Title: Complementary control of sensory adaptation by two types of cortical interneurons.

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Abstract:
Reliably detecting unexpected sounds is important for environmental awareness and survival. By selectively reducing responses to frequently, but not rarely, occurring sounds, auditory cortical neurons are thought to enhance the brain’s ability to detect unexpected events through stimulus-specific adaptation (SSA). The majority of neurons in the primary auditory cortex exhibit SSA, yet little is known about the underlying cortical circuits. We found that two types of cortical interneurons differentially amplify SSA in putative excitatory neurons. Parvalbumin-positive interneurons (PVs) amplify SSA by providing non-specific inhibition: optogenetic suppression of PVs led to an equal increase in responses to frequent and rare tones. In contrast, somatostatin-positive interneurons (SOMs) selectively reduce excitatory responses to frequent tones: suppression of SOMs led to an increase in responses to frequent, but not to rare tones. A mutually coupled excitatory-inhibitory network model accounts for distinct mechanisms by which cortical inhibitory neurons enhance the brain’s sensitivity to unexpected sounds.

Impact Statement: We discover that two distinct types of inhibitory neurons increase the brain’s sensitivity to unexpected acoustic signals by amplifying selective suppression of cortical responses to frequent, but not rare sounds.
Introduction

Across sensory modalities, cortical neurons exhibit adaptation, attenuating their responses to redundant stimuli\textsuperscript{1-5}. Adaptation to stimulus context is thought to increase efficiency of sensory coding under the constraints of limited resources\textsuperscript{6}. Yet the neuronal-circuit mechanisms that facilitate adaptation in the cortex remain poorly understood. In the primary auditory cortex (A1), the vast majority of neurons exhibit stimulus-specific adaptation (SSA, Figure 1). When presented with a sequence of two tones, one of which occurs frequently (termed “standard”) and another rarely (termed “deviant”), the neuron’s response to the standard becomes weaker, but the response to the deviant remains strong\textsuperscript{2, 7-9}. Whereas SSA has also been found in sub-cortical structures, e.g. in the auditory midbrain\textsuperscript{10-12} and the auditory thalamus\textsuperscript{13-16}, it is weak in the lemniscal areas, which project to A1, and stronger in those non-lemniscal areas that receive feedback from A1\textsuperscript{17-19}. Therefore, cortical circuits are proposed to contribute to and amplify SSA in A1\textsuperscript{2, 7, 9, 16, 20}, through a combination of plastic modulation of thalamocortical inputs and intra-cortical inhibitory circuits, which would allow for selective suppression of neuronal responses to specific stimuli\textsuperscript{21}. Our study tests whether and how inhibitory neurons contribute to cortical SSA.

Auditory cortex, like other sensory cortices, contains morphologically and physiologically diverse inhibitory interneurons, which form dense interconnected networks with excitatory neurons\textsuperscript{22, 23}. While different interneuron types have been hypothesized to carry out specialized complementary functions in sensory processing\textsuperscript{22, 24-27}, their function in driving changes in dynamic auditory processing has not been previously established. We hypothesized that the two most common types of interneurons in the cortex, parvalbumin- (PVs) and somatostatin-positive cells (SOMs)\textsuperscript{28, 29}, facilitate SSA in excitatory neurons of A1 in a complementary fashion. PVs, a subset of which receive direct thalamic inputs\textsuperscript{30}, may amplify SSA in excitatory neurons by providing a constant inhibitory drive; equally strong inhibitory drive would attenuate the weak response to standard tones relatively more than the strong response to deviant tones, leading to a greater differential between standard versus deviant tone spiking response. SOMs, which target distal dendrites of pyramidal cells\textsuperscript{31, 32}, have excitatory synapses that exhibit facilitation upon repetitive stimulation\textsuperscript{33, 34}. Therefore, inputs from
SOMs may exert a stimulus-specific increase in suppression of excitatory neurons that is selective to
the standard and does not generalize to the deviant. Alternatively, they may contribute to selective
adaptation in excitatory neurons through differential post-synaptic integration.

To tease apart the function of different inhibitory types in SSA, we tested whether optogenetic
suppression of either PV or SOM interneurons during sound presentation reduced SSA in putative
excitatory neurons in the auditory cortex\(^{35-37}\). We found that both types of interneurons contribute to
SSA in the cortex, with PVs providing constant inhibition, and SOMs increasing their effect with
repeated tones.

Results

Nearly all neurons in A1 exhibit SSA

We recorded spiking activity of neurons as well as local field potentials in A1 in head-fixed
mice under light isoflurane anesthesia. SSA was measured from the firing rate of neurons in response
to tones presented as a series of “oddball” stimuli. Each oddball stimulus consisted of a sequence of
tone pips at one of two frequencies (tones A and B). In each oddball stimulus, one tone was
presented as the rare (deviant) tone while the other was presented as the frequent (standard) tone (A
to B ratio of 90:10 or 10:90, Figure 1a). A third stimulus was also presented (equal stimulus), with
tones A and B being presented equally often (50:50). The frequencies of tone A and B were selected
at 0.39 octave interval, below the typical tuning bandwidth of A1 neurons\(^{38-40}\), such that they activated
the majority of recorded neurons on each session (Figure 1b).

As expected, for a representative neuron recorded in A1, the mean firing rate (FR) in response
to a tone was lower when the tone was presented as the standard than as the deviant (Figure 1c),
exhibiting SSA. To quantify the level of adaptation for each neuron, we computed the index of the
change in FR to the same tone when it was presented as the deviant versus the standard (SSA
index). SSA index is 1 when adaptation is complete (i.e. no response to the standard, and significant
response to the deviant), and 0 when there is no adaptation (i.e. the response to the standard and
deviant are equal). Almost all neurons recorded in A1 exhibited significant SSA (Figure 1d, standard
tone-evoked FR significantly lower than the deviant tone-evoked FR in N = 138 out of 147 neurons, Wilcoxon rank sum test p < 0.05).

**Contribution of thalamocortical inputs to SSA**

We first tested whether SSA is present in inputs from the thalamus. Current source density analysis has been extensively used to quantify inputs from the thalamus\(^7\). We used a linear probe to record local field potentials using electrodes spaced 50 microns apart inserted perpendicularly to brain surface in the primary auditory cortex. The multi-electrode probe is 775 µm long, spanning layers 1-6 of mouse A1. Current source density is computed as the second spatial derivative of the LFPs across the depth of the cortex (Figure 1e, Figure 1—figure supplement 1a, 20 sessions, 15 mice). Typically, in response to tones, CSD exhibits a negative basin, termed sink, within a short delay of tone onset, localized to electrodes in thalamo-recipient layer (Figure 1f, Figure 1—figure supplement 1b)\(^7,41\). The amplitude of current in the sink was taken as a measure of the combined strength of post-synaptic inputs onto layer 4 neurons, which should reflect the strength of the thalamic inputs to the cortex\(^7,41-43\).

We compared the amplitude of the CSD sink for each tone when presented as a deviant or standard, and computed their ratio (Figure 1 f). The sink amplitude was lower for the standard as compared to the deviant tones (Figure 1f, g), suggesting that excitatory signals produced by thalamocortical inputs exhibit stimulus-specific adaptation, consistent with previous findings\(^7\). This finding supports the "adaptation in narrowly tuned inputs" model, which postulates that stimulus-specific adaptation in broadly tuned neurons in A1 reflects adaptation in either thalamocortical inputs, or at the stage of integration of thalamocortical inputs, specific to inputs tuned to the standard tone\(^21,44,45\).

Importantly, across sessions, the SSA index of the granular layer CSD sinks was significantly lower than that of either the non-thalamo-recipient layers (Δ = -28%, p-value from one-sided test after correction (p1) = 6e-4, z = -3.4, Bonferroni corrected for 2 tests (C = 2)) or the SSA index of the mean spiking activity of A1 neurons (Δ = 23%, p1 = 0.029, z = -2.1, C = 2) in each session (N = 20 sessions...
in 15 mice, Figure 1g), suggesting that additional intra-cortical mechanisms may contribute to SSA in the cortex.

Suppression of either PVs or SOMs decreases SSA in putative excitatory neurons

We next tested whether cortical inhibitory interneurons may contribute to stimulus-specific adaptation. Since different inhibitory neuronal subtypes can differentially affect sensory responses of putative excitatory neurons \(^{46-48}\), we separately tested the role of PVs and SOMs. We used targeted viral delivery in the auditory cortex of mice to drive Archaerhodopsin (Arch) expression, which hyperpolarizes neurons when stimulated by light, in either PVs or SOMs\(^{49}\). A modified adeno-associated virus (AAV) encoding anti-sense code for Arch and a fluorescent reporter, under the FLEX cassette, was injected into PV-Cre or SOM-Cre mice\(^{50-54}\) (Figure 2a). Two weeks following virus injection, Arch was expressed selectively in PVs or SOMs in auditory cortex at expected levels \(^{55}\) (Figure 2b, c PV-Cre: N = 250 neurons in 4 mice, specificity = 92 ± 1%, efficiency = 73 ± 5%. SOM-Cre: N = 149 neurons in 5 mice, specificity = 95 ± 2%, efficiency = 86 ± 5%). To activate Arch, a light guide was positioned to cast 180mW/mm\(^2\) 532 nm light onto A1 surface, perpendicular to cortical layers. \textit{In vitro} intracellular recordings from optically identified PVs or SOMs (Figure 2—figure supplement 1, 3) demonstrate that light cast over the auditory cortex \textit{in vitro} drives a strong suppressive current (Figure 2d, Figure 2—figure supplement 1c, d 2c, d) and hyperpolarizes the membrane potential in these neurons (Figure 2—figure supplement 1b, 2b). Assuming a 100 fold attenuation of light over 1 mm of brain tissue \(^{56}\), the estimated irradiance in the deepest cortical layer (1.8 mW/mm\(^2\)) was strong enough to induce hyperpolarizing current in neurons \textit{in vitro} (Figure 2d). \textit{In vivo}, in both PV-Cre and SOM-Cre mice, illuminating the auditory cortex suppressed spiking activity in a small subset of recorded neurons (Figure 2e, f, left, putative inhibitory neurons) and increased activity in a great majority of recorded neurons (Figure 2e, f right, putative excitatory neurons). Shining light over A1 increased spontaneous neuronal activity in the majority of the recorded neurons in both PV-Cre mice (N = 115 neurons, 102 increased, 0 decreased, in 10 mice) (Figure 2g) and SOM-Cre mice (N = 104 neurons, 61 increased, 3 decreased, in 9 mice) (Figure 2h). These
measurements demonstrate that casting light over A1 selectively and effectively suppresses the activity of either PVs or SOMs.

To test the function of PVs and SOMs in SSA, their activity was suppressed during every 5th tone of the oddball stimulus by illuminating A1 (Figure 3a). To directly test the effect of interneuron suppression, we computed the SSA index separately on light-on and light-off trials for neurons responsive to both tones A and B (SSA was found in 63 out of 67 tone-responsive neurons in PV-Cre mice, 42 out of 43 tone-responsive neurons in SOM-Cre mice). Photosuppression of either PVs or SOMs affected the responses of neurons to the tones (Figure 3b, c), resulting in a significant reduction in SSA index across the population (Figure 3e, f, PV-Cre: $\Delta = -41\%$, $p1 = 1e-12$, $t(66) = 8.6$. SOM-Cre: $\Delta = -25\%$, $p1 = 2e-6$, $t(42) = 5.4$). Photo-manipulation affected responses only to the tone during which it was presented, but not to subsequent tones (Figure 3—figure supplement 1).

Additionally, photo-manipulation was limited to cortex since it did not affect thalamo-recipient layer CSD tone responses and SSA (Figure 3 – figure supplement 2). In a control group of PV-Cre or SOM-Cre mice (6 mice), we injected a modified AAV which encoded anti-sense fluorescent reporter alone under the FLEX cassette, and computed the effect of casting light on SSA (SSA was found in 33 out of 37 tone-responsive neurons in control mice). In this control group, SSA was not affected by light (Figure 3d, g, $p > 0.05$, $t(36) = -2.0$), confirming that Arch expression was required for the effect of the light. Therefore, the effects of interneurons are specific to intra-cortical mechanisms. These results demonstrate that both types of interneurons contribute to the reduction of the response of the neuron to the stimulus during SSA.

**PVs and SOMs differentially suppress putative excitatory neuron responses to standard and deviant tones**

A decrease in the SSA index may be due to several factors: (1) an increase in response to the standard only, (2) a decrease in response to the deviant, or (3) an increase in response both to the standard and the deviant, but with a relatively greater increase for the standard. Therefore, we next investigated the effect of interneuron photosuppression on FR of putative excitatory neurons evoked
by the standard and deviant tones separately. The effects of PVs and SOMs diverged; in addition to increasing spontaneous activity ($\Delta = 185\%$, p-value from one-sided t-test after correction (p2) = 3e-11, $t(159) = -7.2$), suppressing PVs led to increased FR to both the standard ($\Delta = 102\%$, p2 = 3e-11, $t(159) = -7.2$) and deviant ($\Delta = 56\%$, p2 = 9e-12, $t(159) = -7.4$) tones (N = 160, Figure 4a, b, c, Figure 4—figure supplement 1a). 83% of neurons exhibited greater FR to the standard and 46% to the deviant during PV suppression (Figure 4—figure supplement 1b). The difference in FR due to suppression of PVs was not significantly different between the standard and deviant tones (p2 > 0.05, t(159) = -0.1, C = 2) but both were greater than the difference in the spontaneous firing rate (Standard: $\Delta = 25\%$, p2 = 0.001, $t(159) = -3.6$, C = 2. Deviant: $\Delta = 26\%$, p2 = 0.039, $t(159) = -2.4$, C = 2), indicating that the change in tone-evoked FR was similar regardless of tone probability (Figure 4b, bottom panel). Because an equal increase in the firing rate produces a weaker relative effect on the response to the deviant (which is higher than to the standard), PV inactivation decreases SSA index (Figure 3e).

By contrast, suppressing SOMs led to an increase in FR for spontaneous activity ($\Delta = 46\%$, p2 = 2e-9, $t(113) = -6.5$) and during the standard ($\Delta = 29\%$, p2 = 2e-8, $t(113) = -6.1$) but not deviant (p2 > 0.05, $t(113) = -0.8$) tone (N = 114, Figure 4d, e, f, Figure 4—figure supplement 1c). 52% of neurons exhibited greater FR to the standard and only 11% to the deviant during PV suppression (Figure 4—figure supplement 1d). The increase in firing rate for spontaneous activity was not different than that during the standard tone (p2 > 0.05, $t(113) = 0.2$, C = 2) and the differences in FR due to suppression of SOMs were stronger for spontaneous activity and the standard tone than the deviant tone (Spontaneous: $\Delta = 390\%$, p2 = 0.004, $t(113) = 3.1$. Standard: $\Delta = 378\%$, p2 = 0.005, $t(113) = 3.1$) (Figure 4e, bottom panel), thereby accounting for the change in SSA with SOM inactivation (Figure 3f). Responses to the equal stimulus evoked consistent, yet weaker effects (Figure 4—figure supplement 2).

PVs and SOMs differ in their density among different layers of the cortex and in laminar sources and targets of their inputs and outputs$^{24, 57, 58}$. The effects of PV and SOM suppression on SSA had differential laminar distribution (Figure 4—figure supplement 3). The effect of PVs on SSA
was equally strong in the supra-granular and infra-granular layers, but stronger in the granular layer, i.e. the thalamo-recipient layer. This differential effect is consistent with the relative proportion of cortical interneurons that are PVs, which is higher in granular than either in infra- or supra-granular layers \(^{29, 59, 60}\). In contrast, suppressing SOMs reduced SSA in the granular and infra-granular, but not supragranular layers. The relative proportion of cortical interneurons that are SOMs is greatest in the granular and infra-granular layers, but still present in supra-granular layers \(^{29, 59, 60}\). As some SOMs predominantly target the distal dendrites of pyramidal neurons \(^{24}\), the effect of suppressing SOMs in supra-granular layers may be evident in recordings of pyramidal neurons with cell bodies in deeper layers, supporting our results. In addition, cortical extracellular recordings may be biased toward neurons in granular and infra-granular layers, precluding adequate sampling of activity in superficial layers. In controls, we did not observe a difference in the effect of light on SSA across layers, demonstrating that the differences are not due to differential artifact of light stimulation.

Our results indicate that both PVs and SOMs affect SSA, but in different ways: (1) The increase in the firing rate of putative excitatory neurons due to PV suppression is constant, either during presentation of the standard or the deviant, and greater than changes in spontaneous activity. Thus, PVs amplify SSA in excitatory neurons by exerting a \textit{relatively} stronger inhibitory drive for the standard than for the deviant. (2) Suppression of SOMs leads to increased putative excitatory neuron activity only during the spontaneous firing or the presentation of the standard, but not for the deviant. This suggests that the strength of SOM-mediated inhibitory drive is not significant in response to the deviant, but increases with repeated presentations of the standard.

In neurons exhibiting SSA, responses to the deviant are stronger than to the standard. This difference might lead to a 'ceiling' effect, reducing the effect of PV photosuppression on FR to the deviant, but not standard\(^{61}\). However, restricting the analysis to two subpopulations of neurons, which have matched mean and standard deviation of FR to the standard versus the deviant tones \(^{17, 62}\), preserved the observed effects of photosuppression (Figure 4—figure supplement 4). Suppressing PVs led to an equal increase in FR to both the standard and the deviant tone \((N = 54 – \text{Standard: } \Delta = 62\%, \ p2 = 6e-8, t(53) = 6.3. \text{Deviant: } \Delta = 55\%, \ p2 = 3e-5, t(53) = 4.5. \text{Standard vs deviant: } p2 > 0.05,\)
t(53) = 0.5). In contrast, suppressing SOMs led to a significant increase in FR to the standard, but no change in FR to the deviant (N = 44 – Standard: Δ = 30%, p2 = 7e-6, t(43) = 5.1. Deviant: p2 > 0.19, t(43) = 1.3. Standard vs. deviant: Δ = 382%, p2 = 6e-4, t(43) = 3.7).

For neurons that responded more strongly to one of the tones (“strong” versus “weak” tone) a ceiling effect would predict that the effect of interneuron suppression would be stronger for the weak than the strong tone. However, PV and SOM suppression exhibited a similar effect on responses to the strong and the weak tones in neurons that exhibited differential responses to two tones (Figure 4—figure supplement 5, 6). Suppressing PVs led to similar increases in tone-evoked FR between weak and strong tones for both deviant (N = 51, p2 > 0.05, t(50) = 1.0) and standard tones (p2 > 0.05, t(50) = -1.9). Suppressing SOMs also led to similar differential effects between strong and weak tones; standard tone-evoked FR increased equally (N = 34, p2 > 0.05, t(33) = 1.1) and deviant tone-evoked FR was equally unchanged (p2 = 0.05, t(33) = -0.1). Combined, these analyses demonstrate that the effect of PV photosuppression on SSA cannot be explained by the ceiling effect for either PVs or SOMs.

Although Arch drove strong currents in both SOM and PV neurons (Figure 2d, Figure 2—figure supplement 1, 2), there might be a difference in expression level or efficacy of Arch between SOM-Cre and PV-Cre mice, leading to a stronger effect of photosuppression in PV-Cre than in SOM-Cre mice on tone-evoked FRs (Figure 4b, e). Alternatively, the difference might be attributable to the morphological or functional differences between SOMs and PVs. To address this confound, we selected tone responses that exhibited matched difference in standard tone-evoked FR between light-on and light-off trials (N = 66, Figure 4—figure supplement 7). Within these matched subpopulations, PV and SOM photosuppression exhibited differential effects similar to those of the whole population. The change in FR due to PV suppression was not significantly different between responses to the standard and deviant (p2 > 0.05, t(65) = -0.3, C = 3). By contrast, the change in deviant tone-evoked FR due to SOM suppression was significantly weaker than that for the standard tone (Δ = -78%, p2 = 0.003, t(3.5), C = 3). By the design of the analysis, the effect of PV or SOM suppression on standard tone evoked FR was nearly identical (p1 > 0.05, t(65) = -0.1, C = 3). However, the change in deviant
tone-evoked FR was greater for PV photosuppression than SOM photosuppression (Δ = 404%, p1 = 0.029, t(65) = 2.4, C = 3). Since the observed differential effects of PV and SOM suppression persisted in subsets of neurons that were matched for photosuppression-induced change in standard tone-evoked FR, these differences are unlikely due to differential expression or efficacy of Arch in the PV-Cre and SOM-Cre mice, but rather reflect functional differences between the two types of interneurons.

SOM-mediated suppression of putative excitatory neurons increases with repeated presentations of the standard tone, whereas PV-mediated suppression remains stable

Within the oddball sequence, after the presentation of the deviant, SSA takes several repeats of the standard to reach an adapted state. Consistent with previous findings, presentation of the deviant tone temporarily reduced SSA without photosuppression (Figure 5a; b and c, dark color bars); Following the deviant tone (T₀) the first two standard tones (T₁ and T₂) evoked elevated FRs compared to the fourth standard tone (T₄) (PV-Cre, Figure 5b – N = 148, T₁: Δ = 60%, p2 = 3e-8, t(146) = 6.3, C = 11, T₂: Δ = 26%, p2 = 0.043, t(146) = 2.9, C = 11. SOM-Cre, Figure 5c – N = 102, T₁: Δ = 72%, p2 = 1e-5, t(101) = 5.2, C = 11, T₂: Δ = 31%, p2 = 0.013, t(101) = 3.3, C = 11). The third standard tone (T₃) and the tone prior to the deviant tone (T₁) evoked responses similar to T₄ (PV-Cre, Figure 5b – T₁ and T₃: p2 > 0.05, t(146) < 2.5, C = 11. SOM-Cre, Figure 5c – T₁ and T₃: p2 > 0.05, t(101) < 2.9, C = 11). Neurons in which response to T₀ did not produce spikes were excluded. Suppressing PVs led to a significant equal increase in FR to four consecutive presentations of the standard following the deviant (Figure 5b, left – for each tone, T₁ through T₄, with light-on compared to T₄ with light-off: Δ > 132%, p2 < 2e-9, t(146) > = 6.8, C = 11. Figure 5b, right – change in FR between light-on and light-off responses to each T₁ through T₃ as compared to T₄: p > 0.05, t(146) < 1.8, C = 5). In contrast with PVs, suppressing SOMs led to a progressively increasing effect on FR to consecutive presentations of the standard following the deviant (Figure 5c, left -- for each standard tone, T₁ through T₄, with light-on compared to T₄ with light-off: Δ > 64%, p2 < 9e-4, t(101) > = 4.1, C =11. Figure 5c, right – difference between FR change in T₁ and T₄ with light-on: p = 0.008, t(101) = -
3.2, \( C = 5 \). Repeated measures ANOVA with tone number \( (T_1 \) through \( T_4 \)) as a factor: \( F(3, 300) = 4.30, \ p = 0.0054 \). These results are consistent with the interpretation that the inhibitory drive from PVs is constant throughout the stimulus regardless of tone history, whereas the effect of SOM modulation increases with repeated presentations of the standard.

The time course of the effect of interneuron photosuppression on FR of the putative excitatory neurons at the beginning of each oddball sequence exhibited similar trends for PVS and SOMs. As expected, on light-off trials, FR decreased in response to the standard tone over the first 20 repetitions of the tone (Figure 5—figure supplement 1). For PV-Cre mice, the difference in FR to the standard between light-on and light-off trials did not change over this time and stayed positive for the remainder of the oddball (Figure 5—figure supplement 1a). Over the first 20 trials, FR adapted with a similar time course for both the light-on and light-off trials, so the change due to PV photosuppression in FR to standard stayed constant (Figure 5—figure supplement 1a, b). In contrast, for SOM-Cre mice, FR on light-on trials increased over the first 40 trials, whereas on light-off trials, it decreased (Figure 5—figure supplement 1a). As a result, the difference due to photo-manipulation in FR to the standard increased over the first 40 trials, and then stayed consistently positive throughout the stimulus presentation (Figure 5—figure supplement 1c). These results demonstrate that the PV-mediated effect on putative excitatory neuronal responses did not change with repeated presentations of the standard, whereas the SOM-mediated effect increased with the repeated stimulus.

**PVs and SOMs exhibit SSA**

In order to understand how PVs and SOM exert the differential control of SSA in putative excitatory neurons, we used optogenetic tagging to identify the specific interneurons and to quantify whether PVs and SOMs exhibited SSA\(^{63} \). Through targeted viral delivery to AC, we drove Channelrhodopsin-2 (ChR2) expression, which depolarizes neurons when stimulated by light, in either PVs or SOMs\(^{49} \) (Figure 6a, d, Figure 6—figure supplement 1a). A modified adeno-associated virus (AAV) encoding anti-sense code for ChR2 and a fluorescent reporter, under the FLEX cassette, was injected into PV-Cre or SOM-Cre mice\(^{50-54} \), and resulted in specific expression of ChR2, localized to
PVs or SOMs (Figure 6—figure supplement 1b, c PV-Cre; N = 183 neurons in 3 mice, specificity = 67 ± 1%, efficiency = 76 ± 5%. SOM-Cre: N = 202 neurons in 4 mice, specificity = 90 ± 3%, efficiency = 81 ± 4%). Neurons were identified as PVs or SOMs if they responded to brief (5 ms) flashes of light with spikes within 1.5-4.5 ms of laser pulse onset Figure 6a, d).

Both PVs and SOMs exhibited SSA, evidenced by a significant reduction in standard tone-evoked FR compared the deviant tone response (Figure 6b, c, e, f, PV: N = 16, Δ = -32%, p2 = 0.023, z = -2.5, C = 2. SOM: N = 28, Δ = -41%, p2 = 0.002, z = -3.3, C = 2. Signed rank test). The SSA index was not significantly different between PVs and SOMs (Figure 6g, neurons responsive to both tones A and B – PV: N = 5, SOM: N = 12. PV and SOM: p2 > 0.05, C = 2. Rank sum test) and both were similar to the mean SSA index in putative excitatory neurons (Figure 6g – Exc: N = 67. Exc vs PV: p > 0.05 , z = 0.7, C = 2. Exc vs SOM: p > 0.05, z = 0.4, C = 2). PVs ad SOMs exhibited some differences in relative response changes between the deviant, the standard and the equal tones (Figure 6, supplement 2b, d); PVs’ response to the equal tones did not decrease significantly as compared to deviant tones (N = 16 p2 > 0.05, z = -1.7, C = 2), whereas SOMs adapted in their response to equal tones (Δ = -36%, p2 = 0.049, z = -2.3, C = 2), and then further to standard tones (N = 28, Δ = -49%, p2 = 0.022, z = -2.6, C = 2). These results suggest that SOMs may adapt at a faster time scale than PVs with repeated presentation of tones.

Adapting inhibitory interneurons facilitate SSA in excitatory neurons in a cortical network model

Our results of recordings from PVs and SOMs present a surprising finding that PVs and SOMs adapt in response to repeated tones, countering our initial hypothesis that SOMs saturate in responses to the deviant, or facilitate with repeated presentation of a tone. How can an adapting interneuron contribute to added adaptation in excitatory neurons? To address this question, we next developed a model of coupled excitatory-inhibitory neuronal populations. Excitatory and inhibitory neurons form tight mutually coupled networks in A1, and we hypothesized that through differential
post-synaptic integration by excitatory neurons, interneurons can amplify adaptation in excitatory neurons.

As a proof-of-principle that would account for our findings that PVs and SOMs exhibit similar magnitude of SSA, yet have a differential effect on SSA in putative excitatory neurons, we constructed a simplified model of mutually coupled inhibitory-excitatory neuronal populations. We tested how responses of the model putative excitatory neurons are affected by manipulation of activity of PVs or SOMs (Figure 7a). Thalamocortical tone-evoked inputs were modeled including an adaptation term and resulted in reduced responses of excitatory, PV and SOM populations to repeated tones (Figure 7—figure supplement 1a, b). The model replicated the differential effects of manipulation of PV and SOM activity on responses to standard and deviant tones in putative excitatory neurons (Figure 7b-e):

When PVs were suppressed optogenetically, the responses to both the standard and the deviant tones increased (Figure 7b, c). By contrast, when SOMs were suppressed, although the spontaneous FR and standard tone-evoked FR were elevated, the responses to the deviant tone remained constant, whereas the responses to the standard tone increased (Figure 7d, e). SOMs have been shown to inhibit PVs \(^{64-66}\). Including inhibition between SOMs and PVs did not affect the model outcome, with suppression of PVs resulting in suppression of excitatory responses to both the standard and the deviant, and suppression of SOMs driving specific suppression of excitatory responses to the standard, but not the deviant (Figure 7 – figure supplement 2).

An explanation for the difference of the effects of PVs and SOMs can be provided by examining the combined transfer function between pre-synaptic inputs and post-synaptic activity of excitatory neurons separately for PVs and SOM suppression (Figure 7a, insets): Light-driven modulation of PV activity has the same effect on excitatory neuron responses at spontaneous, standard-tone evoked and deviant-tone evoked activity (Figure 7a, left inset). Spontaneous, standard and deviant input levels all fall within the linear portion of the transfer function between inputs and change in the excitatory neuron activity. On the other hand, for SOMs, modulation of their activity in the deviant tone-evoked regime drives small to no changes in excitatory neuronal activity, whereas modulation of SOM activity in the spontaneous and standard tone-evoked regime drives significant
changes in excitatory neuronal activity (Figure 7a right inset). The deviant-tone evoked activity falls on
the saturating part of the input-output transfer function, whereas the standard-tone evoked and
spontaneous inputs fall on the linear part of the transfer function. Then, shifts in SOM inputs due to
photosuppression evoke small changes during deviant tone responses, but larger changes during
either standard or spontaneous activity. Either PV or SOM manipulation would result in reduction of
combined SSA of excitatory neurons.

Discussion

The majority of neurons in the auditory cortex selectively reduce their responses to frequent,
but not rare sounds, exhibiting stimulus-specific adaptation. However, the cortical mechanisms
involved in the production and stimulus-specificity of SSA within the auditory cortex are not well
understood. Here we found that, in addition to adaptation at the level of thalamocortical inputs, two
distinct types of interneurons, PVs and SOMs, differentially contributed to SSA in the primary auditory
cortex. Optogenetic suppression of either PVs or SOMs led to a reduction in SSA in putative
excitatory neurons (Figure 3). Suppression of PVs led to an equal increase in the firing rate of the
putative excitatory neurons in response to the standard and the deviant (Figure 4). By contrast,
suppression of SOMs significantly increased the response to the standard, but lacked a significant
effect on the response to the deviant (Figure 4). This series of findings expands on the “adaptation in
narrowly tuned units” model, which proposes that repeated presentation of the standard stimulus
drives adaptation within more narrowly tuned inputs, such as thalamocortical inputs.\textsuperscript{21, 44, 45} Our data
indicate dual effects of cortical inhibition on SSA: (1) PVs contribute to SSA by providing constant
amount of inhibition, resulting in a \textit{relatively} higher inhibitory drive during the presentation of the
standard, as compared to the deviant. Taking into account the non-linear synaptic input to FR output
function of a typical pyramidal neuron, the constant inhibition amplifies the effect of thalamocortical
depression in suppressing the response of the neuron to repeated stimulus (Figure 7a). (2) The
selective increase of the inhibitory drive from SOMs for standard stimulus as compared to the deviant
stimulus responses might be explained by a shift in the non-linear transfer function between inputs to
SOMs and their outputs to excitatory neurons, possibly due to facilitation of SOM-to-excitatory neuron synapses\(^3^4\),\(^3^3\),\(^6^8\) (Figure 7 a).

Surprisingly, we found that, despite the differential effect of PV and SOM suppression on tone-evoked responses in putative excitatory neurons, both PVs and SOMs exhibit stimulus-specific adaptation. This finding is consistent with previous results that found that thalamocortical synapses onto inhibitory neurons and synapses from inhibitory neurons to excitatory cells can be depressing\(^6^7\),\(^6^9\). How does suppression of these interneurons result in differential reduction in SSA in excitatory neurons? Our model provides an intuition for this effect: The mutually coupled excitatory-inhibitory network model demonstrates that the observed differential effects of PV and SOM suppression may be due to their differential action on excitatory neuronal responses in the unadapted and adapted state (Figure 7). Tone-evoked responses of PVs would fall on the linear portion of the transfer function between PV activity and excitatory neuron depolarization, while the same tones maximally affect inputs from SOMs onto excitatory neurons, with stimulus-specific adaptation shifting the inputs to the linear, more sensitive range of inputs from SOMs. SSA may serve thus an additional function: to adjust the responses of neurons in a range that is more sensitive to small changes in the inputs from both excitatory and inhibitory neuronal populations. More generally, the simulation demonstrates that a circuit element, such as PVs or SOMs, that itself adapts may further amplify adaptation in the excitatory neurons.

To estimate the differential contribution of PVs or SOMs inputs to the excitatory neurons, we measured the difference in the firing rate of neurons due to optogenetic partial suppression of their firing. This measurement provides an estimate of the change in the firing rate of the putative excitatory neurons with the change in combined inputs to the inhibitory neurons, thereby allowing estimation of the synaptic transfer function (Figure 7a, insets). A simple biologically plausible network incorporating these transfer functions can reproduce the observed responses (Figure 7). There are several caveats to this interpretation. First, the firing rate may not linearly translate onto synaptic input strength because of the spiking non-linear rectification between the inputs and outputs of the putative excitatory neuron: a small change in FR in the low-FR regime might correspond to a greater change
in the synaptic drive than a similar-sized change in FR in the high-FR regime. However, our findings would still hold were this the case: In examining the effect of SOM suppression on response to the deviant, the actual difference in the synaptic drive between the deviant and the standard would then be even greater than observed. At the other end of the non-linearity, the analysis of neuronal responses sorted based on their firing rate to the standard and the deviant revealed that the “ceiling effect” would not contribute to a decreased effect of photostimulation on the response to the deviant in SOM-Cre mice (Figure 4—figure supplement 4, 5, 6, 7). Second, PVs and SOMs may inhibit not only the excitatory neurons, but also each other. SOMs make synapses onto PVs\textsuperscript{25, 64, 69}, thereby potentially suppressing them with repeated presentation of the standard. Therefore, when SOMs are suppressed, some PVs may be disinhibited, and therefore provide a stronger suppression of excitatory neurons. The null effect on responses to the deviant during SOM suppression could result from a combination of increase in inhibition from dis-inhibited PVs in addition to reduced inhibition of SOMs onto excitatory neurons. Including inhibition from SOMs to PVs in the proof-of-principle model supported experimental findings (Figure 8 – Supportive Figure 2). Third, other interneuron types, such as vasopressin-positive interneuron (VIPs) may be involved in the circuit \textsuperscript{35}, and the changes that we observe may reflect several inhibitory stages of processing.

One must be cautious in translating the data from our experiments as a strict description of neuronal activity in awake animals, as our results were based on recordings from mice under light isoflurane anesthesia. Other forms of anesthesia, such as pentobarbital-based \textsuperscript{70, 71}, ketamine \textsuperscript{72} and high concentrations of isoflurane \textsuperscript{70, 73}, can affect multiple aspects of sound-evoked responses in the auditory cortex. Nonetheless, our results are likely to extend for awake mice, since isoflurane anesthesia-induced effects on neuronal activity decrease as the concentration of isoflurane is reduced to the levels used in our recordings \textsuperscript{74}. In addition, all recordings and manipulations were performed under identical anesthetic conditions, and our conclusions are based on the relative comparison of the effects of suppressing PVs and SOMs, which are expected to hold under awake conditions \textsuperscript{75}. 

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While not demonstrated directly, SSA has been linked to detection of deviant sounds \(^2\), which may be facilitated by a relatively enhanced neuronal response to a change in the ongoing sound \(^{76-78}\). By suppressing the responses to a frequently presented tone, the responses of neurons to a rare stimulus become relatively enhanced. However, whether and how modulating SSA in the auditory cortex affects auditory behavior has not yet been tested. Inhibitory interneurons may prove to have a complementary role in shaping auditory perception in addition to receptive field reorganization driven by synaptic plasticity\(^79\). The use of optogenetic methods to test the function of inhibitory interneurons in SSA overcomes the limitations of lesion or pharmacological studies\(^80, 81\), which only allow for prolonged, non-selective inactivation\(^82\). By combining optogenetic manipulation of interneuron activity with behavioral measurements, future experiments will explore whether interneuron-mediated SSA indeed affects the auditory behavior of the subject, such as enhanced ability to detect unexpected events.
Materials and methods

In vivo experimental preparation:

Animals. All experiments were performed in adult male mice (supplier - Jackson Laboratories; age, 12-15 weeks; weight, 22-32 g; PV-Cre mice, strain: B6;129P2-Pvalbtm1(cre)Arbr/J; SOM-Cre: Sstm2.1(cre)Zjh/J) housed at 28° C on a 12 h light:dark cycle with water and food provided ad libitum. In PV-Cre mice, Cre recombinase (Cre) is expressed in parvalbumin-positive interneurons; in SOM-Cre mice, Cre is expressed in somatostatin-positive interneurons. This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All of the animals were handled according to a protocol approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania (Protocol Number: 803266). All surgery was performed under isoflurane anesthesia, and every effort was made to minimize suffering.

Viral vectors. Modified AAVs were obtained from Penn VectorCore. Modified AAV encoding Archaerhodopsin (Arch) under FLEX promoter was used for selective suppression of PVs or SOMs (catalog number AV-9-PV2432, AAV9.CBA.Flex.Arch-GFP.WPRE.SV40, Addgene22222, serotype 2/9). Modified AAV encoding GFP alone under FLEX promoter was used as a control for the specific action of Arch on the neuronal populations (catalog number AV-9-ALL854, AAV9.CAG.Flex.eGFP.WPRE.bGH, AllenInstitute854, serotype 2/9). Modified AAV encoding Channelrhodopsin (ChR2) under FLEX promoter was used for selective excitation of PVs or SOMs (catalog number AV-9-18917P, AAV9.CAGGS.Flex.ChR2-tdTomato.WPRE.SV40, Addgene18917, serotype 2/9).

Virus injection. 2-3 weeks prior to the start of experimental recordings, a 0.5 mm diameter craniotomy was drilled over primary auditory cortex (2.6 mm caudal and 4.1 mm lateral from bregma) under aseptic conditions while the mouse was anesthetized with isoflurane. A 750 nl bolus of AAV in
water was injected into A1 (1 mm ventral from pia mater) using a stereotaxic syringe pump (Pump 11 Elite Nanomite, Havard Apparatus). The craniotomy was covered with bone wax and a small custom head-post was secured to the skull with dental acrylic.

**Electrophysiological recordings.** All recordings were carried out inside a double-walled acoustic isolation booth (Industrial Acoustics). Electrodes were targeted to A1 on the basis of stereotaxic coordinates and in relation to blood vessels. In electrophysiological recordings, the location was confirmed by examining the click and tone pip responses of the recorded units for characteristic responses of neurons in core auditory areas, as described previously by our group in the rat \(^{84}\) and by other groups in the mouse \(^{27, 39, 85}\). While the electrodes were targeted to A1, some recordings may include data from the anterior auditory field (AAF), adjacent to A1 \(^{86}\). Mice were placed in the recording chamber, anesthetized with isoflurane, and the headpost secured to a custom base, immobilizing the head. After drilling a craniotomy and creating a durotomy exposing auditory cortex, a silicon multi-channel probe (A1x32-Poly2-5mm-50s-177[Poly-2]) or A1x32-tri-5mm-91-121-A32 [Triode], Neuronexus) was slowly lowered to between 750 μm and 1 mm into the cortex, perpendicular to the cortical surface and used to record electrical activity. Raw signals from 32 channels were bandpass filtered at 600-6000 Hz and thresholded for spike analysis, or at 10-300 Hz for local field potential (LFP) and current-source density (CSD) analysis (Poly-2 probe only), digitized at 32 kHz and stored for offline analysis (Neuralynx). Common-mode noise was removed by referencing a probe inserted in the brain outside the auditory cortex. On the Poly-2 probe, two rows of 16 electrodes each on a single shank were arranged such that each electrode site was 50 μm away from all three closest neighbors. This arrangement allowed us to record densely across depth, i.e. one electrode for every 25 μm in depth. On the triode, electrodes were arranged in groups of 3 equidistant sites, forming an equilateral triangle (25 μm separation). The triodes were separated vertically by 91 μm center-to-center distance, spanning 1 mm, with single sites on each end.

**Unit identification.** Spike sorting was performed using commercial software (Offline Sorter, Plexon)\(^{84}\). In order to improve isolation of single units from recordings using low-impedance probes,
spiking activity was sorted across three (Triode, 25 μm separation) or four (Poly-2, 50 μm separation) adjacent electrode sites. We used a stringent set of criteria to isolate single units from multiunit clusters. Single-unit clusters contained <1% of spikes within a 1.0-ms interspike interval, and the spike waveforms across 3 or 4 channels had to form a visually identifiable distinct cluster in a projection onto a three-dimensional subspace. Putative excitatory neurons were identified based on their expected response patterns to sounds and the lack of significant suppression of the spontaneous FR due to light. While this subpopulation may still contain inhibitory neurons, only 2% of all recorded neurons were significantly photo-suppressed at baseline (one-sided paired t-test, significance taken at p<0.05). The low impedance of the extracellular probes precluded us from conducting a more detailed analysis of cortical subpopulations based on the spike waveform.

**Acoustic stimulus.** Stimuli were delivered via a magnetic speaker (Tucker-David Technologies), directed toward the mouse’s head. Speakers were calibrated prior to the experiments to +/- 3 dB over frequencies between 1 and 40 kHz, by placing a microphone (Brüel and Kjaer) in the location of the ear contralateral to the recorded A1 hemisphere, recording speaker output, and filtering stimuli to compensate for acoustic aberrations. First, to measure tuning, a train of 50 pure tones of frequencies spaced logarithmically between 1 and 80 kHz, at 65 dB sound pressure level relative to 20 μPa (SPL), in pseudo-random order, was presented 20 times. Each tone was 100 ms long, with an inter-stimulus interval (ISI) of 300 ms. Frequency response functions were calculated online for several multiunits, and two frequencies (separated by 0.39 octaves), which elicited spiking responses of similar strength, were selected as tone A and B. Next, a series of stimuli composed of tones A and B were presented in interleaved blocks, repeated 4 times. Each oddball stimulus consisted of a train of 653 A and B tones (100 ms long, 300 ms ISI, 65 dB SPL). In oddball stimulus 1, 90% of the tones were A (standard), while 10% of the tones were B (deviant). We used a frozen sequence of standard and deviant tones in pseudorandom order and counterbalanced with respect to the number of standard tones preceding each deviant. In oddball 2, the probabilities of tones A and B were reversed so that tone B was the standard and A the deviant. In the equal probability stimulus, A and B each comprised 50% of tones.
Light presentation. An optic fiber was use to direct 532 nm laser light (Shanghai Laser & Optics Century). After positioning the silicon probe, an optic fiber was placed over the surface of auditory cortex. To limit Becquerel effect artifacts due to light striking electrodes, we positioned the optical fiber parallel to the silicon probe\textsuperscript{55, 95}. During every 5th tone of the oddball and equal probability stimuli, light was cast over A1 to suppress interneurons. The light onset was 100 ms prior to tone onset, and lasted for 250 ms. At 180 mW/mm\textsuperscript{2}, light pulses were intense enough to significantly modulate multiunit activity throughout all cortical layers. The effect of optical stimulation was not significant for responses to subsequent tones (Figure 3—figure supplement 1).

Immunohistochemistry. Brains were post-fixed in paraformaldehyde (4\%, PFA) and cryoprotected in 30\% sucrose. Coronal sections (40\mum) were cut using a cryostat (Leica CM1860), washed in PBS containing 0.1\% Triton X-100 (PBST; 3 washes, 5 min), incubated at room temperature in blocking solution (for PV 10\% normal goat serum and 5\% bovine serum albumin in PBST; for SOM 10\% normal goat serum with 0.1\% sodium azide and 2\% cold water fish gelatin in PBS; 3h), and then incubated in primary antibody diluted in blocking solution overnight at 4°C. The following primary antibodies were used: anti-PV (PV 25 rabbit polyclonal, 1:500, Swant) or anti-SOM (AB5494 rabbit polyclonal, 1:200, Millopopore). After incubation, sections were washed in blocking solution (3 washes, 5 min), incubated for 2hr at room temperature with secondary antibodies (Alexa 594 goat anti-rabbit IgG; for PV 1:1000 and SOM 1:400), and then washed in PBS (3 washes, 5min each). Sections were mounted using fluoromount-G (Southern Biotech) and confocal images were acquired (Leica SP5). Cells were identified in independent fluorescent channels and subsequently scored for colocalization by hand using ImageJ’s cell counter plug-in. Transfection efficiency is the percent of antibody labelled neurons which are co-labelled with GFP. Transfection specificity is the percent of GFP expressing neurons which are co-labeled with the antibody.

In vitro experimental preparation:
**Slice Preparation.** Acute brain slices were prepared from mice using standard techniques essentially as previously described. Mice were anesthetized via inhaled isoflurane and then trans-cardially perfused with 10 mL of oxygenated, ice-cold artificial cerebrospinal fluid (ACSF) at a rate of 5 mL/minute, that contained, in mM: 87 NaCl, 75 sucrose, 2.5 KCl, 1.25 NaH2PO4, 26 NaHCO3, 10 glucose, 0.5 CaCl2, 4 MgSO4. Slices (300 μm thick) were cut on a Leica VT1200S and incubated in cutting solution in a holding chamber at 32°C for approximately 30 minutes followed by continued incubation at room temperature prior to electrophysiological recording, at which point slices were transferred to a submersion type recording chamber attached to the microscope stage. ACSF used for recording contained, in mM: 125 NaCl, 2.5 KCl, 1.25 NaH2PO4, 26 NaHCO3, 10 glucose, 2 CaCl2, and 1 MgSO4. The solution was continuously bubbled with 95% O2 and 5% CO2 throughout cutting, slice incubation, and recording, so as to maintain a pH of approximately 7.4.

**Electrophysiology.** Cells were identified via GFP expression under epifluorescence microscopy, and subsequently visualized using a 40X, 0.8 NA water-immersion objective (Olympus) on an Olympus BX-61 upright microscope equipped with infrared differential interference contrast optics. Recordings were performed using the whole-cell patch clamp technique. Access resistance (Ra) was < 25 MΩ upon break-in; data obtained from a given cell was rejected if Ra changed by > 20% during the course of the experiment. Internal solution contained, in mM: potassium gluconate, 130; potassium chloride, 6.3; EGTA, 0.5; MgCl2, 1.0; HEPES, 10; Mg-ATP, 4; Na-GTP, 0.3; biocytin, 0.1%. Osmolarity was adjusted to 285-290 mOsm using 30% sucrose. Voltage was recorded using a MultiClamp 700B amplifier (Molecular Devices, Union City, CA), lowpass filtered at 10 kHz, digitized at 16-bit resolution (Digidata 1550) and sampled at 20 kHz. pCLAMP 10 software (Axon Instruments) was used for data acquisition, and analysis was performed using the Clampfit module of pCLAMP.

**Optogenetics.** Cells were illuminated with a 561nm solid state laser (Coherent) routed to the standard X-Y galvanometer of a two-photon microscope (Bruker Corporation, Billerica, MA) via a
single mode fiber. Illuminance at the specimen was estimated using a 10μm pinhole aperture (Edmund Optics) and a photodiode power sensor (Thorlabs).

**In vivo neuronal response analysis:**

**Tone response firing rate.** For each putative excitatory neuron, the spontaneous FR and tone-evoked FRs were measured as the mean FR over 50 ms pre and post tone onset, respectively. For each identified interneuron, FRs were measured 100 ms pre and post tone onset. FR was measured separately for each tone, A and B, as standard, deviant and equal probabilities, and for light-off and light-on trials. FR normalization was carried out separately for each tone, A and B, for each neuron by dividing the response under all conditions by the maximum FR (across 5ms bins) of the deviant tone, light-off condition. Performing this normalization by dividing response in all conditions by the mean, rather than maximum FR of the deviant tone, light-off condition did not alter significant results (Figure 4 – figure supplement 8). For all FR analyses, each neuron’s responses to tones A and B were treated separately, and each was only included if the light-off deviant tone-evoked FR was significantly greater than the spontaneous FR (Wilcoxon signed rank test p < 0.05). Further, tone responses were only included in analysis if the neuronal FR during each oddball stimulus exceeded 0.02 Hz, and the neuron was significantly tuned to the tone. Tuning was considered significant if the spike count in response to a tone (A or B) was significantly higher than the pool of spike counts across all tones outside one octave band centered on tones A and B (N = 42, t-test, p1 < 0.05). Population responses in each condition were measured as the mean and standard error of FRs across tone responses in each experimental group.

**Stimulus-specific adaptation index.** For each neuron, SSA index is a measure of the strength of SSA based on its mean FR with respect to tone probability. FRs to tones A and B were summed according to their standard or deviant probability within each oddball stimulus\(^2\). Thus, SSA index was computed as:

$$SSA \text{ Index} = \frac{(D_A + D_B) - (S_A + S_B)}{D_A + D_B + S_A + S_B}$$
Where S and D indicate the mean firing rate for standard and deviant trials, respectively, and their subscripts indicate the tone frequency condition. SSA index was computed separately for light-off and light-on conditions. Population SSA indices were measured as the mean and standard error of SSA indices across all neurons of each population. Criteria for inclusion in the analysis was the same as in tone response FR analysis described above, with the added criterion that the deviant tone-evoked FR must be greater than spontaneous FR for each of tones A and B (Wilcoxon signed rank test p < 0.05).

**Localization of cortical layers and CSD.** To calculate the CSD, the net current density moving through cortical tissue at 32 positions along the cortical axis was calculated based on LFPs of responses to tones recorded on each electrode, by using the second order central finite difference to calculate the second spatial derivative across the LFPs over the vertically arranged electrodes. Across the CSD profile, the deepest current sink corresponds to the thalamo-recipient granular layer allowing us to reconstruct the laminar location of recorded neurons. Neurons recorded on electrodes falling within the deepest sink were assigned to the granular layer, while those superior and inferior were assigned to the supra-granular and infra-granular layers (Figure 1e, f, Figure 4—figure supplement 3). The tone-evoked amplitude of the CSD was measured by first calculating root mean square of each channel during the first 50ms post tone onset, and then calculating the mean across all electrodes determined to fall within either the deepest short latency sink (granular layer) or pooled across all electrodes either above (supra-granular layer) or below (infra-granular layer). For each session, the granular layer CSD amplitude for all tone conditions was normalized across conditions by the deviant tone, light-off condition, and the mean across sessions was statistically analyzed. The SSA index was calculated as described in *Stimulus-specific adaptation index* on the basis of the amplitude.

**Statistical tests.** For all statistical tests in which N>=30, we applied the student’s t-test (Matlab) unless specified otherwise, and reported the p-value, degrees of freedom and t-statistic. For all tests with N < 30, sample variance was tested for normality using the Komogorov-Smirnov test. If any
group’s variance was non-normal, we applied a non-parametric test, e.g. Wilcoxon sign rank or rank sum test (Matlab), and provided the z-statistic for any group with a normal distribution. For all tests, Bonferroni correction was applied for multiple comparisons, and reported as "C=X" where X is the factor by which the p-value was adjusted. Statistical tests were single-tailed if there was a reasonable prior expectation about the direction of the difference between samples. p1 refers to one-sided, and p2 refers to two-sided statistics set. In all figures, single, double and triple stars indicate p < 0.05, 0.01 and 0.001 respectively. Error bars in all figures represent the standard error of the mean, unless otherwise noted.

**Excitatory-inhibitory network model.** We constructed models of the excitatory-inhibitory neuronal circuit to understand the coupling of excitatory interneurons with PV and SOM interneurons. We constructed firing-rate models based on Wilson-Cowan dynamics [28-30]. The parameters were chosen in order to achieve a match to experimental data. The mean activity level of each population was modeled as:

\[
\frac{dE}{dt} = \frac{1}{\tau_E} [-E(t) + (k - r)S(j_{ETone}(t) + S_{inh}(j_{IE}I(t)))]
\]

\[
\frac{dl}{dt} = \frac{1}{\tau_I} [-l(t) + (k - r)S(j_{inh}(t) + j_{ITone}(t) + j_{IE}E(t))]
\]

where \(E(t)\) is the activity of the excitatory population; \(l(t)\) is the activity of the inhibitory population; \(S(x)\) is the transfer function between the combined “synaptic” input and the neuronal FR. \(S(x)\) is linear with respect to intermediate inputs, but imposes a minimum and maximum activation limits. \(S_{inh}(x)\) is the transfer function between the inhibitory firing rate and excitatory post-synaptic current; \(j_{IE}\) and \(j_{IE}\) are excitatory-inhibitory and inhibitory-excitatory synaptic weights (.2 and -1.0 for PVI, .05 and -.39 for SOMs, respectively); \(j_{ETone}(t)\) and \(j_{ITone}(t)\) are tone-evoked input currents to excitatory and inhibitory neurons, respectively, modeled as 50 ms long exponentially decaying inputs of maximum amplitude 3
(delayed by 7ms for SOMs, which do not receive direct thalamic inputs, relative to PVs, which receive
direct thalamic inputs); \( \tau_E \) (10 ms) and \( \tau_I \) (10 ms) are synaptic time constants for excitatory and
inhibitory neurons; \( k \) and \( r \) represent the maximum and minimum FR of neurons respectively \( (k = 15, r = 1) \); \( j_{\text{inh}}(t) \) is the negative input to inhibitory neurons due to Arch. The optogenetic modulation
was modeled as a unitary 250 ms pulse. To capture the differences in inputs due to repeated tone
exposures, we modeled thalamic inputs reflecting the tone inputs with synaptic depression. We
modeled the conductance of the thalamic projections, \( g_{\text{inp}} \), as changing according to the equation:

\[
\frac{dg_{\text{inp}}}{dt} = \frac{(g_0 - g_{\text{inp}})}{T_g} - \frac{(g_{\text{inp}}r)}{T_r}
\]

Where \( g_0 \) is the maximum conductance \( (g_0 = 1) \), \( r \) is the gating coefficient representing tone-evoked
thalamic input, \( T_g \) is the timescale for replenishment \( (T_g = 3 \text{ s}) \), \( T_r \) is the timescale for depletion
\( (T_r = 80 \text{ ms}) \). We took \( r \) to be a step function with an exponential decay (with 40 ms time constant and
amplitude of 3). The full input to auditory cortical neural populations is then equal to \( g_{\text{inp}}r \). In train of
four tones, the first tone-evoked response was taken as the deviant tone, and the fourth tone as the
standard tone.

For the inhibitory-to-excitatory inputs, we used a sigmoidal transfer function and showed the existence
of parameter regimes consistent with our results. For PVs, we used a sigmoid of the form:

\[
S_{PV}(r_{PV}) = \frac{1}{1 + \exp[ - p(r_{PV} - \theta) ]}
\]

where \( p = 0.3 \) and \( \theta = 9 \). This gives a facilitating response at low input levels and a linear response at
high input levels. For SOMs, we used a hyperbolic tangent that provided a saturating non-linearity:

\[
S_{SOM}(r_{SOM}) = \frac{1 - \exp[-2r_{SOM}/s]}{1 + \exp[-2r_{SOM}/s]}
\]

where \( s = 3 \). For visualization, the baseline firing rate of neurons was removed and the peak tone
response to a “deviant” without optogenetic manipulation normalized to 1.

We also constructed a model coupling excitatory neurons and SOM and PV interneurons
using a generalization of the above dynamics, which may be written:
\[ \frac{dN_i}{dt} = \frac{1}{T_i} \left( -N_i + (k - r)S(j_{\text{tone},i}(t) + j_{\text{ext},i}(t) + \sum_k j_{ki} * S_k(N_k)) \right) \]

where \( N_i \) is the firing rate of the \( i \)th population (EXC, PV, SOM), \( T_i = 10 \text{ ms} \) is the time constant for each population, \( k = 15, r = 1, S \) has different maximum and minimum values for each population \((x_{\text{min,E}} = -1, x_{\text{max,E}} = 1.75, x_{\text{min,PV}} = -0.5, x_{\text{max,PV}} = 4, x_{\text{min,SOM}} = 0, x_{\text{max,SOM}} = 3). S_E(x) = x, \) and \( S_{\text{SOM}} \) and \( S_{\text{PV}} \) use the definitions above. \( j_{\text{EE}} = j_{\text{SOM,SOM}} = j_{\text{PV,PV}} = j_{\text{PV,SOM}} = 0, j_{\text{E,SOM}} = .25, j_{\text{SOM,E}} = -0.25, j_{\text{E,PV}} = .4, j_{\text{PV,E}} = -1, \) and \( j_{\text{SOM,PV}} = -1. j_{\text{ext,PV}} = 1.5, j_{\text{ext,SOM}} = 1. \) Tone inputs are the same as described above.

**Author Contributions**

RGN and MNG designed the study and wrote the paper. RGN, LMT, EMG, SJ and MA collected the data. RGN, LMT, EMG, SJ, JJB and MNG analyzed the data.

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References


**Figures**

**Figure 1. Nearly all recorded A1 neurons exhibit stimulus-specific adaptation.**

(a) Diagrams of oddball stimuli; Oddball stimuli are composed of a 2.5Hz train of 100ms long sine-wave tone pips separated by 300ms of silence (gray and red dots). Each tone pip is at one of two frequencies, tone A or B. In oddball stimulus 1, 10% of all pips are tone A and 90% of pips are tone B. In oddball stimulus 2, the tone probabilities are reversed. The less frequent tone is referred to as the deviant tone (red dots). The more frequent tone is referred to as the standard (gray dots).

(b) Left: Diagram of recording. Electrode was lowered perpendicular to the brain surface. Virus was injected in A1. Right: The frequencies of tones A and B (dashed black and gray lines) are selected based on the frequency response functions of neurons of interest. Mean firing rate of 5 co-tuned neurons (colored lines) recorded simultaneously in a single session in response to 65dB tone pips at 50 frequencies logarithmically spaced from 1 to 80kHz. FR is normalized to the peak response of each neuron.

(c) A representative neuron exhibited suppressed responses to a tone presented as a standard (gray raster and PSTH) compared to the same tone presented as a deviant (red raster and PSTH). Left: Responses to tone A, presented as a deviant in oddball stimulus 1, and a standard in oddball stimulus 2. Right: Responses to tone B. Shaded regions indicate standard (gray) and deviant (red) tones trials.

Gray dashed lines indicate tone onset and offset times.

(d) Population histogram of SSA index exhibited by all neurons included in the analysis. Gray and white bars indicate neurons expressing significant and non-significant SSA, respectively. Spike count for response to deviant tones was significantly greater than for response to standard tones (Wilcoxon rank sum test, one tail, p < 0.05). The black marker indicates the population average SSA index.

(e) Left: Diagram of electrode spanning A1. Right: Representative peri-stimulus CSD. Top: Mean response to deviant tones. Bottom: Mean response to standard tones. Gray dashed lines indicate tone onset and offset. Green dashed lines indicate the location of the granular layer. Negative CSD values (blue) indicate current sinks, while positive CSD values (red) indicate current sources.

(f) Mean CSD collected from the thalamo-recipient layer, in response to standard (gray) and deviant (red) tones. Gray dashed lines indicate tone onset and offset.

(g) Mean SSA index across sessions measured from thalamo-recipient granular layer CSD, infra- and supra-granular layer cortical CSD and mean neuronal spiking activity SSA index averaged over sessions.

The following figure supplements are available for Figure 1:

**Figure 1—figure supplement 1.** Local field potentials recorded in A1 exhibit stimulus-specific adaptation.
Figure 1—figure supplement 1. Local field potentials recorded in A1 exhibit stimulus-specific adaptation.
(a) Representative peri-stimulus LFPs across cortical layers. Top: Mean response to deviant tones. Bottom: Mean response to standard tones. Gray dashed lines indicate tone onset and offset. Green dashed lines indicate the margins between cortical layers.
(b) Mean LFP collected from the thalamo-recipient granular layer, in response to standard (gray) and deviant (red) tones. Gray dashed lines indicate tone onset and offset.
Figure 2. Cell type – specific optogenetic suppression of parvalbumin-positive and somatostatin-positive neurons.

(a) Optogenetic methods diagram. Top: A1 was injected with AAV-FLEX-Arch-GFP. During experiments, an optic fiber was positioned to target A1 and neuronal activity was recorded using a multichannel silicon probe in A1. Bottom: Green light (532 nm) suppresses PVs in PV-Cre mice or SOMs in SOM-Cre mice.


(c) Efficiency and specificity of transfection of interneurons with Arch. Bar Plots: Efficiency (Ef) and specificity (Sp) of visual transfection of PVs (top) and SOMs (bottom) with Arch. Ef - Percent of labelled interneurons expressing Arch. Sp -Percent of Arch-expressing cells which are also labelled interneurons.

(d) Mean Arch mediated outward current evoked in response to increasing photostimulation power, recorded in vitro by whole cell patch recording in putative excitatory neurons from PV-Cre (blue, N = 5) and Som-Cre (orange, N = 5) mice. The gray dashed line indicates the level of irradiance expected in in vivo experiments at the deepest recording sites, in cortical layer 6.

(e, f) Tone responses of representative neurons, which are suppressed (left) or activated (right) by photostimulation, from PV-Cre (e) and SOM-Cre (f) mice. Raster plot of spike times (bottom) and PSTH (top) of a single neuron response to a 100 ms long tone (gray dashed lines, shaded region) on light-on (overlapping 250-ms light pulse, green shading) and light-off trials. Light-on trials: green. Light-off trials: black.

(g, h) Modulation of spontaneous FR by interneuron photosuppression recorded in PV-Cre (g) and SOM-Cre (h) mice. Each neuron is represented by a circle that is filled for those with significantly increased (green) or decreased (red) FR or unfilled for those without significant modulation. Gray dashed line – identity line.

The following figure supplements are available for Figure 2:

Figure 2—figure supplement 1. Optogenetic control of PVs in mouse primary auditory cortex via photostimulation of Arch in acute slices.

Figure 2—figure supplement 2. Optogenetic control of SOMs in mouse primary auditory cortex via photostimulation of Arch in acute slices.
Figure 2—figure supplement 1. Optogenetic control of PVs in mouse primary auditory cortex via photostimulation of Arch in acute slices.

(a) Sustained high-frequency firing pattern typical of a PV-positive FS cell (top) in response to rectangular current injection (bottom; 600pA) recorded in vitro via whole cell patch clamp. Inset, epifluorescence (i) and corresponding IR-DIC image (ii) of the depicted cell. Scale bar – 20 μm.

(b) Membrane hyperpolarization mediated by 532nm light.

(c) Outward current mediated by photoactivation of Arch.

(d) Plot of light-induced outward current vs. illuminance (mW/mm2). Error bars – standard deviation.
Figure 2—figure supplement 2. Optogenetic control of SOMs in mouse primary auditory cortex via photostimulation of Arch in acute slices.

(a) Adapting discharge pattern typical of a somatostatin-positive cell (top) in response to rectangular current injection (bottom; 200pA) recorded \textit{in vitro} via whole cell patch clamp. Inset, endogenous GFP fluorescence of the recorded cell illustrating AAV9.Arch.GFP expression (i) filled with Alexa 594 (ii) and imaged using a two-photon microscope. Scale bar – 20 μm.

(b) Membrane hyperpolarization mediated by 532nm light.

(c) Outward current mediated by photoactivation of Arch.

(d) Plot of light-induced outward current vs. illuminance (mW/mm2). Errorbars – standard deviation.
Figure 3. Optogenetic suppression of either PVs or SOMs reduces stimulus-specific 
adaptation in putative excitatory neurons in the auditory cortex.

(a) Diagram of oddball stimuli with light; Two oddball stimuli are presented (as in Figure 1a), with 250 
ms light pulses (green bars) delivered during every 5th tone, starting 100ms before tone onset.
(b-d) Representative neuron PSTH in response to tone A (left) and B (right) as a standard (gray) or 
deviant (red) on light-on (light colors) and light-off trials (dark colors). Neurons recorded in PV-Cre (b, 
e), SOM-Cre (c, f), and control (d, g) mice.
(e-g) Effect of interneuron photosuppression on SSA. Left: SSA index on light-on vs light-off trials. 
Each neuron is represented by a circle that is filled if the neuron exhibits significant SSA i.e. its FR in 
response to deviant tones is greater than that to standard tones. The respective representative 
neuron in b, c, and d is indicated by a red circle. Gray dashed line - identity line. Right: Mean SSA 
index on light-on (green) and light-off (gray) trials over neuronal population.

The following figure supplements are available for Figure 3:

Figure 3—figure supplement 1. Photostimulation during standard tone does not affect SSA during 
subsequent tones on light-off trials.

Figure 3—figure supplement 2. Interneuron photosuppression does not affect thalamo-cortical 
responses to standard or deviant.
Figure 3—figure supplement 1. Photostimulation during standard tone does not affect SSA during subsequent tones on light-off trials.

(a) Diagram of oddball stimuli illustrating post-photostimulation tone number: Tones and light pulses indicated as in Figure 3a. Numbers indicate each tone position relative to light pulses as included in the analysis below. Any tones following deviant tones were excluded from the analysis.

(b) The mean population FR in response to standard (gray) and deviant (red) tones subsequent to light-on trials are not affected by light presentation (dark bars: light-off. light bars: light-on). For each neuron, responses are normalized by the response to the third post-laser standard tone (T3, indicated by blue dashed line). In PV-Cre mice, the standard tone-evoked FR with light-on (T0) and the tone preceding it (T1) were significantly higher than that of standard T3 (N = 159, T0; Δ = 10%, p2 = 0.037, t(158) = 2.9, C = 9. T0: Δ = 170%, p2 = 2e-7, t(158) = 5.9, C = 9), while the two post-light tones (T1 and T2) were not significantly different (N = 159, T1 and T2: p2 > 0.05, t(158) < 2.6, C = 9). In SOM-Cre mice, the standard T0-evoked FR was greater than that of T3 (N = 114, Δ = 54%, p2 = 4e-8, t(113) = 6.4, C = 9) while all light-off tones were not significantly different (T0, T1, and T2: p2 > 0.05, t(113) < 0.9, C = 9). In control mice, no standard tones evoked greater FR than T3, (N = 107, T1 through T2: p2 < 0.05, t(106) < 2.7). In all three groups, deviant tones in all positions evoked greater FRs than standard T3, (Δ > 209%, p2 < 5e-5, t(106) > 4.7, C = 9).

(c) Mean SSA index for each sequential tone position (for T0,1,2,3), calculated based on the pair of standard and deviant tones at each respective position. Each tone response, tone A or B, was used to calculate a separate SSA index:

\[
SSA Index = \frac{D_A - S_A}{D_A + S_A} \text{ or } \frac{D_B - S_B}{D_B + S_B}
\]

Where S and D indicate mean FR evoked by standard and deviant tone probabilities, respectively, and their subscripts indicate the tone frequency condition. Compared to T3, SSA index was significantly reduced only for T0, the only light-on trial, in both PV-Cre and SOM-Cre mice (PV-Cre: Δ = -40%, p2 = 4e-10, t(158) = -6.9, C = 4. SOM: Δ = -29%, p2 = 2e-7, t(158) = -5.8, C = 4), as expected from Figure 3e, f, g. In both PV-Cre and SOM-Cre mice, the SSA index at all of the other sequential tone positions, T1 through T2 was not significantly different than that of T3 (p2 > 0.05, t(113) < 1.9, C = 4), indicating that the effects of photosuppression were not detectable beyond T0. In control mice, the SSA index was not different compared to T3 for any tone position, even T0 (p2 > 0.05, t(106) < 1.8, C = 4). Together, this analysis demonstrates that the optogenetic effects are acute to illumination periods, and unlikely to confound interpretation of effects observed during light-off trials.
Figure 3—figure supplement 2. Interneuron photosuppression does not affect thalamocortical responses to standard or deviant.

(a) In PV-Cre and SOM-Cre mice, the mean granular layer CSD SSA index was not significantly different between the light-off and light-on conditions for standard or deviant tones (p2 > 0.05, for each condition; left, PV-Cre: N = 16. Center, SOM-Cre: N = 12).

(b) In both experimental groups, the mean granular layer CSD amplitude was not significantly different between the light-off and light-on conditions for standard or deviant tones (p2 > 0.05, for each condition; left, PV-Cre: N = 8. Center, SOM-Cre: N = 6).
Figure 4. PVs and SOMs differentially affect response to standard and deviant tones.

(a, d) Top: Mean response to deviant (left, red) and standard (right, black) tones, during light-on (light colors) and light-off trials (dark colors). Bottom: Mean of the difference between responses on light-on and light-off trials for each neuron for deviant (left, red) and standard (right, black) tone. Each trace is a population average of putative excitatory neuron PSTHs normalized to each neuron’s maximum deviant tone-evoked FR on light-off trials. Shaded regions around traces indicate standard error (SE). Dashed lines indicate light onset (green) and tone onset and offset (gray). Neurons recorded in PV-Cre (a), SOM-Cre (d) mice.

(b, e) (Top) Mean population FR on light-on and light-off trials; (Bottom) Mean population FR difference between light-on and light-off conditions for deviant (red) and standard (gray) tones and spontaneous activity (blue). Normalization as in a. Neurons recorded in PV-Cre (b), SOM-Cre (e) mice.

(c, f) Modulation of PV-Cre mouse putative excitatory neuron FR response to tones by interneuron photosuppression. Neuronal responses to each tone are represented by two circles, one for standard (black) and one for deviant (red) tone responses. Filled circles represent significantly increased (gray, pink) or decreased (black, red) response; unfilled circles: responses without significant modulation. Gray dashed line – identity line. Neurons recorded in PV-Cre (c), SOM-Cre (f) mice.

The following figure supplements are available for Figure 4:

Figure 4—figure supplement 1. PVs and SOMs differentially affect response to standard and deviant tones.

Figure 4—figure supplement 2. Consistent effects of PV and SOM suppression in response to equal probability tones.

Figure 4—figure supplement 3. PVs and SOMs have differential effects on SSA across different layers of cortex.

Figure 4—figure supplement 4. Differences between PV and SOM effects on standard and deviant tones are preserved for subsets of neurons matched for FR.

Figure 4—figure supplement 5. Effects of PV suppression are identical for tones that evoke strong or weak responses in putative excitatory neurons.

Figure 4—figure supplement 6. Effects of SOM suppression are identical for tones that evoke strong or weak responses in putative excitatory neurons.

Figure 4—figure supplement 7. Differences between PV and SOM effects on standard and deviant tones are preserved for subsets of neurons matched for strength of laser effects on standard tones.

Figure 4—figure supplement 8. Differences between PV and SOM effects on standard and deviant tone responses are preserved when FRs are normalized by the mean onset response.
Figure 4—figure supplement 1. PVs and SOMs differentially affect response to standard and deviant tones

(a, c) Correlation between standard and deviant tone response change by photostimulation. Each neuron’s response to each tone, A and B, is represented by one circle. Gray dashed line – identity line. Green dashed line – regression line. Neurons recorded in PV-Cre (a) and SOM-Cre (c) mice.

(b, d) Proportion of putative excitatory population exhibiting significantly increased (gray, pink), decreased (black, red), or unchanged (unfilled) FR to standard and deviant tones due to photosuppression. Neurons recorded in PV-Cre (a), SOM-Cre (c) mice.
Figure 4—figure supplement 2. Consistent effects of PV and SOM suppression in response to equal probability tones.

(a) Diagram of equal probability tone stimulus; an equal number of pseudorandom tones A and B are presented with 250 ms light pulses (green bars) delivered during every 5th tone, starting 100ms before tone onset.

(b) Effect of interneuron photosuppression on putative excitatory neuron responses to standard and deviant and equal probability tones. Mean FR of single neuron responses to standard (gray), equal (green) and deviant (red) tones on laser-off (dark colors) vs laser-on (light colors) trials. Top: responses to tone A. Bottom: Responses to tone B. Left: neuron from PV-Cre mouse. Center: neuron from SOM-Cre mice Right: neuron from control mouse.

(c, e) PSTH of FR to equal probability tones, during light-on (light green) and light-off trials (dark green). Each trace is a population average of putative excitatory neuron PSTHs normalized to each neuron’s maximum deviant tone-evoked FR on light-off trials. Shaded regions around traces indicate standard error (SE). Dashed lines indicate light onset (green) and tone onset and offset (gray).

Neurons recorded in PV-Cre (c, N = 160) and SOM-Cre (e, N = 114) mice.

(d, f) Population mean spontaneous FR (50ms prior to tone onset, yellow) and equal-tone evoked FR (50 ms from tone onset, green) for light-off (dark colors) and light-on (light colors) trials. Normalized as in c. Neurons display an increase in control FR and equal tone-evoked FR with light-on for both PV-Cre (d – Spn: Δ105%, p2 = 3e-6, t(159) = -8.1. Eqn: Δ = 41%, p2 = 1e-13, t(159) = 4.8) and SOM-Cre (f, Spn: Δ = 17%, p2 = 0.002, t(113) = -3.1. Eqn: Δ = 17%, p2 = 0.012, t(113) = -2.54) mice.

(g, h) Modulation of PV-Cre mouse putative excitatory neuron FR response to tones by interneuron photosuppression. Left: Circle: Response of each neuron to tone A and/or B. Filled: significantly increased (light green) or decreased (dark green) response; Unfilled: non-significant modulation. Gray dashed line – identity line. Right: Fraction of neuronal tone responses in the population that increased (light green), decreased (dark green) or did not significantly change with light. Neurons recorded in PV-Cre (g), SOM-Cre (h) mice.

(i, k) Mean of the difference between light-on and light-off trials for each neuron for equal probability tones FR response PSTHs. Normalization and dashed lines as in c. Neurons recorded in PV-Cre (i), SOM-Cre (k) mice.

(j, l) Mean population FR difference between light-on and light-off conditions for spontaneous activity (yellow) and equal probability tones (green). Measured and normalized as in d and f. Neurons display a larger increase in equal-tone evoked FR than spontaneous FR with light-on for those recorded in both PV-Cre (j, Δ = 32%, p2 = 0.029, t(159) = 2.2), SOM-Cre (l, Δ = 118%, p2 = 0.047, t(113) = 2.0) mice.
Figure 4—figure supplement 3. PVs and SOMs have differential effects on SSA across different layers of cortex.

(a) Diagram of multi-electrode recording across the supra-granular, granular and infra-granular layers of A1.

(b) SSA index for cortical supra-granular (Sup, cyan), granular (Grn, yellow) and infra-granular (Inf, magenta) layers on light-off (dark colors) and light-on (light colors) trials.

(c) Difference in SSA index between responses on light-on and light-off trials for each layer as shown in b.

Suppressing PVs reduced SSA throughout all cortical layers (b left – Sup: N = 15, Δ = -31, p2 = 0.002. Grn: N = 27, p2 = 2e-4, z = 3.8. Inf: N = 79, Δ = -39%, p2 = 1e-8, z = 5.7). Notably, the effect of PVs was significantly stronger in the granular than in the infragranular layers (c left – Δ = 194%, p = 0.014, C = 2), but was not different between the supragranular and the granular or infragranular layers (p > 0.05, z < 1.8, C = 2). In the controls, SSA index was not significantly reduced between light-on and light-off trials in any layer (b right – Sup: N = 3. Grn: N = 21. Inf: N = 75. For each layer: p2 > 0.05, z <1.4), demonstrating that the light-induced effects required Arch. In contrast, suppressing SOMs reduced SSA in the granular (N = 7, Δ = -42%, p2 = 0.031) and infragranular (N = 63, Δ = -24%, p2 = 6e-7, z = 5.0) layers, but did not have a significant effect on SSA in the supragranular layers (N = 3, p2 > 0.05) (b, center). In SOM-Cre mice and controls, there was no difference between effects of photosuppression on SSA index in different layers (c, center and right – p > 0.05, z < 1.1).

Signed rank test for b and ranked sum test used for c.
Figure 4—figure supplement 4. Differences between PV and SOM effects on standard and deviant tones are preserved for subsets of neurons matched for FR.

(a) Left: Two subsets of neurons recorded in PV-Cre mice with matched FR response magnitude to standard (gray, above x-axis) and deviant (red, below x-axis) tones on light-off trials. Right: Difference between light-on and light-off FR in response to standard (gray) and deviant (red) tones for the respective subsets of neurons.

(b) Same as a for neurons recorded in SOM-Cre mice.
Figure 4—figure supplement 5. Effects of PV suppression are identical for tones that evoke strong or weak responses in putative excitatory neurons.

Each neuron’s response to oddball tones A and B are pooled according to their response strength. The tone which evokes a higher peak FR as a deviant is pooled across neurons as the ‘strong tone’ response, while the tone which evoked a lower peak FR is pooled as the ‘weak tone’ response.

(a-j) Data are presented as in Figure 4. Strong tone response data are presented on the left (a, c, d, g, i) with solid lines and filled bars, and weak tone response data are presented on the right (b, e, f, h, j) with dashed lines and unfilled bars. All data are from PV-Cre mice.

(k) Mean population FR difference between light-on and light-off conditions for deviant (red) and standard (gray) tones and spontaneous activity (blue) for strong (filled) and weak (unfilled) tones. Measured and normalized as in D and F.

Photosuppression of PVs led to increased spontaneous FR (Spn) and standard (Stn) and deviant (Dev) tone-evoked FR for both strong (d – Spn: Δ = 187%, p2 = 4e-7, t(50) = -5.8. Stn: Δ = 71%, p2 = 3e-10, t(50) = -7.8. Dev: Δ = 24%, p2 = 0.002, t(50) = -3.3) and weak tones (f – Spn: Δ = 171%, p2 = 3e-7, t(50) = -5.9. Stn: Δ = 89%, p2 = 2e-8, t(50) = -6.5. Dev: Δ = 58%, p2 = 2e-7, t(50) = -6.0) (N = 51). There were no significant differences between strong and weak tones for the change in spontaneous FR and standard and deviant tone-evoked FR (k, Spn, Stn and Dev: p > 0.05, t(50) < 2.0).
Figure 4—figure supplement 6. Effects of SOM suppression are identical for tones that evoke strong or weak responses in putative excitatory neurons.

(a-k) Data are presented as in Figure 4—figure supplement 5. All data are from SOM-Cre mice.

Photosuppression of SOMs lead to increased spontaneous FR and standard tone-evoked FR, and did not change deviant tone-evoked FR for both strong (d – Spn: $\Delta = 45\%$, $p2 = 7e-7$, $t(33) = -6.1$. Stn: $\Delta = 27\%$, $p2 = 4e-5$, $t(33) = -4.7$. Dev: $p2 > 0.05$, $t(33) = -0.2$) and weak tones (f – Spn: $\Delta = 45\%$, $p2 = 0.001$, $t(33) = -3.5$. Stn: $\Delta = 32\%$, $p2 = 0.003$, $t(33) = -3.2$. Dev: $p2 > 0.05$, $t(33) = -0.1$) ($N = 34$). There were no significant differences between strong and weak tones for the change in spontaneous FR and standard and deviant tone-evoked FR (Spn, Stn, and Dev: $p > 0.05$, 0.28 and 0.95, $t(33) < 1.2$).
Figure 4—figure supplement 7. Differences between PV and SOM effects on standard and deviant tones are preserved for subsets of neurons matched for strength of laser effects on standard tones.

(a) Two subsets of tone responses (N = 66) matched across PV-Cre (above x-axis) and SOM-Cre (below x-axis) mice for standard tone-evoked FR difference between light-on and light-off conditions.

(b) Difference between light-on and light-off FR for spontaneous FR (blue) and standard (gray) and deviant (red) tone-evoked FR and for the PV-Cre (left) and SOM-Cre (right) subsets. With PV photosuppression, spontaneous FR, standard and deviant tone-evoked FR increased (Spn: 20%, p2 =1e-12, t(65) = 8.8, Stn: 19%, p2 = 1e-11, t(65) = 8.2, Dev: 21%, p2 = 0.001, t(65) = 3.3), and there were no significant differences between spontaneous and tone-evoked FR changes (Spn v Stn: p2 > 0.05, t(65) = 0.1, C = 3, Spn v Dev: p2 > 0.05, t(65) = -0.3, C = 3, Stn v Dev: p2 > 0.05, t(65) = -0.3, C = 3). With SOM photosuppression, spontaneous FR and standard tone-evoked FR increased (Spn: 17%, p2 = 1e-8, t(56) = 6.6, Stn: 19%, p2 = 2e-11, t(65) = 8.1), while deviant tone-evoked FR did not change (p > 0.05, t(65) = 0.9). These changes were not significantly different between spontaneous FR and standard tone-evoked FR (Spn v Stn: p > 0.05, t(65) = -1.2), but both were greater than the change in deviant tone-evoked FR (Spn v Dev: 309%, p2 = 0.022, t(65) = 2.8, C = 3, Stn v Dev: 360%, p2 = 0.003, t(3.5), C = 3). By design, the change in standard tone-evoked FR was nearly identical between PV-Cre and SOM-Cre mice (p1 > 0.05, t(65) = -0.1, C = 3). Spontaneous FR was also similarly modulated by PV and SOM photosuppression (p1 > 0.05, t(65) = 0.8, C = 3). However, deviant tone-evoked FR was more strongly modulated by PV photosuppression than by SOM photosuppression (405%, p1 = 0.029, t(65) = 2.4, C = 3).
Figure 4—figure supplement 8. Differences between PV and SOM effects on standard and deviant tone responses are preserved when FRs are normalized by the mean onset response. a, b, c and d as in Figure 4 a, b, d and e, respectively.

(a, b) In PV-Cre mice, spontaneous FR and standard and deviant-tone evoked FR increased with light (b top – Spn: Δ = 210%, p2 = 2e-9, t(159) = -6.4. Stn: Δ = 116%, p2 = 9e-10, t(159) = -6.5. Dev: Δ = 56%, p2 = 5e-11, t(159) = -7.1). For FR changes between light-on and light-off conditions, there was no difference significant difference between standard and deviant-tone evoked FRs (b, bottom – Stn vs Dev: p2 > 0.05, t(159) = 0.7, C = 2), but both were greater than the difference in spontaneous FR (Spn vs Stn: Δ = 34%, p2 = 1e-4, t(159) = -4.1, C = 2. Spn vs Dev: Δ = 26%, p2 = 0.029, t(159) = -2.5).

(c, d) In PV-Cre mice, spontaneous and standard-tone evoked FRs increased with light (d top – Spn: Δ = 46%, p2 = 2e-10, t(113) = -7.0. Stn: Δ = 26%, p2 = 2e-7, t(113) = -5.5), but deviant tone-evoked FRs did not (Dev: p2 > 0.05, t(113) = -1.0). For FR changes between light-on and light-off conditions, there was no difference between spontaneous and standard-tone evoked FR (d, bottom – Stn vs Spn: p2 > 0.05, t(113) = -0.3, C = 2), but both were significantly greater than deviant-tone evoked FR differences (Spn vs Dev: Δ = 298%, p2 = 0.011, t(113) = 2.8 Stn vs Dev: Δ = 282%, p2 = 0.016, t(113) = 2.7, C = 2).
Figure 5. Post-deviant timecourse of interneuron-mediated effect on stimulus-specific adaptation.

(a) Diagram of oddball stimuli illustrating post-deviant tone number used in subsequent analysis; tones and light pulses are as indicated in Figure 3a. Numbers indicate each tone position relative to deviant tones. Responses to any standard tones following light-on standards were excluded from the analysis.

(b, c) Left: Mean population FR in response to standard tones (gray) subsequent to deviant tones (red) within the oddball sequence on light-off (dark colors) and light-on (light colors) trials. All responses are normalized to the response to the fourth post-deviant standard tone on light-off trials (green dashed line). Right: Difference between FR on light-on and light-off trials in response to standard (gray) and deviant (red) tones. (b): PV-Cre mice. (c): SOM-Cre mice.

The following figure supplements are available for Figure 5:

Figure 5—figure supplement 1. Initial timecourse of interneuron-mediated effect on stimulus-specific adaptation.
Figure 5—figure supplement 1. Initial timecourse of interneuron-mediated effect on stimulus-specific adaptation.

The inhibitory influence of PV+ interneurons is persistent while that of SOM+ interneurons builds up over the first 40 tones. (a, b, c, d) Top: Mean population FR in response to consecutive tones of the oddball sequence. Lines represent FR to standard tones on light-off (dark gray) and light-on (light gray) trials, interpolated to continuous lines. Dots represent FR to deviant tones on light-off (red) and light-on (pink) trials. Bottom: Difference between FR on light-on and light-off trials to standard tones of the oddball sequence. Left: Whole oddball sequence. Right: First 50 tones of each sequence. a, b: PV-Cre mice. c, d: SOM-Cre mice.
Figure 6. PV and SOM interneurons exhibit stimulus specific adaptation

(a, d) Optogenetic methods. A1 was injected with AAV-FLEX-ChR2-tdTomato. During experiments, an optic fiber was positioned to target A1 and neuronal activity was recorded using a multichannel silicon probe in A1. Top diagram: Blue light (473 nm) excites PVs in PV-Cre mice or SOMs in SOM-Cre mice. Bottom. Peri-stimulus spike raster of a representative optogenetically identified PV (top) or SOM (bottom). Shaded region – blue light on. (a) PV-Cre. (d) SOM-Cre.

(b, e) PTH of PVs (b) or SOMs (e) FR response to deviant (red) and standard (black) tones. Normalization and dashed lines as in Figure 4a, b.

(c, f) Mean PVs (c) or SOMs (f) FR response over the 100ms of deviant (red) and standard tones (gray), and 100ms of spontaneous activity prior to tone onset (blue). Each line represents a single neuron’s response to each conditions, and its color indicates the magnitude of significant differences between two conditions; Pink, gray, blue and dashed black lines indicate a greater response to deviant tone, standard tone, silence and no significant change, respectively.

(g) Mean SSA index of putative excitatory neurons (gray), PVs (cyan) and SOMs (yellow). Circles represent SSA index values of individual neurons.

The following figure supplements are available for Figure 6:

Figure 6—figure supplement 1. Optical tagging of PVs and SOMs.

Figure 6—figure supplement 2. PVs and SOMs have different adaptation profiles for equal probability tones.
**Figure 6—figure supplement 1. Optical tagging of PVs and SOMs.**

(a) Diagram of optogenetic methods. A1 was injected with AAV-FLEX-ChR2-tdTomato. During experiments, an optic fiber was positioned to target A1 and neuronal activity was recorded using a multichannel silicon probe in A1.

(b, c) Transfection of interneurons with ChR2. Images: Immunohistochemistry demonstrating co-expression of ChR2 and an interneuron-type reporter in A1. Bar Plots: Efficiency (Ef) and specificity (Sp) of visual transfection of PVs (top) and SOMs (bottom) with ChR2. Ef - Percent of labelled interneurons expressing ChR2. Sp -Percent of ChR2 expressing cells which are also labeled interneurons. (b) PV-Cre mouse A1. Green; anti-body stain for parvalbumin. Red; ChR2-tdTomato. Merge; co-expression of ChR2 and PVs. (c) SOM-Cre mouse A1. Green; anti-body stain for somatostatin. Red; ChR2-tdTomato. Merge; co-expression of ChR2 and SOMs. Scale Bar = 25 μm.

(d, e) Fraction of PVs (d) or SOMs (e) exhibiting a greater response to deviants than standards (pink), the reverse (gray), or neither (white).
Figure 6—figure supplement 2. PVs and SOMs have different adaptation profiles for equal probability tones.

(a, c) PTH of PVs (a) or SOMs (c) FR response to standard (black), equal probability (green) and deviant (red) tones. Normalization and dashed lines as in Figure 4a, b.

(b, d) Mean PV (b) or SOM (d) population FR response to standard (gray), equal probability (green) or deviant (red) tones over 100ms tone duration. The mean spontaneous FR (during the 100ms prior to all tones) of oddball and equal probability stimuli was subtracted from respective tone-evoked mean FRs. In PVs, equal probability tones evoked FRs greater than standard tones (b – N = 16, Δ = 110%, p2 = 0.030, z = -2.4, C = 2), and not significantly different that deviant tones (p2 > 0.05, z = -1.7, C = 2). In SOMs, equal tones evoked higher FRs than standard tones (c – N = 28, Δ = 95%, p2 = 0.022, z = -2.6, C = 2), and lower FRs than deviant tones (Δ = -36%, p2 = 0.049, z = -2.3, C = 2). In both types of interneuron, deviant tones evoked higher FRs than standard tones (b, PV - Δ = 188%, p2 = 0.010, z = -2.8, C = 2. c, SOM – Δ = 205%, p2 = 0.002, z = -3.3, C = 2).
Figure 7. Mutually coupled excitatory-PV-SOM neuronal model accounts for differential effects of PVs and SOMs on SSA in putative excitatory neurons.

(a) Center: Diagram of coupled network model. Excitatory (Exc) and two types of inhibitory interneurons (PV and SOM), receive tone-evoked inputs. They make reciprocal connections on each other; Exc makes excitatory synapses on PV or SOM; PV and SOM inhibit Exc. Closed circles: excitatory synapses. Open circles: inhibitory synapses. Orange outlines: excitatory input-output pathway. Purple outlines: PV input-output pathway. Green outlines: SOM input-output pathway. The effect of optogenetic modulation was modeled as an additional input current delivered to inhibitory neuronal populations. Adaptation was modeled as decaying synaptic coefficient with slow adaptation. Left and right inset plots: Combined input-output transfer function that represents the transformation between synaptic inputs and the activity of excitatory neurons. The values of inputs are depicted by arrows for the spontaneous and tone-evoked activity in response to deviant and standard tones under light–off (dark color) and light-on (light color) conditions, with change due to light highlighted by light green arrows.

(b, d) Tone-evoked responses of model neuronal excitatory population to deviant (red) and standard tones (gray), i.e. the first and fourth consecutive tone presented, under light-off (dark colors) and light-on (light colors) conditions. Dashed lines indicate light onset and offset (green) and tone onset and offset (gray). (b) Light suppresses PVs. (d) Light suppresses SOMs.

(c, e) Left: Spontaneous FR (blue) and standard (black) and deviant (red) tone-evoked FRs on light-off (dark colors) and light-on (light colors) conditions. Right: Mean difference between responses on light-on and light-off conditions. (c) Light suppresses PVs. (e) Light suppresses SOMs.

The following figure supplements are available for Figure 7:

Figure 7—figure supplement 1. Adaptation to repeated tones in model excitatory and inhibitory neurons.

Figure 7—figure supplement 2. Excitatory-inhibitory model with inhibitory inputs from SOM to PV population accounts for differential effects of PVs and SOMs on SSA in putative excitatory neurons.
Figure 7—figure supplement 1. Adaptation to repeated tones in model excitatory and inhibitory neurons.

Responses evoked by four consecutive tones Exc (purple), PVs (orange, a) and SOMs (green, b).

Note adaptation in the responses of both excitatory and inhibitory neurons. During 4th tone, there is light-evoked suppression of interneuron activity. Ligh-On: solid; Light-Off: dashed lines. (a) Light suppresses PVs. (b) Light suppresses SOMs.
Figure 7—figure supplement 2. Excitatory-inhibitory model with inhibitory inputs from SOM to PV population accounts for differential effects of PVs and SOMs on SSA in putative excitatory neurons.

(a) Center: Diagram of coupled network model. Model is as in Figure 7, with additional inhibitory inputs from SOM to Exc population.

(b, e) Tone-evoked responses of model neuronal excitatory population to deviant (red) and standard tones (gray), i.e. the first and fourth consecutive tone presented, under light-off (dark colors) and light-on (light colors) conditions. Dashed lines indicate light onset and offset (green) and tone onset and offset (gray). (b) Light suppresses PVs. (e) Light suppresses SOMs.

(c, f) Left: Spontaneous FR (blue) and standard (black) and deviant (red) tone-evoked FRs on light-off (dark colors) and light-on (light colors) conditions. Right: Mean difference between responses on light-on and light-off conditions. (c) Light suppresses PVs. (f) Light suppresses SOMs.

(d, g) Responses evoked by four consecutive tones Exc (purple), PVs (orange) and SOMs (green). Note adaptation in the responses of both excitatory and inhibitory neurons. During the 4th tone, there is light-evoked suppression of interneuron activity. Dark traces: Light-off. Light traces: Light-on. (d) Light suppresses PVs. (g) Light suppresses SOMs.