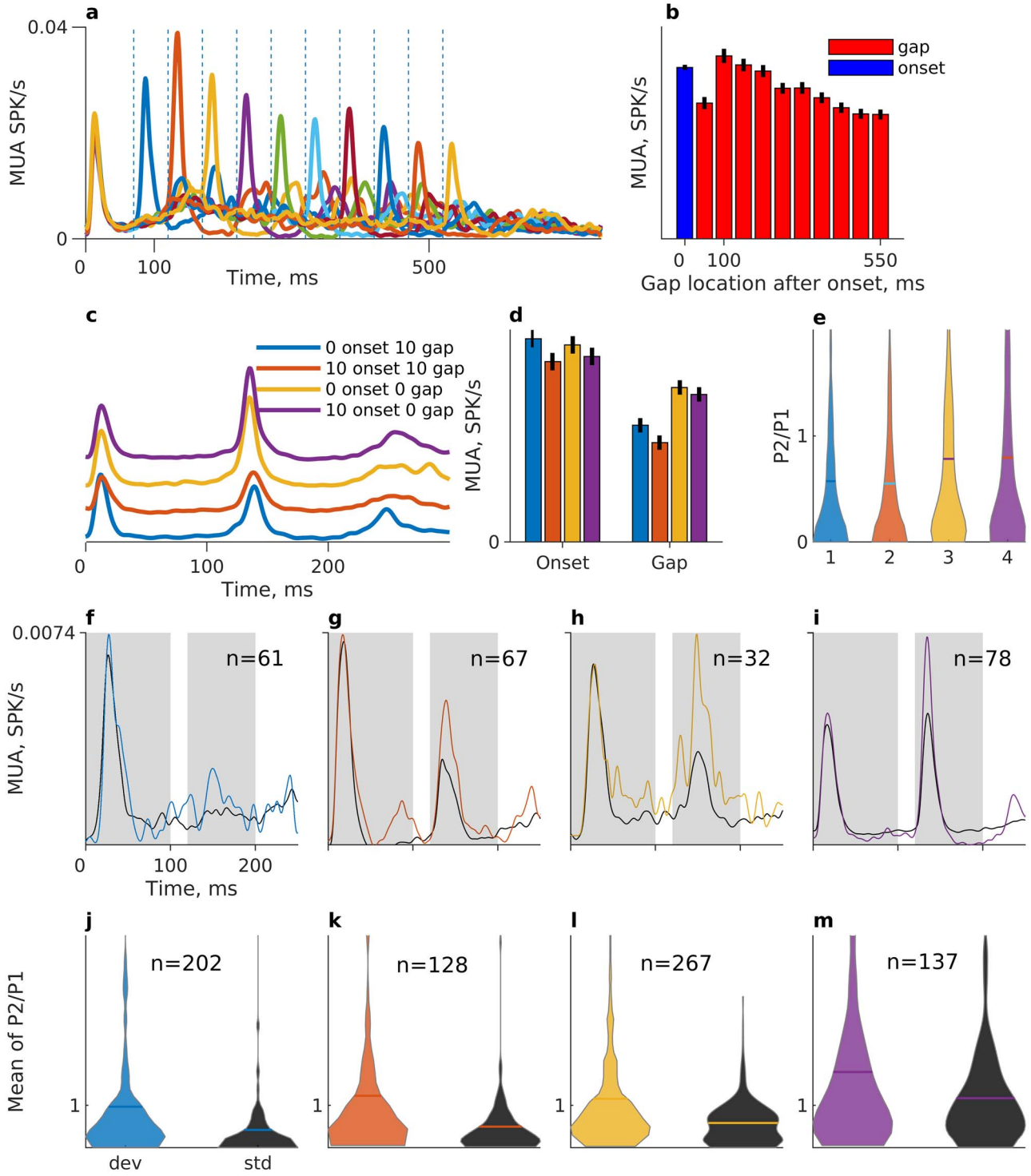

Figure 8. Control and extensions. (*a*) PSTHs computed from the responses of all units in one penetration (average of $n = 176$ units) to gaps with varying duration of the first marker. The onset of the first marker was at time 0 in all trials. The dashed lines indicate the onset of the second marker. (*b*) Average peak responses of all units in all penetrations ($n = 1678$ units). (*c*) PSTH recorded from one site stimuli in varying gating conditions (average of $n = 226$ units). All stimuli had a 20-ms gap. The onset and the gap were gated either with a linear 10-ms ramp or instantaneously (0-ms ramp). The responses in the different conditions are shifted vertically to facilitate the visual comparison between them. (*d*) Average peak responses ($n = 657$ units) for each gating combination. Similar color scheme as in *c*. (*e*) Distribution of P2/P1 (gap termination response normalized by the onset response) for all gating combinations, presented as violin plots showing a smoothed version of the histogram of all relevant values. Similar data and color scheme as in *d*. Horizontal bars indicate means. (*f*–*i*) Average PSTHs for the gap stimuli with different combinations of onset and gap gating, when used as standards (in black) or as deviants (in color, same convention as in *c*). The number of units that were averaged is displayed. These are all units that had a significant positive response at onset and a positive response at the gap. (*j*–*m*) Violin plots of the distributions of all normalized gap termination responses (P2/P1) when standard (black plot) or deviant (colored plot), in the different combinations of onset and gap gatings. Horizontal bars indicate means number of units contributing to each plot is displayed on the corresponding panel.

These data were analyzed using a linear mixed effects model, with the duration of the first marker as a fixed factor and unit identity as a random factor. There was a significant effect of the duration of the first marker ($F(10,16\,769) = 43.7$, $P = 1.7*10^{-86}$). Post-hoc pairwise comparisons between the gap termination responses at all first marker durations (using a Benjamini-Hochberg correction for multiple comparisons with a family-wise detection rate of 0.05) showed that the gap termination response at 100 ms was significantly larger than the gap termination response at 50 ms as well as from the gap termination responses for first marker duration of 200 ms and greater. These results are consistent with the recruitment of synaptic resources by the gap termination responses, with the added information that these effects are largest around 100–200 ms after stimulus onset.

The second extension involved the gating of the gap. In the main experiments, all stimuli had a first marker with a 10-ms linear onset ramp but instantaneous offset, while the second marker had an instantaneous onset and a 10-ms linear offset ramp. It is well known that the shape of the onset and offset gates has strong effects on neuronal responses. Thus, the size of the gap termination responses could be accounted for by the instantaneous gating of the noise at the beginning of the second marker: shorter gates are often more efficient at eliciting neurons responses.

To test the role of the onset gates in shaping gap termination responses, we recorded the responses evoked by a 20-ms gap with all possible combinations of instantaneous (0 ms) and linear ramp (10 ms) gates. The gaps were introduced into a 200-ms long noise burst, with gap onset at 100 ms after stimulus onset, as in the main experiments. The different gate conditions were presented in pseudorandom order with intervals of 2–2.5 s between sound presentations. Responses to all gating conditions are shown in Figure 8*c*, averaged over all responding units within one of the penetrations ($n = 226$). Figure 8*d* shows the average response at the onset and at the gap for the four types of stimuli ($n = 657$ units from seven penetrations in two animals). Figure 8*e* shows the distribution of normalized gap termination response, P2/P1, which we use to control for the effect of the onset response on the gap termination response.

These data were analyzed using a linear mixed effects model. The fixed factors were the response type (onset or gap) and the gate type—instantaneous (0 ms) or linear (10 ms), at onset and at the gap. The model also included all interactions. Unit identity was used as a random factor. There was a significant main effect of response type ($F(1,5248) = 58.6$, $P = 2.3*10^{-14}$), of the onset ramp ($F(1,5248) = 12.4$, $P = 0.00043$), and a significant interaction of the response type (onset or gap) with the gap ramp ($F(1,5248) = 202$, $P = 7.2*10^{-6}$). This last was expected, since the gap ramp should not affect the onset response.

The onset responses with an instantaneous gate were significantly larger than with the 10-ms ramp ($F(1,5248) = 36.8$, $P = 1.4*10^{-9}$; compare the blue and yellow bars with the red and purple bars in Fig. 8*d*, onset). The gap termination responses were also significantly larger when the instantaneous gate was used at the gap ($F(1,5248) = 92.6$, $P = 1.6*10^{-21}$; compare the blue and red bars with the yellow and purple bars in Fig. 8*d*, gap). Importantly, the effect of the gate shape at the gap was significantly larger than at onset ($F(1,5248) = 6.1$, $P = 0.013$). This finding suggests that the responses at onset and at the gap are shaped by different mechanisms, consistent with the notion of recruitment of additional synaptic resources.

In order to study the interaction between the onset and gap gates, we used the normalized responses P2/P1 (Fig. 8*e*). The main determinant of these was obviously the gating of the gap, with the responses to instantaneous gating being substantially larger than with the 10-ms ramp ($F(1,1498) = 16.2$, $P = 5.9*10^{-5}$). However, the onset ramp affected the responses as well ($F(1,1498) = 9.6$, $P = 0.0019$). Importantly, the two were different from each other ($F(1,1498) = 25.9$, $P = 4.1*10^{-7}$) with the gating of the gap affecting the responses substantially more than the gating at onset.

Finally, we tested the effects of the gate shapes on the differential adaptation of the gap termination responses in oddball sequences. We used again all four combinations of gates—instantaneous and a 10-ms ramp, at stimulus onset and at the gap. The other stimulus in these sequences was again a BBN, and its onset gate was matched to the onset gate of the gap in each condition. We calculate the P2/P1 ratio for used as standards and as deviant for each condition. If the recruitment of synaptic resources would be specific to the instantaneous gap, we would not expect to find SSA when both onset and gap were gated with a 10-ms ramp, or when both onset and gap had an instantaneous gate.

Examples of responses are shown in one penetration, averaged over all units (Fig. 8*f*–*i*). The population distribution of the P2/P1 ratios (from seven penetrations in two animals) at the gap when standard and when deviant is displayed in Figure 8*j*–*m*. A linear mixed effects model was fitted with the ramp conditions at onset and at the gap, as well as the probability condition (standard vs. deviant), as fixed factors. Unit identity was a random factor. Clearly, SSA was present in all gating combinations (deviant vs. standard: $F(1,3175) = 379$, $P = 6.6*10^{-80}$). Importantly, the gate at onset did not affect significantly the amount of SSA (when the gap was gated instantaneously, the difference in the amount of SSA between the two onset gates was barely significant, $F(1,3175) = 4.08$, $P = 0.0435$; when the gap was gated with a 10-ms linear ramp, the difference in the amount of SSA between the two onset gates was not significant, $F(1,3175) = 0.243$, $P = 0.62$). Thus, the hypothesized recruitment of additional resources did not depend on the onset gate.

Discussion

Gap detection is an important index of temporal resolution in the auditory system. Gap detection thresholds are associated with consequences beyond mere sensory performance (Fitzgibbons and Wightman 1982; Snell and Frisina 2000; Rosen 2003; Bhatara et al. 2013). Here, we explicitly demonstrate the involvement of cortical mechanisms in shaping gap termination responses. We demonstrate that the gap-related responses, in our hands primarily responses to the onset of the second marker (gap termination responses), engage synaptic resources that are at least partially different than those underlying the onset responses to the first marker. The most surprising feature of these responses, at least in some of the neurons, is their unexpected large excitatory component.

Gaps evoke responses throughout the auditory system. These responses are usually related to one of the two acoustic events that define the gap—the offset of the first marker (gap onset) and the onset of the second marker (gap offset). For example, some neurons in mouse auditory cortex have off responses that are believed to be evoked by synapses distinct from those that evoke the onset response (Scholl et al. 2010). These responses may reflect an off pathway already apparent in

the thalamus (Anderson and Linden 2016). Off responses tend to have longer duration and smaller amplitude than corresponding onset responses (Scholl et al. 2010). Recent work suggested an important role for off responses in shaping sensitivity to gaps, since the summation of the off response of the first marker and of the on responses of the second marker facilitates the responses evoked by the gap.

Here, the vast majority of the responses consisted of onset responses to the second marker. We had little evidence for responses evoked by the offset of the first marker. The predominance of onset responses to the second marker in our hands may reflect a species difference—most recent studies of off responses in rodent auditory cortex are in mice, rather than rats. However, we believe that other differences are more important. Indeed, off responses are much more common when using tonal stimuli (Brugge and Merzenich 1973; Chimoto et al. 2002; Moshitch et al. 2006; Scholl et al. 2010) than when using BBN (Eggermont 2000). In mice, short (≤100 ms) BBN bursts do not evoke much of an off response, and even longer bursts rarely evoke off responses in pyramidal neurons. Most neurons we recorded from in rats are likely to be pyramidal. Thus, our stimuli, consisting of gaps that follow short first markers, most probably did not engage the off pathway (Anderson and Linden 2016) to any substantial degree.

One of the most reproducible finding in auditory cortex is the presence of strong and long-lasting forward suppression—very often, the response to a probe stimulus shortly following a masker tends to be smaller than the responses to the masker (Harris and Dallos 1979; Calford and Semple 1995; Brosch and Schreiner 1997; Wehr and Zador 2005; Bleeck et al. 2006; Scholes et al. 2011). Facilitation does occur sometimes, but mainly when the probe and the marker are spectrally unmatched (Brosch and Schreiner 1997; Brosch et al. 1999; Bartlett and Wang 2005). In click trains, the response to a second click has been reported to be sometimes larger than to the first, but this occurred mostly when interclick interval was around 300 ms (Christianson et al. 2011), substantially longer than the interval between the onsets of the two markers (102–120 ms). Wehr and Zador (2005), using click pairs, reported that at an interval of 128 ms, the response to the second click reached on average ~55% of the response to the first click in their sample of neurons. Their interval of 128 ms was the closest to the interval between the onsets of the first and second markers in our experiments. Wehr and Zador (2005) argued that the suppression they observed in the response to the second click was due to a long lasting synaptic depression.

Here, some single neurons recorded intracellularly as well as extracellular recordings show P2/P1 > 0.55 even for gaps as short as 2 ms, and a substantial number of recordings showed P2/P1 > 1 for gaps of 20 ms, particularly when deviant in an oddball sequence. Such large responses are particularly puzzling since gap stimuli should, if anything, produce more synaptic depression and reduce the time available for recovery relative to the very short stimuli used by Wehr and Zador (2005). Thus, although the mere existence of gap-related responses is well established (Ison 1982; Eggermont 1999, 2000; Khouri et al. 2011; Weible et al. 2014; Anderson and Linden 2016), their substantial size as well as their steep dependence on gap duration under the conditions used here were surprising.

Instead of the expected strong suppression of the excitatory currents due to forward masking, we show here that the excitatory synaptic currents at the onset response were comparable with those evoked at the gap termination response for gaps as short as 20 ms (Fig. 3c). We provide evidence for the engagement of a fresh synaptic population in the generation of the gap termination response.

The main experiments provided a number of observations supporting this conclusion. First, NMDA currents had substantial contribution to P1 but a smaller one to P2 (Fig. 3*d*,*e*). Second, while the excitatory inputs were comparable at the P1 and P2 responses, inhibition was much more variable (Figs 3*f* and 4*c*), and the E/I balance sometimes increased and sometimes decreased at the P2 responses relative to the onset responses. Third, the gap termination responses adapted differentially in oddball sequences, with P2/P1 being larger when gaps were deviants than when they were standards, suggesting that the P2 response involved a different set of synapses than those activated by stimulus onset. When gaps are deviant, these synapses (or their parent neurons) show a change in E/I balance, with excitation become more prominent than when gaps are standard. Adaptation caused by gaps change the short-term plasticity when gap is deviant in oddball sequences.

The control experiments offered further support to this hypothesis. First, we observed that gaps at delays of 100–200 ms following stimulus onset evoked particularly large responses (Fig. 8*a*,*b*). This result provides a time course for the facilitation of the gap termination responses following a short first marker, and places strong constraints on its underlying mechanisms. Second, we showed that the strong gap termination responses were not due only to the difference between onset gate (a 10-ms linear ramp) and the gap gating, which was instantaneous. Indeed, even when both the onset and the gap were gated in the same way (either both by a 10-ms linear ramp, or both instantaneously), there was a robust specific adaptation of the gap termination responses when the gaps were standard, leading to significant SSA (Fig. 8*f*–*m*).

We describe here one speculative mechanism that may support these observations. We suggest that the differential synaptic fingerprints of the onset and gap termination responses may be due to the way VIP+, SST+, and PV+ interneurons balance inhibition and disinhibition in the cortical network (Kuchibhotla et al. 2016). In the mouse, both VIP+ and SST+ interneurons inhibit PV+ neurons (Pi et al. 2013). Furthermore, while PV+ neurons are activated early by direct thalamic inputs, SST+ interneurons (and presumably the VIP+ interneurons) show longer response latencies (Li et al. 2015). If a similar disinhibitory circuit operates in rats as well, it may happen that an excitatory neuron in cortex is suppressed by fast inhibition from PV+ interneurons at stimulus onset, but not at the time of the gap, when SST+ and VIP+ inhibition is operative. Such disinhibition could provide the additional synaptic resources that are reflected in the large gap-induced excitatory currents. Since SST+ interneurons also inhibit directly the excitatory neurons, the net E/I balance during the gap termination responses would depend on the idiosyncratic distribution of inputs to each individual neuron, resulting in the heterogeneous distribution reported here (Figs 3*f* and 4*c*). If this mechanism is indeed responsible for the substantial gap termination responses, we document here, the time course of the facilitation (Fig. 8*b*) would reflect the time course of the disinhibition.

Cortical responses to gaps are the consequence of processing throughout the auditory system, with significant contributions of subcortical integration mechanisms. Inhibitory mechanisms shape gap-related responses as early as the inferior colliculus (Khouri et al. 2011). Off responses have been shown to be crucial for shaping gap-related responses, presumably by compensating for some of the synaptic depression of the synapses providing

the onset input (Scholl et al. 2010; Anderson and Linden 2016). Here, we show that a mechanism with a similar flavor—the recruitment of an additional synaptic population—may underlie large gap termination responses even when off responses are weak or absent. This finding therefore suggest the existence of a general cortical principle, by which synaptic resources are released for stimulation only when needed, presumably in order to keep a constant overall level of responsiveness to important sensory cues in the presence of synaptic depression.

Funding

Ministry of Science, Technology and Space through Tsvi Yanai grant to B.A. European Research Council (ERC) (advanced grant GA-340063) (project RATLAND); FIRST (grant no. 1075/2013); Israel Science Foundation (personal grant no. 390/13).

Author Contributions

B.A. and I.N. designed the research, analyzed the data, and wrote the paper. B.A. conducted the experiments. M.J. performed the recordings using the Neuropixels probes.

Competing Interests

The authors declare no competing financial interests.

References

American Speech-Language-Hearing Association. 2005. Central auditory processing disorders[Technical Report].

Anderson LA, Linden JF. 2016. Mind the gap: two dissociable mechanisms of temporal processing in the auditory system. *J Neurosci*. 36:1977–1995.

Bamiou DE, Musiek FE, Luxon LM. 2001. Aetiology and clinical presentations of auditory processing disorders - a review. *Arch Dis Child*. 85:361–365.

Bartlett EL, Wang X. 2005. Long-lasting modulation by stimulus context in primate auditory cortex. *J Neurophysiol*. 94:83–104.

Bellis TJ, Ferre JM. 1999. Multidimensional approach to the differential diagnosis of central auditory processing disorders in children. *J Am Acad Audiol*. 10:319–328.

Bertoli S, Smurzynski J, Probst R. 2002. Temporal resolution in young and elderly subjects as measured by mismatch negativity and a psychoacoustic gap detection task. *Clin Neurophysiol*. 113:396–406.

Bhatara A, Babikian T, Laugeson E, Tachdjian R, Sininger YS. 2013. Impaired timing and frequency discrimination in high-functioning autism spectrum disorders. *J Autism Dev Disord*. 43:2312–2328.

Bishop DVM, McArthur GM. 2005. Individual differences in auditory processing in specific language impairment: a follow-up study using event-related potentials and behavioural thresholds. *Cortex*. 41:327–341.

Bleeck S, Sayles M, Ingham NJ, Winter IM. 2006. The time course of recovery from suppression and facilitation from single units in the mammalian cochlear nucleus. *Hear Res*. 212:176–184.

Brosch M, Schreiner CE. 1997. Time course of forward masking tuning curves in cat primary auditory cortex. *J Neurophysiol*. 77:923–943.

Brosch M, Schulz A, Scheich H. 1999. Processing of sound sequences in macaque auditory cortex: response enhancement. *J Neurophysiol*. 82:1542–1559.

Brugge JF, Merzenich MM. 1973. Responses of neurons in auditory cortex of the macaque monkey to monaural and binaural stimulation. *J Neurophysiol*. 36:1138–1158.

Bruner AP, Sato EI, Pereira LD. 2009. Central auditory processing in patients with systemic lupus erythematosus. *Acta Reumatol Port*. 34:600–607.

Calford MB, Semple MN. 1995. Monaural inhibition in cat auditory cortex. *J Neurophysiol*. 73:1876–1891.

Chermak GD, Musiek FE. 1997. *Central auditory processing disorders: new perspectives*. San Diego, CA: Singular Publishing Group.

Chimoto S, Kitama T, Qin L, Sakayori S, Sato Y. 2002. Tonal response patterns of primary auditory cortex neurons in alert cats. *Brain Res*. 934:34–42.

Christianson GB, Sahani M, Linden JF. 2011. Depth-dependent temporal response properties in core auditory cortex. *J Neurosci*. 31:12837–12848.

Cohen-Kashi Malina K, Jubran M, Katz Y, Lampl I. 2013. Imbalance between excitation and inhibition in the somatosensory cortex produces postadaptation facilitation. *J Neurosci*. 33:8463–8471.

Eggermont JJ. 1999. Neural correlates of gap detection in three auditory cortical fields in the cat. *J Neurophysiol*. 81: 2570–2581.

Eggermont JJ. 2000. Neural responses in primary auditory cortex mimic psychophysical, across-frequency-channel, gap-detection thresholds. *J Neurophysiol*. 84:1453–1463.

Fitzgibbons PJ, Wightman FL. 1982. Gap detection in normal and hearing-impaired listeners. *J Acoust Soc Am*. 72:761–765.

Fitzpatrick DC, Kuwada S, Kim DO, Parham K, Batra R. 1999. Responses of neurons to click-pairs as simulated echoes: auditory nerve to auditory cortex. *J Acoust Soc Am*. 106:3460–3472.

Fournier P, Hébert S. 2013. Gap detection deficits in humans with tinnitus as assessed with the acoustic startle paradigm: does tinnitus fill in the gap? *Hear Res*. 295:16–23.

Gomez R, Condon M. 1999. Central auditory processing ability in children with ADHD with and without learning disabilities. *J Learn Disabil*. 32:150–158.

Green DM. 1971. Temporal auditory acuity. *Psychol Rev*. 78:540–551.

Harris DM, Dallos P. 1979. Forward masking of auditory nerve fiber responses. *J Neurophysiol*. 42:1083–1107.

Hershenhoren I, Taaseh N, Antunes FM, Nelken I. 2014. Intracellular correlates of stimulus-specific adaptation. *J Neurosci*. 34:3303–3319.

Higgins NC, Storace DA, Escabí MA, Read HL. 2010. Specialization of binaural responses in ventral auditory cortices. *J Neurosci*. 30:14522–14532.

Hirsh IJ. 1959. Auditory perception of temporal order. *J Acoust Soc Am*. 31:759–767.

Ison JR. 1982. Temporal acuity in auditory function in the rat: reflex inhibition by brief gaps in noise. *J Comp Physiol Psychol*. 96:945–954.

Jun JJ, Steinmetz NA, Siegle JH, Denman DJ, Bauza M, Barbarits B, Lee AK, Anastassiou CA, Andrei A, Aydin Ç, *et al*. 2017. Fully integrated silicon probes for high-density recording of neural activity. *Nature*. 551:232–236.

Katz J, Stecker N, Henderson D. 1992. *Central auditory processing: a transdisciplinary view*. St. Louis (MO): Mosby-Year Book, pp. 81–91.

Keller CH, Kaylegian K, Wehr M. 2018. Gap encoding by parvalbumin-expressing interneurons in auditory cortex. *J Neurophysiol*. 120:105–114.

Khouri L, Lesica NA, Grothe B. 2011. Impaired auditory temporal selectivity in the inferior colliculus of aged Mongolian gerbils. *J Neurosci*. 31:9958–9970.

Kilgard MP, Merzenich MM. 1999. Distributed representation of spectral and temporal information in rat primary auditory cortex. *Hear Res*. 134:16–28.

Kuchibhotla KV, Gill JV, Lindsay GW, Papadoyannis ES, Field RE, Sten TAH, Miller KD, Froemke RC. 2016. Parallel processing by cortical inhibition enables context-dependent behavior. *Nat Neurosci*. 20:1–14.

Las L, Stern EA, Nelken I. 2005. Representation of tone in fluctuating maskers in the ascending auditory system. *J Neurosci*. 25:1503–1513.

Li LY, Xiong XR, Ibrahim LA, Yuan W, Tao HW, Zhang LI. 2015. Differential receptive field properties of Parvalbumin and Somatostatin inhibitory neurons in mouse auditory cortex. *Cereb Cortex*. 25:1782–1791.

Moshitch D, Las L, Ulanovsky N, Bar-Yosef O, Nelken I. 2006. Responses of neurons in primary auditory cortex (A1) to pure tones in the halothane-anesthetized cat. *J Neurophysiol*. 95:3756–3769.

Musiek F, Gollegly K, Lamb L, Lamb P. 1990. Selected issues in screening for central auditory processing dysfunction. *Semin Hear*. 11:372–383.

Phillips E, Chrisotph S, Hasenstaub A. 2017. Cortical interneurons differentially regulate the effects of acoustic context. *Cell Rep*. 20:771–778.

Pi H-J, Hangya B, Kvitsiani D, Sanders JI, Huang ZJ, Kepecs A. 2013. Cortical interneurons that specialize in disinhibitory control. *Nature*. 503:521–524.

Pratt H, Bleich N, Mittelman N. 2005. The composite N1 component to gaps in noise. *Clin Neurophysiol*. 116:2648–2663.

Riccio CA, Hynd GW, Cohen MJ, Hall J, Molt L. 1994. Comorbidity of central auditory processing disorder and attention-deficit hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry*. 33:849–857.

Rosen S. 2003. Auditory processing in dyslexia and specific language impairment: is there a deficit? What is its nature? Does it explain anything? *J Phon*. 31:509–527.

Scholes C, Palmer AR, Sumner CJ. 2011. Forward suppression in the auditory cortex is frequency-specific. *Eur J Neurosci*. 33:1240–1251.

Scholl B, Gao X, Wehr M. 2010. Nonoverlapping sets of synapses drive on responses and off responses in auditory cortex. *Neuron*. 65:412–421.

Snell KB. 1997. Age-related changes in temporal gap detection. *J Acoust Soc Am*. 101:2214–2220.

Snell KB, Frisina DR. 2000. Relationships among age-related differences in gap detection and word recognition. *J Acoust Soc Am*. 107:1615–1626.

Stach BA, Spretnjak ML, Jerger J. 1990. The prevalence of central presbyacusis in a clinical population. *J Am Acad Audiol*. 1:109–115.

Taaseh N, Yaron A, Nelken I. 2011. Stimulus-specific adaptation and deviance detection in the rat auditory cortex. *PLoS One*. 6:e23369.

Tan AYY, Brown BD, Scholl B, Mohanty D, Priebe NJ. 2011. Orientation selectivity of synaptic input to neurons in mouse and cat primary visual cortex. *J Neurosci*. 31: 12339–12350.

Turner JG, Brozoski TJ, Bauer CA, Parrish JL, Myers K, Hughes LF, Caspary DM. 2006. Gap detection deficits in rats with tinnitus: a potential novel screening tool. *Behav Neurosci*. 120: 188–195.

Ulanovsky N, Las L, Nelken I. 2003. Processing of low-probability sounds by cortical neurons. *Nat Neurosci*. 6:391–398.

Wehr M, Zador AM. 2003. Balanced inhibition underlies tuning and sharpens spike timing in auditory cortex. *Nature*. 426:442–446.

Wehr M, Zador AM. 2005. Synaptic mechanisms of forward suppression in rat auditory cortex. *Neuron*. 47:437–445.

Weible AP, Moore AK, Liu C, Deblander L, Wu H, Kentros C, Wehr M. 2014. Perceptual gap detection is mediated by gap termination responses in auditory cortex. *Curr Biol*. 24: 1447–1455.

Yaron A, Hershenhoren I, Nelken I. 2012. Sensitivity to complex statistical regularities in rat auditory cortex. *Neuron*. 76:603–615.

Yin SK, Feng YM, Chen ZN, Wang J. 2008. The effect of noise-induced sloping high-frequency hearing loss on the gap-response in the inferior colliculus and auditory cortex of Guinea pigs. *Hear Res*. 239:126–140.

Zatorre RJ, Belin P, Penhune VB. 2002. Structure and function of auditory cortex: music and speech. *Trends Cogn Sci*. 6:37–46.

Zhao Y, Xu X, He J, Xu J, Zhang J. 2015. Age-related changes in neural gap detection thresholds in the rat auditory cortex. *Eur J Neurosci*. 41:285–292.