Gap encoding by parvalbumin-expressing interneurons in auditory cortex.

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Abstract

Synaptic inhibition shapes the temporal processing of sounds in auditory cortex, but the contribution of specific inhibitory cell types to temporal processing remains unclear. Here we recorded from parvalbumin-expressing (PV+) interneurons in auditory cortex to determine how they encode gaps in noise, a model of temporal processing more generally. We found that PV+ cells had stronger and more prevalent on-responses, off-responses, and post-response suppression compared to presumed pyramidal cells. We summarize this pattern of differences as "deeper modulation" of gap responses in PV+ cells. Response latencies were also markedly faster for PV+ cells. We found a similar pattern of deeper modulation and faster latencies for responses to white noise bursts, suggesting that these are general properties of on- and off-responses in PV+ cells rather than specific features of gap encoding. These findings are consistent with a role for PV+ cells in providing dynamic gain control by pooling local activity.

New & Noteworthy

Here we found that parvalbumin-expressing (PV+) interneurons in auditory cortex showed more deeply modulated responses to both gaps in noise and bursts of noise, suggesting that they are optimized for the rapid detection of stimulus transients.

Introduction

A fundamental task faced by the auditory system is the temporal processing of dynamic acoustic streams such as music and speech. Age-related speech processing deficits affect more than half of the population over 65. These speech processing deficits can occur even with completely normal audiometric hearing, and are instead associated with temporal processing deficits in the central auditory system (Helfer and Vargo 2009; Ben-David et al. 2011; Walton 2010). Temporal processing deficits are also correlated with developmental deficits in language and reading comprehension (Tallal, Stark, and Mellits 1985). Age-related loss of synaptic inhibition occurs at multiple levels of the auditory system, including the cochlear nuclei, inferior colliculus (IC), and auditory cortex (Caspary et al. 2008). This loss of inhibition is thought to be involved in temporal processing deficits (Caspary et al. 2008), but how inhibitory circuits contribute to temporal processing remains unclear.

Gap detection — the rapid detection of brief gaps in background noise — is a well-established model for temporal acuity, such as that required for phoneme discrimination. Age-related gap detection deficits are well-correlated with speech comprehension deficits (Glasberg, Moore, and Bacon 1987; Schneider et al. 1994; Fitzgibbons and Gordon-Salant 1996; Snell and Robert Frisina 2000). Inhibitory circuits in auditory cortex are clearly involved in gap detection, because optogenetic manipulation of either parvalbumin-expressing (PV+) or somatostatin-expressing interneurons enhances or impairs gap detection, depending on the sign and the timing of the manipulation (Weible, Moore, et al. 2014; Weible, Liu, et al. 2014). Yet it remains unclear
precisely how inhibition shapes cortical activity during gap detection, and whether specific inhibitory cell types have distinct roles in gap detection or temporal processing in general. No one has recorded from inhibitory interneurons during gap detection, so it is unknown whether or how they respond to gaps.

How might inhibitory interneurons contribute to temporal processing in auditory cortex? Here we focus on PV+ cells, the most prevalent class of cortical interneurons. PV+ cells appear to pool local excitatory synaptic input rather indiscriminately, which tends to broaden their tuning for stimulus features compared to pyramidal neurons (Kerlin et al. 2010; Hofer et al. 2011; Moore and Wehr 2013). PV+ cells also make fast, powerful synapses onto the soma, proximal dendrites, and axons of pyramidal neurons. These properties of both their input and output connectivity make PV+ cells likely candidates to provide cortical gain control. Cortical synaptic inhibition has also been shown to sharpen spike timing (Wehr and Zador 2003), which could improve temporal acuity by enhancing the temporal precision of gap encoding. Inhibition has also been proposed to shape gap responses by implementing a temporal comparison between ongoing and recent activity (Weible, Moore, et al. 2014). Finally, if PV+ cells are tuned for specific gap durations, they could confer gap duration tuning to pyramidal cells by suppressing responses away from the target cell’s preferred gap duration. To examine these possible roles, we recorded from cortical PV+ inhibitory interneurons in auditory cortex to characterize how they encode gaps and thereby contribute to temporal acuity.

Methods

All procedures were in accordance with the National Institutes of Health guidelines, as approved by the University of Oregon Institutional Animal Care and Use Committee.

Mice. We used optogenetic tagging to identify parvalbumin-positive interneurons that expressed Channelrhodopsin2 (ChR2) and responded to light (Lima et al. 2009; Moore and Wehr 2013). All mice were 7-12 weeks of age at the time of surgery (and 10-15 weeks at the conclusion of recordings), and were bred to the C57Bl6/J background strain. We used offspring (n = 9 total, n= 3 males, 4 females, 2 unknown) of a cross between homozygous Pvalb-IRES-Cre (“PV”, 008069; The Jackson Laboratory) and homozygous CAG-ChR2-eYFP (“ChR2”, 012569; Ai32, The Jackson Laboratory) lines. In these mice (PV x ChR2), ChR2 was expressed in parvalbumin-expressing (PV+) interneurons, with 97% specificity (Moore and Wehr 2013). All mice had normal startle responses and behavioral gap detection and showed no evidence of C57BL/6J age-related hearing loss, which is generally not seen until after 12 weeks of age (Ison et al. 2007).

Surgery. We implanted mice with an array of 8 tetrodes for recording as well as an optic fiber for laser activation of PV+ interneurons. We administered dexamethasone (0.1 mg/kg) and atropine (0.03 mg/kg) pre-operatively to reduce inflammation and respiratory irregularities. Surgical anesthesia was maintained with isoflurane (1.25–2.0%). The tetrode array was inserted vertically through a small craniotomy (2 mm x 1 mm) dorsal to the left auditory cortex, and cemented into place with Grip Cement (Dentsply, Milford, DE). For optogenetic excitation, a
single 200 μm optic fiber was implanted overlying auditory cortex. A thin coating of anti-bacterial
ointment was applied over the dura, and the whole area covered with Grip Cement. Ketoprofen
(4.0 mg/kg) was administered post-operatively to minimize discomfort. Mice were housed
individually after the surgery and allowed 7 days of post-operative recovery.

Stimulus delivery and data acquisition. All data were collected in a sound-attenuating chamber.
Mice were allowed to freely move about within a 15 cm diameter plastic arena with mouse litter
covering the floor. Sounds were delivered from a free-field speaker directly above the animal.
The speaker was calibrated to within ±1 dB using a Brüel and Kjær 4939 1/4-inch microphone
positioned horizontally in the center of the arena at a height of 2 cm above the arena floor
(mouse ear height), without the animal present. Sound intensity varied by no more than 8.5 dB
at different positions around the arena floor. To identify PV+ interneurons, we presented pulses
of blue light (100 ms duration, 445 nm wavelength, 5-30 mW total power, corresponding to 160-
950 mW/mm² irradiance measured at the fiber tip). Pulses were presented interleaved with 50
ms white noise (WN) bursts and silent periods with an intertrial interval of 500 ms. We then
tested responses to WN bursts of various durations (1 - 256 ms) at 70 dB SPL. WN bursts were
presented every 1000 ms in random order and repeated 10-30 times. To test responses to
gaps, silent gaps of duration 1-256 ms were inserted into continuous background white noise
(80 dB SPL). Each gap duration was presented in random order, 15-30 repetitions, separated
by an intertrial interval of 1 s. Sound onsets and offsets for WN bursts and gap stimuli (including
gap onsets and terminations) had rise and fall times of zero. Note that this did not introduce any
spectral splatter because of the white noise carrier.

Single neuron recording. We implanted an array of 8 tetrodes passed through a 1 X 4 array of
28 gauge stainless steel hypodermic tubing, with 2 tetrodes per tube. Tetrodes were made of 18
μm (25 μm coated) tungsten wire (California Fine Wire). The entire array was mounted on a
custom microdrive. Tetrode data were acquired with 32-channel RH2000 hardware (Intan
Technologies) and Open Ephys software (http://open-ephys.org). A minimum threshold of 50 μV
was set for collection of spiking activity. Spiking activity of individual neurons was isolated offline
using the open source spike sorting software packages Simpleclust
(http://jvoigtsscripts.mit.edu/blog/simpleclust-manual-spike-sorting-in-matlab) and MClust
(http://redishlab.neuroscience.umn.edu/MClust/MClust.html). Measures of peak and trough
waveform voltage, total energy, and first principal component were used as waveform
separation parameters in 2-dimensional cluster space. Cells were accepted for analysis only if
they had a cluster boundary completely separate from adjacent cluster boundaries, and
completely above threshold, on at least one 2-D view.

Data analysis and statistics. We characterized neuronal responses to laser pulses, white noise
stimuli and gap-in-noise stimuli. Spike width was measured from 20% of the peak to 20% of the
trough (Figure 1D inset). Latencies to the half-maximum stimulus-evoked firing rate were
calculated from PSTHs of spiketimes accumulated by repeated presentation of WN bursts or
gaps in noise and smoothed by convolution with a Gaussian (sigma = 6 ms), as described in
Moore and Wehr (2013). Population-averaged PSTHs across cells for each stimulus were
obtained from the baseline-subtracted PSTHs after normalization to the maximum firing rate for
that cell across all stimuli. For comparison between responses to stimuli of differing durations, we binned spiking data at 5 ms. We quantified excitatory responses as the spike count in a 50 ms window immediately following sound onset or sound offset, and we quantified suppressive responses in a window from 70 to 140 ms after onset or offset. Note that for short gaps these time periods overlap, which provides information about temporally overlapping responses to sound offset and onset. We tested for significant gap responses using a paired t-test, comparing spike counts during a given response window to spike counts on interleaved trials without a gap. Similarly, we tested for significant WN responses using a paired t-test, comparing spike counts during a given response window to spike counts in a silent window of the same duration prior to the stimulus. A cell was only considered to have a response if the same sign of significant change occurred for two consecutive durations. For example, a cell with a significant excitatory response to the onset of a 4 ms gap must have also exhibited a significant excitatory response following the onset of either the 2 ms or 8 ms gap, following the criterion established by (Walton, Barsz, and Wilson 2008). We used the Kolmogorov-Smirnov goodness-of-fit hypothesis test to compare latency distributions, the $\chi^2$ test to compare percentages of cells responsive under differing conditions, and the Wilcoxon rank-sum test to compare tuning widths. A significance criterion of $p < 0.05$ was established for each of these tests before the analyses. We calculated Pearson correlation coefficients to compare tuning within and between tetrodes. All statistical tests were carried out in MATLAB (R2015b, the Mathworks).

**Histology.** All brains were sectioned coronally. We verified that optic fibers and recording tetrodes accurately targeted auditory cortex using the structure of the hippocampus and the rhinal fissure as rostrocaudal and dorsoventral landmarks (section thickness: 100 μm).

**Results**

We isolated and characterized responses from 627 neurons in the auditory cortex of 9 PV-ChR2 mice. Of these, 187 neurons (30%) responded with short latency spiking to 100 ms pulses of blue laser light, indicating that they were parvalbumin-positive (PV+) interneurons (Lima et al. 2009; Moore and Wehr 2013). PV+ cells generally responded to light in two distinct ways — either with a transient response (Figure 1A, left panel) or a sustained response (Figure 1A, right panel). To investigate these differences further, we separated ‘transient’ from ‘sustained’ neurons (Figure 1B) by comparing their firing within the first 25 ms of photostimulation to firing within the third 25 ms of photostimulation (Fig. 1B shows the log of this ratio). Cells showing sustained responses (light red dots in Figure 1B; 162 of 187 PV+ cells or 87%) generally had log-ratio values around zero (i.e. their firing remained roughly constant), whereas transient neurons (dark red dots enclosed within an ellipse in Figure 1B; 13%) showed more negative values (after an initially strong response to photostimulation, their firing diminished greatly).

We next looked at the spike waveform for each cell. We found that spike waveforms generally fell into two distinct categories: broad spikes and narrow spikes, similar to previous reports (Moore and Wehr 2013; Kawaguchi and Kubota 1997; Markram et al. 2004). As expected from previous work, nearly all transient and sustained PV+ cells had narrow spikes. Most PV- cells...
fired broad spikes, but a subset (13%) had narrow spikes. Thus we initially examined 4
categories of cells (transient PV+, sustained PV+, broad spiking PV-, and narrow spiking PV-) to
determine if they had any further distinguishing characteristics. Figure 1C shows the spike
amplitude ratio (ratio of the magnitudes of the spike peak and trough) plotted against the spike
width. Consistent with previous reports, PV+ interneurons of both the 'transient' and 'sustained'
subgroups had relatively short duration spikes (dark and light red dots/curves in Figs. 1B-E),
with a mean spike width of 0.55 ms (+/- 0.015 ms s.e.m.) and 0.57 ms (+/- 0.008 ms),
respectively (Fig. 1C-E). In contrast, the majority of cells unresponsive to photostimulation
showed broader spikes (383 PV- 'broad', 61% of total cells, green dots and curves), with a
mean spike width of 0.74 ms (+/- 0.004 ms). Figure 1C, however, shows that a subset of PV-
neurons (57 PV- 'narrow', 9% of total cells) had narrow spike widths (0.56 ms +/- 0.009 ms)
similar to those of PV+ interneurons (orange dots enclosed within the ovoid of Figure 1C). The
average spike waveform for each category of cell (Fig. 1D) demonstrates the clear similarity of
waveforms between PV+ 'sustained', PV+ 'transient' and PV- 'narrow' putative cell types, and
their separation from the average waveform of the PV- 'broad' cell type.

PV+ interneurons are also known as 'fast spiking' cells because of their capacity for short
latency responses and sustained high spike rates (Markram et al. 2004). Average interspike
interval histograms for each cell type (Figure 1F) and plots of their spontaneous firing rates
(Figure 1G) show that PV+ 'sustained', PV+ 'transient' and PV- 'narrow' cells all fired at similarly
high rates compared to PV- 'broad' cells. In summary, we found no response differences
between transient and sustained PV+ cells (other than their response to light). Similarly, when
we compared the sound-evoked responses (to WN bursts or gaps in noise) of these two
subcategories of PV+ cells, we found that they were indistinguishable (as described below).
Therefore, in all subsequent figures, we pool 'transient' and 'sustained' PV+ interneurons into a
single PV+ group (in red). In contrast, PV- 'narrow' and PV- 'broad' cell types were clearly
differentiable in both their firing properties and their responses to WN bursts and to gaps in
noise, and we therefore plot them separately in the figures to follow (PV- 'narrow' in orange and
PV- 'broad' in green). Indeed, apart from being non-responsive to light, PV- 'narrow' cells were
nearly indistinguishable from PV+ cells both in their firing properties and their sound-evoked
responses.

Gap encoding

We presented mice with continuous white noise that was periodically interrupted by brief gaps
ranging in duration from 1 to 256 ms (in octave steps), as well as a control condition without a
gap. Most neurons responded with a brief burst of spikes both at the sound offset (start of the
gap) and at sound onset (gap termination). Figure 2A shows an example of a PV+ neuron
responding to gaps. Each horizontal panel shows the response to one gap duration (indicated to
the left within each panel). Sound offsets and onsets are denoted by vertical red lines, with
responses aligned to sound onset (gap termination). Excitatory response windows (50 ms
duration) are color-coded for off-responses (red) and on-responses (green). For this cell, both
128 and 256 ms gaps evoked robust and separable excitatory responses to both sound offset
and sound onset. Figure 2B shows the gap duration tuning curve for this cell, based on spike
counts within each response window. Filled circles indicate responses that were significantly
different from the ‘no gap’ firing rate (p < 0.05, paired t-test). Although both off- and on-
responses were robust and separable at long gap durations, for gaps ≤ 64 ms these responses
overlapped in time, so that for shorter gaps it is not possible to assign spikes as having been
evoked by offset or onset. However, spikes following sound onset have been shown to play a
role in gap detection behavior (Weible, Moore, et al. 2014) and thus, for shorter gap stimuli (<32
ms), we report these spikes following sound onset with the understanding that they may result
from both sound offset and onset. This cell had significant on-responses for all durations ≥ 4
ms. This cell also showed a prominent suppression of spiking following the on-response
(analyzed in a 70-140 ms window after sound onset; blue curve in Fig. 2B).

Both PV+ and PV- ‘narrow’ cells were more responsive to gaps than were PV- ‘broad’ cells. A
higher percentage of PV+ and PV- ‘narrow’ cells showed significant responses to at least one
gap duration (Figure 2C) when compared to PV- ‘broad’ cells. Excitatory responses to sound-
offset for 256 ms gaps (for which responses to offset and onset could be clearly discriminated)
were significantly more common for PV+ and PV- ‘narrow’ cells (PV+ vs. PV- ‘broad’: p=0.0002,
PV- ‘narrow’ vs. PV- ‘broad’; p=0.0003, \(\chi^2\) test). Cells showing suppression following these
excitatory responses were less common overall, but still more common amongst PV+ and PV-
‘narrow’ cells (PV+ vs. PV- ‘broad’: p=0.005). Many more cells of all three cell classes showed
responses to sound onset (Figure 2C, right panel). As with responses to sound offset, a higher
percentage of PV+ and PV- ‘narrow’ cells showed significant excitatory and suppressive
responses to sound onset (excitation: PV+ vs. PV- ‘broad’: p=0.007, PV- ‘narrow’ vs. PV-
‘broad’: p=0.02; suppression: PV+ vs. PV- ‘broad’: p<0.00001, PV- ‘narrow’ vs. PV- ‘broad’:
p<0.00001).

Considering cells with significant responses to brief gaps in noise, PV+ and PV- ‘narrow’ cells
showed a more strongly modulated response than did PV- ‘broad’ cells. Population average
responses to gaps of 256 ms (Figure 2D, top panel) clearly showed strong off- and on-
responses and some degree of suppression for all 3 cell types. However, whereas PV+ (red)
and PV- ‘narrow’ (orange) cells showed very similar, deeply-modulated response profiles, PV-
‘broad’ (green) cells showed relatively weaker off- and on-responses and negligible suppression
following the on-response. For shorter gaps (8 ms, shown in bottom panel), off- and on-
responses overlapped in time, but showed much the same between-cell class pattern as for
responses to longer gaps.

For all three cell types, the latencies of firing in response to gaps were broadly distributed
across cells. However, PV+ and PV- ‘narrow’ cells had significantly shorter latencies to sound
onset (Figure 2E, right panel) than did PV- ‘broad’ cells (p<0.01 Kolmogorov-Smirnov
goodness-of-fit hypothesis test). Latencies to sound offset for all three cell types were broadly distributed (Figure 2E, left panel).

Figure 2F summarizes how on-responses change with gap duration, illustrating the deeper modulation of PV+ and PV- ‘narrow’ cells. The excitatory on-responses (y-axis) are plotted against the suppressive responses (x-axis) for each cell class and each gap duration. Gaps of only one or two milliseconds elicit weak excitatory on-responses with no following suppression, in all three cell classes. Gap durations of 4 or 8 ms already show stronger excitation of PV+ and PV- ‘narrow’ cells, but still little or no suppression. With gap durations of 16 ms or longer, PV+ and PV- ‘narrow’ cells show increasing suppression and a plateaued excitatory response, where PV- ‘broad’ cells reach a slightly lower peak response without suppression. The strongest excitatory responses belong to the PV- ‘narrow’ cell class.

Across the population, a significantly higher percentage of PV+ and PV- ‘narrow’ cells responded to short gaps (Figure 3A; filled circles indicate a significant difference from PV- broad). Similarly, PV+ and PV- ‘narrow’ cells had shorter preferred gap durations than PV- broad cells (Figure 3B). Previous studies have shown that PV+ cells are more broadly tuned than PV- cells for some stimulus features, such as orientation (in mouse visual cortex), but not for other features such as frequency (in auditory cortex) (Moore and Wehr 2013; Kerlin et al. 2010). This is thought to arise from local pooling of inputs by PV+ cells, and thus to depend on whether neurons in the local neighborhood show heterogenous tuning (as they do for orientation) or similar tuning (as they do for frequency). To examine this for gap duration tuning, we first measured tuning width. For each cell, we aligned the gap duration tuning curve to the preferred gap duration, and then computed population-averaged tuning curves. We found that PV+ cells were slightly but significantly more broadly tuned to gap duration than PV- ‘broad’ cells (Figure 3C, width at half-height: 3.5 octaves (PV+) versus 2.9 octaves (PV- broad), rank-sum p=0.014). To examine whether gap duration tuning of neighboring neurons is similar or heterogeneous, we asked whether the preferred gap duration of a given cell had any predictive value about the preferred duration of neighboring cells recorded on the same tetrode. Preferred gap duration was uncorrelated with that of neighboring cells (r=0.03, p=0.84, n=39 simultaneously recorded cells), suggesting that gap duration tuning is heterogeneous among the local population.

Noise burst encoding

Are these response differences among cell classes specific to gaps, or are they general features of on- and off-responses? In order to test the generality of on- and off-responses for different cell classes, we presented each cell with white noise bursts of varying duration (1 - 256 ms, in octave steps). Overall, responses to gaps in noise and to WN bursts were quite similar for each cell class despite the difference in temporal structure of the two stimulus types. Latency distributions revealed faster response times for PV+ and PV- ‘narrow’ cells compared to PV- ‘broad’ cells (Figure 4A; p<0.01 KS-test). Significantly more PV+ and PV- ‘narrow’ cells showed excitatory responses to sound onsets (Figure 4B, left; p<0.05 χ² test) when considering all burst durations. Similarly, more PV+ and PV- ‘narrow’ cells showed excitatory responses to sound
offsets, considering only longer (128, 256 ms) durations in which sound onset and sound offset
responses were clearly separable (Figure 4B, right; p<0.05 $\chi^2$ test). For each duration tested,
we found a significantly greater proportion of PV+ and PV- ‘narrow’ cells to be responsive to
sound onsets (Figure 4C, left) compared with PV- ‘broad’ cells. Suppressive responses
following the initial excitatory response were also significantly more common in PV+ and PV-
‘narrow’ cells (Figure 4C, right) for longer (128, 256 ms) burst durations.

We found that PV+ and PV- ‘narrow’ cells had higher spontaneous firing rates than PV- ‘broad’
cells (Fig. 1G), and also that they showed stronger and more prevalent suppressive responses
(Fig. 4B). We wondered whether the stronger suppression we observed for PV+ cells could be a
trivial consequence of the higher spontaneous rate, because suppression can only be detected
as a reduction below the spontaneous rate. To test this, we used an analysis of covariance to
ask how the strength of suppression depended on spontaneous firing rate and cell type. In
addition to significant effects of cell type (F=17, p=3.9x10$^{-5}$) and spontaneous rate (F=178,
p=1.7x10$^{-35}$), we found that there was a significant interaction between cell type and
spontaneous rate (F=32, p=1.9x10$^{-8}$), indicating that the stronger suppression seen in PV+ cells
is cell-type specific and not simply explained by their higher spontaneous rate.

As with responses to gaps in noise, population-averaged responses to WN bursts were more
strongly modulated for PV+ and PV- ‘narrow’ cells than for PV- ‘broad’ cells (Figure 5A).
Responses to sound onset and offset were easily separable during 256 ms WN bursts (Figure
5A, left), and the population-averaged responses of PV+ and PV- ‘narrow’ were quite similar to
each other. They each showed stronger excitatory responses to both sound onset and sound
offset than did PV- ‘broad’ cells. PV+ cells showed significant suppression following the
excitatory response to sound onset, whereas PV- ‘narrow’ cells showed weaker suppression,
and PV- ‘broad’ cells showed no suppression. On- and off-responses began to merge as the
burst duration shortened. At intermediate durations (Figure 5A, middle panels), temporal
summation of on-evoked suppression with off-evoked excitation appeared to weaken PV+
responses more than it did PV- ‘narrow’ responses. At short burst durations (4 ms, Figure 5A
right panel), responses of PV+ and PV- ‘narrow’ cells were indistinguishable, with strong
excitatory responses followed by weak suppression. By comparison, PV- ‘broad’ responses
were much weaker.

Despite the differences between cell classes in the percentage of cells with significant excitatory
responses to burst offset, all three cell classes had similarly broad distributions of ‘best’
excitatory durations (Figure 5B). Tuning width for noise bursts was no different across cell types
(Figure 5C, rank-sum test p> 0.10). Preferred burst duration for a given cell was uncorrelated
with that of neighboring cells recorded on the same tetrode (r=-0.05, p=0.76, n=35).

Discussion

Here we recorded from PV+ interneurons in auditory cortex to determine how they encode gaps
in noise, a model of temporal processing more generally. We found that, in response to gaps,
PV+ cells have stronger and more prevalent on-responses, off-responses, and post-response suppression compared to PV- cells with broad spikes, which we presume to be pyramidal neurons (PNs). We summarize this pattern of differences as "deeper modulation" of gap responses in PV+ cells. Compared to PNs, PV+ cells were more likely to respond to short gaps, and had shorter preferred gap durations. Response latencies were also markedly faster for PV+ cells, consistent with previous findings (Moore and Wehr 2013; Atencio and Schreiner 2008). We found a similar pattern of deeper modulation and faster latencies for responses to white noise bursts, suggesting that these are general properties of on- and off-responses in PV+ cells rather than specific features of gap encoding. These findings are consistent with a role for PV+ cells in providing dynamic gain control by pooling local activity. Although gain control is important for temporal processing, these results argue against any specialized role of PV+ cells in gap detection.

Synaptic inhibition has been proposed to play a number of functional roles in temporal processing. Based on the effects of optogenetic manipulations on gap detection behavior, we recently proposed a model in which cortical inhibitory interneurons contribute to gap detection by performing a temporal comparison between ongoing and recent cortical activity (Weible, Moore, et al. 2014). This model predicts that gap responses of inhibitory interneurons should resemble low-pass filtered responses of PNs — that is, interneuron gap responses should be slower and more prolonged than those of PNs. The gap responses of PV+ cells are clearly inconsistent with this model, because they had faster latencies than PNs, and had population responses no more prolonged than those of PNs. More generally, inhibition has been proposed to sculpt the tuning of target cells by suppressing responses to non-preferred stimuli. Although we found that PV+ cells were tuned for specific gap durations, we found no evidence to support the idea that they confer gap duration tuning to PNs. The preferred gap duration of a PV+ cell had no predictive value about the tuning of neighboring PNs recorded on the same tetrode.

PV+ cells have been widely proposed to provide dynamic gain control for the cortical network, by non-selectively pooling input from local excitatory neurons (Kerlin et al. 2010; Hofer et al. 2011; Atallah et al. 2012; Moore and Wehr 2013). PV+ cells receive dense input from nearby excitatory cells (Thomson and Lamy, 2007), and their tuning for stimulus features approximates the average tuning of the local network. Thus PV+ cells in auditory cortex are well-tuned for frequency, because local neurons share similar frequency tuning, whereas PV+ cells in visual cortex are quite broadly tuned for orientation, because they average across local neurons with heterogeneous orientation tuning (Zariwala et al. 2011; Hofer et al. 2011; Kerlin et al. 2010; Moore and Wehr 2013). Here we found that PV+ cells were slightly but significantly more broadly tuned for gap duration than PV- cells. This result is consistent with local pooling by PV+ cells, which therefore predicts that the preferred gap duration tuning of local excitatory cells should be heterogeneous. Indeed, we found that the preferred gap duration of a given cell had no predictive value about the tuning of neighboring cells recorded on the same tetrode. Together with a growing body of work in visual, somatosensory, and auditory cortex, these results suggest that PV+ neurons pool input from the local network, consistent with a role in providing dynamic gain control. We found that PV+ cells respond faster and more strongly to transient inputs than presumed PNs, indicating that the gain control they provide is both rapid
and powerful, in striking contrast to the slower time scale of adaptation that would be involved in
a temporal comparison between ongoing and recent cortical activity.

We found that PV+ cells (and PV- cells with narrow spikes) had markedly faster response
latencies than PNs, as well as stronger and more prevalent driven responses, and that these
were true for responses to either gaps or noise bursts. These findings agree with previous
studies of PV+ and fast-spiking cells, and likely arise from both faster membrane time constants
(Cardin, Palmer, and Contreras 2007) as well as powerful thalamocortical and intracortical
synaptic drive (Swadlow 2003; Hofer et al. 2011). Although we did not measure the frequency
tuning of PV+ cells in this study, previous work has shown that they have similar width of
spectral tuning as non-PV cells (Moore and Wehr 2013), indicating that their greater sensitivity
to broadband stimulus transients is not explained by broader spectral tuning. Rather, these
response properties appear to be general features of PV+ cells, and suggest that they are
optimized for the rapid detection of stimulus transients, and thereby provide rapid feedforward
inhibition to PNs. This fast and powerful inhibition has been shown to enhance the temporal
precision and reliability of PN spiking in auditory cortex (Wehr and Zador 2003). Such precise
and reliable spiking responses have a considerable impact on gap detection, because gap
responses in auditory cortex make a critical contribution to the perceptual detection of brief gaps

We found that PV+ cells had narrow spike waveforms compared to PV- cells, although the spike
width distributions were partially overlapping, in agreement with previous work (Atencio and
Schreiner 2008; Niell and Stryker 2008; Wu et al. 2008; Runyan et al. 2010; Moore and Wehr
2013). A small subset of PV- cells (13%) also had narrow spikes, and these cells were
indistinguishable from PV+ cells in every aspect we tested (both sensory and biophysical). Thus
the meaningful categories here were narrow-spiking cells (PV+ and PV-) and broad-spiking cells
(PV-, presumed PNs). The identity of PV- narrow-spiking cells is not clear, although it’s possible
that they are PV- basket cells, which might express somatostatin (for review, see Markram et al.
2004; Yavorska and Wehr 2016). Although no previous studies have investigated gap encoding
in inhibitory neurons (whether identified genetically or by spike waveform), it’s important to note
that previous findings have not always agreed about frequency tuning width (Moore and Wehr
2013; Atencio and Schreiner 2008; Li et al. 2015). Those differences are likely due to laminar
differences and the genetic or waveform criteria used to categorize cells, and could very likely
be influenced by anesthesia. A strength of the chronic tetrode approach we used here is that it
permits recordings from awake mice, but a limitation is the lack of reliable laminar depth
information. These factors will be important to consider when comparing to future work.

We found that late, sound-evoked suppression was stronger in PV+ and PV- ‘narrow’ cells than
PV- ‘broad’ cells. The latency of this suppression was too long for it to affect the initial,
excitatory gap responses of PV+ or other cells, but it could affect temporal processing for more
prolonged or repetitive signals (such as amplitude-modulated noise, click trains, or prolonged
natural sounds). This suppression could be caused by synaptic depression following the
synaptic release that drives excitatory responses (Wehr and Zador 2005). Alternatively, it could
be due to network suppression mediated by somatostatin-expressing interneurons originating
from distant frequency-tuned areas that are activated by the white noise transients (Kato et al. 2017). This suppression may not be evident in recordings from anesthetized animals, which generally show low levels of spontaneous activity, because suppression may only be revealed by high spontaneous rates typically seen in awake animals. This suppression could enhance signal-to-noise in either the temporal domain or the spectral domain, which could be especially important for the processing of temporally or spectrally complex signals.

The picture that emerges from these results is that PV+ cells are optimized to encode rapid stimulus transients, and thereby play a fundamental role in controlling the gain and timing of cortical spiking responses. This appears to be generally true for responses to gaps in noise, noise bursts, tones, and other sounds. Thus, despite the absence of any specific role in gap detection, PV+ cells clearly have a great impact on temporal processing. A loss of PV+ cells or a reduction in their synaptic efficacy would be expected to lead to a corresponding loss of dynamic gain control and temporal precision, which would in turn cause deficits in temporal processing in general and gap detection in particular. A growing body of work has shown that hearing loss reduces cortical inhibitory function, whether due to acoustic trauma, conductive hearing loss, or age (Scholl and Wehr 2008; Caspary et al. 2008; Martin del Campo, Measor, and Razak 2012; Takesian, Kotak, and Sanes 2012). It seems plausible that these effects, and the resulting deficits in gap detection and speech comprehension, could be mediated specifically by impairments to PV+ cell function.

Figure legends

Figure 1

Identification of cell types. A) Examples of transient (left) and sustained (right) PV+ responses to laser. Upper raster plot is summarized below as a peri-stimulus time histogram. Illumination (100 ms) indicated by blue shading. Insets: spike waveform of each cell. B) For each PV+ cell, the persistence of spiking throughout the duration of the laser pulse is plotted against the cell's spontaneous spike rate. "Persistence" is quantified as the log (signal-to-noise-ratio (SNR) for the third 25 ms after laser onset / SNR for the first 25 ms after laser onset). We clustered PV+ cells into two subsets: transient (dark red dots within black ellipse) and sustained (light red dots). C) Spike peak-to-trough amplitude ratio plotted against peak-to-trough width. Inset shows how spike width was measured. A subset of "narrow" spiking PV- cells (orange dots within black ellipse) that overlapped with the main cluster of PV+ cells was identified for further analysis. D) Average spike waveform (solid lines: mean across cells; s.e.m. shown with lighter shading) for each of the identified cell types. Each cell's average waveform was normalized before averaging across cells. E) Distribution of spike widths for each cell type. In C,D,E, only cells with spikes recorded with a leading positive phase were included for analysis. F) Average interspike interval histogram (solid lines; s.e.m. shown with lighter shading) for each cell type. Histograms were
normalized for each cell before averaging. G) Histograms of spontaneous firing rate for each cell type. Number of cells for each analysis (provided in each panel) varied slightly after screening for insufficient data.

Figure 2

PV+ cells show more deeply modulated gap responses. A) Raster and PSTH plots for an example PV+ cell’s responses to gaps (delimited by red vertical lines) with durations 1-256 ms (red numbers at left), aligned to gap termination. Red shading indicates the excitatory ‘off-response’ analysis window, green indicates the excitatory ‘on-response’ analysis window. B) Gap duration tuning curve of the cell shown in A) for off-responses (red), on-responses (green) and a window 70 to 140 msec following the on-response, where suppression is often seen (blue). Solid circles indicate significant differences from spontaneous (dotted line). Off-responses are only shown for gaps >=32 ms where they are separable from on-responses. C) Following sound offset (left panel, only 256 ms gaps) or sound onset (right panel, all gap durations), percentage of each cell type showing significant excitatory responses within 0-50 ms and/or suppressive responses from 70-140 ms. Brackets above histograms indicate significant differences (p < 0.05, \( \chi^2 \) test). D) Population-averaged PSTHs for each cell type to a 256 ms gap (top panel) or 8 ms gap (bottom panel), compiled from all cells with a significant (excitatory) response to any gap duration. All cells with significant responses were included (numbers of cells are in parentheses). Shading denotes the standard error of the mean (s.e.m.). Black horizontal bar denotes white noise background, red vertical lines indicate the gap beginning and end. Before averaging, a cell’s PSTH with spontaneous level subtracted was normalized to its maximum response across all gap durations. E) PV+ and PV- ‘narrow’ cells had shorter response latencies for gaps in noise. We measured latencies as time from sound offset (left) or sound onset (right) to half peak-height for 128 and 256 ms gaps (combined), and smoothed with gaussian convolution (\( \sigma=5 \) ms). All cells with significant responses and peak responses within 128 ms of sound offset (or onset) were included. F) For each cell type and each gap duration, the mean (+/- s.e.m.) excitatory on-response (0-50 ms after sound onset) is plotted against post-response suppression (70-140 ms after sound onset). Lightly shaded arrows show the trajectory of change in the means as gap duration lengthens. For reference, the off-responses for 256 ms gaps are shown with dotted lines (with excitatory response and suppression calculated similarly as for on-responses).

Figure 3

Tuning of on-responses to gap duration for each cell class. A) Percentage of cells with significant on-responses to each gap duration (filled circles: p<0.05 significantly different from PV- broad, \( \chi^2 \) test). B) Distribution of best gap durations for cells that had significant on-responses (filled circles: p<0.10 significantly different from PV- broad, \( \chi^2 \) test). C) Population-averaged gap duration tuning curves. Duration tuning curves for excitatory (left) or suppressive (right) responses were aligned to each cell’s best gap duration (solid line: mean; shaded area: standard error of the mean). Baseline firing rates correspond to z-scores of zero. Gap durations are represented in octaves from best gap duration (i.e. in log_2 units of the duration in ms).
Figure 4
Responses to white noise bursts. A) Latency from stimulus onset to the time when firing first reached half the height of the PSTH peak. Latencies were compiled across cells and burst durations with significant responses. B) Percentage of cells with significant on-responses (left panel) or off-responses (right panel) to white noise bursts to any of the tested durations. Bars above histograms indicate significant differences ($p < 0.05$, $\chi^2$ test). C) Percentage of cells with significant excitatory (left panel) or suppressive (right panel) on-responses for each burst duration (filled circles indicate significant difference from PV-broad cell response, $p < 0.05$ $\chi^2$ test).

Figure 5
Tuning of responses to white noise burst duration. A) Normalized post-stimulus time histograms to white noise bursts averaged across cells that showed significant responses (shaded area shows s.e.m.) to this burst duration. Four burst durations are shown (duration is shown in upper left of each panel). The stimulus is indicated by the black horizontal bar. B) Distribution of best durations for cells with significant excitatory responses to burst offset. Note that responses to durations $\leq 32$ ms include spikes evoked by both sound onset and offset. Responses to 32 and 64 ms bursts include both excitatory responses and suppressive responses evoked by sound onset. Responses to 128 and 256 ms bursts include only off-responses. The percentage of cells responding to a given duration were not significantly different across cell types (all $p > 0.05$, $\chi^2$ test).


Figure 1
Figure 2

A. PV+ Responses to Gaps in Noise (cell #9)

B. Gap Duration Tuning (cell #9)

C. Percentage Cells with Significant Responses

D. Average PSTH to 256 ms Gap

E. Latency to Gaps

F. On-response Excitation vs Suppression (mean +/- s.e.m.)
Figure 3
Figure 4
Figure 5