Hour-long adaptation in the awake early visual system

Carl R. Stoelzel,1 Joseph M. Huff,1 Yulia Bereshpolova,1 Jun Zhuang (庄骏),1 Xiaojuan Hei (黑晓娟),1 Jose-Manuel Alonso,1,2 and Harvey A. Swadlow1,2

1Department of Psychology, University of Connecticut, Storrs, Connecticut; and 2Department of Biological Sciences, State University of New York, New York, New York

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Stoelzel CR, Huff JM, Bereshpolova Y, Zhuang J, Hei X, Alonso JM, Swadlow HA. Hour-long adaptation in the awake early visual system. J Neurophysiol 114: 1172–1182, 2015. First published June 24, 2015; doi:10.1152/jn.00116.2015.—Sensory adaptation serves to adjust awake brains to changing environments on different time scales. However, adaptation has been studied traditionally under anesthesia and for short time periods. Here, we demonstrate in awake rabbits a novel type of sensory adaptation that persists for >1 h and acts on visual thalamocortical neurons and their synapses in the input layers of the visual cortex. Following prolonged visual stimulation (10–30 min), cells in the dorsal lateral geniculate nucleus (LGN) show a severe and prolonged reduction in spontaneous firing rate. This effect is bidirectional, and prolonged visually induced response suppression is followed by a prolonged increase in spontaneous activity. The reduction in thalamic spontaneous activity following prolonged visual activation is accompanied by increases in 1) response reliability, 2) signal detectability, and 3) the ratio of visual signal/spontaneous activity. In addition, following such prolonged activation of an LGN neuron, the monosynaptic currents generated by thalamic impulses in layer 4 of the primary visual cortex are enhanced. These results demonstrate that in awake brains, prolonged sensory stimulation can have a profound, long-lasting effect on the information conveyed by thalamocortical inputs to the visual cortex.

MATERIALS AND METHODS

Recordings were obtained from the LGN of three awake female adult Dutch-Belted rabbits. The general surgical procedures for chronic recordings have been described earlier (Bezdudnaya et al. 2006; Stoelzel et al. 2008, 2009; Zhuang et al. 2014) and are reported only briefly here. All experiments were conducted with the approval of the University of Connecticut Animal Care and Use Committee in accordance with U.S. National Institutes of Health guidelines.

Animal preparation. Initial surgery was performed under ketamine-acepromazine anesthesia using aseptic procedures. After removal of the skin and fascia above the skull, the bones of the dorsal surface of the skull were fused together using stainless-steel screws and acrylic cement. A stainless-steel rod was oriented in a rostrocaudal direction and cemented to the acrylic mass. The rabbit was held rigidly by this rod during the electrode implantation and recording sessions. Silicone rubber was used to buffer the wound margins from the acrylic cement on the skull. Following at least 10 days of recovery, recordings of neuronal activity were obtained in the awake state through a small hole in the skull.

Recording and electrophysiological signal acquisition. Extracellular single-unit recordings were obtained from the LGN of awake rabbits. Single-unit activity was studied using fine-diameter (40 μm), quartz-insulated platinum/tungsten electrodes tapered and sharpened to a fine tip (impedance, 1.5–3 MΩ). A group of seven such electrodes was chronically implanted in a concentric array (200 μm separation), with tips initially located just above the dura. Each of these electrodes was independently controlled by a miniature microdrive (Swadlow et al. 2005). Multiunit activity from superficial layers of the superior colliculus (SC) was recorded simultaneously via one to three electrodes of the same type but lower impedance (<1.5 MΩ), controlled by a similar three-channel microdrive system. Hippocampal EEG was recorded, using two electrodes implanted above and below the cornu ammonis 1 layer, and used, along with cortical EEG, for monitoring brain states. All electrophysiological activity was acquired using a

THE VISUAL SYSTEM IS HIGHLY dynamic, able to scale neuronal responses across several orders of magnitude of mean luminance and to alter tuning specificity based on recent visual experience. Adaptations of neural responses, lasting from seconds (Baccus and Meister 2002; Brown and Masland 2001; Rieke 2001) to minutes (Dragoi et al. 2000; Giaschi et al. 1993; Hammond et al. 1988; McLelland et al. 2009; Ohzawa et al. 1988; Vautin and Berkley 1977), have been described, with the longest lasting adaption ~10 min (Dragoi et al. 2000). Yet, the perceptual effects of long-duration adapting stimuli can last considerably longer [see, for example, Dong et al. (2014)]. In addition, multiple forms of experience-dependent plasticity are thought to be permanent (Fox and Wong 2005; Levelt and Hubener 2012), and in some preparations, adaptation can be experimentally induced well past the critical periods of early development (Cooke and Bear 2012; Hess and Thompson 2013; Heynen and Bear 2001; Kuo et al. 2009).

Here, we describe a novel form of adaptation, following prolonged visual stimulation, in which the spontaneous activity of neurons in the lateral geniculate nucleus (LGN) of awake rabbits is reduced to as little as 10% of pre-adaptation baseline activity levels and slowly recovers over a period of ~1 h. We demonstrate that this adaptation, which is cell specific and retinotopically precise, is accompanied by an increase in the reliability (reduced Fano factor) and detectability [increased area of receiver operator characteristic (ROC) functions] of visual responses and by an increase in the ratio of evoked-to自发 firing rates. We also show that during the adapted period, monosynaptic currents, generated in layer 4 by thalamic impulses, are greatly increased, providing a mechanism for further enhancing the saliency of visual stimuli. Finally, we show that visual stimuli that suppress cell responses for a prolonged period of time can have the opposite effect and generate a prolonged increase in thalamic spontaneous activity. We speculate that these cell-specific adaption effects are sufficiently robust to alter visual perception in a retinotopic manner following prolonged stimulation of a small region of visual space.

Address for reprint requests and other correspondence: H. A. Swadlow, Dept. of Psychology (U-1020), Univ. of Connecticut, 406 Babbidge Rd., U-1020, Storrs, CT 06269 (e-mail: harvey.swadlow@uconn.edu).
Plexon data acquisition system. For a subset of LGN cells, spike-
triggered cortical field potentials were studied, obtained using 16-
channel silicon probes (NeuroNexus Technologies, Ann Arbor, MI),
inserted into the retinotopically aligned region of the primary visual
cortex (V1). Probe sites were separated vertically by 100 μm, had
surface areas of 700 μm², and had impedances of 0.3–0.8 MΩ. Spike
data from the probe sites usually consisted of low-amplitude, multi-
unit activity, used for plotting receptive fields (RFs). Field potentials
at each site were filtered at 2 Hz–1.9 kHz (half-amplitude) and
sampled continuously at 5 or 10 kHz. The depth of layer 4 was
determined by identifying the reversal point in currents generated from a brief, full-field visual flash (Stoelzel et al. 2008).

Spikes from single LGN neurons were isolated during the experi-
ment and verified offline by using Plexon cluster analysis software.
Spikes were required to be well isolated. To ensure this, we required <0.1% of spikes to have interspike intervals (ISIs) under 1.2 ms to be
classified as a single unit. All LGN cells were studied for at least 1 h
and usually much longer. After initial testing of the RF, all cells were studied for at least 20 min of baseline recording, for 5–30 min of
visual stimulation (the adaptation period), and for at least 20 min poststimulation (mean poststimulation period was 68 min). All data
analysis was then performed with NeuroExplorer (Nex Technologies,
Madison, AL) and MATLAB (MathWorks, Natick, MA).

EEG states. Hippocampal EEG activity provided the index of alert
vs. nonalert states. Hippocampal activity in the rabbit can generally be
separated into theta activity (4–8 Hz) and high-voltage, irregular
(HVIR) activity. Hippocampal theta activity is associated with cortical
desynchronization and an aroused, alert state, whereas HVIR activity is
associated with cortical synchronization and a nonaroused, nonalert
state (Bereshpolova et al. 2011; Green and Arndui 1954; Swadlow and
Gusev 2001). During our recordings, rabbits often alternated spontaneously between theta and HVIR activity. Low-intensity audi-
ory or tactile stimulation was used occasionally to convert HVIR
activity quickly into theta activity. After the recording session, event
records were manually segmented into “alert” segments containing theta
and “nonalert” segments containing HVIR activity. Segmentation of
hippocampal EEG was then evaluated through fast Fourier transform
(FFT) analysis of the selected segments, and the power in the theta
range (5–7 Hz) was compared with the power in the range of 2–4 Hz
(Bezdudnaya et al. 2006; Cano et al. 2006; Swadlow and Gusev
2001). For alert periods, the peak power in the theta range (5–7
Hz) was required to be 2.5 times the peak power in the low-frequency
range (1–3 Hz), whereas for nonalert periods, the peak power in the
theta range could not exceed 10% higher than the peak power in the
low-frequency range. We also regularly examined the EEG recorded
on cortical channels to confirm that the cortical and hippocampal EEG
were correlated appropriately (above).

Visual stimulation. All visual stimuli for LGN cells were presented
on a cathode ray tube monitor (primary monitor, NEC MultiSync;
40 × 30 cm; mean luminance, 48 cd/m²; refresh rate, 160 Hz). The
cells’ RFs were mapped by sparse noise stimulation. High-contrast
light and dark squares (1–3°, mostly 2°) were presented pseudoran-
domly in a grid of 30 × 22° on the primary monitor. Each grid space was
1°, and each square was presented for 18.75 or 31.25 ms.
The cell’s raw ON and OFF RF matrices were generated by the reverse
correlation method (Jones and Palmer 1987). After mapping, the cell’s
RF center was constantly tracked by dynamically mapping the RF
position of multunit recordings in the SC, and all of the visual stimuli thereafter were presented to the cell’s RF center (see below).

Cell classification. LGN neurons were classified as concentric
sustained, concentric transient, or directionally selective (DS). In the
rabbit LGN, the response of concentric neurons to stationary stimuli can be classified as either “sustained” or “transient,” and this distinc-
tion is robust and bimodal (Bezdudnaya et al. 2006). However, the
sustained response is severely attenuated when animals are not alert in
both LGN (Swadlow and Weyand 1985) and V1 (Swadlow and
Weyand 1987; Zhuang et al. 2014). Therefore, we classified LGN
neurons as sustained or transient based on tests that were done in the
alert state. The cell’s sustained/transient property was measured with
flashing stationary stimuli, which were optimized to elicit the stron-
gest response possible. The stimulus was either a circle or rectangle
(for directional cells), optimized for size, orientation, and contrast
polarity (dark or light). The stimulus was presented either 1 s on and
1 s off or 2 s on 2 s off. “Bursts” were identified as clusters of two or
more spikes with ISIs of ≤4 ms, where the initial spike of the burst
had a preceding ISI of at least 100 ms (Lu et al. 1992).

LGN neurons were assessed initially for directionally responses
using moving bar stimuli. Those showing directionality were then
tested quantitatively using circular drifting gratings that were optim-
tized to the spatial frequency, temporal frequency, size, and contrast.
The orientation tuning was measured with gratings drifting in one of
the eight, 12, or 24 randomly interleaved directions while keeping
other parameters optimal. Each presentation of a particular grating
orientation lasted for 3–8 s with a 4-s gap in between.

In a subset of cells, contrast-tuning measures were obtained every 10 min by pseudorandomly presenting eight different gratings of
varying luminance contrast (ranging from 1 to 95%). Each contrast
presentation was presented twice, each time lasting for 2 s, with a 4-s
gap in between. Spike rate data during the 1.5 min required to complete the contrast-tuning protocol were not used in the determi-
nation of spontaneous rates or bursting levels either during baseline
recordings or the recovery phase.

The first harmonic component (F1) of the peristimulus time his-
ogram (PSTH) responding to drifting gratings stimuli was calculated by
FFT analysis. F1 responses were fitted by a hyperbolic model (Al-
brecht and Hamilton 1982; Naka and Rushton 1966) as follows:
y = A·x^n·(C + x^n), where A is the response amplitude, x is contrast, and
C is the contrast at half-maximum response. Reliability was measured
for each stimulus contrast as the Fano factor (variance/mean), with a
bin size equal to the period of the stimulation.

ROC curves were calculated independently for each stimulus
contrast (Cohn et al. 1975; Green and Swets 1966). For each cycle of
the drifting grating stimulation, the response was calculated as the
total number of spikes within a bin size equal to the period of
stimulation. An equal number of random time windows were selected
from the spontaneous spike train with a bin size equal to the stimu-
lation window, and spike counts were counted within these spontaneous
windows. For each point in the ROC curve, a family of criterion
response levels was chosen from all of the observed spike rates at that
contrast. Each of these criterion response levels was tested to see
which yielded the greatest number of correct detections (y-axis, an
index of “sensitivity”) and the fewest number of false positives
(x-axis, “1 – specificity”).

Monitoring eye position. The eye position of the awake rabbit is
generally very stable (Bezdudnaya et al. 2006; Collewijn 1971; Fuller
1981; Swadlow and Weyand 1985, 1987; Zhuang et al. 2014). During
recording sessions, the eye position was monitored continuously by
mapping the SC multiunit RF center position with sparse noise on a
second liquid-crystal display monitor (Acer AL1516; 30 × 23 cm;
mean luminance, 36 cd/m²; refresh rate, 75 Hz). For most cells, the
second monitor was placed 40 cm from the eye. If an eye movement occurred during testing, then the
relation between the RF centers of the LGN cell and SC multiunit RF
center was used to place dynamically the stimulus on the LGN RF
center. During the offline analysis, we discarded data recorded within
15 s around the eye movement by sliding a time window of 25–30 s
with a step of 5 s over time and detecting the steps in which the SD
was correlated appropriately (above).
Spike-triggered current source-density analyses. Depth profiles of the axonal and monosynaptic fields generated by spikes of LGN neurons were generated using methods described previously (Stoelzel et al. 2008, 2009; Swadlow et al. 2002). Spike-triggered averages of the cortical field activity were generated from the “spontaneous” impulse activity of thalamocortical neurons. This method yields a view of the presynaptic axonal currents, as well as postsynaptic currents generated by single axons. Notably, the presynaptic component of the response follows the LGN spike at intervals consistent with thalamocortical axonal conduction times, has a very brief rise time (~0.22 ms) (Stoelzel et al. 2008), and is unaffected by α-amino-3-hydroxy-5-methyl-4-isoxazolepropanionic acid (AMPA)/kainate antagonists or by variations in the firing rate of the thalamocortical neuron (Swadlow and Gusev 2000). By contrast, the postsynaptic component follows the onset of the axonal component by ~0.5 ms, has a somewhat slower rise time (~0.45 ms) (Stoelzel et al. 2008), is reversibly blocked by AMPA/kainate antagonists, and is very sensitive to the preceding ISI, consistent with the depressing nature of these synapses (Swadlow and Gusev 2000). Notably, although both axonal and postsynaptic components of the response are restricted to the aligned region of the cortex, the temporal and depth distributions of these responses are very different for neighboring thalamic neurons. We limited our analysis of the postsynaptic component of the response to the initial 1 ms to ensure that we are studying the monosynaptic thalamocortical response [for further discussion of these issues, see Jin et al. (2008, 2011b), Stoelzel et al. (2008, 2009), and Swadlow et al. (2002)].

Spike-triggered current source-density (ST-CSD) profiles were generated from the field profiles, according to the method described by Freeman and Nicholson (1975). First, we duplicated the uppermost and lowermost field trace (Vaknin et al. 1988), which converted our 16 recording channels to a total of 18 channels. Next, we smoothed (Freeman and Nicholson 1975) to reduce high spatial-frequency noise components. This eliminated two of the 18 traces.

\[
\text{Smoothing: } \rho(r) = \frac{1}{4} \left( \rho(r + h) + 2 \rho(r) + \rho(r - h) \right)
\]

where \( \rho \) is the field potentials; \( r \) is the coordinate perpendicular to the layers; and \( h \) is the sampling interval (100 μm).

Next, we calculated the second derivative, and this yielded a total of 14 traces.

\[
\text{Second derivative: } D = \frac{1}{h^2} \left( \rho(r + h) - 2 \rho(r) + \rho(r - h) \right)
\]

In the ST-CSD profiles, current sinks are indicated by downward deflections and sources by upward deflections. To facilitate visualization of ST-CSD profiles, we generated color image plots, linearly interpolated along the depth axis, with red and blue representing current sinks and sources, respectively. Green is approximately zero, normalized to the 1-ms period before the thalamic spike.

RESULTS

We studied 22 sustained-concentric cells, 22 transient-concentric cells, and four DS LGN neurons in awake rabbits (Table 1). For each cell, we first recorded spontaneous activity for 20 min or more using a full-field gray background to establish baseline levels of activity. Next, an optimal drifting grating or flashing spot was presented over the RF for a period lasting between 5 and 30 min. Immediately after the visual stimulation was terminated, we continued monitoring spontaneous activity (the poststimulation period).

The firing rates from an example transient LGN neuron, which we studied for 3 h, are shown in Fig. 1A. This transient cell had a spontaneous firing rate of ~8.3 spikes/s, which was stable for 40 min. When a drifting grating was presented over the receptive center, the cell responded with an increased rate of 40.1 spikes/s for 20 min. Next, the gray background was presented again, and the cell became nearly silent (spontaneous rate = ~0.5 spike/s in the initial 5 min). Thirty minutes into the poststimulation period, the spontaneous rates still had not exceeded a rate of two spikes/s but recovered to near prestimulation firing rates after 1 h. Spikes were required to remain very well isolated during these extended recording sessions. To illustrate this, all spikes collected during the first 5 min and last 5 min of this recording session are shown in Fig. 1, A–C. Another example of a sustained LGN cell can be seen in Fig. 1B and was recorded for nearly 3 h. This cell had a baseline spontaneous firing rate of 13.5 spikes/s, which was stable for the 50 min of initial recording. The drifting grating stimulus raised the firing rate to 24.8 spikes/s, and this elevated rate was maintained for 20 min. When the gray background was presented again, the spontaneous firing rate dropped to 4.5 spikes/s and did not return to prestimulus levels during 100 min of the poststimulation period.

It is important to know if this effect is bidirectional (i.e., if prolonged suppression of spike rate by a visual stimulus results in a subsequent increase in spontaneous activity), but it is difficult to suppress the spontaneous firing rate of concentric LGN cells for prolonged periods. However, the LGN of the rabbit contains DS neurons that increase their firing rate to stimulation in the preferred direction and decrease their rates to stimulation in the null (opposite) direction (Hei et al. 2014; Levick et al. 1969; Swadlow and Weyand 1985). Therefore, we asked if prolonged suppression of cell firing (by continuous stimulation in the null direction) results in a prolonged elevation of spontaneous firing rates at the termination of stimulation. This was proven to be the case. Figure 1C shows a DS neuron that was studied for 2½ h. After 30 min of baseline activity, a drifting grating was presented at the null direction for another 30 min. During this period, the firing rate was reduced to an average value of 3.23 spikes/s. Following this stimulation, spontaneous firing increased considerably (from 15.9 to 29.6 spikes/s) and recovered gradually over the next 20 min. Next, we presented a drifting grating of the preferred direction over the RF center for 30 min, generating firing rates of 39.48 spikes/s. Following this, the spontaneous firing rate dropped to 2.5 spikes/s and after 30 min of monitoring, had only recovered to a firing rate of 6.7 spikes/s. We repeated this experiment in three additional DS neurons, balancing the order of preferred and nonpreferred directions. In all four DS neurons, we saw an increase in the spontaneous firing following prolonged visual stimulation in the null direction and a decrease in the spontaneous firing following prolonged visual stimulation with the preferred stimuli (see Fig. 1D), showing that the prolonged effect of visual stimulation on spontaneous activity was, indeed, bidirectional.
To examine what factors contribute to the degree to which spontaneous firing rate is reduced following prolonged stimulation, we varied the visual stimuli either by using a less-optimal stimulus or by changing the presentation time. We studied 56 different manipulations where an excitatory visual stimulus was either by using a less-optimal stimulus or by changing the presentation time. We studied 56 different manipulations where an excitatory visual stimulus was held over the RF center for a prolonged period of time. In all cases, spike rate was reduced significantly in the window from 2.5 to 10 min later (60.6%, $P < 0.001$, paired $t$-test). In the first of these experiments (Fig. 1E), we asked how the duration of prolonged stimulation affects the reduction in spontaneous rate. In this experiment, we used visual stimuli that drove visual firing rates at least 2.5 times higher than the baseline rates. We used prolonged visual stimulation for 5, 10, 15, 20, or 30 min. For each cell, we measured the reduction in mean firing rate in the 2.5- to 10-min window of the poststimulation period and normalized that rate to prestimulation levels. This neuron was then visually driven for 30 min in the preferred direction, and this was followed by a reduction in firing lasting $>1$ h. D: effect of prolonged suppressive and excitatory visual stimulation on DS neurons. For 4 DS neurons, we examined prolonged (30 min) visual stimulation with drifting gratings (black bars) of either the preferred direction (left) or nonpreferred direction (right). Spontaneous firing rates (open bars) were measured 2.5–10 min following each prolonged stimulation. All 4 rate measures were normalized to prestimulation baseline firing rates. Neurons were allowed to recover to baseline spontaneous rates and then tested again. E: effect of stimulation duration on subsequent reduction in spontaneous firing. For neurons where visually evoked firing rates were at least 2.5 times the baseline (nonstimulation) firing rates, a firing-rate reduction ($\gamma$-axis) was calculated by comparing the spontaneous firing rates from 2.5 to 10 min into the poststimulation period with the firing rates observed before visual stimulation. F: effect of response amplitude during stimulation on subsequent reduction in spontaneous firing. For neurons where we visually stimulated for 10 or 15 min, we examined the relationship between the ratio of the firing rate during visual stimulation to baseline firing rates and the firing rate reduction seen following prolonged visual stimulation.
to the prestimulation baseline firing rate. The effect of stimulus duration was cumulative in that longer periods of prolonged stimulation led to greater reductions in spontaneous firing rates (Pearson’s correlation, $r = -0.542$, $P < 0.01$; Fig. 1E).

Next, we wanted to know how much the magnitude of the increase in firing rate contributed to the poststimulation rate reduction (Fig. 1F). To address this question, we presented prolonged visual stimuli between 10 and 15 min and varied the contrast. Thus we asked to what extent the increase in activity during visual stimulation contributed to a later decrease in spontaneous rate. We calculated a firing ratio by normalizing the visually evoked firing rate to the baseline firing rate and found a significant correlation between this firing ratio and the reduction in spontaneous rate seen during the poststimulation period (Pearson’s correlation, $r = -0.776$, $P < 0.001$; Fig. 1F).

Since the suppression of spontaneous firing following stimulation depends on the duration and strength of the elicited responses (Fig. 1, E and F), it would follow that this effect is retinotopically local and that precise alignment of the stimulus with the RF is required. To examine this directly, on four occasions, we recorded a second LGN neuron simultaneously with our primary cell, and the RFs of the two cells were separated by 5–10°. In all four cases, the visually driven cell showed a reduced spontaneous activity in the poststimulation period, whereas the secondary cell remained at baseline firing rates. Therefore, the adaptation was cell specific and involved only cells that were accurately aligned with the stimulus and strongly driven by it.

**EEG state.** Because EEG state has a strong influence on the spontaneous firing and response gain of thalamic neurons (Berespolova et al. 2011; Bezdudnaya et al. 2006; Cano et al. 2006; Stoelzel et al. 2008; Swadlow and Gusev 2001), we asked if brain state influences the reduction in firing rates seen during the poststimulation period. To examine this, we selected segments of alert and nonalert periods based on hippocampal EGG (Berespolova et al. 2011; Bezdudnaya et al. 2006; Cano et al. 2006; Stoelzel et al. 2009; Swadlow and Gusev 2001; Zhuang et al. 2014) and then calculated the firing rate for each state independently. Figure 2A shows the same transient LGN neuron as Fig. 1A, with spike rates for alert and nonalert periods shown. Consistent with our previous observations, baseline spontaneous firing rates of LGN neurons are higher in the alert state (alert = 15.64 ± 4.18 spikes/s to nonalert = 9.26 ± 3.71 spikes/s; Fig. 2B, paired $t$-test, $P < 0.001$). However, following prolonged periods of visual stimulation (Fig. 2B), the effect of brain state on spontaneous rates disappeared (alert = 3.38 ± 2.23 spikes/s to nonalert = 3.85 ± 1.99 spikes/s, paired $t$-test, $P = 0.0792$).

Thalamic neurons show much more bursting when spontaneous firing is reduced due to sleep or nonalertness (Bezdudnaya et al. 2006; Sherman and Guillery 2002; Stoelzel et al. 2008; Swadlow and Gusev 2001; Weyand et al. 2001). Therefore, it was important to examine bursting during the reduced

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Fig. 2. EEG state does not explain the reduction in LGN firing after visual stimulation. A: for the same example used in Fig. 1A, the spontaneous firing rate during alert (red) and nonalert (blue) periods is shown for each 5-min bin. Firing rate is plotted on a log scale. B: before visual stimulation, firing rates were much higher in the alert state (left). The effect of state on firing rate was absent in the poststimulation period (right). C: bursts were defined based on the criteria of Lu et al. (1992), and burst fractions (proportion of all thalamic spikes that are found in bursts) were calculated in both states before and after visual stimulation. Baseline burst fractions were significantly higher when nonalert and increased (in both states) in the poststimulation period.
activity following prolonged stimulation. The burst fraction was calculated as the number of spikes generated by the cell that were a part of a burst, divided by the total number of spikes generated by the cell (Fig. 2C). Burst fractions were increased significantly following prolonged intense visual stimulation (baseline burst fraction = 8.79 ± 1.6%; poststimulation burst fraction = 20.30 ± 3.8%, \(P < 0.01\)), and this effect was seen in both alert (baseline burst fraction = 1.05 ± 0.36%; poststimulation burst fraction = 5.65 ± 1.88%, paired \(t\)-test, \(P < 0.01\)) and nonalert states (baseline burst fraction = 24.0 ± 2.21%; poststimulation burst fraction = 33.6 ± 3.13%, paired \(t\)-test, \(P < 0.01\)).

A shift toward a greater percentage of time spent in the nonalert state could help explain the reduction in spike rate that we observed. To examine this, the proportion of time spent alert in relation to nonalert was compared in both the baseline period and in the time window following prolonged visual stimulation used to measure the effect of stimulus duration and strength in Fig. 1 (2.5–10 min). However, there was no significant difference between the amount of time in the alert (baseline alert = 36.65 ± 1.88%; poststimulation alert = 40.63 ± 2.31%; paired \(t\)-test, \(P = 0.18\)) or nonalert state (baseline nonalert = 36.31 ± 1.51%; poststimulation nonalert = 32.58 ± 2.17%, paired \(t\)-test, \(P = 0.16\)), before and after prolonged visual stimulation.

**Visual responsiveness during period of reduced spontaneous activity.** Next, the effect of prolonged stimulation on visual responding was examined using the protocol described in Fig. 3A. During an initial baseline period (>36 min), repeated measurements of both spontaneous activity and the contrast response functions were made. To measure the contrast response function, we presented 2-s periods of drifting gratings with different contrasts, separated by 4 s of gray screen. Each measure of the contrast response function was followed by 10 min of gray screen to measure spontaneous activity. Importantly, these initial probes of contrast tuning functions had no measurable effect on the firing rate of the cell, which showed stable, spontaneous measures between each of the 4- or 10-min baseline recordings. These baseline measures were followed by a period of intense, prolonged stimulation with an optimal, high-contrast grating over the RF center that lasted 10–15 min. This resulted in at least a doubling of the average firing rate of the cell over this entire period (mean increase = 164 ± 16%). Ten minutes after stopping the prolonged visual stimulation, contrast tuning was tested again. Example contrast response
functions from before and after the prolonged stimulation can be seen in Fig. 3B.

This experiment was repeated in seven neurons (mean reduction in spontaneous rates = 53.7%, measured from 10 to 36 min into the poststimulation period). All seven neurons showed a reduced visual responsiveness during the poststimulation period (maximum response prestimulation 47.81 ± 5.67 spikes/s to maximum response poststimulation 34.80 ± 5.30 spikes/s, paired t-test, P < 0.01; see Fig. 3C). However, there was no shift in the contrast sensitivity function [mean contrast at one-half saturation (C50) prestimulation 8.49 ± 3.49%; mean C50 poststimulation 8.25 ± 3.55%, paired t-test, P = 0.58, not significant]. Since the reduction in visual responsiveness (27.2%) was less than the reduction in spontaneous rate (53.7%), our results suggest that prolonged visual stimulation increased the signal-to-noise ratio of the visual responses. To quantify this finding, the ratio of the peak (F1) response in the contrast response function to the spontaneous firing of the LGN neuron was calculated, for both baseline measures (prestimulation period) and during the poststimulation period (Fig. 3D). This ratio increased in all cells (baseline = 3.752 ± 0.483; poststimulation = 6.291 ± 0.909, paired t-test, P < 0.01).

To test if the reduction in visual-evoked responses after prolonged stimulation affected the response reliability, we calculated the Fano factor of the response to each stimulus contrast before and after the period of prolonged visual stimulation. As the results demonstrate, the Fano factor decreased significantly with increased stimulus contrast [Fig. 4A; FContrast = 8.694, degrees of freedom (df) 7,42, P < 0.001, mixed ANOVA] but not with visual adaptation (FAdaptation = 4.484, df 1,42, P = 0.079, mixed ANOVA). However, there was a significant interaction between contrast level and adaptation (FAdaptation·Contrast = 2.437, df 1,42, P = 0.034, mixed ANOVA), such that the Fano factor did not differ between adapted and nonadapted states at the lowest contrast levels, but it was significantly lower after adaptation at the two highest contrasts (post hoc Tukey’s test: P < 0.05).

To exclude the possibility that changes in Fano factor were the result of the reduction in response rate, we compared the Fano factor between visual responses with similar firing rate before and after adaptation (mean rate before adaptation = 39.925 ± 4.755 spikes/s; mean rate after adaptation = 37.793 ± 4.700 spikes/s). The result of this comparison indicates that adaptation reduces the Fano factor even in conditions with similar firing rate (Fano factor before adaptation = 0.975 ± 0.134; Fano factor after adaptation = 0.541 ± 0.0741, paired t-test, P < 0.05, Fig. 4B).

Finally, to investigate if the observed changes in signal-to-noise ratio and reliability could translate into a change in signal detectability, we examined the ROC curves for each of these cells. To do this, we compared the probability distributions of spike density counts obtained during periods of spontaneous activity and periods of visual stimulation. This was performed independently for each stimulus contrast tested. The ROC curves allowed us to measure the extent in which variations in spike rate can be used to distinguish the presence or absence of a drifting grating. Figure 4C shows the ROC curves for a single neuron (same as in Fig. 3B), generated before and after prolonged visual stimulation with the highest contrast. The ROC...

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**Fig. 4.** Lasting alterations in reliability and detectability of visual processing by LGN neurons following prolonged stimulation. A: the Fano factor, as calculated from each level of stimulus contrast for both baseline (black dots) and poststimulation (white dots). *P < 0.05 from post hoc analysis (Tukey’s test). B: comparison of mean Fano factor for response to high-contrast drifting grating poststimulation (white bar) and the rate-matched condition from the baseline tuning curve (black bar). Inset: schematic of how data were selected. Solid line shows an idealized contrast response function before adaptation, dashed line shows the idealized contrast response function after adaptation, and shaded boxes highlight rate-matched selected conditions for comparison. C: receiver operator characteristic (ROC) curves calculated, comparing spontaneous rate with responses to each value of stimulus contrast before (solid line, black circles) and after (dashed line, open circles) prolonged visual stimulation with the highest contrast. The diagonal solid line shows the unity line. D: ROC curves were quantified by calculating the area under the curves for both the baseline (black circles) and poststimulation (white circles) curves.
curve shifted to the left following prolonged visual stimulation. To quantify this shift in the ROC curves, we calculated the area under the curves, a measure that ranges from zero to one, with values closer to one reflecting better signal detection and values <0.5 reflecting signal detection no better than noise. This was repeated for each level of stimulus contrast (see Fig. 4D). The area under the curves increased as stimulus contrast was increased (F_{Contrast} = 21.768, df 7,42, P < 0.001, mixed ANOVA) and did so at each contrast tested. The analysis of ROC area indicates that prolonged visual stimulation improves signal detection (F_{Adaptation} = 20.121, df 1,42, P = 0.004, mixed ANOVA), whereas there was no interaction between stimulus intensity and adaptation (F_{Adaptation\times Contrast} = 0.788, df 7,42, P = 0.601, mixed ANOVA).

Thalamocortical monosynaptic impact on the cortex during altered state of reduced firing. Thalamocortical synapses in awake subjects are in a chronic state of synaptic depression (resulting from their typically high spontaneous firing rates), and reductions in activity allow for a recovery from the depression (Boudreau and Ferster 2005; Castro-Alamancos 2002; Castro-Alamancos and Oldford 2002; Ramcharan et al. 2000; Stoelzel et al. 2008; Swadlow and Gusev 2001; Swadlow et al. 2002). Because of this, it would be expected that the prolonged reduction in spontaneous rate should result in an increased synaptic drive on their cortical targets. To test this prediction, we used the method of single-axon ST-CSD analysis (Bereshpolova et al. 2006; Jin et al. 2008, 2011a, b; Stoelzel et al. 2008, 2009; Swadlow et al. 2002) to examine the synaptic impact of LGN impulses in layer 4, both before and after the reduced firing induced by prolonged stimulation. This yielded a measure of the presynaptic (axon terminal response) and monosynaptic-postsynaptic currents, generated through the cortical layers by the impulses of the single thalamic neuron under study. A detailed description of how such measures are obtained from the ST-CSD can be found in Stoelzel et al. (2008) and Swadlow et al. (2002) and is summarized in MATERIALS AND METHODS. Spontaneous firing rates and ST-CSDs were obtained before and after periods of intense visual stimulation.

An example baseline ST-CSD depth profile of the currents elicited in the cortex by the spontaneous spikes of a single LGN neuron can be seen in Fig. 5A. The time of the thalamic spike is shown at time zero. Current sinks are downward going, and sources are upward going. A color map is applied for ease of visualization, with current sinks shown. The depth of layer 4 was measured separately by examining the reversal potential in currents, generated from a brief full-field flash, and is indicated in the colorized panel. The channel in layer 4 with the maximal postsynaptic impact strength (channel 7) is expanded in Fig. 5A. Following a 60-min baseline measure, this neuron was visually stimulated to double its firing rate for 20 min. Spontaneous spikes were then recorded, and ST-CSD traces (for channel 7) are shown for each 20-min period following the prolonged visual stimulation (Fig. 5A).

**Fig. 5.** Lasting alterations in firing rate affect the postsynaptic impact of LGN neurons on the visual cortex. A: the baseline spike-triggered current source-density (ST-CSD) depth profile (left) from a sustained LGN neuron was generated from spontaneous spikes during 1 h of recording. A color map is applied to visualize the activation (middle). The vertical, dashed lines indicate the time of the LGN action potential, the solid, horizontal arrow in the ST-CSD indicates the channel that corresponded to the reversal point of the field potential generated by a diffuse flash stimulus, and layer 4 is indicated by the bracket in the colorized ST-CSD. Current sinks are shown in red. A single ST-CSD channel from layer 4, which had the largest postsynaptic response (channel 7 of the depth profile), is expanded to the top right. Following this, we stimulated the RF of the neuron with a flashing visual stimulus, which doubled its average rate for 20 min. The 3 ST-CSD traces to the bottom right were obtained from spontaneous spikes for each of 3, 20-min windows into the poststimulation period. These traces are noisier than the trace on the left, because far fewer spikes are averaged (31,155 spikes during the 1-h baseline period and 2,402, 4,613, and 5,223 spikes during the 3 subsequent 20-min periods). Gain settings for all traces to the right are identical. B: for each of the 4 traces in A, right, the peak amplitude of the postsynaptic current that occurred during the 1st 1 ms of the postsynaptic response was measured (closed circles). The amplitude of the axon terminal component of the response was also measured from peak to peak (open circles), and the spontaneous firing rates (triangles) are also shown. C: for 5 LGN neurons, measures of layer 4 postsynaptic currents (closed circles), axon terminal response (open circles), and spontaneous firing rates (triangles) were obtained and were normalized to baseline measures. Error bars show ±SE.
The peak amplitude during the first millisecond of the postsynaptic current sink, generated in layer 4, was measured for spikes that occurred during each successive 20-min time period following the visual stimulation (0–20, 20–40, and 40–60 min; Fig. 5B). Notably, the amplitude of the postsynaptic current sink increased in the poststimulation period 53.8% above baseline (from 364 to 560 μV/mm²), whereas the LGN spontaneous rates decreased by 77.0% (from 8.7 to 2.0 spikes/s). The elevated postsynaptic current sink amplitude was still present 40–60 min into the poststimulation period (16.2%). Importantly, the amplitude of the axon terminal response did not change (Fig. 5B). We measured changes in the strength of the layer 4 postsynaptic current sink caused by prolonged stimulation in five LGN neurons (Fig. 5C; all measured for at least 40 min following the offset of the prolonged visual stimulation and two of them measured for 60 min). Clearly, the observed increases in postsynaptic response amplitude mirror the reduced, spontaneous firing, with no changes seen in the amplitude of the presynaptic (axon terminal) component of the response.

Since LGN visually evoked firing decreases over time during prolonged visual stimulation, we examined the ST-CSD/LGN spontaneous rates decreased by 77.0% (from 8.7 to 2.0 spikes/s). The elevated postsynaptic current sink amplitude was still present 40–60 min into the poststimulation period (16.2%). Importantly, the amplitude of the axon terminal response did not change (Fig. 5B). We measured changes in the strength of the layer 4 postsynaptic current sink caused by prolonged stimulation in five LGN neurons (Fig. 5C; all measured for at least 40 min following the offset of the prolonged visual stimulation and two of them measured for 60 min). Clearly, the observed increases in postsynaptic response amplitude mirror the reduced, spontaneous firing, with no changes seen in the amplitude of the presynaptic (axon terminal) component of the response.

DISCUSSION

Our results show that prolonged (>10 min) and strong (two- to fivefold increase in firing rate) visual stimulation of an LGN neuron causes a long-lasting reduction in its spontaneous firing rate and increase in its bursting activity that last in excess of 1 h. The duration and magnitude of this effect are related to the duration and intensity of the visual drive and are retinotopically constrained to the area of visual stimulation. Furthermore, it is cell specific in that neighboring neurons with different response properties will be affected differently by prolonged stimulation (e.g., sustained vs. transient-concentric cells or directional neurons with differing directional preferences). Finally, the effect is bidirectional: spontaneous activity is suppressed after prolonged response enhancement and increased after prolonged response suppression.

The profound and long-lasting changes in neuronal spontaneous firing that we describe have very significant consequences for visual processing. The reduced neuronal activity was associated with increased reliability in visual responding of LGN cells (decreased Fano factor) and improvement in accuracy with which LGN signals could be detected (increased ROC area). Notably, the reduction in response amplitude (27.75%) was considerably smaller than the reduction in spontaneous activity (51.7%), resulting in a strongly enhanced ratio of thalamic visual signal to spontaneous activity [e.g., Sherman and Guillery (2002)] following the prolonged period of stimulation (Fig. 3D). Following prolonged stimulation, the response amplitude was shifted, but the value of the C50 remained constant, consistent with a response gain model. Similarly, we have previously shown that shifts in awake brain state result in changes in visual-response amplitude in contrast response functions, with little change in the C50 responses (Cano et al. 2006).

It has recently been suggested that adaptation shifts detectability away from low-contrast stimuli in favor of higher contrasts (Ollerenshaw et al. 2014); however, our results suggest that adaptation increases the detectability of a grating stimulus across different stimulus contrasts. As Ollerenshaw and colleagues (2014) point out in their paper, it is likely that the effects of adaptation are highly task specific. Whereas the task in Ollerenshaw et al. (2014) involved detecting a single deflection in a vibrissa after presenting a brief adapting stimulus for 1 s, the task in our experiments involved detecting a drifting grating after presenting a prolonged adapting stimulus for 10–15 min.

The marked prolonged reduction in spontaneous activity following visual stimulation allowed LGN impulses to generate a much stronger postsynaptic response in layer 4 of V1. The reduced visual responding seen at the end of prolonged stimulation allowed for an enhanced postsynaptic response in the cortex. These are an expected consequence of two known characteristics of the thalamocortical pathway: 1) thalamocortical synaptic transmission is depressing (i.e., impulses with short preceding ISIs generate weaker postsynaptic responses than impulses with longer preceding ISIs) (Boudreau and Ferster 2005; Castro-Alamancos and Oldford 2002; Gil et al. 1997; Stoezel et al. 2008; Swadlow and Gusev 2001; Swadlow et al. 2002), and 2) LGN neurons have high rates of spontaneous activity in awake animals (e.g., range 2–25 spikes/s, mean ~10 spikes/s in rabbits) (Bershadplova et al. 2011; Bezdudnaya et al. 2006; Stoezel et al. 2009). Thus thalamocortical synaptic transmission is normally chronically depressed when spontaneous firing rates are high (Castro-Alamancos and Oldford 2002; Ramcharan et al. 2000; Stoezel et al. 2008; Swadlow and Gusev 2001; Swadlow et al. 2002), and the reduced, spontaneous firing rate, following prolonged visual stimulation, allows thalamocortical synapses to recover more fully from the synaptic depression. Additionally, the increased proportion of bursting in LGN neurons after adaptation could benefit from temporal summation of multiple burst spikes, resulting in improved reliability of transmission across the thalamocortical synapse.

The observed reduction in spontaneous firing could be due to a variety of mechanisms, including a reduced excitatory drive from the retina or cortex or an enhanced inhibitory drive from thalamic reticular nucleus (TRN) or intrinsic inhibitory neurons, or due to factors intrinsic to LGN neurons (e.g., alteration in membrane properties). It is unlikely that a reduced retinal input could explain our findings, since both fast and slow adaptation in retina (Baccus and Meister 2002; Brown and Masland 2001; Zaidi et al. 2012) has been shown to recover in just a few minutes; however, the effects of prolonged stimulation on retinal activity have not been well studied. Our findings are suggestive of a homeostatic control of synaptic inputs for stabilizing neuronal output around a target level (Miller 1996; Turrigiano and Nelson 2004), possibly due to an activity-dependent potentiation of the group I metabotropic glutamate receptor (Govindiaiah et al. 2012) or inhibitory potentiation (Sieber et al. 2013). In particular, a lasting potentiation of TRN inputs onto LGN could generate a tonic hyperpolarization that could account for many of our findings (e.g.,
increased bursting of thalamic neurons in the alert state, reduced spontaneous activity, and the lack of brain-state modulation that followed the prolonged stimulation (Sherman and Guillery 2002; Weyand et al. 2001).

The adaptation changes that we observed in the contrast response functions are considerably longer than those described in the visual cortex (Carianini et al. 1998; Maffei et al. 1973; Movshon and Lennie 1979; Ozawa et al. 1985), which have been thought to contribute to the perceived contrast reduction following prolonged viewing of patterned stimuli, as reported by human subjects (Blakemore et al. 1973; Hammett et al. 1994; Snowden and Hammett 1996). However, psychophysical studies have also shown a number of adaptations and afterimage effects that can last for hours to months following induction (Bao and Engel 2012; Jones and Holding 1975; McCollough 1965). Here, we show that the effect of prolonged visual stimulation can last for >1 h in the thalamus. Moreover, psychophysical studies have described a relationship between stimulus duration and recovery time from adaptive effects, termed the duration-scaling law (Bao and Engel 2012; Greenlee et al. 1991; Greenlee and Magnussen 1987; Leopold et al. 2005), which is consistent with our measurements from thalamic neurons (Fig. 1E). However, both the effect of stimulus intensity on the magnitude of adaptation (Fig. 1F) and the bidirectional effects observed for DS neurons (Fig. 1, C and D) are consistent with challenges to duration scaling that suggest a more complicated role of visual history in adaption (Patterson et al. 2013; Wark et al. 2009).

Whereas many previous studies investigated sensory adaptation to stimuli that increase neuronal responses, few have examined the effect of adaptation to stimuli that reduce neuronal responses. Subsets of cells responding to repeated visual stimulation with sensitization have been described in the retina (Kastner and Baccus 2011), LGN (Camp et al. 2009), and cortex (Wissig and Kohn 2012). Psychophysical experiments have demonstrated enhanced sensitivity following stimulus deprivation (Zhang et al. 2009), which may be related to the increased spontaneous rate that we found after prolonged neuronal response suppression.

Repetitive visual stimulation is frequently used to induce perceptual learning (Ball and Sekuler 1982; Fiorentini and Berardi 1980; Karni and Sagi 1991; Watanabe et al. 2001) and treat visual brain disorders, such as amblyopia (Li et al. 2011b). It is has been assumed that repetitive stimulation increases visual responsiveness in the long term and that this response increase is required for the treatment of amblyopia (Li et al. 2011a). Our results suggest that this may not always be the case. Instead, repetitive visual stimulation may improve perception by reducing spontaneous rates and increasing the accuracy of signal detection and the postsynaptic impact of visually induced thalamic signals entering the input layers of the visual cortex, a novel mechanism that is described for the first time in our experiments.

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Present address of J. Zhuang: Dept. of Neural Coding, Allen Institute for Brain Science, 551 N 34th St. #200, Seattle, WA 98103.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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