Multiplicative Mechanism of Lateral Interactions Revealed by Controlling Interhemispheric Input

Thomas Wunderle, David Eriksson and Kerstin E. Schmidt

Research Group: Cortical Function and Dynamics, Max-Planck-Institute for Brain Research, 60528 Frankfurt, Germany

Address correspondence to Thomas Wunderle, Max-Planck-Institute for Brain Research, Deutschordenstraße 46, 60528 Frankfurt, Germany. E-mail: thomas.wunderle@brain.mpg.de.

Long-range horizontal connections are thought to modulate the responsiveness of neurons by supplying contextual information. A special type of long-range connections are interhemispheric projections, linking the 2 cerebral hemispheres. To investigate the action of those projections in a causal approach, we recorded in cat primary visual cortex while deactivating corresponding regions on the contralateral hemisphere. Interestingly, the action of callosal projections turned out to depend on the local and global composition of the stimulus: Full-field stimulation with gratings revealed moderate rate decreases (modulation index $-0.24$) and some significant increases ($+0.21$), whereas with lesser salient random dot textures, much more neurons were affected and reacted with pronounced rate decreases ($-0.4$). However, orientation and direction selectivity of those neurons were only slightly influenced by callosal input. This invariance could be achieved by scaling responses multiplicatively. Indeed, we could quantify the action of callosal input as a multiplicative scaling of responses, but additive scaling also occurred, especially for grating stimulation. We conclude that the quantitative action of long-range horizontal connections is by no means fixed but depends on how the network is driven by an external stimulus. Qualitatively, those connections seem to adjust the response gain of neurons, thereby preserving their selectivity.

Keywords: corpus callosum, gain modulation, long-range connections, orientation selectivity, reversible deactivation

Introduction

Neurons in the cortex usually integrate over hundreds to thousands of synaptic inputs in order to create an output spike train. Interestingly, a large fraction of synapses in early visual areas are formed by local (intra-areal) connections projecting horizontally over short or long distances (Gilbert and Wiesel 1979; Martin and Whitteridge 1984; Stepanyants et al. 2009) or by feedback connections from higher areas (Felleman and Van Essen 1991). Stimulus-driven responses of a neuron are therefore also influenced by the intracortical network the neuron is embedded in. Therefore, the question arises whether this influence alters the tuning properties of that neuron. To preserve tuning properties, a modulatory input usually scales the responses of a neuron multiplicatively rather than by acting additively. Indeed, experimental studies suggested multiplicative scaling of tuning curves induced by a change in spatial attention (McAdams and Maunsell 1999), stimulus contrast (Anderson et al. 2000), and feedback from higher areas (Wang et al. 2007). Models describing such a scaling have been proposed for response normalization (Carandini and Heeger 1994) and gain modulation (Murphy and Miller 2003; Ayaz and Chance 2009). However, modulation by lateral connections has so far not been quantified in a causal approach. This is probably because it is difficult to investigate them in the intact brain; therefore, many conclusions rely on in vitro or modeling studies. A second issue of scientific debate is to which extent response modulation mediated by lateral connections depends on local and global features of the driving stimulus.

To address these issues, we combined multielectrode recordings in one hemisphere with reversible thermal deactivation of topographically corresponding regions on the contralateral side, thereby controlling lateral input via the corpus callosum. This enabled us to causally deactivate and reactivate a large part of lateral input to the primary visual cortex while stimulating with 2 distinct stimuli.

In the visual system, the corpus callosum connects the 2 hemispheres especially between regions representing the central visual field along the vertical meridian (VM). Early studies therefore suggested that callosal connections (CCs) serve to integrate the representation of the 2 visual hemifields along the VM into a unified percept (Hubel and Wiesel 1967). Anatomical studies confirmed that CCs are predominantly excitatory in nature (e.g., Conti and Manzoni 1994) and have much in common with long-range lateral connections like patchy terminal arbor (Houzel et al. 1994) coinciding with iso-orientation domains (Schmidt et al. 1997; Rochefort et al. 2009). Therefore, CCs may be seen as an extension of the long-range intrinsic lateral network across the 2 hemispheres (Voigt et al. 1988).

Previous work demonstrated that CCs can increase and/or decrease the activity of neurons, observed for both spiking activity (Payne et al. 1991; Sun et al. 1994; Schmidt et al. 2010) and local field potentials (Carmeli et al. 2007; Makarov et al. 2008). Furthermore, a recent report suggested a contribution of the CCs to the response specificity of neurons, especially for those preferring cardinal contours (Schmidt et al. 2010).

By taking advantage of the callosal system, providing well-defined synaptic input from a spatially separated region, we found a stimulus feature specific modulation of firing rates during cooling deactivation. Moreover, the stronger this modulation, the more it resembled a multiplicative scaling and, as predicted, preserved the tuning properties of the neuron. Our results therefore provide a direct insight into the nature of modulating synaptic input from lateral connections in an in vivo system, supporting previous theoretical and experimental work.

Materials and Methods

**Surgical Procedures**

Eleven adult cats bred at the Institute’s colony were used in this study. All experimental procedures have been performed in accordance with...
the guidelines of the Society for Neuroscience and the German law for the protection of animals.

Anesthesia was initiated intramuscularly with 10 mg/kg ketamine hydrochloride (Ketamin; CP-Pharma, Germany) and 1 mg/kg xylazine hydrochloride (Rompun; Bayer, Germany) supplemented with 0.1 mg/kg atropine sulfate (Atropin; B. Braun, Germany) and was maintained after tracheotomy by artificial ventilation with a mixture of 0.6%/1.1% halothane (for recording/surgery, respectively) and N2O/O2 (70%/30%). After all surgical procedures had been terminated, the animals were paralyzed by continuous intravenous infusion of pancuronium bromide (0.15 mg/kg/h, Pancuronium, DeltaSelect, Germany). Depth of anesthesia was controlled by continuously monitoring the electrocardiogram and CO2 level.

A craniotomy was performed on both hemispheres in topographical correspondence, leaving a ridge of bone above the superior sagittal sinus intact. The position of the craniotomy was chosen to cover a portion of both area 17 or 18 and the 17/18 border region (centered on Horsley–Clarke coordinates AP 0 to −2, ML +2). A recording chamber was implanted over one of the craniotomies and, after removing the dura, filled with silicon oil (50 cs, Boss Products, Elizabethtown, KY) for optical imaging. After the optical recordings, the silicon oil was removed, and 2 microelectrode arrays were implanted for electrophysiological recordings.

A surface cryoloop (e.g., Lomber et al. 1999) was placed on the contralateral hemisphere and covered with clear agar (Agarose type XI, Sigma, Germany) to allow visual inspection of the correct position throughout the experiment. Loop dimensions were approximately 5 × 3 mm². Because cells stop firing at a temperature around 20 °C (Lomber et al. 1999), and the temperature gradient is between 10 and 15°C/mm (Payne et al. 1991; Lomber et al. 1999), this corresponds to a deactivated region of about 8 × 6 mm². To monitor the temperature, a thermocouple sensor was attached to the basis of the loop. At the end of the experiment, animals were killed with a lethal dosis of pentobarbital (Narcorn, Merial, Germany).

Visual Stimulation

For visual stimulation, the eyes were fitted with contact lenses, and the pupils were dilated with topical application atropine sulfate (1%; Atropine-POS, Ursapharm, Germany) and phenylephrine (5%; Neosynephrin, Ursapharm, Germany). Eye alignment was checked throughout the experiment and corrected with a prism if necessary.

Visual stimuli were presented on a 21” CRT monitor in 57 cm distance from the animal’s eyes covering 20° of both visual fields (using in house stimulation software). Two sets of stimuli were used: High contrast square wave gratings moving in 8 directions (45° steps) orthogonal to their orientation and coherently moving random dot textures (RDTs) moving in 12 directions (30° steps) with reduced contrast (Michelson contrast: 0.44) to prevent motion streaks. Spatial frequency and speed of the gratings as well as size and speed of the random dots were chosen depending on the cortical area in which the recording was performed. A18: 0.15 cycles/°, 16°/s for gratings and 0.6° diameter and 20°/s for random dots. A17: 0.5 cycles/°, 4°/s for gratings and 0.4°/diameter and 13°/s for random dots. Both stimuli were held stationary for 500 ms (static phase) after a blank of the same duration and then moved for 1000 ms (dynamic phase). Each condition was randomly presented 20–30 times with an interstimulus interval of 1.5 s. We have chosen this type of stimuli because they exhibit different types of features: Gratings are optimized for the receptive field properties of primary visual cortex and exhibit a fundamental spatial and temporal frequency (as well as its odds harmonics) with a strong orientation component orthogonal to their direction of motion. Random dots, on the other hand, exhibit a broad spatiotemporal frequency spectrum without an axis of orientation but a strong motion component. We used moderate dot speeds, which usually result in a preferred direction similar to stimulation with gratings but a somewhat broader tuning. Furthermore, by using full-field stimulation, we could record a large population of neurons with distributed receptive fields simultaneously.

As a control, we introduced a third type of stimulus in 3 of the 11 cats: moving random bar textures (RBs). The bars’ width was the same as the dots, but they were 3 times as long. We designed this type of stimulus to exhibit features of both grating and random dot stimuli. The individual elements are still randomly positioned, but because of their elongation in one axis each element contains an orientation component orthogonal to its direction of motion. RDTs, RBs, and gratings were presented randomly in 8 directions and repeated 20 times each. Additionally, we adjusted the contrast of the gratings and bars in this new set of stimuli, in order to match evoked baseline firing rates of all 3 sets of stimuli. To this end, we first obtained average contrast response functions by stimulating with gratings/bars of 6 different contrasts (3.125%, 6.25%, 12.5%, 25%, 50%, and 100% contrast, at each unit’s optimal orientation) averaged over a set of selected units. We then created the grating and bar stimuli according to this response function at a contrast where firing rates matched that of the dots.

Optical Imaging of Intrinsic Signals

Optical imaging of intrinsic signals was performed to functionally define the 17/18 border as described in Bonhoeffer et al. (1995) allowing subsequent positioning of the electrode arrays in either the 17/18 transition zone (TZ) or close by in area 17 or 18 (Bonhoeffer et al. 1995). This method is superior to the mapping of the VM with multiple electrode penetrations because it is comprehensive but noninvasive and therefore does not damage the cortical tissue. Two sets of grating stimuli adjusted to optimally stimulate either area 17 or 18 (see Visual Stimulation) were presented. Single condition maps (0°, 45°, 90°, and 135°), obtained by stimulating with a grating of high spatial frequency, were summed and divided by the sum of the same set of maps obtained by low spatial frequency stimulation. The 17/18 border was defined as the region of equal brightness on this map.

Electrophysiological Recordings

Two 4 × 4 tungsten microelectrode arrays (1 MΩ; Microprobes, Gaithersburg, MD) with an electrode spacing of 400 (6 cats) or 250 μm (5 cats) were positioned in a region of the primary visual cortex representing the central visual field as described above. The majority of cells (n = 418) were located at the 17/18 border close to the VM or in area 18 (n = 366) with receptive fields a few degrees away from the VM (Supplementary Fig. 3). A few cells were recorded also in area 17 (n = 55) with receptive fields still close to the VM and therefore grouped with the cells recorded in the TZ. Electrodes were lowered 200–600 μm into the cortex using a microdrive (Narishige, Tokyo, Japan) in order to target the superficial layers. The craniotomy was subsequently covered with agar and bone wax. The recorded signals were amplified (1000-fold), band-pass filtered (0.7–6 kHz), digitized, and thresholded around 4 standard deviations above noise level to obtain spike time stamps using a Plexon acquisition system (Plexon Inc., Dallas, TX) and custom written Acquisition software (SPASS by Sergio Neuenschwander, in LabView, National Instruments). A recording session consisted of baseline, cooling, and recovery period, each containing 20–30 repetitions per condition. Recording during the cooling period was initiated after the cooling loop reached a stable temperature of 3 ± 1.5° for 5 min by pumping chilled methanol through the lumen of the cooling probe. The recovery was started ~20 min after cooling was terminated.

Data Analysis

Offline analysis was done using custom written software in LabView and Matlab (Mathworks Inc., Natick, MA). From 32 simultaneously recorded channels, only those exhibiting reliable spiking activity were used for further analysis according to the following criteria: 1) A 2-factor analysis of variance was performed to test if the response amplitude (measured in spikes per second) to a drifting stimulus was significantly larger than within the prestimulus period (blank), and if there was a significant difference between stimulus directions (both P < 0.05). 2) The direction or orientation tuning of the channel reached a certain threshold (VAm or VAqm > 0.2, for definition of tuning indices, see Tuning Selectivity).

For each remaining unit (n = 491 for grating and n = 348 for RDT stimulation), only responses to the preferred direction were considered in subsequent analyses.
We calculated, for each unit and state, a peristimulus time histogram (PSTH) smoothed with a Gaussian kernel of \( \sigma = 10 \) ms and normalized it by the maximum response in the dynamic phase. Individual PSTHs were then averaged over all animals and units. Statistical significance was assessed by a t-test \((P < 0.05)\) corrected for multiple comparisons using the false discovery rate, which controls the proportion of false positives at a specific level (we allowed for 5% false positives; Benjamini and Hochberg 1995).

To quantify changes in neuronal activity, the spike rate was averaged over all trials in the 500 ms prestimulus window (spontaneous activity) and in a 1000 ms window following stimulus motion onset (stimulus-driven activity). We calculated a modulation index between cooling/baseline \((\text{MI}_{\text{cooling}})\) for the spike rate \((R)\):

\[
\text{MI}_{\text{cooling}} = \frac{R_c - R_b}{R_c + R_b}; \quad \text{MI} \in \{-1 \ldots 1\}.
\]

Here, \(R_c\) and \(R_b\) denote the average spike rate in the cooling and baseline period, respectively. The measurement always relates the responses to the baseline, with values \(>0\) indicating a decrease and a value \(>1\) an increase compared with baseline (substituting \(R_t\) with the rate during the recovery period indicate if the effect of deactivation is reversible).

To correct for differences in evoked baseline firing rates between gratings and RDT stimulation, we applied a mean matching procedure in order to equalize their population means (Churchland et al. 2010). In brief, for both types of stimuli, the rate distribution for the baseline recordings across all units was binned at 1 sp/s. Then, the largest common distribution across both stimuli was taken. Subsequently, for each bin and stimulus, the same units exceeding this common distribution were randomly discarded in the baseline, cooling, and recovery period. The average firing rate for all 3 periods was then calculated on this matched sample. This procedure was repeated 300 times, and the obtained mean values were averaged.

Statistical significance between 2 samples was assessed by t-tests \((\alpha = 5\%)\) unless otherwise stated. Error bars in figures denote ±1 standard error of the mean. Significance level is indicated by stars: n.s., not significant; \(^* P < 0.05; ^{**} P < 0.001; ^{***} P < 0.0001,\) unless stated otherwise.

### Response Scaling Mechanism

To describe how removal of callosal input scales neuronal responses, direction tuning curves obtained during baseline and cooling were averaged for each recorded unit. First, directional responses for RDTs and gratings were interpolated to 16 directions using piecewise cubic interpolation (producing a smoother result than linear interpolation, included in the Matlab function ‘interp1’; see also Fritsch and Carlson 1980). Interpolation was done to equalize the number of conditions of each stimulus, allowing a direct statistical comparison; however, the results of subsequent analyses were not changed qualitatively. Then, the effect of cooling on the tuning curve was expressed with the linear regression model:

\[
R(\theta) = \beta_0 + \beta_1 \cdot R(\theta)_h \quad \text{(full model)},
\]

by minimizing the sum of squares between data and model, with \(R(\theta)_h\) and \(R(\theta)\) representing the response to direction \(\theta\) in baseline and cooling, respectively. The parameter \(\beta_0\) shifts the whole tuning curve up or down along the response axis (additive scaling), while the parameter \(\beta_1\) scales the tuning curve by a constant factor (multiplicative scaling). Only units with an \(R^2 > 0.8\) for the linear regression were kept for further analysis. To compare the amount of additive and multiplicative scaling, we also fitted the tuning curves with reduced models:

\[
R_e(\theta)_h = \beta_0 + R(\theta)_h \quad \text{(additive model)}
\]

and

\[
R_m(\theta)_h = \beta_1 \cdot R(\theta)_h \quad \text{(multiplicative model)}.
\]

The amount of additive or multiplicative scaling was then obtained by calculating a reduced model index \((\text{RMI})\) of the residual sum of squares (RSS) for the 2 reduced models:

\[
\text{RMI} = \frac{\text{RSS}_a - \text{RSS}_m}{\text{RSS}_a + \text{RSS}_m}; \quad \text{RMI} \in \{-1 \ldots 1\}
\]

\(\text{RSS}_a\) and \(\text{RSS}_m\) are the residual sum of squares for the multiplicative and additive model, respectively. The \(\text{RMI} = 1\) for a perfect multiplicative scaling, \(-1\) for an additive, and \(0\) if both models describe the scaling of the tuning curve equally well. We further tested for each unit, if one or the other model performs significantly better or if they perform equally well. To this end, we calculated the probability that one or the other model is correct using Akaike’s information criterion (Akaike 1974\(^{1}\)). This method, based on information theory, is suitable for comparing non-nested models. When comparing 2 models, A and B, it gives the probability \(P\) that A is more likely than B, with the corresponding probability \(1 - P\) that B is more likely than A. A probability of \(P = 0.5\) indicates that both models are equally probable. We selected a threshold of \(P < 0.025\) and \(P > 0.975\) indicating that there is a probability \(>95\%\) that the 2 models are significantly different from each other.

### Tuning Selectivity

Tuning selectivity was obtained for direction and orientation tuning curves. For orientation, the responses of opposite directions were averaged to obtain tuning curves with angles between 0 and 180°. A direction selectivity index \((\text{DI})\) was defined as

\[
\text{DI} = 1 - \frac{R_{\text{null}}}{R_{\text{rect}}}; \quad \text{DI} \in \{0 \ldots 1\},
\]

where \(R_{\text{rect}}\) is the maximum average firing rate and \(R_{\text{null}}\) is the response in the null direction, 180° apart. DI is 0 for a cell responding to both directions similarly and 1 for a pure direction selective cell. Accordingly, we defined an orientations selectivity index \((\text{OI})\), where \(R_{\text{rect}}\) is the maximum firing rate of the orientation tuning curve and \(R_{\text{null}}\) is the activity orthogonal to \(R_{\text{rect}}\) 90° apart. Additionally, we quantified direction and orientation tuning by calculating the (normalized) vector average \((\text{VA})\) across responses (Swindale 1998):

\[
\text{VA}_{\text{dir}} = \left[ \frac{\sum_k R_k \cos \theta_k}{\sum_k R_k} \right]; \quad \text{VA}_{\text{dir}} \in \{0 \ldots 1\},
\]

where \(R_k\) is the firing rate at direction \(k\) and \(\theta_k\) is the direction in radians (ranging from 0 to 2π). When plotted in a polar diagram, a VA of 0 would describe a circle, while a VA of 1 would describe responses, which are zero everywhere but for one direction.

### Results

**Action of Interhemispheric Input on Moderately Stimulus-Driven and Spontaneous Firing Rates Is Mainly Excitatory**

In a first attempt, the trial averaged firing rate, at each neurons preferred stimulus direction was compared between baseline (native) and cooling (deactivation) condition. An example recording of a cell stimulated with gratings (Fig. 1A) and RDTs (Fig. 1B) shows a reduction in firing rate while blocking callosal input by cooling deactivation. Even though stimulation with RDTs evoked a smaller response compared with grating stimulation, the decrease during cooling deactivation was more pronounced for RDTs. This holds true for the population data as depicted in Figure 1C (grating) and Figure 1D (RDT) for the normalized PSTHs averaged across all recorded units. Firing rates decreased significantly \((t\text{-test}, P < 0.05,\) corrected for multiple comparisons) during cooling in both static and dynamic phase for both stimuli and recovered to baseline level after rewarming (recovery), but the decrease in the dynamic phase for RDT stimulation was much stronger compared with grating stimulation. Because the cooling effect did not change
throughout the dynamic phase, we averaged the firing rate across time for subsequent analysis. The average firing rate decreased significantly from 116 to 99 sp/s (−14%) for grating ($P < 0.05$) and from 75 to 44 sp/s (−42%) for random dot stimulation ($P < 0.001$). Although, averaged firing rates did not fully recover (110 and 72 sp/s for grating and dot stimulation), recovered rates were not significantly different from baseline rates ($P > 0.1$). This demonstrates a robust effect of cooling deactivation on neurons in the primary visual cortex, with more pronounced rate decreases for RDT than for grating stimulation.

In order to find a measure comparing the effects on individual units, we calculated for each unit a MI between baseline and cooling (eq. 1). For grating stimulation, 55% of the units showed a significant modulation (Fig. 1E; Mann–Whitney $U, P < 0.05$) by cooling (black area of the histogram) with decreases being more frequent (48.3% of units, median MI: −0.24) than increases (6.7% of units, median MI: +0.21). In contrast, for RDT stimulation, 73.3% of the units showed a significant modulation by cooling (Fig. 1F), with by far most units (73%, median MI: −0.4) showing a rate decrease. Only one unit increased its firing significantly.

For the static presentation of stimuli, the average effect was also a decrease in firing rate of −20% for grating and −34% for RDT. This difference between the 2 stimuli was not as large as for dynamic presentation (Supplementary Fig. 1), and the number of significantly effected units was smaller for static than for dynamic stimuli. However, there was a positive correlation between the amplitude of rate change for the static and dynamic presentation (Supplementary Fig. 1C,D).
Thus, although the major effect of cooling deactivation was a decrease in firing rate for all stimuli, we noted 2 differences between grating and RDT stimulation: First, both the number of significantly affected cells and the strength of the effect were larger for RDT stimulation. Second, rate increases were much more frequent for grating stimulation.

We also quantified effects on spontaneous activity by averaging the ongoing activity in the 500 ms before stimulus onset pooling data from both stimulus protocols. The median spontaneous firing rate decreased significantly from 2.7 to 1.7 sp/s (8%) during cooling ($P < 0.0001$, Wilcoxon signed-rank test; Supplementary Fig. 2A). This finding indicates that even in the absence of a visual stimulus, driving the neurons in both hemispheres, corticocortical connections exert a tonic, excitatory influence on their target cells.

According to the anatomy of CCs, namely extending more densely in the 17/18 TZ, we expected the cooling effect to be region specific. However, all cells were recorded in the central visual field, with most receptive fields extending no more than 10° into the periphery (for an example of the most peripheral recording, see Supplementary Fig. 3B). Within this range, recorded neurons were influenced at all eccentricities tested (Supplementary Fig. 3C). Nevertheless, we could observe a tendency to more pronounced effects for neurons recorded close to the VM, especially for RDT stimulation (Supplementary Fig. 3B,C). On average, firing rates decreased more strongly for neurons recorded close to the VM than for those in area 18 (both stimulus protocols, $P < 0.01$). However, the ratio between rate increases and decreases was relatively similar across all azimuths tested (Supplementary Fig. 3D).

**Excitatory and Inhibitory Action of Interhemispheric Input Depends on Local and Global Stimulus Features**

In order to demonstrate that the stimulus-specific differences were indeed due to inherent features of the stimuli and not an artifact of higher baseline firing rates for gratings than for RDTs, we performed 2 controls. First, we applied a mean matching method in order to equalize the rate distributions of gratings and dots. This procedure led to identical rates for grating and RDT stimulation in the baseline (68.3 sp/s; Fig. 2A). As for nonmatched data, rates decreased significantly during cooling deactivation to 57.3 sp/s for grating and 39.5 sp/s for RDT stimulation ($P < 0.05$). Interestingly, the rate decrease remained much stronger for RDT stimulation than for grating stimulation ($P < 0.0001$). Using the modulation ratio between baseline and cooling instead of the absolute firing rate, confirmed this result and yielded similar results than for nonmean-matched data (data not shown).

Second, for another set of units obtained from 3 more animals, we tried to match the average firing rates evoked by grating and RDT stimuli already during acquisition. This was done by lowering the contrast of the gratings until the rate was close to that for RDTs. However, on average, the firing rate for the contrast-reduced protocols was still higher than for the RDT (18 sp/s more) and RBT protocols (20 sp/s), but this difference was not statistically significant ($P > 0.05$). Because the number of units in this control set ($n = 92$) was smaller than for the previous analysis, we randomly subsampled 92 units for high contrast grating stimulation from the complete data set of 491 units. Although low contrast grating stimulation resulted in a slightly stronger rate decrease during cooling than high contrast stimulation, the rate decrease for RDT stimulation was still significantly stronger (Fig. 2A). Supporting a particular behavior for gratings, stimulating with RBTs revealed similar rate decreases under cooling as stimulating with random dots. The amount of rate change for a particular cell during RDT presentation was positively correlated with the amount of rate change during grating ($r = 0.53$) and RBT stimulation ($r = 0.81$). This means that some neurons are more influenced by callosal input than others, independent of the stimulation protocol. However, for a given degree of rate change, RDT stimulation led to the profoundest changes, indicating contextual dependency.

This strongly indicates that the way in which corticocortical connections modulate their target cells in primary visual cortex depends on the local and global composition of the stimulus driving the system and less on the presence of an orientation component.

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**Figure 2.** Controlling for differences in baseline firing rate between grating, RDT, and RBT stimulation. (A) Population average of firing rates after mean matching. Note the identical baseline firing for grating and RDT stimulation. However, the rate decrease for RDT stimulation compared with grating stimulation is much more pronounced. (B) Rate matching through contrast adjustment. Cooling-induced changes in spike rate are expressed by the MI between cooling and baseline as well as between recovery and baseline. Data for contrast-adjusted recordings are shown in gray, whereas data for high contrast stimulation are black. A sketch of the stimuli is depicted on top of the graph. Note that neurons stimulated with RDTs and RBTs decrease about the same amount, whereas stimulation with high and low contrast gratings also behave similarly but less pronounced. **$***P < 0.0001,** analysis of variance.
**Direction and Orientation Selectivity Are Only Moderately Influenced by Interhemispheric Connections**

Given the strong influence of callosal projections on their target cells, the feature specificity of those cells might also be influenced. One of the main features of cells in the primary visual cortex is direction/orientation selectivity. Therefore, we investigated if these features are modulated by cooling deactivation and if there is a relation between tuning selectivity and the additive/multiplicative scaling of tuning curves (see next paragraph). We used 3 measures to quantify tuning selectivity (see Materials and Methods): The ratio between preferred and null responses of direction (DI) and orientation (OI) tuning curves and a measure of the circular variance expressed as the vector average across the tuning curve, VA_{dir} (for definition, see eqs. 6 and 7). Figure 3 depicts those measures for baseline, cooling, and recovery period. Apparently, cooling-induced changes are very small but reach significance for a decrease in direction selectivity when presenting RDTs (paired t-test, P < 0.001). It should be noted that subtracting spontaneous activity from driven responses prior to calculation of the tuning indices did not alter the results presented above. In general, we could observe a trend toward a stronger multiplicative scaling for broadly tuned neurons (for RDT stimulation), but this correlation was very weak (r = 0.12; P = 0.028 for correlating the multiplicative component with VA_{dir}, spearman rank correlation). Individual units, which changed their tuning selectivity, were those with strong additive scaling. This is a consequence of how tuning indices are defined: Shifting the tuning curve up or down changes the ratio of preferred to nonpreferred firing and therefore its selectivity.

In summary, direction and orientation tuning of neurons in area 17 and 18 does not substantially depend on interhemispheric input, in accordance with a predominantly multiplicative scaling of tuning curves.

**Interhemispheric Input Scales Tuning Curves in a Multiplicative Manner**

We have demonstrated that corticocortical connections influence their target cells by predominantly enhancing their activity. But which responses are exactly modulated and how?

We first tested, if the impact of cooling deactivation on orientation tuning curves can be described by a linear regression model (eq. 2). Figure 4A illustrates the orientation tuning curve (baseline, green) for an example cell recorded in the 17 zone in response to grating stimulation. Cooling deactivation led to a decrease in the tuning curve’s amplitude (cooling, blue). Fitting the responses during baseline and cooling to the linear model (middle plot in Fig. 4A) gives the fit coefficients of the model. The intercept of the regression line with the ordinate returns the parameter β₀ (additive scaling), while the parameter β₁ (multiplicative scaling) is equal to the slope of the regression line. For the particular example cell, the linear regression gives an additive scaling component of 1.1 sp/s and a multiplicative scaling of 0.62. From this fit coefficients, the tuning curve during cooling can be derived from that during baseline. The linear model nearly perfectly describes the effect of cooling deactivation as depicted by the overlap of the model (black in the right plot) with the true curve obtained during cooling (blue), with an R² of 0.99.

We have chosen a threshold of R² > 0.8 of the regression for a successful description of the cooling effect on the tuning curves by the linear model. This is important because regression parameters are only meaningful if the model describes the data sufficiently well. Applying this criterion, the effect was well described for 90% of units stimulated by gratings (n = 444) and for 77% of units stimulated by RDT (n = 269). Units that did not pass this criterion usually had a broader and noisier tuning curve (the fit of a model function such as a Gaussian to this units was worse than for units with R² > 0.8 and their mutual information was also smaller, data not shown). For all units passing the criterion, the median multiplicative component (MC) was 0.84 for grating and 0.56 for RDT stimulation. Because the additive component (AC) is in units of spikes per second, we normalized each unit’s tuning curve by the response to the preferred direction before applying the linear model. The median normalized AC was 0.006 and 0.02 for grating and RDT stimulation, respectively. We emphasize that the conclusions drawn from the following analysis are independent of the normalization scheme. To better understand the combinations of additive and multiplicative scaling, we plotted the MC against the AC of each unit (Fig. 4B,C) and averaged the normalized tuning curves for each of the 4 possible combinations (AC positive/MC smaller 1, AC positive/MC larger 1, AC negative/MC larger 1, and AC negative/MC smaller 1) separately (Insets in Fig. 4B,C). The influence of the AC can be best observed at the nonpreferred orientations of the difference curve (black) between baseline (green) and cooling (blue). Upscaling or downscaling by the

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**Figure 3.** Direction (VA_{dir}, DI) and orientation (OI) selectivity during baseline (gray), cooling (black), and recovery (striped) for grating (A) and RDT stimulation (B). Note the significant decrease in direction selectivity for RDT presentation. However, this decrease was very small compared with overall selectivity.
MC dominates in all 4 cases and determines if the response at the preferred direction increases or decreases. However, the tails of the tuning curve are either lifted up or pulled down by the AC.

Although the linear model of the cooling effect describes the AC and MC quantitatively, one still cannot directly judge which of the 2 components dominates. Therefore, we constructed 2 reduced models (eqs. 3 and 4) as exemplified in Figure 5A, expressing the tuning curve during cooling as either a pure additive or a pure multiplicative scaling of the baseline. For the same example cell as in the previous figure, the additive model returns a line parallel to the unity line with slope 1 and an intercept of $-6.8$ sp/s, the multiplicative model a line with a slope of 0.66 and an intercept of zero (middle figure part). For each model, one gets an estimation of the tuning curve during cooling deactivation (right figure part) based on the baseline tuning. The AC simply shifts the whole tuning curve down during cooling, whereas the MC multiplies the baseline tuning curve with a constant in order to fit with the cooled tuning curve. For this cell, it is obvious that the multiplicative model describes the data much better than the additive model. This holds also for the averaged data and for both types of stimuli (Fig. 5B, C).

The question arises whether the amount of additive/multiplicative scaling depends on the overall impact of cooling deactivation. To address this question, we binned the rate changes (in spikes per second for the preferred direction) and averaged the RMI for each group separately (Fig. 6C). First, the bigger spike rate changes were the more a change in the tuning curve resembled a multiplicative scaling, independently of rate increase or decrease (Note that the number of units contributing to the rate increases $>15$ sp/s for RDT stimulation is very small, making an estimation of the RMI unreliable.) This relationship also holds when comparing the RMI across different azimuths: Stronger rate changes close to the VM are accompanied by more pronounced multiplicative scaling. Furthermore, rate increases had a smaller RMI, indicating that cells being inhibited by CCs scale their tuning curves in an additive manner. Consequently, the rate changes close to the VM are accompanied by more pronounced multiplicative scaling. Furthermore, rate increases had a smaller RMI, indicating that cells being inhibited by CCs scale their tuning curves in an additive manner.

So far, we have quantified the amount of additive/multiplicative scaling dependent on the preference of the recorded neurons for a certain stimulus direction/orientation. Because
CCs preferentially link together neurons of similar orientation preference, the amount of synaptic input, coming through the callosum, should depend on the stimulus, that is, its orientation or direction of motion, respectively. That is, a neuron firing strongly in response to its preferred stimulus should also receive strong input from the callosum, whereas for non-optimal stimulation, this input should be weaker. We therefore tested, if a predominant multiplicative response modulation is a consequence of this stimulus dependency or a general feature of response modulation. To this end, we analyzed the static phase from RDT stimulation protocols, evoking an unspecific activation of the recorded cells, and therefore, on average, a constant (unselective) input from the callosum. We then estimated the amount of additive/multiplicative scaling by computing the linear regression between the firing rates in the baseline against those obtained during cooling, across the population of neurons recorded (Fig. 6D). This analysis reveals a significant multiplicative scaling of responses (MC: 0.73, \( P < 0.05 \); AC: \(-0.41\), \( P = 0.43\); using robust regression, bootstrap \( t\)-test), in accordance with the results shown above. Moreover, applying the same analysis to spontaneously recorded data revealed the same result (Supplementary Fig. 2B).

Thus, the multiplicative interaction seems to be independent of a certain use of the specific callosal anatomy. This result generalizes our findings and renders them unlikely to be a consequence of coactivating in the 2 hemispheres neurons of similar response properties and thus interconnected neurons.

**Discussion**

In this study, we investigated the role of interhemispheric projections in shaping the output of neurons in cat primary visual cortex. We compared firing rates of neurons before and after thermal deactivation of corresponding parts on the contralateral hemisphere. Although our results speak in favor of a predominant excitatory influence, they clearly demonstrate that both overall impact as well as the ratio between suppressive and facilitatory influences on the callosal recipient zone depend on the composition of the stimulus. The mechanism summarizing all rate modulations can be described as a mainly multiplicative scaling of different sign and magnitude, largely preserving tuning properties. Interestingly, spontaneous activity and activity evoked by unstructured visual

**Figure 5.** (A) Applying the reduced models to the same example neuron as shown in Figure 4. The additive model (upper row) gives a shifted version of the baseline tuning curve in order to fit with the cooling one. The multiplicative model (lower row) multiplies the baseline tuning curve with a constant factor. The multiplicative model clearly outperforms the additive one. (B,C) Population average of the (normalized) tuning curves during baseline and cooling for grating (B) and RDT (C) stimulation together with the predictions of the additive and multiplicative model. As for the single example, the multiplicative model fits the data much better than the additive one.
stimuli were—though less pronounced—also affected in a multiplicative manner. Our results indicate that the gain of V1 is dynamically regulated, increasing the impact of modulatory input for lesser salient stimuli.

**Stimulus-Dependent Qualitative and Quantitative Effects of Interhemispheric Input**

In this study, we used whole-field stimuli covering both visual hemifields. Consequently, both callosal sending and recipient zone were activated by the (same) global stimulus enabling us to study the modulating impact of the callosal system on the largely active network. This is in contrast to stimulating the receptive field of a neuron with a single bar or a Gabor patch which leads to a specific and spatially restricted feedforward activation of that neuron without any context mediated by horizontal (recurrent) or feedback connections (for review, see Angelucci and Bressloff 2006). Both grating and RDT stimuli activate the visual cortex uniformly but presumably in a different manner because of their different spatiotemporal properties, local and global composition as well as orientation and direction components. We therefore asked if this distinction also affected the modulation through the callosal system.

On average, we found facilitatory actions, in line with previous experimental results (Payne et al. 1991; Payne 1994; Sun et al. 1994; Schmidt et al. 2010) and the predominately excitatory nature of those connections (Shoumura 1974; Fisket et al. 1975; for review, see Conti and Manzoni 1994). The visual stimulus used to drive the system nevertheless determined the qualitative and quantitative characteristics of this modulation. For the range of oriented grating stimuli used in this study, we found both weak suppressive and facilitative actions, whereas for RDTs, we observed an almost exclusive and strong excitatory influence.

One reason for those differences might be that tuning curves obtained with moving RDTs are usually broader than those obtained with grating stimulation (Skottun et al. 1988; Wörgötter and Eysel 1989). Thus, RDTs presumably activate a larger population of neurons. A certain neuron may therefore receive more input from the contralateral hemisphere than with grating stimulation. However, our results for the RBTs render this argument unlikely (Fig. 2B). By extending the size of the dots along one dimension, we created a stimulus containing properties of both gratings and RDTs. That is, we introduce an orientation component, leading to a more selective activation of neurons but keep the variable spatiotemporal property. Interestingly, stimulation with this stimulus led to comparable results as stimulation with RDTs, indicating that larger rate decreases during cooling are not explained by the absence of the orientation component and thus an unselective recruitment of neurons.

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**Figure 6.** (A,B) Distribution of the RMI (eq. 5) for grating (A) and RDT (B) stimulation. A value smaller than zero indicates additive, a value bigger than zero multiplicative scaling. Unit counts with a significant difference between the 2 models are shown in black, nonsignificant units in gray. The percentage of significantly different units is given in the upper left corner. Arrows denote the median for the RMI. (C) Average RMI for different groups of spike rate change between cooling and baseline (negative values indicate rate decrease, positive values rate increase due to cooling deactivation) for grating and RDT stimulation. Note the clear increase in RMI with a more pronounced rate change. (D) Linear regression across neurons for the static response of RDTs. Each dot represents the (trial averaged) firing rate of one recorded unit. The regression line intersects the ordinate near zero, indicating a pure multiplicative scaling mechanism.

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A more likely explanation is a stimulus-dependent gain. By gain, we mean here the slope of the relationship between input amplitude and spike output of a neuron. Changing this relationship is a powerful mechanism because small changes in membrane potential can be transformed to large changes in spike rate. Via this mechanism, the intracortical circuitry can amplify small signals such as weakly tuned input from LGN neurons (Ben-Yishai et al. 1995; Sompolinsky and Shapley 1997). Given the anatomical situation of highly recurrent connections especially in layer II/III of visual cortex (Binzegger et al. 2004), both strong recurrent excitation and inhibition seem to be critical for amplification (Somers et al. 1995; Salinas and Abbott 1996). Moreover, recent experimental and modeling evidence suggest that in cortical circuits excitation is tightly balanced by inhibition (Marino et al. 2005; Okun and Lampl 2008; Stimberg et al. 2009). This is important because the total (background) synaptic input regulates the neuronal gain in a divisive manner (Chance et al. 2002; Shu et al. 2003). A high contrast grating, supposed to evoke a high level of background synaptic input, thus sets the cortex in a low gain state. The lower salient RDT stimulus, on the other hand, presumably evokes lower levels of background synaptic input. The cortex therefore operates here in a high gain regime. Other mechanisms such as activity dependent synaptic depression (Thomson 1997) or shunting inhibition (Borg-Graham et al. 1998) maybe also at work. In any case, synaptic input from CCs in the high gain regime should have a larger impact. This is exactly what we observed.

We controlled for the overall level of activity by lowering the contrast of the grating stimulus. In this case, we still observed a significant difference between gratings and dots, although individual neurons showed a tendency for more pronounced rate decreases at low contrast (unpublished results). Therefore, we conclude that the distinct properties inherent to the stimuli determine the cortical gain. Support for stimulus-dependent gain modulation comes from a study performing intracellular recordings in vivo (Cardin et al. 2008). A broadband stimulus with a variable spatiotemporal frequency had a different effect on the membrane potential than a sinusoidal grating resulting in distinct gain control for that neuron.

As a second result, we observed a qualitative difference between grating and RDT stimulation. Gratings evoked significant, although fewer, rate increases during removal of callosal input, whereas for RDT stimulation, we observed only rate decreases. A likely reason could be that, though mainly excitatory (but see Buhl and Singer 1989), callosal projection cells can target inhibitory neurons in the recorded hemisphere (Somogyi et al. 1983). Those inhibitory neurons can target both iso-oriented and cross-oriented neurons as also suggested by a model in the former study of Makarov et al. (2008). A disynaptic activation of local interneuronal circuits provides the possibility of a net effect of inhibition through callosal activation. In accordance, electrical stimulation of callosal afferents evoked a monosynaptic EPSP, usually followed by a delayed IPSP (Toyama et al. 1974). Those inhibitory neurons may need a large feedforward drive to be activated, explaining the larger number of rate increases for grating stimulation compared with RDT. Such high threshold inhibitory interneurons were proposed to explain contrast dependent surround modulation (Shushruth et al. 2012).

The absolute amount of inhibitory effects observed in cat and ferret spiking data (Schmidt et al. 2010) seems to be much less than reported for LFP data obtained in ferrets (Makarov et al. 2008) indicating that not all of the inhibitory effects become suprathreshold. Interestingly, in the former study, the number of increased LFP responses was also higher for grating stimuli, which stimulated the 2 hemifields in a coherent manner.

Our spike data strongly indicate that inhibitory circuits are recruited to a variable extent, depending critically on the feedforward stimulus drive of the network. In detail, we could show that the fine-tuning of modulations through CCs is dependent on the properties of the visual stimulus.

**Impact of Interhemispheric Input on Tuning Curves**

Despite strong rate changes, the tuning properties of target cells were only slightly affected. We observed a weakly significant influence on direction selectivity and only when stimulating with RDTs, the stimulus that exerted the biggest rate decreases. It is also plausible that direction—not orientation—selectivity was preferentially influenced because the representation of coherent motion across the visual midline requires the integration of the 2 hemispheres.

Previous studies had reported slightly larger orientation selectivity for neurons close to the VM than for neurons in the far periphery (Wilson and Sherman 1976; Payne and Berman 1983), indicating a contribution of the callosal system to orientation selectivity. We do not have much evidence for drastic changes of orientation selectivity during cooling. In addition, our recordings were made always near the center of the visual field, so that we cannot make comparisons to contributions of CCs to orientation selectivity in the far periphery.

In Schmidt et al. (2010), it has been shown that subpopulations of neurons preferring cardinal contours were more and differently affected than others in their responsiveness. This could be observed in the population data of this study as well (data not shown). Real changes in orientation and direction selectivity observed during deactivation constituted only a small fraction of the total selectivity in both the present cat and the previous ferret study (Schmidt et al. 2010; Fig. 7).

In order to understand how neurons kept largely their tuning selectivity while changing their responsiveness, we explored here the scaling mechanism of the corresponding tuning curves. It turned out that changes were linear and could be well described by the multiplication of the tuning curve with a constant factor. The simple addition of a constant provided a worse fit to the data, especially for units with strong rate changes. However, a few units showed a pronounced additive shift, especially with grating stimulation, which was often accompanied by rate increases. Interestingly, additive shifts were also reported for the attention dependent scaling of contrast response functions in human (Buracas and Boynton 2007) and monkey (Thiele et al. 2009) visual cortex.

Multiplicative scaling preserves the ratio of preferred to nonpreferred firing rates. As a consequence, the neuron’s response selectivity is preserved and invariant to changes in the level of synaptic input (Finn et al. 2007). Such multiplicative scaling is a widespread phenomenon and can be inferred for the action of feedback (Wang et al. 2007), contextual modulation (see Fig. 2 in Li et al. 2000), and shifts in spatial attention (McAdams and Maunsell 1999; Treue and Martinez Trujillo 1999).
Although not affecting tuning width downscaling an orientation tuning curve may still have an influence on the performance of orientation discrimination (Li et al. 2000). Recordings in cat and monkey visual cortex indicate that the discriminative capability of a neuron is a combination of mean firing rate, variance, and tuning width (Scobey and Gabor 1989; Snowden et al. 1992). Furthermore, it was suggested that for fine orientation discrimination the high-slope region of a tuning curve is most informative (under the condition of low noise, i.e., low neuronal firing variability) because this region is most sensitive to small changes in orientation offset (Butts and Goldman 2006; Scolari and Serences 2009). A divisive scaling of a tuning curve decreases its peak as well as its maximal slope, thereby decreasing its sensitivity to coarse and fine orientation differences. Thus, the callosal system—albeit exerting only a minor influence on tuning width—is nevertheless capable of supporting orientation discrimination in primary visual areas. We consider it likely that this conclusion holds also for other short- and long-range lateral connections.

**How Can Feedforward and Lateral Inputs Be Combined in a Multiplicative Manner?**

Traditionally, a neuron is thought to integrate over its synaptic inputs calculating a weighted sum of the total input. In addition, a couple of biophysical mechanisms have been described introducing nonlinearities to the integration mechanism, enabling a single neuron to perform even more powerful mathematical operations (for review, see Silver 2010). However, our results can be explained by a rather simple mechanism: If the callosal input is similarly tuned as the geniculocortical, a simple addition of the 2 will resemble a multiplicative scaling (Fig. 7). There is indeed good evidence that callosal fibers interconnect preferentially domains of similar orientation preference (Schmidt et al. 1997; Rochefort et al. 2007; Rochefort et al. 2009) and that the input from the callosum is therefore orientation tuned (Lepore and Guillemtot 1982). The callosal input (CI) may be as narrow tuned as the geniculocortical input (GI), but its amplitude is likely to be only a fraction (F_C) of it (Berardi et al. 1987). This fraction can be inferred from the MC in our regression analysis by the relation $F_C = 1/\beta_1 - 1$. Furthermore, if one assume CI = GI + $F_C$, then $F_C$ becomes crucial for the observed multiplicative effect can be the result of either an input modulation due to the tuning and functional connectivity or an output modulation due to nonlinear cellular and network properties of neurons in visual cortex.

**Can One Generalize Our Findings to Other Corticocortical Connections?**

The effects of callosal projections, although strongest in the 17/18 TZ, could be observed in regions of area 17 and 18 several millimeters away from the TZ. Early anatomical studies report a widespread of callosal terminals into large parts of the lateral and posterior lateral gyrus (Sanides and Albus 1980; Payne and Siwek 1991). Thus, the observed effects can be attributed largely to direct removal of callosal synaptic input to the recorded neurons. However, spike rate decreases might have been additionally caused via an indirect disynaptic pathway. Using the horizontal network of short- and long-range horizontal connections neurons within the TZ could...
spread response decreases to more lateral parts of area 17 and 18. Such a lateral spread of information is thought to underlie perceptual effects of center–surround interactions (for review, see Angelucci and Bressloff 2006). The impact of the spread could be amplified by the local recurrent network (Shushruth et al. 2012). Taken together our results support a more general role of CGs, more in the sense of completing the long-range intrinsic network across the hemispheres, merging the 2 visual hemifields along the VM (Hubel and Wiesel 1967).

One difference between callosal and long-range horizontal connections could be the fact that in the absence of the feedforward loop, callosal projections can directly drive their target cells (Choudhury et al. 1965; Berlucchi and Rizzolatti 1968; Rochefort et al. 2007), whereas intrinsic ones are believed to be only modulatory (e.g., Hirsch and Gilbert 1991). However, under certain conditions, for example, adult plasticity, they might take over a driving role. A recent report documents that a stimulus presented outside the classical receptive field can occasionally induce spikes (Chavane et al. 2011). For the intrinsic network, this cannot be tested easily in a causal approach like in our study. Our finding that cooling deactivation of interhemispheric connections also affects spontaneous activity supports a driving and not exclusively modulatory role. Changes in spontaneous activity have also been observed for different attentional states (Williford and Maunsell 2006) usually believed to be modulatory as well as in feedback deactivation studies (Wang et al. 2007).

In summary, we assume that the change from modulatory multiplicative to driving is a continuum rather than a discrete step for all mentioned types of corticocortical connections and depends critically on the actual contribution of the other possible input sources.

Our results provide, for the first time, a detailed and quantitative description of spike rate modulation in primary visual areas through a corticocortical network. This modulation is dependent on how an external stimulus drives the cortical network and supports earlier theoretical work on gain modulation in cerebral cortex.

Funding
Max Planck Society.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/

Notes
We are grateful to Dr Anne Schmidt and Christiane Peiker for their help in some of the experiments. Conflict of Interest: None declared.

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