Stimulus dependent transformations between synaptic and spiking receptive fields in auditory cortex

Kyunghhee X. Kim, Craig A. Atencio & Christoph E. Schreiner

Auditory cortex neurons nonlinearly integrate synaptic inputs from the thalamus and cortex, and generate spiking outputs for simple and complex sounds. Directly comparing synaptic and spiking activity can determine whether this input-output transformation is stimulus-dependent. We employ in vivo whole-cell recordings in the mouse primary auditory cortex, using pure tones and broadband dynamic moving ripple stimuli, to examine properties of functional integration in tonal (TRFs) and spectrotemporal (STRFs) receptive fields. Spectral tuning in STRFs derived from synaptic, subthreshold and spiking responses proves to be substantially more selective than for TRFs. We describe diverse spectral and temporal modulation preferences and distinct nonlinearities, and their modifications between the input and output stages of neural processing. These results characterize specific processing differences at the level of synaptic convergence, integration and spike generation resulting in stimulus-dependent transformation patterns in the primary auditory cortex.
Auditory cortical circuitry shapes spectral processing by nonlinearily integrating converging auditory information across frequency and time. Synaptic inputs are integrated and subsequently transformed into a spiking output. As a consequence, cortical spectral tuning properties, such as bandwidth of tonal receptive fields (TRFs), can differ from their inputs, widely vary, and demonstrate nonlinearly facilitated or suppressed responses (e.g., in two-tone stimuli-derived by combining single pure tone responses. Thus, spectral integration and the cellular transformation of information of more complex natural or dynamically modulated artificial sounds should be affected by these nonlinear processes. This suggests that receptive fields derived from complex sounds could differ significantly from those derived by combining single pure tone responses.

Auditory cortex neurons exhibit diverse and dynamic receptive fields in response to dynamically changing stimuli. Synthetic stimuli that contain essential properties of natural sounds are effective tools for estimating the response properties of auditory cortex neurons because they are under full experimental control and can be modified to allow for the analysis of nonlinear response features. The dynamic moving ripple (DMR) is a complex sound that contains the essential modulation features common to many natural sounds. Unlike many natural sounds, which are often non-Gaussian, the DMR is globally uncorrelated. This aspect of DMRS supports rigorous estimates of receptive fields and associated nonlinear input–output functions by event-triggered receptive field estimation.

Nonlinear interactions between stimulus elements preclude the use of certain methodologies to estimate the degree of spectral integration by quantifying receptive field features such as the spectral bandwidth. In the auditory cortex, spectral bandwidths for subthreshold responses have been examined predominantly for TRFs. TRF bandwidths for subthreshold responses were found to be slightly broader than TRF bandwidths obtained from spikes. This indicates that the subthreshold convergence of various excitatory and inhibitory inputs can be further refined by the spike-generation process. The mechanisms that underlie subthreshold and suprathreshold difference in TRF bandwidths between responses may not apply directly to more complex sounds. However, very few studies have related suprathreshold and subthreshold integration for stimuli with different sound statistics.

The main goal of this study is to apply a quantitative, comparative approach to the different stages of information transformation at the neuronal level. For that purpose, we examine spectral and temporal tuning by comparing the suprathreshold and subthreshold receptive fields with in vivo whole-cell recordings using the blind patching approach. We find that the spectral tuning of spectrottemporal receptive fields (STRFs) in both subthreshold and suprathreshold responses is often much narrower than that of TRFs. The nonlinearities associated with sub- and suprathreshold STRFs reveal distinct differences. This suggests that spectral tuning in the primary auditory cortex (A1) is determined by different underlying influences when processing pure tones and complex stimuli. Furthermore, the best spectral and temporal modulation frequencies in STRFs from small subthreshold events are usually higher than for large subthreshold responses and spikes, suggesting that A1 neurons receive diverse inputs with respect to the modulation preferences that shape their output patterns. Examination of synaptic events underlying the generation of post-synaptic potentials (PSPs) reveals clear distinctions in excitatory and inhibitory STRFs further constraining the information transformation in A1.

Results

Tonal receptive fields. We studied the responses of A1 neurons to pure tones and dynamic broadband stimuli estimating both TRFs and STRFs. Recordings were obtained largely at depths corresponding to layer 4, the main hub receiving lemniscal thalamic inputs from the ventral medial geniculate body. Tone-evoked membrane potentials (e.g., Fig. 1a, b; n = 66) typically resulted in V-shaped TRFs for subthreshold PSPs, with a distinguishing trough at the minimum sound level needed to evoke a response and increasing bandwidth with increasing stimulus intensity. PSPs for tonal responses were identified based on their onset latencies (5–50 ms) relative to tone onset and response magnitudes (>4 × standard deviation above baseline). For pure tones the maximum PSP amplitude was 16.3 ± 4.8 mV (mean ± s.d., n = 66) (Fig. 1a, b; mean ± s.d., n = 66; PSP decay time (90–10%): 86 ± 20 ms (mean ± s.d., n = 66)). Some recordings showed a high responsiveness where almost every PSP generated spikes (Fig. 1a), whereas others had only a few spikes (Fig. 1b) thus limiting the ability to obtain a spike-based TRF sufficiently reliable to estimate the spectral bandwidth. Therefore, we quantified the ratio of the number of spikes to the number of PSPs as the normalized-driven ratio (see “Methods” section). The distribution of normalized-driven ratios resembled an exponential decay with a larger number of neurons near 0 and very few near 1 (Fig. 1d). The range of encountered resting membrane potentials (−85 to −59 mV) was consistent with previous observations.

Methods
distributions for non-spiking events were either bimodal (Fig. 2a, b, top right; magenta and green) or unimodal in 60% (n = 24/40) and 40% (n = 16/40) of the recorded neurons, respectively. For bimodal PSP histograms, PSP amplitudes were subdivided into large (magenta) and small (green) events at the trough between the two maxima. When a PSP histogram was unimodal, large and small PSP amplitudes were divided at approximately 50% of the number of non-spiking PSPs. Both large and small PSPs (Fig. 2) likely represent the integration of multiple synchronous synaptic inputs from many synapses, since unitary synaptic inputs usually have an amplitude of ~1 mV22,23.

STRFs were estimated for the three different response events by extracting peak timing information for spikes (black tick marks below the voltage traces), and non-spike related large (magenta), and small (green) PSPs (Fig. 2a, b, bottom). Very small events (marked gray in amplitude histogram) indicate events less than...
4 × s.d. of unresponsive baseline segments and were excluded from further analysis. Most neurons (75%, n = 30/40) showed significant STRF subfields for spiking, large PSP, and small PSP events (Fig. 2a, bottom; example with a best frequency of ~21.7 kHz and a significant STRF bandwidth of ~0.28 octaves). For these neurons, the mean amplitudes from large PSPs ranged from 13.8 to 28.8 mV and the mean amplitudes from small PSPs were between 7.2 and 11.5 mV. The two amplitude ranges did not overlap. A second group (25%, n = 10/40) lacked significant spike-based STRF subfields but showed significant PSP-based STRF subfields (Fig. 2b, bottom; example with two peak frequencies at ~12.1 and ~20.2 kHz). This grouping was independent of the uni- or bimodal nature of the PSP peak amplitude distributions.

**Different mean DMR intensities have only minor effects on STRF.** The frequency extent of TRFs is strongly intensity dependent (Fig. 1a, b). We tested for the effects of variations in DMR intensity on STRFs by comparing three sound intensities (38, 54, and 69 dB SPL) in each of five neurons that showed stable recordings over more than 35 min (Supplementary Fig. 3). For all three event types, STRF shape remained quite similar although the STRF magnitude occasionally was reduced at the higher intensity (Supplementary Fig. 3a). Different intensities did not yield significant changes in peak latency (Supplementary Fig. 3b, middle) or STRF bandwidths for individual or double frequency peaks (Supplementary Fig. 3b, right). Thus, broadband

**Response reliability over extended recording durations is high.** For a subset of neurons, the same DMR stimulus was presented a second time (e.g., Supplementary Fig. 2a). Correlations between the resulting STRFs for both large and small PSPs were usually high and significantly exceeded those for spike STRFs (Supplementary Fig. 2b). This fairly high test-retest reliability indicates that the extended recording period required for obtaining STRFs did not compromise the quality of the functional characterization.

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**Fig. 2 Spectrotemporal receptive fields obtained from in vivo current-clamp whole-cell recordings to the DMR stimulus. a** Top left, a segment of a neuron response to the DMR stimulus. The short black line marks indicate spike times. Horizontal scale: 0.3 s; vertical scale: 15 mV. The magenta and green marks indicate peak times of PSPs with large and small amplitudes, respectively. Top right, an amplitude histogram of spikes and PSPs was obtained from the left recording and shows a bimodal PSP peak amplitude distribution. Bottom, the spike STRF (first), the STRF for large PSPs (second), and the STRF for small PSPs (third) resulted from spike times (black in the histogram), peak times of large PSPs (magenta in the histogram), and peak times of small PSPs (green in the histogram), respectively. The number of peaks included in computing each STRF was 1226 for spikes, 1437 for large PSPs, and 1162 for small PSPs. Bottom right, the color bar goes from the overall maximum (red) to the minus maximum (blue) on the same absolute scale of three STRFs from spikes, large PSPs, and small PSPs. **b** As in (a), but from a different neuron. Top left, horizontal scale: 0.3 s; vertical scale: 20 mV. Top right, an amplitude histogram shows a bimodal PSP peak amplitude distribution. The number of peaks included in computing each STRF (bottom) was 532 for spikes, 1009 for large PSPs, and 863 for small PSPs.
stimulation reduces robust intensity effects on frequency selectivity for both sub- and suprathreshold events that are commonly observed for narrowband stimuli, likely due to corticocortical influences24–26.

Double-peaked STRFs with harmonic frequency relationships are more common for PSPs. Among all recorded neurons, 50% (Fig. 3f; n = 20/40) showed single-peaked STRFs while 50% (Fig. 3f; n = 20/40) had double-peaked responses in either the spiking or the subthreshold STRFs (Fig. 3a–c). Double-peaked responses in spike STRFs were less common (Fig. 3d; n = 7/40; 17.5%). Harmonic relationships between the peaks, i.e., whole-number ratios of a higher frequency to a lower frequency (e.g., 2/1, 3/2, etc.), were common. The observed frequency ratios (n = 20) of doubled-peaked STRFs obtained for all event types were assigned to the closest of five whole-number ratio categories (Fig. 3e; i.e., 1.25, 1.33, 1.5, 1.67, and 2). The actual ratios fell within 4.5% of these categories and reflected a wide range of observed frequency ratios, from 5/4 to 2/1. A ratio of 1.67 (i.e., 5/3) was most prevalent (Fig. 3e; n = 6/20). This indicates that convergence of low-frequency harmonic components is common in subthreshold activity but is reduced by ~61% in spiking outputs (double-peaked STRFs for all events, n = 20/40; double-peaked PSP STRFs, n = 18/40; double-peaked spike STRFs, n = 7/40).

STRF modulation properties can differ between subthreshold and spiking events. Because spectral and temporal modulations are inherent features of natural sounds, it is important to
understand how modulation information carried by synaptic and spiking activity differs, and how it may be transformed by the spike generation process. STRFs with significant subfields (e.g., Fig. 2a; \(n = 30/40\)) were transformed into ripple transfer functions (RTFs; see the “Methods” section) that reflect the preferred spectral and temporal modulations present in the stimuli. The spike-based STRFs (Fig. 4a, top left) showed a significant high-energy subfield (red) combined with a longer-latency suppression subfield (blue). By contrast, the STRF for small PSPs showed a short as well as a long-latency suppression subfield indicating potential temporal modulation diversity (Fig. 4a, top right). These STRF differences are reflected in RTF differences (Fig. 4a, bottom).
Table 1 Functional properties of spiking and PSP STRFs.

| STRF properties                     | Spikes (mean ± s.d.) | PSPs (large) (mean ± s.d.) | PSPs (small) (mean ± s.d.) | p-value |
|-------------------------------------|----------------------|----------------------------|----------------------------|---------|
| Bandwidth [single bands] (octaves)  | 0.32 ± 0.12 (n = 31) | 0.28 ± 0.11 (n = 31)      | 0.25 ± 0.07 (n = 31)      | 0.1127  |
| Bandwidth [all bands] (octaves)     | 0.50 ± 0.32 (n = 30) | 0.57 ± 0.33 (n = 30)      | 0.50 ± 0.32 (n = 30)      | 0.0016**|
| Latency (ms)                        | 42.7 ± 11.1 (n = 30) | 48.7 ± 11.8 (n = 30)      | 45.2 ± 8.2 (n = 30)       | 0.1874  |
| Duration (ms)                       | 43.0 ± 24.1 (n = 30) | 43.6 ± 18.8 (n = 30)      | 34.5 ± 10.3 (n = 30)      | 0.7493  |
| bTMF (cyc/s)                        | 0.55 ± 0.74 (n = 31) | 0.15 ± 0.04 (n = 30)      | 0.7493                    |         |
| Feature selectivity index           | 0.61 ± 0.87 (n = 30) | 0.57 ± 0.87 (n = 30)      | 0.55 ± 0.74 (n = 30)      | 7 × 10⁻⁶**|
| Nonlinearity threshold, Θ (s.d.)    | 1.25 ± 0.48 (n = 30) | 1.59 ± 0.27 (n = 30)      | 0.7493                    |         |
| Nonlinearity transition, σ (s.d.)   | 1.25 ± 0.48 (n = 30) | 1.59 ± 0.27 (n = 30)      | 0.1888                    |         |

Statistical testing was performed using two-tailed paired Student’s t-test; significant differences are indicated by *p < 0.05 and **p < 0.005.

Frequency selectivity differences between TRFs and STRFs. The transformation of subthreshold inputs to spiking outputs is a fundamental computational task performed by neurons and may be stimulus-dependent. Thus, we compared the frequency preference and selectivity of sub- and suprathreshold events for tonal and DMR receptive fields. STRF best frequencies and TRF characteristic frequencies were well correlated (Fig. 5a). For STRFs with two best frequencies (Fig. 3), frequencies closest to characteristic frequencies of their corresponding TRFs were chosen for the analysis. Best frequencies from spike-based STRFs were closely matched to STRF-derived estimates for both large and small PSPs (Fig. 5b; n = 30/40) with no between-group difference (one-way ANOVA, p = 0.99). Therefore, the dominant preferred frequency of neurons is essentially identical for sub-threshold and suprathreshold activity, and is independent of the test stimulus (i.e., narrowband versus broadband stimuli).

We next explored whether the range of spectral integration or frequency selectivity of STRFs differs between neuronal PSPs and spikes. We found four general relationships (Fig. 5c): (i) spike-based STRFs with a single frequency band (e.g., Fig. 2a; Fig. 5c, bottom left region) commonly were sharply tuned with bandwidths below ~0.5 octaves (n = 20/30; 67%). The PSP-based bandwidths of 65% (n = 13/20, large PSPs) and 75% (n = 15/20, small PSPs) of these neurons were also below ~0.5 octave; (ii) 30% (n = 9/30, large PSPs) and 20% (n = 6/30, small PSPs) showed much wider total bandwidths above ~0.5 octaves for PSP-based STRFs largely due to secondary frequency peaks (Fig. 5c, upper left region); (iii) spike-based STRFs with total bandwidths > 0.6 octaves (n = 8/30, single or double peaked) had similar bandwidths to the corresponding PSP-based STRFs (Fig. 5c, upper right region); and (iv) one spike-based STRF with two peaks had single-peaked STRFs for both types of PSP events with bandwidths < 0.5 octaves (Fig. 5c, bottom right region). Thus, differences in total frequency bandwidth between spiking- and PSP-STRFs are largely due to the emergence or dropping-out of secondary, usually harmonically related frequency components (Fig. 3).

Subthreshold and spiking STRF bandwidths need to be compared to assess whether there is a transformation of the local frequency selectivity, as has been indicated for pure-tone frequency selectivity (Fig. 1f–h). Contrasting individual frequency peaks from spike-based STRFs to their corresponding bandwidths of large PSPs were not statistically different while the bandwidths derived from small PSPs were slightly narrower than for both...
Fig. 5 Bandwidth comparison between TRFs and STRFs. a Relationship between TRF characteristic frequencies and STRF best frequencies derived from all events ($n = 40/66$ neurons with paired recordings for both, pure tones and DMR stimuli; Pearson’s $r = 0.73$, $p = 10^{-7}$). b Relationship between spike-based STRF best frequencies and PSP-based TRF best frequencies ($n = 30/40$ neurons with significant STRF subfields for spiking, large PSP, and small PSP events; large PSPs (closed triangles), small PSPs (open triangles)). c Relationship between spike-based STRF bandwidths and PSP-based STRF bandwidths ($n = 30/40$ neurons with significant STRF subfields for spiking, large PSP, and small PSP events; large PSPs (closed triangles), small PSPs (open triangles); single-peaked STRFs (black), double-peaked STRFs (red) for spikes, or large PSPs, or small PSPs). Note that red color indicates the presence of a second peak in small PSP-, or large PSP-, or spike-based STRFs. d Relationship between STRF bandwidths and TRF bandwidths ($n = 30/40$ neurons with significant STRF subfields for spiking, large PSP, and small PSP events). e Relationship between TRF Q30s and STRF Qs derived from all events ($n = 40/66$ neurons with paired recordings for both, pure tones and DMR stimuli; two-tailed paired Student’s t-test, $p = 10^{-9}$). Open squares indicate the relationship between spiking-based TRF Q30s (normalized-driven ratio $\geq 0.3$) and STRF Qs derived from all events ($n = 15/40$ neurons with paired recordings for TRFs (normalized-driven ratio $\geq 0.3$) and STRFs; two-tailed paired Student’s t-test, $p = 0.0025$). f Bandwidth differences ($\Delta$bandwidth) between PSP-based TRFs and STRFs. $\Delta$bandwidths (mean ± s.d., $n = 30/40$ neurons with significant STRF subfields for spiking, large PSP, and small PSP events) were 0.99 ± 0.45 octaves (for spikes), 0.92 ± 0.44 octaves (for large PSPs), and 0.99 ± 0.44 octaves (for small PSPs). The median $\Delta$bandwidth values, the middle line between hinges, were 1.02 for spikes, 1.01 for large PSPs, and 0.91 for small PSPs. The lower and upper hinges are at the 25th and 75th percentiles. Minimum and maximum values are indicated by whiskers.

Comparing the spectral bandwidths obtained with narrowband and broadband stimuli can illuminate the influence of distant frequency components on neuronal frequency integration and selectivity. Most STRF-derived bandwidths were substantially narrower than TRF-derived bandwidths based on both spiking and PSP events (Fig. 5d–f). PSP-based TRF bandwidths were on average ~1 octave wider than total STRF bandwidths for all three STRF event types with no significant group differences (Fig. 5f; one-way ANOVA, $p = 0.81$). Q factors ((best frequency)/bandwidth), another estimate of sharpness of frequency tuning, showed corresponding differences with STRF Q values from all events significantly higher than TRF Q30 values for PSP TRFs and spike TRFs (Fig. 5e).

Overall, the effective spectral integration seen in synaptic responses clearly differed between narrowband and broadband stimuli. The transformational effect of each stimulus type on the corresponding outputs, however, was fairly small. It increased TRF frequency selectivity slightly more than STRF selectivity for individual peaks.

### STRF nonlinearity

In linear-nonlinear filter models of a neuron, the nonlinearity determines the response rate (or probability of an event) as a function of the similarity between the stimulus and a linear filter, which is often modeled by the STRF[27]. The nonlinearity depicts the number of events as a function of the correlation (or projection value) between the stimulus spectrogram preceding an event and the linear filter (STRF). These z-scored projection values are plotted. Nonlinearity characteristics can capture important features of a cell’s input–output transformation (Fig. 6a).

We parametrically described the nonlinearities by fitting an expansive power-law function[19,28,29]. The fitted function has two main parameters: Threshold designates the lowest projection value indicative of a driven response. High thresholds require a close match between stimulus and STRF to achieve a response,
corresponding to high feature selectivity; Transition is the smoothness of the nonlinearity transition across threshold. When transition is 0, the function describes hard rectification with little leakage from poorly matched stimuli. High transition values reflect more smoothly varying transitions from absent or weak stimulus/STRF matches to strong matches, indicative of a noisier or leaky thresholding process.

The thresholds of spike nonlinearities were significantly higher than for PSPs (Fig. 6b; Table 1) indicative of a process that transforms noisy synaptic inputs with lower feature-selectivity into less noisy spiking outputs with higher feature selectivity. Additionally, the transition measure for spike nonlinearities is smaller than for either PSP type (Fig. 6c; Table 1). This difference points to a harder rectification process at the spike generation level further reducing the influence of low stimulus/STRF similarities or random events and, thus, enhancing feature selectivity and reducing response variability and contamination. For spikes, nonlinearity threshold and transition covaried (Fig. 6d; Pearson’s r = 0.68, p = 0.0294 for large PSPs; Pearson’s r = 0.39, p = 0.0294 for large PSPs; Pearson’s r = 0.30, p = 0.1045 for small PSPs). Relationship between transitions and feature selectivity indexes (n = 30/40 neurons with significant STRF subfields for spiking, large PSP, and small PSP events; power fit, Pearson’s r = −0.67, p = 4 × 10^-5 for spikes; Pearson’s r = −0.50, p = 0.0045 for large PSPs; Pearson’s r = −0.54, p = 0.0019 for small PSPs). Relationship between event rates and feature selectivity indexes (n = 30/40 neurons with significant STRF subfields for spiking, large PSP, and small PSP events; power fit, Pearson’s r = −0.63, p = 0.0002 for spikes).

**Fig. 6 Nonlinearities of STRFs.** a Representative example illustrates different nonlinearities for spikes and PSPs. b Histograms of thresholds (n = 30/40 neurons with significant STRF subfields for spiking, large PSP, and small PSP events; Pearson’s r = 0.024 and p = 0.9 between small PSPs and spikes; Pearson’s r = 0.075 and p = 0.7 between large PSPs and spikes). The width of a gray bar corresponds to a bin size. Three histograms for spikes, large PSPs, and small PSPs are plotted together in each bin. c As in b, but for histograms of transitions (n = 30/40 neurons with significant STRF subfields for spiking, large PSP, and small PSP events; Pearson’s r = −0.007 and p = 0.97 between small PSPs and spikes; Pearson’s r = −0.078 and p = 0.68 between large PSPs and spikes). d Relationship between thresholds and transitions (n = 30/40 neurons with significant STRF subfields for spiking, large PSP, and small PSP events). e Relationship between thresholds and feature selectivity indexes (n = 30/40 neurons with significant STRF subfields for spiking, large PSP, and small PSP events; linear fit, Pearson’s r = 0.68, p = 2 × 10^{-5} for spikes; Pearson’s r = 0.39, p = 0.0294 for large PSPs; Pearson’s r = 0.30, p = 0.1045 for small PSPs). f Relationship between transitions and feature selectivity indexes (n = 30/40 neurons with significant STRF subfields for spiking, large PSP, and small PSP events; power fit, Pearson’s r = −0.67, p = 4 × 10^{-5} for spikes; Pearson’s r = −0.50, p = 0.0045 for large PSPs; Pearson’s r = −0.54, p = 0.0019 for small PSPs). g Relationship between event rates and feature selectivity indexes (n = 30/40 neurons with significant STRF subfields for spiking, large PSP, and small PSP events; power fit, Pearson’s r = −0.63, p = 0.0002 for spikes).
These relationships are clearly expressed for the spiking events (Fig. 6c–g). PSPs indicate similar relationships although the correlations were weaker or not significant.

Higher threshold and lower transition values for spiking versus PSP events signify an essential transformation from active subthreshold information integration to higher, suprathreshold information selection in auditory cortical neurons. Neither the nonlinearity thresholds nor the transition values were correlated between PSPs and spiking events, reflecting that the input and output transformations accomplished by synaptic integration and the spike-generation mechanisms are largely independent from each other and specific to each neuron.

**DMR-evoked excitatory and inhibitory synaptic currents.** The observed distinctions in sub- and supra-threshold information processing, particularly reflected in the differences between the derived nonlinearities, raise the question of distinct synaptic contributions. We recorded successfully 31 neurons in voltage-clamp mode and obtained 27 excitatory DMR responses and 12 inhibitory traces with eight neurons yielding both components (e.g., Fig. 7a, b). The amplitude distributions of peak currents (>3 s.d. above baseline) were unimodal for excitatory postsynaptic currents (EPSCs) and inhibitory postsynaptic currents (IPSCs) and we constructed STRFs across all significant events. For the eight paired recordings, excitatory and inhibitory best frequencies were closely matched (Fig 7c). Double-peak STRFs were observed in 35% (Fig. 7d; n = 11/31) of the current traces, a similar proportion as for small PSPs (Fig. 3d; n = 14/40), and about twice as high as for spiking STRFs (Fig. 3d; n = 7/40).

Previous studies with tones suggested that inhibition and excitation are generally co-tuned although slightly broader frequency tuning of the inhibitory inputs was noted. This is also observed here for the STRF bandwidths of EPSCs and IPSCs (Fig. 7e). The inhibitory bandwidth exceeded the excitatory bandwidth by ~27% (Fig. 7e; Table 2). These spectral tuning differences were also reflected in the RTFs by showing lower bSMFs of IPSC versus EPSC STRFs (Fig. 7f).

By contrasting subthreshold and spiking events emanating from distinctly different stimulus classes, tones and DMRs, we made three main observations: (1) Assessment of STRFs of excitatory and inhibitory synaptic currents revealed a much higher response selectivity for inhibitory inputs, a wider spectral bandwidth of inhibitory versus excitatory STRFs, and higher temporal modulation capacities than for high-amplitude PSPs and spiking events. (2) STRFs derived separately for high- and low-amplitude PSPs differed in their temporal preferences, but not in spectral preferences. (3) Spectral tuning assessed with broadband stimuli was substantially sharper than seen with narrowband stimuli for non-spiking PSPs and spiking events. Combined, we characterized a set of stimulus-dependent aspects of integration and transformation between auditory cortical inputs and outputs.

We distinguished between two types of PSP events based on response magnitude. The high similarity of modulation preference between large PSPs and spiking events indicates that the properties of small PSP events do not directly shape the effective functional sensory–output transformation. The differences in the modulation preferences between large and small PSPs suggest two parallel input pathways and/or synaptic networks serving different synaptic populations. Studies have established the convergence of various thalamo-cortical and cortico-cortical pathways to A1 neurons. The higher temporal following capacity of the small PSP events might indicate that its main source is thalamic in origin, which often prefers faster amplitude modulation rates and higher spectral modulations than cortical neurons.

Two types of synapses have been shown to affect auditory cortical neurons, providing two modes of transmission tuned for specific roles. The low probability synapses showed low success probability, small current amplitudes, a low degree of short-term synaptic depression and higher temporal precision. In contrast, the high probability synapses illustrated high success probability, larger current amplitudes, marked short-term depression and lower temporal precision. It can be speculated that the small PSPs observed here may be driven by the low probability synapses and the large PSPs by the high probability synapses. Since small PSP events, in contrast to large PSPs, prefer higher spectral modulation stimuli, it appears that the convergence of spectral tuning from the two synaptic networks may also differ.

EPSCs did not reveal corresponding bimodal magnitude or temporal modulation distributions but showed a bTMF distribution similar to small PSPs. One potential contribution to the selective reduction of faster temporal modulations for large PSP events could be a higher synchrony between phase-locked excitatory and inhibitory events that is more likely to occur at high temporal modulations. By contrast, responses to low temporal modulations may be accompanied by a timing mismatch of phase-locked excitatory and inhibitory currents, thus failing to effectively suppress excitatory inputs.

The manner (“how”) in which PSPs and spikes are generated substantially differs and is largely reflected in differences in their nonlinearities. PSP event had low thresholds (~0.6 s.d.) and EPSC thresholds were even lower (~1.14 s.d.), resulting in noisy trains with many events that mark stimuli with little similarity to the STRF. In the spiking responses, the distribution of
nonlinearity thresholds ($\theta$) was centered at approximately 0.6 s.d., implying that the stimulus-filter similarity had to be sufficiently high for spike rates to be discernible. This mean threshold is slightly below that for spikes in cat auditory cortex (1.5 s.d.)\textsuperscript{19} or monkey visual cortex (~1.0 s.d.)\textsuperscript{36}, potentially due to species-specific or anesthesia-related differences. Since the precision of stimulus envelope phase-locking in mouse cortical neurons is usually less than in cats or monkeys\textsuperscript{37}, the reduced threshold-values are not unexpected given that STRFs depend on high event-time precision. The surprisingly high nonlinearity thresholds and, consequently, high stimulus selectivity of IPSCs (1.26 s.d.) appears to effectively curtail the transmission of equally
Fig. 7 Bandwidths, modulation properties, and nonlinearities of STRFs from in vivo voltage-clamp whole-cell recordings to the DMR stimulus. a A representative example of excitation. Top left, a segment of excitatory responses at a holding potential of −70 mV. Scale bar, 0.5 s. The short black line marks below current responses indicate excitatory current peak times. Top right, an amplitude histogram of excitatory currents was obtained from the left recording. Bottom, STRF (left), RTF (center), and nonlinearity (right) for the excitatory responses of this neuron. 

b A representative example of inhibition. Top left, a segment of inhibitory responses at a holding potential of 0 mV. Scale bar, 1 s. The short black line marks below current responses indicate inhibitory current peak times. Top right, an amplitude histogram of inhibitory currents was obtained from the left recording. Bottom, STRF (left), RTF (center), and nonlinearity (right) for the inhibitory responses of this neuron. 

c Relationship between paired inhibitory and excitatory STRF best frequencies (n = 8/31 neurons with paired recordings of excitation and inhibition). 

d Quantification of double-peaked responses for excitation (n = 9/27) and inhibition (n = 5/12). 

The bandwidth comparison among current-clamp (CC; n = 40/66 neurons with paired recordings between pure tones and DMR stimuli) and voltage-clamp (VC; n = 31 neurons from 24 mice); excitation (n = 27/31), inhibition (n = 12/31), paired recordings of excitation and inhibition (n = 8/31) STRFs. The lower and upper hinges are at the 25th and 75th percentiles. The median bandwidth values are indicated by the middle line between hinges. Whiskers indicate minimum and maximum values. Asterisks indicate outliers. 

Table 2 Functional properties of excitatory and inhibitory synaptic STRFs.

| STRF properties | Excitation (mean ± s.d.) | Inhibition (mean ± s.d.) | Comparison with CC* (mean ± s.d.) | p-value |
|-----------------|--------------------------|--------------------------|----------------------------------|---------|
| Bandwidth [all bands] (octaves) | 0.65 ± 0.30 (n = 27) | 0.89 ± 0.34 (n = 12) | 0.64 ± 0.37 (n = 40) | 0.0496* |
| Latency (ms) | 35.2 ± 10.9 (n = 27) | 35.3 ± 14.8 (n = 12) | 0.64 ± 0.37 (n = 40) | 0.0399* |
| Duration (ms) | 41.5 ± 16.9 (n = 27) | 45.6 ± 29.0 (n = 12) | 0.0917 | |
| bTMF (cyc/s) | 11.2 ± 6.0 (n = 27) | 12.0 ± 6.9 (n = 12) | 0.06591 | |
| bSMF (cyc/oct) | 0.62 ± 0.47 (n = 27) | 0.25 ± 0.21 (n = 12) | 0.7132 | |
| Event rate (Hz) | 3.72 ± 0.74 (n = 27) | 1.06 ± 0.61 (n = 12) | 7.862 | |
| Feature selectivity index | 0.09 ± 0.03 (n = 27) | 0.13 ± 0.05 (n = 12) | 0.0242* | |
| Nonlinearity threshold, \( \Theta \) (s.d.) | −1.14 ± 0.87 (n = 27) | 1.26 ± 0.70 (n = 12) | 8 × 10^{-12}** | |
| Nonlinearity transition, \( \sigma \) (s.d.) | 2.21 ± 0.49 (n = 27) | 1.03 ± 0.31 (n = 12) | 8 × 10^{-9}** | |

Statistical testing was performed using two-tailed unpaired Student’s t-test; significant differences are indicated by *p < 0.05 and **p < 0.005.

*Current-clamp (CC) recordings.

**STRFs for all events.

*STRFs for spikes.

**STRFs for large PSPs.

**STRFs for small PSPs.
well-matched, fast excitatory inputs. It also reflects distinct differences in what drives the excitatory and inhibitory inputs to a neuron. The low noise-level and high feature-selectivity of IPSCs indicates the dominance of a functionally more restricted pathway, such as via parvalbumin-expressing interneurons. By contrast, the low threshold values of EPSCs contribute some functional aspects only loosely related to the stimulus features reflected in the STRF. Such feature-independent inputs may be the result of the convergence of top-down inputs from higher-order cortical areas or other sensory areas, and may represent activity that encodes for higher-order, state-dependent and context-driven auditory functions including stimulus probabilities, predictive signaling, expectations, motivation, decision-making, memory and other task-related information.

Nonlinearity transition values for PSPs were relatively high and also resulted in noisier event trains compared to spikes. EPSC transitions were equal or higher than for PSPs. By contrast, IPSC transitions were even lower than for spikes, again reflecting input trains with low noise contamination. The threshold and transition differences between EPSCs and IPSCs are similar. But more pronounced are the first-order statistics for spikes with more excitatory and inhibitory neurons in cat A1. Both, nonlinearity threshold and transition, were negatively correlated, and control the degree of change in response specificity between input and output. Feature selectivity index was higher for neurons with lower event rates, higher thresholds, and lower transition values (Fig. 6c–g). Spiking events altogether have higher feature selectivity index than large PSPs but not small PSPs. EPSC and IPSC values were similar to those of the PSPs. Thus, both input–output transformations (from PSCs to PSPs to spikes) increase the signal-to-noise ratio and provide improved stimulus-feature selectivity, thereby reducing spurious stimulus-filter matches, and enhancing the ability to detect, transmit and eventually identify signals.

Tonal stimuli are short, onset-heavy and spectrally restricted, whereas DMRs are characterized by their long duration, relative lack of sharp onset features and broad frequency extent. These stimulus classes represent extremes along a continuum of properties including stimulus probabilities, predictive signaling, expectations, motivation, decision-making, memory and other task-related information.

Methods

Animal preparation. In vivo whole-cell recordings were obtained from neurons in A1 and A2 of mice (female C56BL/6 mice, Charles River) between 4 and 11 weeks of age. Mice were housed in standard cages (1–5 mice per cage) for 1–4 weeks. Mice were anaesthetized with ketamine (90 mg/kg) and xylazine (12 mg/kg) by intraperitoneal injection. This mixture was supplemented by one third of the initial dose to maintain the mouse anesthesia. Dexamethasone (5 mg/kg) and atropine (0.1 mg/kg) were administered to reduce brain swelling and atropine and salivary secretions, respectively. Lidocaine (2 mg/kg) was applied at surgical sites to relieve pain. Artificial tears were applied on the animal eyes. The animal was kept on a feedback-controlled heating pad to maintain the body temperature. The mouse head was fixed by attaching a metal head-frame to the skull with an adhesive material (C&B Metabond, Parkell). After the head-frame was secured, the cranium was removed by a small transversal incision over the stereotaxic coordinates of auditory cortical regions. The brain surface was covered with 2% agarose in saline after the dura was removed. Before we performed in vivo whole-cell recordings, multiunit recordings with a 1 MΩ tungsten electrode (MicroProbes) were obtained at a depth of ~400 μm to confirm tonotopic pro-
amplitude difference to nearby minima (~3 mV for current-clamp recordings; ~20 mV for temporal and SMF, respectively)19. The nonlinearities were calculated and parameterized following our previous approach19. Each stimulus segment, s, that preceded a spike was correlated with the STRF by projecting it onto the STRF via a trigger average analysis was used to calculate the STRF. Spikes and amplitude fluctuations whose effects were masked by using the complete subthreshold response without regard to the size of the amplitude fluctuation, we analyzed the subthreshold selectivity of each event type, we calculated a feature selectivity indexigraphic framework. We mitigated the signal masking that might occur if the complete amplitude range was used. Further, since we used events, we were able to apply, in a straightforward manner, classical event-triggered averaging techniques. Additionally, since the stimulus that we employed was globally uncorrelated, we were able to make unbiased receptive field estimates.

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Author contributions
K.X.K., C.A.A., and C.E.S. designed the study. K.X.K. conducted all experiments. K.X.K., C.A.A., and C.E.S. analyzed data and wrote the paper.

Competing interests
The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to K.X.K.

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