Somatostatin Interneurons Control a Key Component of Mismatch Negativity in Mouse Visual Cortex

Graphical Abstract

Highlights

- Visual mismatch negativity (MMN, reduced in schizophrenia) is reproduced in awake mice
- Two MMN components (adaptation and deviance detection) are differentiated in neurons
- Silencing somatostatin-containing interneurons (SOMs) specifically impairs deviance detection
- Silencing SOMs also alters MMN-related theta/alpha activity, resembling patient MMN

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In Brief
Hamm and Yuste develop a mouse model of mismatch negativity, a classic EEG biomarker of schizophrenia. They pinpoint two components of this marker within visual cortex (adaptation and deviance detection); revealing that pharmacogenetic suppression of somatostatin inhibitory neurons specifically eliminates deviance detection, the higher-order component critically deficient in patients.
INTRODUCTION

Schizophrenia involves fundamental aberrations in perception and cognition. Deficient neural processing of environmentally salient events may underlie these core symptoms (Javitt and Freedman, 2015). Such deficiencies have been traditionally quantified in schizophrenia patients using electroencephalography (EEG) while employing “oddball” sensory stimulation paradigms. In such experiments, evoked cortical responses to a repetitive or “standard” auditory stimulus are compared to a deviant or target stimulus. The resulting difference in EEG potentials between the redundant and the deviant stimulus is quantified and termed mismatch negativity (MMN). While psychiatrically healthy individuals react to the new, deviant stimulus with an amplified cortical response (or MMN, peaking between 150 and 250 ms post-stimulus onset), schizophrenia patients show a strongly reduced MMN, as if they did not register the target stimulus as deviant. A reduced MMN response is a widely replicated biomarker of schizophrenia, showing invariance across sensory modalities and even predicting illness onset in pre-morbid individuals (Garrido et al., 2009; Light and Näätänen, 2013).

More specifically, the MMN difference potential consists of at least two more basic underlying components: (1) a simple adaptation (reduction) of neural responses to a redundant or repeated stimulus (stimulus-specific adaptation, or SSA), and (2) an amplified deviance detection response, indicating that the rare stimulus violates the expected contextual regularity (Harms et al., 2016). Animal studies show that SSA is present both cortically and subcortically and may reflect synaptic depression in feedforward excitatory inputs (Farley et al., 2010; Garrido et al., 2009). But the neural mechanisms underlying deviance detection, and thus the salience processing deficits thought to underlie the MMN biomarker of schizophrenia, remain unknown.

Understanding whether and how deviance processing is generated in sensory cortex could help link quantifiable biomarkers to the fundamental disease pathophysiology and symptoms. While non-SSA components of MMN are dependent on NMDA receptor neurotransmission (Farley et al., 2010; Javitt and Freedman, 2015), little consistency exists regarding the contribution of GABAergic inhibition (Garrido et al., 2009). Examining the causal roles of specific inhibitory interneuron subtypes, rather than GABAergic cells in general, may help advance our understanding of MMN. In particular, somatostatin-containing interneurons (SOMs) display abnormalities in post-mortem brain samples of schizophrenia patients (Hashimoto et al., 2008). SOMs are strong candidates for MMN processing given their (1) late input/output facilitation (or amplification) in the MMN time range (Karnani et al., 2014), (2) capacity for both inhibition and disinhibition of neighboring pyramidal neurons (Cottam et al., 2013), and (3) preferential inhibition of the local neuronal network to redundant stimuli compared to deviants (Natan et al., 2015). These characteristics contrast those of other more commonly studied interneuron types, such as parvalbumin (PV)-containing cells (Cottam et al., 2013; Karnani et al., 2014; Natan et al., 2015; Zhu et al., 2015).

The use of genetically engineered cre-driver mice would enable the investigation of neocortical SOM involvement in MMN directly (Cottam et al., 2013). Studies employing basic oddball paradigms have shown that MMN-like waveforms (deviant minus redundant) are present in mouse EEG recordings (Featherstone et al., 2015), but an analytical and mechanistic differentiation of higher-order deviance processing from basic sensory adaptation (SSA) is absent, leaving the translational relevance to human MMN unclear (Harms et al., 2016). Some studies...
focusing on SSA have used mouse strains that allow interneuron access with optogenetics or florescent microscopy (PV-cre, SOM-cre). Natan et al. (2015) quantified SSA from extracellular spiking in auditory cortex (A1) of anesthetized mouse and showed that both SOMs and PVs play a role in SSA; however, deviance detection was not examined in this study, and a link to MMN was never claimed. Using patch recordings, again in anesthetized mouse A1, Chen et al. (2015a) focused mainly on SSA but found some evidence of subthreshhold deviance detection in pyramidal neurons in tertiary experiments. Spiking outputs nevertheless failed to show deviance detection in this setup, leaving the question of whether rodent neocortical circuits generate or even passively relay deviance-related, human-like MMN signals uncertain at best.

RESULTS

Here we demonstrate that key MMN components, SSA and deviance detection, are quantitatively and mechanistically differentiable in mouse cortical circuits. Unlike past work, we study awake animals, a potentially important experimental difference because the activity of SOMs in particular is strongly suppressed under anesthesia relative to awake conditions (Gentet et al., 2012). Furthermore, we focus on primary visual cortex, because neurons of specific orientation selectivity are intermixed in mouse primary visual cortex (V1) (Niell and Stryker, 2008), allowing for localized recordings of both adapted and facilitated populations simultaneously in a simple oddball paradigm. Using targeted pharmacogenetics (designer receptors exclusively activated by designer drugs, or DREADDs), we further show that a deficit in SOMs, a known component of schizophrenia pathophysiology, could be causally related to the MMN biomarker and affect related oscillatory dynamics in the theta/alpha band.

**Stimulus Adaption and Deviance Detection Can Be Found in Mouse Cortical Circuits**

We performed (1) multielectrode recordings with current source density (CSD) analysis (described later) to measure localized synaptic activities (Buzsáki et al., 2012) and (2) fast two-photon resonance imaging (30 Hz) of florescent calcium indicators (GCaMP6s/f) to infer spiking activity in populations of individual neurons simultaneously and stably in awake, head-fixed mice (C57BL/6 background; n = 31; Figure 1A; Figure S1). We included three 10- to 15-min stimulus presentations (Figure 1B): (1) oddball with 12.5% deviant full-field square-wave gratings of 45° intermixed with 87.5% redundants of 135°, (2) oddball with stimulus reversal (135° deviants), and (3) many standards control sequence with eight orientations (0°–180°), each occurring 12.5% of the time. We then quantified cortical responses to the same stimulus across contexts wherein it is (1) redundant, (2) rare and contextually deviant, and (3) rare but not deviant (many standards control), equating the number of trials across conditions. Comparing presentation 1 to presentation 3 yields an estimate of SSA, whereas presentation 2 compared to presentation 3 estimates deviance detection (Harms et al., 2016).

Local field potentials (LFPs) were recorded from 16 linearly arranged sites on a silicon probe (spaced 50 μm for 750 μm; Figure 1G). Past studies of MMN in humans have typically compared EEG response magnitudes between deviant and redundant stimuli directly or generated a difference score to be compared between diagnostic groups (Javitt and Freedman, 2015). Here, average stimulus-evoked LFP traces (Figure 1D; Figure S1A) showed a protracted deviant greater than the redundant effect in mid- to late time latencies (40–240 ms, t(13) = –2.19, p < 0.05), which similar to human MMN, peaked around 120–240 ms (LFP-MMN; Figure 1D, right; plots and analyses are computed by averaging over stimulus orientations unless otherwise stated). The LFP, like EEG, is spatially imprecise, convolving activity from 0.5 to 1 cm² involving neighboring layers, columns, and in mice, other brain regions (Buzsáki et al., 2012). To examine underlying components of this effect at the circuit level, LFPs were transformed to CSD by computing the discrete second spatial derivative (Figure 1D). Consistent with previous CSD recordings in mouse V1, we identified a large current sink approximately 400 μm from the surface, flanked by later current sources in superficial and deeper regions, which may correspond to granular, supragranular, and infragranular cortical regions, given their concordance with previously verified histological work and high-pass filtered multunit activity (see Experimental Procedures; Figure S1A). Regardless of cortical depth, we found clear evidence of SSA, mainly within an earlier range (40–80 ms), and in a later range (120–240 ms), we found clear evidence of deviance detection (Figure 1E). After rectifying averages and combining across layers, we confirmed statistically smaller responses to redundant (t(13) = 3.24, p < 0.01) and greater responses to deviant (t(13) = –2.94, p < 0.01) than control (putative SSA and deviance detection; Figure 1F).

We then used two-photon calcium imaging (2P-Ca²⁺) to measure spiking activity of populations of neurons in layer 2/3 of V1 (820 neurons across 18 mice; Figure 1G). We quantified frame-to-frame changes in florescence (delta-f) converted to Z scores based on baseline signal fluctuation (see Experimental Procedures). Approximately 19% of all recorded neurons (n = 160) displayed an increase in activity of greater than 1.67 SD above the pre-stimulus baseline (one-tailed probability, p < 0.05; Figure 1H; Figure S2) to one of the two key stimulus orientations in either the control or the deviant condition. Among these cells, we observed a clear reduction in activity, relative to control, when its preferred stimulus was redundant (t(159) = 5.40, p < 0.01; Figures 1I and 1J; Figure S2) and a marked increase in responses when its preferred stimulus was contextually deviant (t(159) = –2.73, p < 0.01; Figures 1I and 1J; see Figures S1C and S1D for further validation). These effects were consistent with electrophysiological multunit activity (unsorted population spiking) acquired with the multielec- trode (Figure S1B). Thus, using this visual oddball paradigm, an MMN-like LFP response was present, and key underlying components, SSA and deviance detection, could be measured and differentiated in mouse V1.

**Silencing SOMs Disrupts Deviance Detection but Not SSA**

Given our ability to measure and differentiate MMN components in this mouse model, we next explored potential local circuit elements contributing to these phenomena. Given the specialized functional properties of SOMs compared to other interneuron
types (noted earlier), we tested whether the MMN-like response we observed in mouse V1 depended on SOM activity by using a pharmacogenetic approach (DREADDs), virally targeting SOMs in a SOM-cre mouse line to express inhibitory channel hM4D (Figure S3). Activation of hM4D by clozapine N-oxide (CNO; a naturally inert ligand) functionally silences host cells primarily through the suppression of synaptic current amplitude and release (see Supplemental Information). We quantified CSDs and 2P-Ca²⁺ dynamics before and 30 min after subcutaneous injection of CNO (12 mg/kg CNO) in SOM-hM4D mice (n = 11) or controls (n = 9). Immunohistochemistry confirmed the specificity of hM4D-mCherry construct to SOM interneurons across all cortical depths (Figure S3). The 2P-Ca²⁺ dynamics recording in a dark room during non-locomotive rest confirmed a generalized disinhibition of the local network after SOM suppression (Figures S4A–S4C) but did not significantly alter the frequency of locomotion (F(1,10)SOM-hM4D = 0.82, p = 0.38).

Traditional MMN analysis of average LFP values (deviant minus redundant) in the 120- to 240-ms range suggested that SOM suppression disrupted MMN-like responses (Figure 2), showing a group-treatment interaction (F(1,7) = 6.08, p < 0.05; F(1,4)CNO-control = 0.04, p = 0.85; F(1,4)SOM-hM4D = 8.53, p < 0.05; Figures 2A, 2B, 2F, and 2G). Early latency SSA (40–80 ms) was not affected by SOM suppression (control minus redundant; group-treatment interaction; F(1,7) = 0.13, p = 0.72; F(1,3)CNO-control = 1.54, p = 0.30; F(1,4)SOM-hM4D = 0.00, **p < 0.01. Error bars, SEM.

Figure 1. Circuit-Level Components of MMN Are Present in Visual Cortices of Awake Mice

(A and B) Head-fixed mice viewed square-wave gratings while running on a treadmill (A) during oddball and many-standards control of stimulus frequency (B). (C–F) The 16-channel multielectrode recordings in left visual cortex (C) reveal a significant deviant versus redundant effect in the peak LFP channel with a time course similar to human MMN (D). CSD profiles with an initial large current sink occurring in a putative granular layer (E) evinced SSA and deviance detection (DD) across all depths (averaged over orientations; F). Significant SSA occurred early (40–80 ms), while DD occurred later (120–240 ms; plots cross-animal averages, and bars reflect area under the curve [AUC] for these time ranges averaged across all depths).

(G–J) 2P-Ca²⁺ of GCaMP6-expressing cells (G and H) demonstrate SSA and DD in the 19% neurons significantly activated by stimuli (only response to the preferred stimulus is plotted or analyzed for each cell; H–J); (I) Averaged across 159 neurons (0–1 s AUC).

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p = 0.97; Figures 2C, 2D, 2H, and 2I). However, deviance detection, particularly in the time range of the MMN (120–240 ms), was strongly diminished after SOM suppression (deviant minus control; group-treatment interaction; $F(1,7) = 5.75, p < 0.05$; $F(1,4)_{\text{SOM-hM4D}} = 7.56, p = 0.05$; $F(1,3)_{\text{CNO-control}} = 0.03, p = 0.87$; Figures 2C, 2E, 2H, and 2J). This pattern of effects was mirrored in $2P$-$\text{Ca}^{2+}$ (top 40 neurons plotted in Figures 3A and 3B). The proportion of active neurons expressing deviance detection, but not SSA, was decreased after CNO treatment only in SOM-hM4D ($\chi^2 = 6.16; F(1,42)_{\text{SOM-hM4D}} = 7.81, p < 0.01$), which was driven by a treatment effect in the SOM-hM4D group only ($F(1,13)_{\text{SOM-hM4D}} = 12.0, p < 0.01$; $F(1,29)_{\text{CNO-control}} = 0.36, p = 0.55$; Figure 3E, normalized by pre-treatment response). These results held true for non-normalized values (Figures S4G and S4H). SOM suppression did not alter evoked calcium transients to control stimuli in responsive neurons (first 500 ms, $F(1,64)_{\text{SOM-hM4D}} = 1.66, p = 0.20$; Figures S4D–S4H), but it did produce a trend-level increase in responses to non-preferred stimulus orientations ($F(1,89)_{\text{SOM-hM4D}} = 3.24, p = 0.07$; repeated-measures ANOVA on magnitudes $90^\circ$ from peak response; Figure S4D). Previous work has reported either small increases (Zhu et al., 2015) or no change in neuronal responses to visual stimuli after SOM suppression (Chen et al., 2015b), consistent with their concurrent roles in subtractive inhibition and pyramidal cell disinhibition in V1 (Cottam et al., 2013).

These data thus demonstrated that blocking SOM function selectively reduces deviance detection in cortical circuits without substantively affecting average cell or circuit-level responses at baseline or to redundant stimuli.

**Deviance Detection Responses Are Generated by SOMs in the Theta/Alpha Band**

LFP or EEG recordings of neocortical sensory-evoked responses capture not only large transient events but also dynamic changes in oscillatory power, which reflect population-level synchrony at multiple timescales and frequencies. While other GABAergic interneurons, particularly fast-spiking PV-containing cells, generate population-level synchrony in the gamma band,
converging evidence suggests that SOMs may play a similar role in lower bands, including theta/alpha (4–14 Hz) or beta (15–30 Hz; Womelsdorf et al., 2014). In awake mice, SOM interneurons show spontaneous and evoked firing rates in the 4- to 20-Hz range (Cottam et al., 2013; Gentet et al., 2012). We reasoned that if SOMs were supporting human-like MMN and deviance detection, it should be most apparent in LFP recordings of stimulus-elicited oscillations in subgamma bands.

We adopted a spectral analytical approach previously optimized on clinical EEG data from schizophrenia patients to maximize translational potential (see Supplemental Information). We converted single-trial LFP traces from putative granular layers (the location of the strongest LFP response; Figure S1) to time-frequency power spectra using a modified Morlet wavelet approach (4–120 Hz, 2-Hz steps, 1–14 cycles, 5-ms steps from /C0 to 450 ms post-stimulus, averaged across trials and within conditions in Figure 4A). For SSA, redundants evoke significantly lower (30–450 ms) gamma power (34–60 Hz) than responses to controls (\(F(1,8) = 22.5, p < 0.01\); Figures 4B–4D), but there was no effect of SOM suppression (i.e., no interaction effects; Figure 4B–4D). For deviance detection responses, there was a stimulus-treatment interaction only for theta/alpha power (deviants versus control, pre- versus post-CNO; \(F(1,8) = 8.56, p < 0.05\)). Significantly enhanced low-frequency power for deviants was present before (\(t(4) = –2.70, p < 0.05\)) but not after (\(t(4) = 1.27, \text{not significant; Figures 4E and 4F}\)). Together, these findings further establish (1) the quantitative and mechanistic differentiability of SSA and deviance detection and (2) a stronger link of SOMs to schizophrenia pathophysiology observed in human EEG studies (Clementz et al., 2016).

**DISCUSSION**

Our results demonstrate that deviance processing, a critical component of the MMN biomarker of psychosis, is present in V1 circuits in awake mice and depends on the activity of SOMs. These findings suggest a causal link in schizophrenia between SOM abnormalities seen in post-mortem studies, low-frequency population oscillatory dynamics, and deficits in salience processing in patients. Future work is needed to identify new inroads for therapeutic rescue of sensory deficits and may uncover further fine-scale mechanistic details for how SOMs exert this control. It is unlikely that PV-containing cells contribute similarly to MMN (Natan et al., 2015), but other neocortical cell types (e.g., vasoactive interstitial peptide neurons; Karnani et al., 2014) and modulatory neurotransmitters (e.g., acetylcholine; Chen et al., 2015b) may play key mediating or moderating roles in the SOM-deviance detection relationship.

Although most studies in patients have quantified MMN in the auditory domain, visual MMN and visual processing are not spared in schizophrenia (Garrido et al., 2009; Javitt and Freedman, 2015). Nevertheless, caution should be taken when equating sensory modalities in a one-to-one manner between species; our results present an animal model for investigating psychotic biomarkers and sensory-cortical microcircuitry in general. As an additional point of consideration, some slight but non-significant, long-term, pre- versus post-CNO decreases in evoked activity responses were seen across all measures (LFP, CSD, and calcium transients), which may reflect longer-term adaptation effects (Figure 2; Figures S4F–S4I) or even CNO administration (though this is unlikely; Roth, 2016). These did not differ as a function of condition (i.e., SOM suppression) and clearly did not alter the relative magnitudes of the constructs of interest (MMN, deviance detection, and SSA), but they may be important topics for future study.

By linking the SOMs to deviance detection, and thus the generation of human-like MMN, our data provide a mechanistic entry point into the pathophysiology of schizophrenia and associated sensory or cognitive dysfunction. SOMs and MMN most directly affect low-frequency bands in the evoked response, which is in line with most EEG research, suggesting that theta/alpha disruptions in schizophrenia are more consistent, are more heritable,
and carry larger effect sizes when compared to beta or gamma disruptions (Moran and Hong, 2011). Gamma-band power was neither altered by SOM suppression nor tracked with deviance detection, which is also in line with the notion that PV interneurons do not play a similar role in these phenomena (Womelsdorf et al., 2014). Linking specific pathophysiology like SOM abnormalities to empirical biomarkers such as the MMN may help future attempts at reclassifying psychotic disorders (Clementz et al., 2016) and potentially enable therapeutic approaches at the local microcircuit level.

**EXPERIMENTAL PROCEDURES**

Detailed descriptions of experimental procedures and rationale are provided in Supplemental Experimental Procedures.

**Animals, Surgery, and Training**

All experimental procedures were approved by and carried out in accordance with Columbia University institutional animal care guidelines. Experiments were performed on adult PV-cre (n = 10) or SOM-cre transgenic mice (n = 21; Jackson Laboratory, C57BL/6 background, 22–32 g, N = 31), at postnatal day (P) 60 to P90. Virus injection, titanium head plate fixation (see Supplemental Information), and skull thinning or craniotomy were carried out in that order of the course of 4 weeks. A glass capillary pulled to a sharp micropipette was advanced with the stereotaxic instrument (coordinates from lambda: x = 2,500 μm, y = 200 μm), and a 75-μl solution of 1:1 diluted AAV1/Syn:GCaMP6s/f and AAV8hSynDI0mM4D(Gi) mCherry (University of North Carolina [UNC] vector core; n = 11 SOM-cre mice) was injected into putative layer 2/3 over a 5-min period at a depth of 200–300 μm from the pial surface using a UMP3 microsyringe pump (World Precision Instruments).

On the day of the experiment, the mouse was anesthetized again with isoflurane. For LFP recordings, a small region (approximately 0.5 mm in diameter) was removed over left V1 centered just anterior to the injection site. The skull was thinned until the bone, moistened with saline, was transparent enough that the underlying vasculature was visible to the naked eye (usually 10 min of drilling). The mouse was then allowed to wake up and was transferred to the wheel for recordings.

**Multielectrode Recordings**

Extracellular electrophysiological data are reported on 14 mice (8 female; 22–28 g; 5 PV-cre, 9 SOM-cre). The 16-channel linear silicon probes (spaced at 50-μm intervals; model a1x16-3mm50-177, Neuronexus Technologies) were inserted perpendicular to and with the top electrode aligned just at the pial surface (visually confirmed with an adjustable miniature digital microscope; Adafruit). Recordings were referenced to the skull above prefrontal cortex and grounded to the head plate. Continuous data were acquired with a MiniDigi amplifier and software (Plexon). LFP signals were filtered from 0.5 to 300 Hz and sampled at 1 kHz. Single electrodes with the peak negative deflection 50–200 ms post-stimulus near putative granular cortex were reserved for LFP analysis, or the whole array was analyzed as CSD (described later). Locomotion was recorded as stripes in the running wheel crossed the path between an infrared light emitting diode and photo-Darlington pair (see Supplemental Information).

**2P-Ca**

The activity of cortical neurons was recorded by imaging fluorescence changes under a two-photon microscope (Bruker) excited with a Ti:sapphire laser (Chameleon Ultra II; Coherent) tuned at 940 nm and scanned with resonant galvanometers through a 20× (0.95 numerical aperture) water immersion objective lens (Olympus). To ensure stability of the imaging meniscus for long imaging sessions, a small volume (approximately 1 ml) of Aquasonic ultrasound gel (Parker Laboratories) was centrifuged and dolloped onto a moistened, thinned skull in lieu of water. Scanning and image acquisition were controlled by Prairie View software (~30 frames per second for 256 × 256 pixels, 200–225 μm beneath the pial surface). On imaging days (before and after treatment), mice were allowed 1 hr on the wheel before imaging began. Imaging consisted of a visual stimulation condition (15 min), followed by 20–40 min of awake rest in a dark room with the monitor off. Data are reported on 18 mice (11 female; 7 PV-cre, 11 SOM-cre).

Both 2P-Ca** and LFP recording sessions occurred between 11 a.m. and 3 p.m. Mice were monitored by the experimenter to ensure they were awake...
during data collection. Locomotion was detected as voltage deflections in the photo-Darlington readout. While previous work has suggested that locomotion enhances visual processing in V1 in mice, most of our mice did not exhibit enough locomotion to enable thorough examination of this effect in our paradigm (>10% of frames or trials in LFP). Therefore, when detected, frames or trials during locomotion periods were excluded, along with the previous and subsequent 60 frames (2 s). Large eye movements are uncommon in mice except during periods of locomotion, and because our experiments employed near-full-field visual stimulation, the input for the recorded V1 retinotopic subfields was likely unaltered across trials and conditions.

Visual Stimulation

Visual stimuli were generated using the MATLAB (MathWorks) Psychophysics Toolbox and displayed on a liquid crystal display monitor (19-inch diameter, 60-Hz refresh rate) positioned 15 cm from the right eye, roughly at 45° to the long axis of the animal (Figure 1A). Stimuli were static full-field square-wave gratings (100% contrast, 0.04 cycles/degree) oriented in two separate orientations for the oddball paradigm (45° and 135°) or in eight orientations for the many-standards control (30°, 45°, 60°, 90°, 120°, 135°, 150°, and 180°). Stimuli were presented for 500 ms, followed by an interstimulus interval of 1,000–1,500 ms of mean luminescence gray screen. In the oddball sessions, the standard stimulus was presented at a minimum of three sequential trials, followed by a linearly increasing probability of the target stimulus on each successive trial to yield an overall 12.5% probability of targets. These sessions lasted 10 min and were repeated with the standard and target stimulus reversed. In the many-standards sessions, stimuli of six separate orientations each lasted 10 min and were repeated with the standard and target stimulus reversed. For SSA, the discrete first derivative on lowess-smoothed traces was scored as delta-f (within-cell versus single-cell comparison) and used to compute a CSD map. For deviance detection, the cross-trial average LFP response from 40 to 240 ms post-stimulus was averaged across mice and compared between redundant and deviant stimuli with a paired-samples t test. SSA and deviance detection were confirmed with CSD and 2P-Ca(i).

CSD

LFP data were manually pre-screened for excessive artifact (e.g., signal greater than 8 SDs), and aberrant trials were removed and noisy channels were interpolated if present (never more than two channels, and never two adjacent channels). Data were then digitally filtered from 0.1 to 300 Hz (band-pass least-squares finite impulse response [FIR]) with a 60-Hz notch filter. Average CSD was computed either from the average LFP (for Figure 1D) or on single trials and averaging across trials (number equalized across conditions; between 32 and 68) by taking the discrete second derivative across the electrode sites and interpolating to produce a smooth CSD map (Buzsaki et al., 2012; Neill and Stryker, 2008). Putative laminar subregions (three adjacent channels) were defined, based on CSD demarcations previously published and histologically verified, in mouse V1 for each mouse separately based on average CSD plots (see Supplemental Information). The presence of MMN-like responses and their DREADD modulation did not appear to differ dramatically as a function of layer, so statistical analyses and subsequent conclusions focus more generally on local (but not layer-specific) processing in a V1 column. That is, we calculated a rectified CSD for each layer domain and averaged across domains within each mouse for statistical comparisons (Figures 1 and 2). Average CSD waveforms were then averaged across stimulus orientations.

Image Analysis

Imaging datasets were scored similarly to previous reports (see Supplemental Information). Regions of interest were selected using a semi-manual algorithm assisted by principal-component analysis with halo subtraction (see Supplemental Information). The discrete first derivative on lowess-smoothed traces was scored as delta-f (within-cell versus single-cell comparisons). A 2- to 10-s baseline window was manually selected for each that contained no apparent calcium transients. The mean and SD was calculated on the delta-f values in this window for each cell for the whole experiment, which was used to compute a z-scored delta-f for visualizing and combining activity across cells and determine activation thresholds (see Supplemental Information).

Single-Cell Analyses

Condition averages of normalized delta-f values for redundant (fourth in sequence), deviant, and control stimuli were calculated separately for each stimulus type for each neuron. All analyses focused on the first ten trials to equate across conditions, cells, and mice with varying numbers of available trials. Initial analyses focused on neurons showing, during control or deviant conditions, an average post-stimulus (0–1 s) response of 1.67 SDs above the pre-stimulus baseline (equating to a one-tailed p value of 0.05; n = 160, or 18.9% of all imaged cells; responsive cells). Only responses to one stimulus orientation were considered for each cell (i.e., the orientation with greater magnitude). A minority of mice expressed GCaMP6f (five mice, three of which were SOM-cre), while the rest expressed GCaMP6s (see Supplemental Information).

Pharmacogenetic Suppression of Interneurons

On the day of recording, mice viewed visual stimuli with intermittent rest periods as described earlier. Then, hM4D-SOM mice and an equivalent number of control mice each received a subcutaneous injection of CNO (12 mg/kg, within the range of previously reported doses; Roth, 2016; see Supplemental Information), followed by a repeat of visual stimuli and rest periods during imaging or LFP recording 30 min later. In the presence of CNO, hM4D(Gl) activation functionally silences host cells primarily through the suppression of synaptic current amplitude and release (Roth, 2016).

Statistical Procedures

All significance values for t tests are two-tailed. For establishing the presence of MMN-like potentials, the cross-trial average LFP response from 40 to 240 ms post-stimulus was averaged across mice and compared between redundant and deviant stimuli with a paired-samples t test. SSA and deviance detection were confirmed with CSD and 2P-Ca(i).

CSD

Paired t tests were computed on mouse-wise averages. For SSA, we compared responses between control and redundant stimuli (using the fourth redundant to normalize for trial counts and for relative time during the run) in the early range (40–80 ms). For deviance processing, we compared responses between deviant and control stimuli in the late range (120–240 ms; Chen et al., 2015a).

2P-Ca(i)

Responses were quantified as described earlier (Single-Cell Analyses). Initial demonstration of SSA and deviance detection were established with paired t tests on cell-wise averages of post-stimulus activity (0–1 s) from responsive cells. For SSA, we compared responses between redundant and control stimuli for deviance processing, we compared responses between deviant and control stimuli. Trial numbers were equated between stimulus conditions. All standard error (SE) bars in all figures reflect within-subjects versus within-cells SEs. For determining the effect of SOM suppression, a slightly different statistical approach was employed.

LFP/CSD

For LFP estimates of MMN (deviant minus redundant responses, 120- to 240-ms range), CSD estimates of SSA (redundant minus control, 40–80 ms), and CSD estimates of deviance detection (deviant minus control, 120–240 ms), difference values for each mouse were subjected to 2 by 2 mixed ANOVA, with group (CNO-control; SOM-hM4D) as the between-subject variable and treatment (pre-stimulus versus post-stimulus, or pre/post) as the within-subject variable. One-way repeated-measures ANOVAs within groups were used to describe interaction effects. As an exploratory step, we next focused on stimulus-evoked oscillatory power in the LFP of the SOM-hM4D group. For SSA and deviance detection separately and for each frequency band, we carried out 2 by 2 repeated-measures ANOVA on evoked power within mice, with stimulus (redundant or deviants; control) and treatment (pre/post) as within-subject variables. Follow-up paired t tests for interactions were computed within treatment conditions.

2P-Ca(i)-SOM Suppression

Two complimentary statistical approaches tested for single-cell effects of SOM suppression. First, focusing on responsive cells during the main SSA time window (0–500 ms post-stimulus, control only; number of cells: CNO-control/pre/post = 60/63, SOM-hM4D/pre/post = 60/60), we computed the proportion of cells showing a redundant response of less than X SDs below the control response average (for X = 0.25 to 2.5). Then, focusing on responsive cells during the main deviance detection time window (150–750 ms post-stimulus, control or deviant; number of cells: CNO-control/pre/post = 82/90,
SOM-hM4Dpre/post = 100/103), we computed the proportion of cells showing a deviant-stimulus response of greater than X SDs above the control response average (for X = 0.25 to 2.5 in 0.1 SD steps). Thus, each of the four conditions (pre, post, control, or SOM) had one SSA curve and one deviance detection curve. We then computed a log rank test on these curves for SSA and deviance detection separately and for CNO-control and SOM-hM4D separately.

Second, we focused on the cells with the top 10% magnitude average responses to the control stimulus (for SSA) or to the deviant stimulus (for deviance detection) in the pre-treatment run. Responses to the redundant stimulus and responses to the deviant stimulus were divided by the average response to the control stimulus for each of the four conditions. Then, separately for SSA and deviance detection (DEV), 2-by-2 mixed ANOVA on individual cells with group (CNO-control; SOM-hM4D) as the between-subject variable and treatment (pre/post) as the within-subject variable was computed. One-way repeated-measures ANOVAs within groups were used to describe interaction effects. Pre-normalized values were also reported in Figure S4i, confirming the effects did not depend on changes in baseline response magnitudes.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2016.06.037.

AUTHOR CONTRIBUTIONS


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