Hour-long adaptation in the awake early visual system

Running head: Long lasting adaption in the LGN

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 2, 1, Harvey A. Swadlow 1, 2

1Department of Psychology, University of Connecticut,
406 Babbidge Road, Storrs, Connecticut, 06269, USA.

2Department of Biological Sciences, State University of New York, 33 West 42nd Street, New York, New York 10036, USA.

Corresponding author: Harvey A. Swadlow, Department of Psychology (U-1020), The University of Connecticut, 406 Babbidge Road, U-1020, Storrs, Connecticut 06269

Phone (860)486-2252; fax (860)486-2760; email: harvey.swadlow@uconn.edu

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Abstract

Sensory adaptation serves to adjust awake brains to changing environments on different time scales. However, adaptation has been traditionally studied under anesthesia and for short time periods. Here, we demonstrate, in awake rabbits, a novel type of sensory adaptation that persists for more than one hour and acts on visual thalamocortical neurons and their synapses in the input layers of the visual cortex. Following prolonged visual stimulation (10 – 30 minutes), cells in the dorsal lateral geniculate nucleus (LGN) show a severe and prolonged reduction in spontaneous firing rate. This effect is bi-directional and prolonged visual response suppression is followed by prolonged increase in spontaneous activity. The reduction in thalamic spontaneous activity following prolonged visual activation is accompanied by increases in (a) response reliability, (b) signal detectability, and, (c) 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 primary visual cortex (V1) 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 visual cortex.
Introduction

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. 1985; Vautin and Berkley 1977) have been described, with the longest lasting adaptation being around 10 minutes (Dragoi et al. 2000). Yet the perceptual effects of long duration adapting stimuli can last considerably longer (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 percent of pre-adaptation baseline activity levels, and slowly recovers over a period of >1 hour. We demonstrate that this adaptation, which is cell-specific and retinotopically precise, is accompanied by an increase in both 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-spontaneous 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.

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; Stoelzel et al. 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 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 d 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 Mohm). 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 simultaneously recorded via
1–3 electrodes of same type but lower impedance (<1.5 Mohm) controlled by a similar three-channel microdrive system. Hippocampal EEG was recorded using two electrodes implanted above and below the CA1 layer and used, along with cortical EEG, for monitoring brain states. All electrophysiological activity was acquired using a Plexon data acquisition system. For a subset of LGN cells, spike-triggered cortical field potentials were studied, obtained using 16-channel silicone probes (NeuroNexus Technologies, Ann Arbor, MI) inserted into the retinotopically aligned region of 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. Field potentials at each site were filtered at 2 Hz to 1.9 kHz (half-amplitude) and sampled continuously at 5 or 10 kHz. The depth of layer IV 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 experiment and verified off-line by using Plexon cluster analysis software. Spikes were required to be well isolated. To ensure this we required < 0.1% of spikes to have interpik intervals under 1.2 ms in order to be classified as a single unit. All LGN cells were studied for at least one hour, and usually much longer. After initial testing of the receptive field, all cells were studied for at least 20 minutes of baseline recording, for 5 – 30 minutes of visual stimulation (the adaptation period), and for at least 20 minutes post-stimulation (mean post stimulation period was 68 minutes). All data analysis was then performed with NeuroExplorer (Nex Technologies) and MATLAB (The MathWorks).

**EEG states**

Hippocampal EEG activity provided the index of alert versus non-alert states. Hippocampal activity in the rabbit can generally be separated into theta activity (4–8 Hz) and high-voltage, irregular activity (HVIR). Hippocampal theta activity is associated with cortical desynchronization and an aroused,
alert state, whereas HVIR is associated with cortical synchronization and a non-aroused, non-alert state (Bereshpolova et al. 2011; Green and Arduini 1954; Swadlow and Gusev 2001). During our recordings, rabbits often alternated spontaneously between theta and HVIR activity. Low-intensity auditory or tactile stimulation was occasionally used to quickly convert HVIR activity into Theta activity. After the recording session, EEG records were manually segmented into “alert” segments containing theta and “non-alert” segments containing HVIR activity. Segmentation of hippocampal EEG was then evaluated through FFT analysis of the selected segments, and the power in the theta range (5–7 Hz) was compared to the power in the range of 2–4 Hz (Bezdudnaya et al. 2006; Cano et al. 2006; Swadlow and Gusev 2001). For the 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), where as for non-alert 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 assure ourselves that the cortical and hippocampal EEG were appropriately correlated (above).

**Visual Stimulation**

All visual stimuli for LGN cells were presented on a CRT monitor (primary monitor, Nec MultiSync, 40 x 30 cm, mean luminance, 48cd/m², refresh rate: 160 Hz). The cells’ RFs were mapped by sparse noise stimulation. High-contrast light and dark squares (1 to 3 degrees, mostly 2 degrees) were presented pseudorandomly in a grid of 30 x 22 degrees on the primary monitor. Each grid space was 1 degree and each square was presented for either 18.75 or 31.25 ms. The cell’s raw ON and OFF receptive field (RF) matrices were generated by reverse correlation method (Jones and Palmer 1987). After mapping, the cell’s RF center was constantly tracked by dynamically mapping the RF position of multiunit recordings in the Superior Colliculus (SC), and all 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. In the rabbit LGN, the response of concentric neurons to stationary stimuli can be classified as either “sustained” or “transient,” and this distinction 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 strongest 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 interspike intervals (ISIs) of $\leq 4$ ms, where the initial spike of the burst had a preceding ISI of at least 100 ms (Lu et al. 1992).

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

In a subset of cells, contrast tuning measures were obtained every ten minutes by pseudorandomly presenting 8 different gratings of varying luminance contrast (ranging from 1 to 95%). Each contrast presentation was presented twice, each time lasting for 2 seconds, with a 4 second gap in between. Spike rate data during the minute and a half required to complete the contrast tuning
protocol was not used in the determination of spontaneous rates or bursting levels either during baseline recordings or recovery phase.

The first harmonic component (F1) of the PSTH responding to drifting grating stimuli was calculated by Fourier analysis. F1 responses were fitted by a hyperbolic model (Albrecht and Hamilton 1982; Naka and Rushton 1966) as follows: \( y = A \cdot x^n/(C^n + x^n) \); 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 stimulation window, and spikes were counted within these spontaneous windows. For each point in the ROC curve a family of criterion response levels was chosen from all 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 1987; 1985; Zhuang et al. 2014). During recording sessions, the eye position was continuously monitored by mapping the SC multiunit RF center position with sparse noise on a second LCD monitor (Acer AL1516, 30 x 23 cm, mean luminance, 36cd/m2, refresh
rate, 75 Hz). For most cells, the pupil position and size were also simultaneously monitored by a high-frequency (220 Hz) infrared camera system (ViewPoint EyeTracker system, Arrington Research) placed 40 cm from the eye. If an eye movement occurred during testing, the relation between the RF centers of the LGN cell and SC multiunit RF center was used to dynamically place the stimuli 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 standard deviation of SC RF centers was more than 1 degree. Visual responses to drifting gratings were measured by generating peristimulus time histograms (PSTHs) during stable eye periods, and the PSTH peaks were aligned across the different periods to correct for small differences in response phase.

Spike-triggered current source-density analyses

Depth profiles of the axonal and monosynaptic fields generated by spikes of LGN neurons were generated using methods previously described (Stoelzel et al. 2008; Stoelzel et al. 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 either 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 ~½ 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 interspike interval, 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 cortex, the temporal
and depth distribution 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. 2011b; Jin et al. 2008; Stoelzel et al. 2008; Stoelzel et al. 2009; 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 (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.

$$\text{Smoothing: } \bar{\varphi}(r) = \frac{1}{4}(\varphi(r+h) + 2\varphi(r) + \varphi(r-h))$$

Where: $\varphi$ is the field potentials; $r$ is the coordinate perpendicular to the layers; $h$ is the sampling interval (100 μm).

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

$$\text{Second derivative: } D = \frac{1}{h^2}(\bar{\varphi}(r+h) - 2\bar{\varphi}(r) + \bar{\varphi}(r-h))$$

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, red and blue representing current sinks and sources, respectively. Green is approximately zero, normalized to the one millisecond period prior to the thalamic spike.
Results

We studied 22 sustained concentric cells, 22 transient concentric cells, and 4 directional selective LGN neurons in awake rabbits (table 1). For each cell, we first recorded spontaneous activity for 20 or more minutes using a full field grey background to establish baseline levels of activity. Next, an optimal drifting grating, or flashing spot was presented over the receptive field for a period lasting between 5 and 30 minutes. Immediately after the visual stimulation was terminated, we continued monitoring spontaneous activity (the post-stimulation period).

The firing rates from an example transient LGN neuron which we studied for three hours is shown in figure 1A, each dot showing the average firing rate during a five minute period. This transient cell had a spontaneous firing rate of ~ 8.3 spikes/second, which was stable for 40 minutes. When a drifting grating was presented over the receptive center, the cell responded with an increased rate of 40.1 spikes/second for 20 minutes. Next, the gray background was presented again and the cell became nearly silent (spontaneous rate = ~ 0.5 spikes/second in the initial 5 minutes). 30 minutes into the post-stimulation period, the spontaneous rates still had not exceeded a rate of 2 spikes/second, but recovered to near pre-stimulation firing rates after an hour. Spikes were required to remain very well-isolated during these extended recording sessions. To illustrate this, all spikes collected during the first five minutes, and last five minutes of this recording session are shown at the bottom left and right side, respectively of Fig. 1A-C. Another example of a Sustained LGN cell can be seen in figure 1B, and was recorded for nearly 3 hours. This cell had a baseline spontaneous firing rate of 13.5 spikes/second, which was stable for the 50 minutes of initial recording. The drifting grating stimulus raised the firing rate to 24.8 spikes/second, and this elevated rate was maintained for 20 minutes. When the gray background was presented again, the spontaneous firing rate dropped to 4.5 spikes/second, and did not return to pre-stimulus levels during 100 minutes of the post-stimulation 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 directionally selective (DS) neurons which 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). We asked, therefore, 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 proved to be the case. Figure 1C shows a directional selective neuron that was studied for two and half hours. After 30 minutes of baseline activity, a drifting grating was presented at the null direction for another 30 minutes. During this period, the firing rate was reduced to an average value of 3.23 spikes/second. Following this stimulation, spontaneous firing increased considerably (from 15.9 spikes/second to 29.6 spikes/second) and recovered gradually over the next 20 minutes. Next, we presented a drifting grating of the preferred direction over the receptive field center for 30 minutes, generating firing rates of 39.48 spikes/second. Following this, the spontaneous firing rate dropped to 2.5 spikes/second and, after 30 minutes of monitoring, had only recovered to a firing rate of 6.7 spikes/second. We repeated this experiment in three additional directional selective neurons, balancing the order of preferred and non-preferred directions. In all four directional selective 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 figure 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 held over the receptive field center for a prolonged period of time. In all cases, spike rate was significantly reduced in the initial 2.5 minutes of the post-stimulation period (average reduction: 70.3%, p < 0.001, paired t-test), and continued to be reduced in the window from 2.5 to 10 minutes later (60.6%, p < 0.001, paired t-test). In the first of these experiments (Fig. 1E), we asked: how does the duration of prolonged stimulation affect 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 minutes. For each cell, we measured the reduction in mean firing rate in the 2.5 to 10 minutes window of the post-stimulation period and normalized that rate to the pre-stimulation 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 post-stimulation rate reduction (Fig. 1F). To address this question, we presented prolonged visual stimuli between 10 to 15 minutes and varied the contrast. Thus, we asked to what extent the increase in activity during visual stimulation contributed to 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 post-stimulation period (figure 1F, Pearson’s correlation, r = -0.776, p<0.001).

Since the suppression of spontaneous firing following stimulation depends on both the duration and strength of the elicited responses (Fig. 1E,F), it would follow that this effect is retinotopically local, and that precise alignment of the stimulus with the receptive field is required. To examine this directly, on 4 occasions we recorded a second LGN neuron simultaneously with our primary cell, and the receptive fields of the two cells were separated by 5 to 10 degrees. In all four cases, the visually driven
cell showed a reduced spontaneous activity in the post-stimulation period, while 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 (Bereshpolova 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 post-stimulation period. To examine this, we selected segments of alert and non-alert periods based on hippocampal EEG (Bereshpolova 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 figure 1A, with spike rates for alert periods in red and non-alert periods in blue. Consistent with our previous observations, baseline spontaneous firing rates of LGN neurons are higher in the alert state (alert = 15.64 ± 4.18 spikes/second to non-alert = 9.26 ± 3.71 spikes/second, Figure 2B, left, paired t-test, p<0.001). However, following prolonged periods of visual stimulation (Fig. 2B, right), the effect of brain state on spontaneous rates disappeared (alert = 3.38 ± 2.23 spikes/second to non-alert = 3.85 ± 1.99 spikes/second, paired t-test, p=0.0792).

Thalamic neurons show much more bursting when spontaneous firing is reduced due to sleep or non-alertness (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 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 (figure 2C). Burst fractions were significantly increased following prolonged intense visual...
stimulation (baseline burst fraction= 8.79 ± 1.6 % to post stimulation burst fraction = 20.30 ± 3.8 %, p<0.01), and this effect was seen in both alert (baseline burst fraction = 1.05 ± 0.36 % to post stimulation burst fraction = 5.65 ± 1.88 %, paired t-test, p<0.01) and non-alert states (baseline burst fraction = 24.0 ± 2.21 % to post stimulation burst fraction = 33.6 ± 3.13 %, paired t-test, p<.01).

A shift toward a greater percentage of time spent in the non-alert state could help explain the reduction in spike rate we observed. To examine this, the proportion of time spent alert in relation to non-alert 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 figure 1 (2.5 -10 minutes). However, there was no significant difference between the amount of time in either alert (Baseline alert = 36.65 ± 1.88 %, post stimulation alert = 40.63 ± 2.31 %, paired t-test, p=0.18) or non-alert state (Baseline non-alert = 36.31 ± 1.51 %, post stimulation non-alert = 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 Figure 3A. During an initial baseline period (> 36 minutes), repeated measurements of both spontaneous activity and the contrast response functions were made. To measure the contrast response function, we presented two-second periods of drifting gratings with different contrasts separated by four seconds of grey screen. Each measure of the contrast response function was followed by ten minutes of grey 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 four or ten-minute 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 minutes. 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 figure 3B.

This experiment was repeated in seven neurons (mean reduction in spontaneous rates = 53.7%, measured from 10 to 36 minutes into post-stimulation period). All seven neurons showed a reduced visual responsiveness during the post-stimulation period (maximum response pre-stimulation 47.81 ± 5.67 spikes/second to maximum response post-stimulation 34.80 ± 5.30 spikes/second, see figure 3C, paired t-test, p<0.01). However, there was no shift in the contrast sensitivity function (mean C50 pre-stimulation 8.49 ± 3.49%, mean C50 post-stimulation 8.25 ± 3.55%, paired t-test, p=0.58 NS). 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 (pre-stimulation period) and during the post-stimulation period (Figure 3D). This ratio increased in all cells (baseline = 3.752 ± 0.483, post-stimulation = 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, both before and after the period of prolonged visual stimulation. As the results demonstrate, the Fano factor decreased significantly with increased stimulus contrast (Figure 4A, $F_{Contrast} = 8.694$, Df 7,42. p<0.001, mixed ANOVA) but not with visual adaptation ($F_{Adaptation} = 4.484$, Df 1,42, p=0.079, mixed ANOVA). However, there was a significant interaction between contrast level and adaptation ($F_{Adaptation*con} = 2.437$, Df 1,42, p=0.034, mixed ANOVA), such that the Fano factor did not differ between adapted and non-
adapted states at the lowest contrasts levels, but it was significantly lower after adaption at the two highest contrasts (Post-hoc Tukey 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/second, mean rate after adaptation = 37.793 ± 4.700 spikes/second). 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, figure 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 figure 3B) generated before (solid line, black circles) and after prolonged visual stimulation with the highest contrast (dashed line, open circle). The ROC 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 0 to 1, with values closer to 1 reflecting better signal detection, and values below 0.5 reflecting signal detection no better than noise. This was repeated for each level of stimulus contrast (see figure 4D). The area under the curves increased as stimulus contrast was increased ($F_{\text{Contrast}} = 21.768$, Df 7,42. p<0.001, mixed ANOVA), and did so at each contrast tested. The analysis of ROC area indicate that prolonged visual stimulation improves signal
Thalamocortical mono-synaptic impact on 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 spike-triggered current source density analysis (ST-CSD analysis, (Bereshpolova et al. 2006; Jin et al. 2011a; Jin et al. 2011b; Jin et al. 2008; Stoelzel et al. 2008; Stoelzel et al. 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 Swadlow et al. 2002; Stoelzel et al. 2008, 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 cortex by the spontaneous spikes of a single LGN neuron can be seen in Figure 5A (left). The time of the thalamic spike is shown by the dashed vertical line at time zero. Current sinks are downward-going, sources are upward going. A color map is applied for ease of visualization (middle) with currents sinks shown in red. The depth of layer 4 was separately measured by examining the reversal potential (horizontal arrow) in currents...
generated from a brief full field flash and is indicated by the bracket on the left of the colorized panel. The channel in layer 4 with the maximal postsynaptic impact strength (channel # 7) is expanded in Figure 5A right top. Following a 60 minute baseline measure, this neuron was visually stimulated to double its firing rate for 20 minutes. Spontaneous spikes were then recorded, and ST-CSD traces (for channel 7) are shown for each twenty minute period following the prolonged visual stimulation (Figure 5A right bottom three panels).

The peak amplitude during the first millisecond of the post-synaptic current sink generated in layer 4 was measured for spikes that occurred during each successive 20-minute time period following the visual stimulation (0 – 20 minutes, 20 – 40 minutes, 40 – 60 minutes, Figure 5B, closed circles). Notably, the amplitude of the post synaptic current sink increased in the post-stimulation period 53.8 % above baseline (from 364 μV/mm² to 560 μV/mm²) while the LGN spontaneous rates (triangles) decreased by 77.0 % (from 8.7 spikes/second to 2.0 spikes/second). The elevated postsynaptic current sink amplitude was still present 40-60 minutes into the post-stimulation period (16.2 %). Importantly, the amplitude of the axon terminal response did not change (Figure 5B, open circles). We measured changes in the strength of the layer 4 postsynaptic current sink caused by prolonged stimulation in 5 LGN neurons (figure 5C; all measured for at least 40 minutes following the offset of the prolonged visual stimulation, and 2 of them measured for 60 minutes). Clearly, the observed increases in postsynaptic response amplitude (closed circles) mirror the reduced spontaneous firing (triangles), with no changes being seen in the amplitude of the presynaptic (axon terminal) component of the response (open circles).

Since LGN visually evoked firing decreases over time during prolonged visual stimulation, we examined the ST-CSD generated by these LGN neurons at the beginning (0-5 minutes) and near the end (15-20 minutes) of prolonged visual stimulation. Visually evoked firing rates decreased 21.8% between
these two time periods (0-5 minutes = 17.931 ± 2.225 spikes/second, 15-20 minutes = 13.604 ± 1.114 spikes/second, p<0.05) and this corresponded with a significant increase (14.4%) in the amplitude of the post-synaptic potential (from 250 ± 71 μV/mm² to 286 ± 77 μV/mm², paired t-test, p<0.05).

DISCUSSION

Our results show that prolonged (> 10 minutes) and strong (2 – 5 fold increase in firing rate) visual stimulation of an LGN neuron causes a long lasting reduction in its spontaneous firing rate and increase in their bursting activity that last in excess of an hour. The duration and magnitude of this effect is related to both the duration and intensity of the visual drive and is retinotopically constrained to the area of visual stimulation. Furthermore, it is cell-specific in that neighboring neurons with different responses properties will be differently affected by prolonged stimulation (e.g., Sustained vs. Transient concentric cells, or directional neurons with differing directional preferences). Finally, the effect is bi-directional: 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 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 points out in their paper, it is likely, that the effects of adaption are highly task specific. Whereas the task in Ollerenshaw et al. involved detecting a single deflection in a vibrissa after presenting a brief adapting stimulus for 1 second, the task in our experiments involved detecting a drifting grating after presenting a prolonged adapting-stimulus for 10-15 minutes.

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 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 interspike intervals generate weaker postsynaptic responses than impulses with longer preceding interspike intervals, (Boudreau and Ferster 2005; Castro-Alamancos and Oldford 2002; Gil et al. 1997; Stoelzel 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/second, mean ~10 spikes/second in rabbits, (Bereshpolova et al. 2011; Bezdudnaya et al. 2006; Stoelzel 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; Stoelzel 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 more fully recover 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 TC 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, enhanced inhibitory drive from TRN or intrinsic inhibitory neurons, or 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 mGluR1 receptor (Govindaiah et al. 2012) or inhibitory potentiation (Sieber et al. 2013). In particular, a lasting potentiation of TRN inputs onto LGN could generate a tonic hyper-polarization 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 (Carandini et al. 1998; Maffei et al. 1973; Movshon and Lennie 1979; Ohzawa 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 which 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 more than one hour 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) that 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 bi-directional effects observed for directional selective neurons (Fig 1C-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).

While 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 be always the case. Instead, repetitive visual stimulation may improve perception by reducing spontaneous rates and increasing the accuracy of signal detection and the post-synaptic impact of visually induced thalamic signals entering the input layers of the visual cortex, a novel mechanism that is described for first time in our experiments.
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Figure Legends

**Figure 1**: Prolonged and intense visual stimulation leads to a long lasting reduction in spontaneous firing in LGN neurons. A Transient (A) and a Sustained (B) LGN cell, each monitored for several hours. Each dot shows the average firing rate over a five minute period, presented on a log scale. After a baseline firing rate was established (~30-40 minutes), neurons were visual driven by either an optimal drifting grating stimuli or flashing spot stimuli over their receptive field center for 10 to 30 minutes (yellow bar). Next, spontaneous firing was monitored again until the cell returned to its pre-stimulation firing rates or was lost. Insets, Spike waveforms for first five minutes (left) and last five minutes (right). Shadowed areas represent mean ±SD. The temporal window for spike waveform is 0.8 ms. C) A directional selective LGN neuron. After 30 minutes of baseline recording, the firing rate of the neuron was suppressed for 30 minutes by presenting a drifting grating in the null direction over the receptive field. Immediately following the termination of this stimulation the spontaneous firing rate of the neuron increased for ~20 minutes before returning to pre-stimulation levels. This neuron was then visually driven for 30 minutes in the preferred direction, and this was followed by a reduction in firing lasting over one hour. D) Effect of prolonged suppressive and excitatory visual stimulation on Directional selective neurons. For four Directional selective neurons we examined prolonged (30 minute) visual stimulation with drifting gratings (black boxes) of either the preferred direction (left) or non-preferred direction (right). Spontaneous firing rates (open boxes) were measured 2.5 to 10 minutes following each prolonged stimulation. All four rate measures were normalized to pre-stimulation 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 (non-stimulation) firing rates, a firing rate reduction (y-axis) was calculated by comparing the spontaneous firing rates from 2.5 to 10 minutes into the post-stimulation period to 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 minutes, 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.

Figure 2: EEG state does not explain the reduction in LGN firing after visual stimulation. A) For the
same example used in Figure 1A, the spontaneous firing rate during alert (red) and non-alert (blue)
periods is shown for each five minute bin. Firing rate is plotted on a log scale. B) Prior to visual
stimulation, firing rates were much higher in the alert state. The effect of state on firing rate was absent
in the post-stimulation period. 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 non alert,
and increased (in both states) in the post-stimulation period.

Figure 3: Lasting alterations in visual processing by LGN neurons following prolonged stimulation. (A)
schematic of how we examined the effect of prolonged stimulation on LGN visual responses. First, we
studied baseline spontaneous activity and contrast tuning functions for an extended period (> 36
minutes). During this period, we presented two-second periods of various contrast drifting gratings,
separated by four seconds of grey screen, for a minute and half, followed by ten minutes of grey screen
to assess spontaneous activity. This was repeated 3 or more times to establish a baseline spontaneous
firing rate, and a baseline contrast tuning function. These contrast response tests had no measurable
effect on the spontaneous firing rate of the cell, which showed stable spontaneous measures between
each of the three or more ten-minute baseline recordings. Next came a period of intense, prolonged
stimulation, during which the cell was stimulated for 10 – 15 minutes with an optimal, high-contrast
grating over the RF center. Next, we waited for 10 minutes before we began to test contrast tuning and
spontaneous firing rates again, as was done during the baseline period. B) Example contrast tuning
function from a transient LGN neuron during both the baseline (solid) and post-stimulation (open circles,
dashes) period. Lines show the sigmoidal fit, and circles show the F1 of the neuron’s firing during
drifting grating stimulation of a given contrast (x-axis). C) For each cell we calculated the maximum
response of the contrast response function during our baseline assessment (black bar) and 10 to 30
minutes into the post-stimulation period (white bar). In this and subsequent graphs, error bars show
±SEM. D) The ratios of the visually evoked responses to the spontaneous rates were measured by
dividing the maximum responses from the contrast response functions during the baseline and post-
stimulation periods by the spontaneous rates during those periods.

Figure 4: Lasting alterations in reliability and detectability of visual processing by LGN neurons
following prolonged stimulation. A) The Fano factor as calculated from at each level of stimulus
contrast for both baseline (black dots) and post-stimulation (white dots). B) Comparison of mean Fano
factor for response to high contrast drifting grating post-stimulation (white bar) and the rate-matched
condition from the baseline tuning curve (black bar). Inset shows schematic of how data were selected,
solid line showing an idealized contrast response function before adaptation, the dashed line showing
the idealized contrast response function after adaption, and the yellow boxes highlighting rate-matched
selected conditions for comparison. C) ROC curves calculated comparing spontaneous rate with
responses to each value of stimulus contrast. D) ROC curves were quantified by calculating the area
under the curves for both the baseline (black bar) and post-stimulation (white bar) curves.
Figure 5: Lasting alterations in firing rate effect the post-synaptic impact of LGN neurons on visual cortex. A) The baseline ST-CSD depth profile (Left) from a sustained LGN neuron was generated from spontaneous spikes during one hour of recording. A color map is applied to ease visualization (middle). The vertical dashed line indicates 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. A single ST-CSD channel from layer 4 which had the largest post synaptic response (channel 7 of the depth profile) is expanded to the right top. Following this we stimulated the receptive field of the neuron with a flashing visual stimulus, which doubled its average rate for 20 minutes. The three ST-CSD traces to the right bottom were obtained from spontaneous spikes for each of three twenty minute windows into the post-stimulation period. These traces are noisier than the trace on the left because far fewer spikes are averaged (31155 spikes during the 1-hour baseline period, and 2402, 4613, and 5223 spikes during the three subsequent 20-minute periods). Gain settings for all traces to the right are identical. B) For each of the 4 traces on the right side of Fig. 4A, the peak amplitude of the post synaptic current that occurred during the first one 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 post-synaptic currents (closed circles) and axon terminal response (open circles), and spontaneous firing rates were obtained and were normalized to baseline measures. Error bars show ±SEM.
Table 1: Summary of sustained, transient and directional selective neurons recorded from each subject.

<table>
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<th>Rabbit 3</th>
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<tr>
<td>Directional Selective</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td></td>
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</table>
Firing Rate Ratio (% baseline)

0 1 2 3

Firing Rate Reduction

0.0 0.2 0.4 0.6 0.8 1.0

r = -.776, p < 0.01

Sustained
Transient
Directional

Stimulus Duration

5 10 15 20 25 30

Firing Rate Reduction

0.0 0.1 0.2 0.4 0.6 0.8 1.0

r = -.542, p < 0.01

During-stimulation
Post-stimulation

During
Post-stim

During
Post-stim

Preferred
Non-preferred

Firing Rate (spk/sec)

Time (minutes)

0 1 2 3 4

Firing Rate Ratio during Visual Stimulation

Non-preferred Preferred

Preferred

Firing Rate (spk/sec)

Time (minutes)

0 1 2 3 4

Firing Rate Ratio during Visual Stimulation

0 45 90 135 180

0.1 1 10 100

Firing Rate (spk/sec)

Time (minutes)

0 50 100 150 200

0.1 1 10 100

Firing Rate (spk/sec)

Time (minutes)

0 50 100 150 200

0.1 1 10 100
A

Baseline
Prolonged stimulation
Post-stimulation period

36 minutes+
10-15 minutes
36 minutes+

B

Visual Response (spk/sec)
Contrast (%)

Baseline
Post-stimulation

C

Contrast tuning maximum (spk/sec)

Baseline
Post-stimulation

D

Evoked/Spontaneous Ratio

Baseline
Post-stimulation

p < 0.01
A. Depth (mm) vs. Time (ms) showing axon terminal and postsynaptic sink and source.

B. CSD amplitude (μV/mm²) over baseline 0-20, 20-40, 40-60 minutes post-stimulation period.

C. Normalized spike rate (% baseline) over baseline 0-20, 20-40, 40-60 minutes post-stimulation period.