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Selective interhemispheric circuits account for a cardinal bias in spontaneous activity within early visual areas

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Abstract

Ongoing brain activity exhibits patterns resembling neural ensembles co-activated by stimulation or task performance. Such patterns have been attributed to the brain's functional architecture, e.g. selective long-range connections. Here, we directly investigate the contribution of selective connections between hemispheres to spontaneous and evoked maps in cat area 18 close to the 17/18 border. We recorded voltage-sensitive dye imaging maps and spiking activity while manipulating interhemispheric input by reversibly deactivating corresponding contralateral areas. During deactivation,

spontaneous maps continued to be generated with similar frequency and quality as in the intact network but a baseline cardinal bias disappeared. Consistently, neurons preferring either horizontal (HN) or vertical (VN), as opposed to oblique contours, decreased their resting state activity. HN decreased their rates also when stimulated visually.

We conclude that structured spontaneous maps are primarily generated by thalamo- and/or intracortical connectivity. However, selective long-range connections through the corpus callosum - in perpetuation of the long-range intracortical network – contribute to a cardinal bias, possibly, because they are stronger or more frequent between neurons preferring horizontal and/or cardinal contours. As those contours are easier perceived and appear more frequently in natural environment, long-range connections might provide visual cortex with a grid for probabilistic grouping operations in a larger visual scene.

Keywords

Corpus callosum, Transition zone, Orientation preference, Resting state, VSD imaging.

Introduction

Accumulating evidence from the last few decades has been changing the perspective of the brain as a mere input-output system. It is known that the brain does not passively react to external stimulation but rather expresses a diversity of active ongoing processes. Variations in spontaneous activity prior to stimulus presentation account for inter-trial variability in motor cortex activation and human behavior (Fox et al., 2006, 2007) as well as differences in the

precision of stimulus detection (Schölvinck et al., 2012; Emadi et al., 2014), facial recognition (Hesselmann et al., 2008) and auditory perception (Sadaghiani et al., 2009). Pre-stimulus activity also allows for predictions of choice in specific tasks (Bode et al., 2012; Colas and Hsieh, 2014) as well as general patterns of behavior (Davis et al., 2013). Summarizing, it seems likely that external stimuli do not determine brain activity but instead modulate spontaneous activity finally giving rise to output such as perception or behavior (Fiser et al., 2004).

Ongoing brain activity is not random. It has spatial features resembling neural ensembles co-activated by stimulation or task performance. Spontaneous neuronal firing is strongly correlated between functionally related neurons (Tsodyks et al., 1999). Evoked spiking patterns for specific sound frequencies seem to replay from a wider set of possible spontaneous patterns (Luczak et al., 2009). Spontaneous activation of a large population of neurons is capable to generate modular maps of iso-orientation preference in the primary visual cortex (Kenet et al., 2003). Even larger networks of ongoing activity between cortical areas, resembling evoked activity have been observed (Smith et al., 2009). These networks are often referred to as intrinsic connectivity networks (ICNs) or resting-state networks (RSNs) (Sadaghiani and Kleinschmidt, 2013).

There is manifold evidence that neurons in the visual cortex responding to contours of the same orientation are preferentially linked by long-range patchy connections (Gilbert and Wiesel, 1989; Malach et al., 1993; Bosking et al., 1997; Schmidt et al., 1997a; Stettler et al., 2002). A close relationship between the anatomical configuration of that patch system as determined by tracer

injections, and the structure of maps acquired through visual stimulation has been suggested (Muir et al., 2011). Along this line, also spontaneous maps like the ones found by Kenet et al. (2003) have been associated with the architecture of long-range intra- and corticocortical intrinsic and/or feedback connections.

The aim of the present work was to causally investigate the contribution of selective connections to intrinsically generated modular orientation maps. Interhemispheric connections between the two primary visual cortices of cats are thought to perpetuate the intrahemispheric network across the visual field's midline, are also patchy (Houzel et al., 1994) and selectively link neurons of similar orientation (Schmidt et al., 1997b; Rochefort et al., 2007) and direction preference (Peiker et al., 2013). As visual interhemispheric connections (VIC) can be easily manipulated by reversible deactivation without directly interfering with the recorded ipsilateral responses, we chose them as a model for a selective long-range connection between homotopic visual areas.

While imaging central parts of contralateral cat area 18 including the 17/18 areal border using voltage-sensitive dyes (VSD) with and without visual stimulation we confirm that modular maps resembling evoked orientation preference maps occur spontaneously. During deactivation of contralateral input these spontaneous maps continue to be generated but the commonly observed cardinal bias in their frequency of occurrence disappears. As spontaneous cortical activity of a single neuron can be predicted by the spatial pattern of spontaneous population activity in a large cortical area (Tsodyks et al., 1999) we also examine spiking activity during these conditions. In line with the VSD

data spontaneous spiking activity of neurons preferring horizontal and vertical contours decreases during contralateral deactivation.

Material and Methods

Surgical Procedures

All procedures were performed in accordance with the guidelines of the Society for Neuroscience and the university ethics committee for the use of animals (CEUA-UFRN). Eight adult cats, five males and three females, received a craniotomy on both hemispheres covering a portion of both areas 17 and 18 as well as the 17/18 border region. For surgery, animals were initially anesthetized with an intramuscular injection of ketamine (10mg/kg) and xylazine (1mg/kg) supplemented with atropine (0.1mg/kg). Anesthesia 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 surgery each animal was paralyzed by a bolus injection of 1mg pancuronium bromide followed by continuous intravenous infusion (0.15mg/kg/h).

Over the left craniotomy, a recording chamber was implanted, fixed with dental cement and, after removal of the dura mater, filled with silicon oil for intrinsic signal and voltage-sensitive dye (VSD) imaging. We refer to this left to be recorded hemisphere as the ipsilateral one in the following. In case of VSD imaging, the cortex was first stained with a commercially available blue dye (RH 1838, Optical Imaging Inc) and then covered with silicon oil. Over the right hemisphere, a surface cryoloop (Lomber et al., 1999) was placed and covered with clear agar (Figure 1A). We refer to this right to be deactivated hemisphere as the contralateral one in the following. The cooling probes were made of

hypodermic stainless steel tubing shaped to fit the marginal gyrus centered on the 17/18 border (Horsley Clarke coordinates AP 0, L -3.5) including the adjacent central parts of area 17 and area 18. The dimensions of the loop used in the present experiments were 7 x 3.4 mm. The stainless steel tubing was connected to Teflon tubing, through which chilled methanol was pumped. The methanol was chilled by dry ice to -65°C . The temperature of the cooled cortical surface was regulated by the speed of the pump. A thermometer connected to a sub-miniature connector, attached to a copper wire soldered to the union of the loop was used for temperature monitoring. Considering the cooling spread on brain tissue, the critical temperature to cease activity and the cryoloop dimensions, we set the cryoloop temperature to around 2°C in order to get a deactivated area of about $10 \times 5 \text{ mm}^2$.

Visual Stimulation

Visual stimuli were presented randomly on a 21" monitor placed 57 cm in front of the cats' eyes. We used intrinsic signal imaging in order to identify the 17/18 border. To this end, gratings of four different orientations in 45 degree steps and a spatial frequency of 0.5 cyc/deg (for area 17) or 0.1 cyc/deg (for area 18) and a velocity of 4 deg/sec (for area 17) or 16 deg/sec (for area 18). Gratings were moved back and forth for 3000 ms changing direction every 500 ms. For VSD imaging, the stimulus set consisted of 12 conditions; two conditions presented a random rapid sequence of static whole-field gratings at 8 different orientations in 22.5 degree steps (0.1 cyc/deg, 2 Hz) each lasting 250 ms, eight conditions presented only one of the eight orientations for 250 ms followed by 3750 ms of dark screen, and two conditions of 4000 ms presenting

a dark screen (used for spontaneous activity recording). In a similar second stimulus set, stimulus presentations were prolonged to 500 ms each. Stimulus conditions were presented in pseudo-randomized order. In each trial, the recording began 500 ms before the presentation of the stimulus, totaling 4500 ms of recording for all trials. After recording of intrinsic signal and VSD baseline (intact network, no interhemispheric deactivation) imaging the cryoloop was cooled and, 5 min after reaching a stable temperature, VSD imaging was repeated. Cooling would then be switched off and 30 min later a new VSD imaging would take place. We refer to each of these recording sessions as baseline, cooling and recovery. In order to keep non-cooling related signal decay within one session small, each stimulus condition was repeated only 5 times in one session, with 25 s of inter-stimulus interval (ISI), adding to about 30 minutes imaging time per session. For one staining we normally ran three repetitions of the cycle baseline–cooling–recovery, and we stained twice per animal.

For electrophysiology, we determined beforehand receptive field position, extent, orientation and direction preference of all recorded units by presenting whole-field bars moving in 16 different directions (22.5° steps) at a width of 1° and a speed of 20 degrees/s for 2000 ms as done by Botelho et al. (2014). The test stimuli for cooling were the same as for VSD imaging, but with an ISI of 2 s and 20 repetitions for each condition.

During all recordings, background illumination and background noise in the lab were reduced to a minimum aiming to create a quiet and dark ambient for spontaneous activity traces.

Recording procedures

Recorded frames were acquired with a 12mm CCD camera (Dalsa 1M60) through a macroscope fitted with a 1x objective (Imager 3001, Optical Imaging Inc, New York, USA). Rate of acquisition was 5Hz and resolution 512 x 512 pixels for intrinsic imaging and 160Hz, 256 x 256 pixels for VSD imaging. Intrinsic signal imaging was only used to define the transition zone (TZ) between areas 17 and 18. Two sets of gratings with different spatial and temporal frequencies were presented for an optimal response of either area 17 or 18. The subtraction of the signal obtained by presentation of these two sets made the transition zone between the two areas evident (for details see supplementary figure 3 of Wunderle et al., 2013).

For electrophysiology, 4x4 Tungsten microelectrode arrays (1M Ω , 50 μ m diameter, 250 μ m spacing, Microprobes, Gaithersburg, USA) were implanted into both areas 17 and 18, right next to the border, and into the TZ. For this study, we considered only electrodes matching the imaged area on the area 18 side and within the transition zone, which is the area from Horsley-Clarke coordinates 0 to 4 AP and 0 to 5 ML. Electrodes were lowered between 200-600 μ m below cortical surface using a Microdrive (Narishige, Tokyo, Japan) and subsequently, the craniotomy was covered with agar. 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 and spike-wave forms using a Plexon acquisition system (Plexon Inc., Dallas, TX) and custom written Acquisition software (SPASS by Sergio Neuenschwander, in LabView, National Instruments).

Data Analysis

All analyses were performed in Matlab (Mathworks Inc., Natick, MA, USA). All, evoked and spontaneous, VSD imaging data were subjected to a noise removal procedure in order to make the signal more evident. To this end, we subtracted the first frame acquired through evoked activity from the whole 4.5 s recording on the single trial level (first frame correction). Secondly, we removed the heartbeat and respiration artifacts from the signal. Two strategies were used to extract heartbeat noise, one for the stimulation set of 250 ms gratings and another for the set of 500 ms gratings. In the first case, a reject band filter covering frequencies between 2.5 and 3.5 Hz was applied while in the latter case, a band pass filter isolated the heart artifact which was then subtracted from the original signal. The filtered frequency interval varied (1.5-3.5 Hz) for different subjects and depended on the heart frequency. In each case, the same filters were applied to spontaneous trials of the respective data set. A Discrete Wavelet Transform was applied to remove respiration noise. Considering that the camera has a sample rate of 160 Hz, we were able to remove frequencies between 0 and 0.625 Hz (respiration was never faster than 0.3 Hz) at the seventh decomposition level.

Subsequently, we selected trials that were stimulated with gratings of the same orientation and averaged frames between 175 and 500 ms after stimulus onset. For each trial we subtracted the stimulus onset frame from the whole trial, a procedure known as first frame correction. We also clipped the values, setting as upper threshold the mean pixel value plus and as lower threshold the mean pixel value minus two times the standard deviation. This enabled us to calculate preference maps for each orientation (eight single condition maps in total).

Next, we summed up all single condition maps and subtracted the cocktail-blank, the average of the closest trials from all eight orientations. In order to avoid negative pixel values, the absolute of the lowest pixel value was summed to the whole trial and finally pixel values were divided by the mean pixel value. This last normalization distributed pixel values around 1 and reduced the natural dye bleaching effect on the data. These procedures - with exception of clipping - are presented in the following equation:

$$F_n = ((F - F_0) - F_{cb}) + |F_{min}| \div F_{av}$$

Where F_n is the normalized signal, F is the raw signal, F_0 the first frame, F_{cb} the cocktail-blank, F_{min} is the lowest pixel value and F_{av} the average pixel value.

Finally, we applied two spatial filters: a square high pass filter, 20 pixel in size for each side, to correct for uneven luminance (since the recorded area is curved) and a circular low pass filter with a 5 pixel radius to remove intrinsic camera noise.

Subsequently, regions of interest (ROIs) - areas of cortex void of blood vessels and modulated by the stimuli – were selected from the maps. According to Tusa (Tusa et al. 1979), these ROIs covered a part of the lower central visual field representation in area 18 (azimuth 0 to 7 degree, elevation 0 to -5 degree, approximately) including the TZ. Based on these ROIs we then calculated the spatial Pearson correlation coefficient of frames acquired through grating stimulation or spontaneous frames with the averaged single condition maps. This resulted in eight correlation values for each frame, evoked or spontaneous. All analyses were done separately for baseline, cooling and recovery recordings.

Since single trials, and especially single frames, may contain a lot of noise (activity not related to stimulation or patterned activity waves), erroneously indicating structure, we calculated the vector sum of all eight raw correlation coefficients produced by each frame when correlated with each of the eight single orientation maps. These coefficients were disposed in a circle (with correlation coefficients calculated from orthogonal maps in opposite directions) and the resultant vector was calculated (Fig 1B). The resultant vector position defined which map structure the frame mostly resembled and the vector magnitude (referred to as tuning index) defined how well it matched the respective single orientation maps. Note that orthogonal orientation maps are complementary to each other and therefore both, positive (between similar maps) and negative correlation coefficients (between orthogonal maps) are signatures of modular structures.

The comparison of baseline and cooling recordings should be sufficient to detect and describe an effect of interhemispheric deactivation. However, it is possible that the structure observed in spontaneous maps is an artifact generated by data processing instead of real spontaneous structure. To be sure that this was not the case we used as a control maps artificially generated by the convolution of pseudorandom images and single condition maps. After convolution we made circular shifts on these artificial maps in order to obtain an equal number of control images and recorded frames. The same spatial filter applied to recorded frames was applied to artificial maps. This control is similar to the one used by Kenet et al. (2003).

In order to classify spontaneous frames without any prior assumption of their putative structure we applied an algorithm devolving self-organizing maps

(Kohonen, 1990). Self-organizing maps have an initial random pixel values distribution, i.e. there is no detectable pattern in these maps. They can, however, be trained by an input (our spontaneous data in this case), and, with each step of training, patterns present in the input start to become evident in these self-organizing maps. This analysis serves i) to avoid any circular logic of selecting over-the-threshold evoked and spontaneous frames and then correlating them to single condition maps that were used to define the threshold in the first place and ii) to reveal a subtle pattern or effect that was present in frames that didn't achieve the correlation threshold to single condition maps.

The network consisted of 100 nodes, organized as a 10 by 10 network, and was trained by either all spontaneous or all evoked frames (training input). In order to determine the distance between the nodes within the network we used a hexagonal topology and a Euclidean distance function defined by:

$$D_{i,j} = \sqrt{\sum_{k=1}^P (n_{i,k} - n_{j,k})^2}$$

where $D_{i,j}$ is the distance between nodes i and j , $n_{i,k}$ is the k^{th} element from a total of P elements of node i . Training was performed with an initial neighbourhood of 10 nodes and 10 steps. Within each training step q , the node N with higher proximity to the input frame $\mathcal{F}$ and its entire neighborhood is modified to resemble the input even more by following the learning rule:

$$N_i(q) = N_i(q-1) + \alpha(\mathcal{F}(q) - N_i(q-1))$$

where α is the learning rate set to decreasing values from 1 to 0.01. The neighborhood gets smaller with each training step, until only one node learns from the input. This process was repeated 40 times (i.e. epochs) for each input frame. Finally, we calculated the correlation coefficient and tuning index for each trained node, i.e. self-organizing map, with the single condition maps the same way we did for recorded frames.

For the analysis of the electrophysiological data, units situated in the same cortical region as the map ROIs were considered. Multi-unit activity was sorted through *Waveclus*, a spike sorting toolbox developed by Quiroga et al. (2004). Based on wavelet decomposition of spike waveforms the toolbox separates single units recorded with the same electrode.

For each recorded single-unit, we computed orientation-tuning curves based on the spiking responses to the oriented bar of the RF mapping procedure and subsequently, an orientation (OI) and a direction index (DI) (as in Wunderle et al. 2013). Further analysis included only spiking responses of units with an OI or DI higher or equal to 0.2 ($n = 163$ single units).

We then calculated the mean firing rate of each selected unit in 500 ms time windows after presentation of the static grating for each of the 8 orientations ($n = 1280$ baseline responses > 0) and equally sized windows of spontaneous activity recording from the test protocol. As instantaneous spontaneous firing rate varies spontaneously independent of the cooling manipulation, spontaneous windows were selected according to the following procedure. For comparison with the stimulus evoked windows we selected an equal number of windows of high, intermediate, and low level spontaneous

activity compared to the overall level of spontaneous activity in all three states. To this end, we established as a threshold for high-level activity the mean firing rate of all baseline trials plus one standard deviation and for low level activity the mean firing rate of all baseline trials minus 0.1 standard deviation. For every time point detected above (below) threshold, we considered the activity from 100 ms before until 400 ms after that time point, thus defining 500 ms windows of high/low spontaneous activity considered for analysis. Intermediate level activity windows were defined between the two thresholds. This was done for baseline, cooling and recovery recording with the thresholds always calculated on baseline data.

The deactivation effect was measured by the modulation index (MI) of the measured and computed variables (D), a dimensionless quantity:

$$MI_D = \frac{D_{cooling} - D_{baseline}}{D_{cooling} + D_{baseline}}; MI \in \{-1 \dots 1\}$$

A negative MI means that D (correlation frequency of the VSD frames with an average orientation preference map, average spike rate, map and spike tuning) decreased during the cooling session pointing towards an excitatory influence of interhemispheric connections (and vice versa an inhibitory influence in case of MI increase).

Results

Spontaneous modular maps continue to be generated during deactivation of lateral interhemispheric input

During deactivation of visual interhemispheric input, the overall layout of the average evoked single orientation maps did not change (Figure 2, left column). In agreement with this average result, single evoked frames closely resembling the average map could be traced during all three states (baseline, cooling, recovery) (example recording in Figure 2, second column). As Kenet et al. (2003) we detected also frames of spontaneous activity modulated in a similar manner as the evoked map. Most interestingly, these spontaneous modulations continued to occur during deactivation of VIC (Figure 2, third column) although this procedure removes considerable input from the adjacent half of the visual field representation and lowers the overall activation as indicated by a lower contrast in the maps.

In order to quantify the similarity of the single frames with the averaged map we calculated the distribution of all correlation coefficients with the average evoked map for each of the eight orientations and each state. This procedure revealed both high positive and negative correlation coefficients compatible with frames being very similar (positive correlation) to the average columnar pattern evoked by a certain orientation and complementary to the pattern evoked by its orthogonal orientation (negative correlation). We repeated the same procedure for control maps (see methods on their calculation).

Correlations during evoked and spontaneous epochs did not differ significantly from each other during baseline ($p = 0.9627$), cooling ($p = 0.6828$) or recovery ($p = 0.9926$, Kolmogorov-Smirnov two-sample test for all three comparisons), Figure 3A, red, evoked; blue, spontaneous), but clearly from the control maps (black trace). This suggests that images acquired during spontaneous phase during all three states present a clear orientation map

pattern. In order to provide evidence that the structure indicated by high correlation coefficients results from true orientation tuning of the spontaneous maps we calculated the vector sum of all eight raw correlation coefficients (Figure 3B). The resultant vector amplitudes (tuning indices) were not significantly different between evoked and spontaneous maps during baseline ($p = 0.1181$), cooling ($p = 0.2865$) or recovery ($p = 0.1804$, Wilcoxon rank sum test for all three comparisons, Figure 3B). They were much higher than values obtained with control frames (black trace, Figure 3B), which served as a reference for what we considered as sufficiently high to be considered for further analysis in the next section.

Deactivation of VIC had no significant effect on the latencies of evoked activity. This holds true for both the latency of the raw optical signal (Figure 3C) and the latency with which correlation coefficients between single frames and averaged single condition maps increased during stimulation (Figure 3D). On average, the maximum response between stimulus onset and offset was achieved after 153 ms for baseline and 162 ms for cooling ($p = 0.32$, Wilcoxon rank sum test, $n = 12$). At the maximum, the raw optical signal was 18% stronger during baseline than during cooling, but not significantly ($p = 0.4$, Wilcoxon rank sum test, $n = 12$). It took, on average, 181 ms for baseline trials and 187 ms for cooling trials ($p = 0.37$, Wilcoxon rank sum test, $n = 12$) until the maximum correlation coefficient was achieved. Maximum correlation was only 2% higher for baseline trials than cooling trials with no statistical significance ($p = 0.59$, Wilcoxon rank sum test, $n = 12$). This tiny difference between correlation coefficients confirms that there was no significant difference in the pixels'

orientation preference although the average signal was 18% weaker during deactivation.

Absence of lateral interhemispheric input lowers the frequency of spontaneous but not evoked cortical maps with cardinal orientation preference

Kenet (Kenet et al., 2003) had observed that spontaneous frames resembling maps for cardinal orientation preferences were generated with a higher frequency than frames resembling maps for oblique orientation preferences. This is in accordance with earlier observations that in the central visual field representation stimulus-driven responses to cardinal orientations are stronger (e. g. Pettigrew et al., 1968), more frequent (Li et al., 2003) and lead to larger cortical representations of those orientations (e.g. Dragoi et al., 2001).

Frames with a low similarity with single condition maps might introduce noise into an orientation preference analysis, since a low tuning index can also indicate no similarity with any of the single condition maps. In order to quantify any bias in the occurrence of frames resembling maps of a certain orientation preference we thus considered only frames with a tuning index above 90% of the control distribution as indicated in Figure 3B (all frames with tuning strengths past the dotted vertical line). During baseline recordings, on average 39.51% of the frames acquired during stimulation had a tuning index above this threshold. During cooling recordings, 36.75% of the frames surpassed the threshold and 37.11% during recovery. For spontaneous activity, 34.37% of baseline frames were above threshold while 31.94% of cooling frames and

33.6% of recovery frames had tuning indices that exceeded the control threshold.

Figure 4 demonstrates the probabilities of a high correlation of single stimulus-evoked frames with an average map for a certain preference angle. Confirming the previous study (Kenet et al. 2003), 68.51% of the evoked frames were classified as either horizontal, $157.5^\circ < x \leq 22.5^\circ$, or vertical, $67.5^\circ < x \leq 112.5^\circ$ (i.e. cardinal) rather than oblique, $112.5^\circ < x \leq 157.5^\circ$ and $22.5^\circ < x \leq 67.5^\circ$ (Figure 4A). Deactivating VIC did not change this classification for the evoked (63.44% cardinal) activity. Interestingly, this percentage of frames resembling maps of either horizontal or vertical orientation preference was much larger for spontaneous baseline frames (Figure 4B, black traces). Surprisingly, this bias of spontaneous frames tuned to cardinal orientations dropped dramatically during cooling. In contrast to the evoked activity, the frequency of “cardinally tuned” frames decreased significantly from 74.84% to only 58.68%.

Figure 4C summarizes the impact of VIC on the angular preference of single frames, expressed as a modulation index (with positive numbers indicating an increase in the frequency for this angular preference during cooling, and vice versa for negative numbers).

For evoked activity (black), the number of frames classified as vertically, obliquely or horizontally preferring maps did not change significantly during cooling. For spontaneous activity, the average number of frames classified as vertical or horizontal, as compared to the average number of frames classified as oblique, dropped significantly (Wilcoxon signed-rank, 12 datasets, * $p = 0.0137$, 0.002 and 0.0039 for horizontal, oblique and vertical spontaneous

modulation respectively) significantly decreasing the cardinal predominance. All distributions recovered to baseline during rewarming.

In Figure 4D, we illustrate for the example of Figure 4B how the temporal sequence of the preferred angles of spontaneous frames is affected by VIC deactivation. In accordance with the previous finding of Kenet (Kenet et al., 2003), the preference angles of the spontaneous frames changes in small steps prevailing for a longer time at cardinal angles. During cooling, angles continue to change smoothly but the bias shifts to different angles.

In order to examine whether the redistribution of preference maps was caused or accompanied by an overall drop of orientation selectivity, or by a redistribution of maximum tuning indices to different angles we plotted the evoked and spontaneous tuning indices of the “good” maps as a function of the best preference angle obtained for the respective frame (Figure 5AB). Supporting the baseline cardinal bias it turned out that vertically and horizontally preferring frames produced the highest tuning indices for both evoked and spontaneous activity. Cooling deactivation of VIC did not alter these distributions (Figure 5C) indicating that the redistribution of preference angles of spontaneous frames was due to a drop of the frequency - but not the quality - with which spontaneous maps for cardinal orientation preference angles occurred.

Evoked and spontaneous maps training a Kohonen model

In order to quantify the dynamics of spontaneous population activity without explicitly using any stimulus-derived orientation map, we applied a

Kohonen map algorithm (Kohonen, 1990) to compute self-organizing templates trained by evoked or spontaneous frames (as in Kenet et al., 2003). We used all recorded frames as input for training, disregarding the tuning index threshold considered for previous analysis. When compared to real evoked maps, the neural network consisting of 100 nodes, i.e. trained templates, exhibited a cardinal bias, a similar pattern to the one found by directly correlating recorded frames with average single condition maps (Figure 6A). The Kohonen algorithm produced that result also when being fed exclusively with spontaneous baseline data (Figure 6B, dark gray). Unexpected from the previous analysis, the number of evoked frames assigned to horizontal but not vertical orientation preference dropped (Fig. 6A, light gray). This drop however, maybe due to a large dispersion in data, was not significant (Fig. 6C). One reason for the discrepancy between recorded and trained frames results could be that this bias towards vertical orientations exists and we removed it by restricting the former analysis to the frames with a tuning index above threshold. Another possibility is that the bias is due to noisy frames entering the Kohonen analysis.

For spontaneous templates, the Kohonen analysis reproduced quite closely the result we retrieved from correlation analysis with real data. The number of templates classified as cardinally preferring decreased during VIC deactivation while the number of obliquely preferring frames increased (Fig. 6B, C, light gray). Both, templates trained with either evoked or spontaneous data turned out to be as well tuned as the real data frames. Cooling induced changes as a function of orientation preference were not significant (data not shown).

This confirms that modulated maps resembling the functional architecture of that cortical area continue to be generated frequently and with good

selectivity. Only the frequency with which certain orientation preference columns get activated spontaneously changes when part of the lateral network is disrupted.

Effects of VIC input on evoked and spontaneous spiking activity depend on the orientation preference

Previously, it has been reported that single neuron activity can be predicted by the activity of the spontaneous population in a large cortical area (Tsodyks et al., 1999; Schölvink et al., 2015). In order to examine the influence of the lateral network on spontaneous spiking activity we recorded multi-unit activity during VIC deactivation in the same area the VSD images were taken from. 161 tuned (see methods) single-units from 7 experiments were included in the analysis.

In contrast to the seemingly stable layout of evoked and spontaneous modulated frames in both presence and absence of VIC input, average firing rates decreased significantly during cooling of VIC (14.6 ± 10.6 % for activity evoked by the preference orientation $\pm 30^\circ$, 12.6 ± 6.55 % for spontaneous activity). This has been observed before (Schmidt et al., 2010; Wunderle et al., 2013) and is in agreement with the overall drop of map contrast.

In previous work, pre-stimulus activity of neurons driven by contours moving into the cooled hemifield had turned out to be less influenced than that of neurons preferring horizontal contours or such moving out of the cooled

hemifield (Peiker et al., 2013). However, a systematic comparison with neurons preferring or stimulated by oblique contours as well as with real spontaneous activity in a long inter-stimulus interval was missing.

Here, we observed that the exact combination of changes in spontaneous and stimulus-derived activity depends on the orientation preference of the neurons under study.

Figure 7 depicts the PSTHs of three representative example single units, one of vertical (A), one of oblique (B), and one of horizontal orientation preference (C). On average, evoked activity of the unit preferring vertical contours (VN) moving into the cooled hemifield does not change much during cooling (Figure 7A). This is in agreement with previous findings that vertical units depend less than horizontal units on input derived from the deactivated hemifield depending on their direction preference (see Peiker et al., 2013). Also, the obliquely preferring unit loses only few spikes (Figure 7B) but on average more than the pool of all vertically preferring units (Figure 8C). Finally, the horizontally preferring unit (HN) presents a prominent spike rate decrease during VIC deactivation (Figure 7C). Interestingly, when comparing this picture to the evoked VSD maps it matches the lack of templates assigned by Kohonen learning to horizontal as opposed to vertical orientation preference maps during cooling (Figure 6A).

For spontaneous activity, however, both VN and HN lose more spikes than the obliquely preferring neurons (Figure 7, compare A and C with B). This supports the loss of the cardinal bias in the angular distribution of spontaneous maps (Figures 4C, 6C).

Figure 8 demonstrates the mean modulation indices of all firing rates of 163 single units. 24 conditions rendered no baseline spike and did not enter the analysis resulting in 1280 responses. MIs for spontaneous activity were significantly more negative than for evoked activity (Figure 8A). Supporting the observation made for VSD maps, units preferring cardinal contours decreased their spontaneous firing rates significantly more than units preferring oblique contours ($n = 76$) but there was no significant difference between their evoked firing rates (Fig 8B). A more detailed analysis revealed, that HN ($n = 47$) as opposed to VN ($n = 40$) decreased their spiking activity both during grating stimulation and spontaneous periods (Figure 8C). Thus, on the single cell level horizontally tuned units are more affected than vertically tuned units as they drop both evoked and spontaneous activity. A likely explanation is that the visual context imposed by the grating stimulus stabilizes the response of the vertically tuned units more than it does for the horizontally tuned units (see discussion and Peiker et al., 2013).

Discussion

Upon deactivation of the lateral connection from contralateral primary visual cortex (VIC), well-modulated spontaneous maps continue to be generated with the same probability as in the intact network. However, a cardinal bias observed in baseline, i.e. a higher frequency of spontaneous frames being correlated with vertical or horizontal orientation preference maps (Kenet et al., 2003), disappears.

In accordance, decrease of spontaneous firing rates is significantly larger for neurons preferring cardinal as opposed to oblique contours. Thereby,

neurons preferring horizontal contours (HN) decrease their rates independently of whether being stimulated or not. This is not the case for neurons preferring vertical contours (VN), which exhibit stable evoked but not resting state rates upon VIC deactivation.

Interhemispheric input does not generate spontaneous modular maps

Although transient removal of VIC input can lead to stimulus-dependent de- and increase of spiking (Payne et al., 1991; Schmidt et al., 2010; Wunderle et al., 2013) evoked VSD maps maintained their layout. In agreement, stimulus-derived maps in ferrets deteriorated only in vigor but not in layout when deactivating VICs (Schmidt et al., 2010). Also, feedback from the motion-sensitive posterior-medial suprasylvian cortex did not instruct orientation but only direction preference maps (Galuske et al., 2002). This indicates that the feed forward loop and the intrahemispheric network provide sufficient structured input to maintain orientation maps close to the visual field's midline representation when callosal (or feedback) inputs are absent.

More surprisingly, patterned spontaneous maps also continue to be generated with the same frequency and tuning. When these ongoing fluctuations were first observed (Kenet et al., 2003) they were attributed to patterned intracortical connections, i.e. patchy long-range lateral axons interconnecting neurons responding to the same feature space, e.g. orientation preference (Gilbert and Wiesel, 1989; Malach et al., 1993; Bosking et al., 1997; Schmidt et al., 1997b; Stettler et al., 2002). Accordingly, ongoing population activity in cortex would be biased toward states that reflect the spatial

configuration of that patterned connections (Kenet et al., 2003; Muir et al., 2011).

We expected to drastically disturb spontaneous maps by manipulating VIC inputs, because these connections are likely to perpetuate the selective intracortical network across the hemispheres (Schmidt et al., 1997b; Rochefort et al., 2009; Schmidt, 2013) and extend much farther into more peripheral parts of cat area 18 than covered by the transition zone only (Segraves and Rosenquist, 1982; Rochefort et al., 2007). Instead, spontaneous fluctuations continued to regularly activate iso-orientation domains. This might have the following reasons.

Firstly, the remaining local intrinsic network is sufficient to generate maps. Models confirm that orientation maps could indeed be generated by local recurrent connections (Somers et al., 1995; Sompolinsky and Shapley, 1997; Ernst et al., 2001; Tao et al., 2006), and intracellular recordings indicate that selectivity arises from the local connectivity context neurons are embedded in (Frégnac et al., 2003). In agreement, the range of functional connectivity – apart from being input-dependent - has been shown to clearly decay with cortical distances larger than 1-1.5 mm (Nauhaus et al., 2009; Chavane et al., 2011).

Secondly, orientation maps could emerge spontaneously within the thalamocortical loop. There is evidence that geniculocortical afferents exhibit orientation selectivity at their target structures in layer IV (Chapman et al., 1991). During early development, orientation maps emerge even after disrupting lateral connectivity in close vicinity (Löwel and Singer, 1990). In agreement, orientation selective responses turned out to be quite robust when

cooling cortex in the immediate vicinity of the recorded neuron (Girardin and Martin, 2009).

Thirdly, intrahemispheric feedback could contribute to spontaneous activity because it has considerable impact on stimulus-driven activity in area 18 (Wang et al., 2000; Galuske et al., 2002). However, anatomical studies indicate that feedback connections exhibit a lesser degree of orientation selectivity (Stettler et al., 2002) than intracortical axons and deactivation studies did not reveal pronounced specificity.

Taken together it is likely that not only long-range connections contribute to the generation of spontaneous patterns but that other circuits embedding primary visually cortically neurons into the visual stream, in particular, local and intracortical connections are also involved.

Lateral connections account for a cardinal bias in spontaneous activity

A cardinal bias in cortical structures goes along with improved psychophysical performance for cardinal contours in humans (Appelle, 1972; Orban et al., 1984) and monkeys (Boltz et al., 1979; Krebs et al., 2000), known as the oblique effect. In agreement, cells responding to cardinal orientations respond more vigorously (cats: Pettigrew et al., 1968; Frégnac and Imbert, 1978; Orban et al., 1981; Leventhal and Schall, 1983; monkeys: Mansfield and Ronner, 1978; Blakemore et al., 1981) and are greater in number (Li et al., 2003).

Further, cardinal contours produce larger cortical representations in imaging studies (Chapman and Bonhoeffer, 1998; Coppola et al., 1998; Dragoi et al.,

2001; Wang et al., 2003; Yacoub et al., 2008) and ongoing fluctuations appear to be more often correlated with cardinal than with oblique maps (Kenet et al., 2003). Our data largely confirm the latter observation. It is noteworthy that a human imaging study describes the representational bias in early visual areas to change with the stimulus' contrast (Maloney and Clifford, 2015) pointing towards an adaptive circuit feeding it.

Interestingly, in the present study, the cardinal bias was eliminated from spontaneous but not evoked activity when deactivating VICs. This is in agreement with previous observations that spontaneous or responses to low contrast are more sensible to the influence of lateral connections than responses saturated by high contrast stimulation via feedforward input (Nauhaus et al., 2009). Cooling activation of VIC removes a large part of selective lateral connections and also changes contrast sensitivity of many neurons (Wunderle et al., 2015). It is likely, that the high contrast stimulation sufficiently drove a rest cardinal bias in the remaining feed forward and intracortical connections.

We assume that a true redistribution of probabilities took place rather than improvement or impoverishment of different maps as tuning indices of single frames did not change significantly. If the interrupted network produces spontaneous cardinal maps less often the selective loss of spikes in these domains is explained as spiking is intrinsically linked to the underlying fluctuations (Arieli et al., 1995; Tsodyks et al., 1999).

Our finding thus indicates that synapses conveyed by long-range lateral connections – at least those passing through the corpus callosum - are more numerous or stronger between neurons preferring cardinal contours.

Anisotropy between networks linking neurons preferring horizontal, vertical or oblique contours

A previous study (Peiker et al., 2013) pointed towards an anisotropy of selective networks interconnecting HN and VN in the vicinity of the 17/18 border (for review see Schmidt, 2016). VN reacted less to VIC removal when driven by a stimulus moving into the cooled hemifield, i.e. expecting movement processed by the intact hemisphere. This anticipatory drive would be delivered by a VN interconnecting circuit supposedly elongated parallel to the 17/18 border.

Here, we additionally observe, that during VIC removal evoked rates of VN on the area 18 side of the 17/18 border decrease not only less than those of HN but also of obliquely preferring neurons. A likely explanation is that the elongated circuit processing oblique contours, naturally crosses the midline, as does the one processing horizontal contours. However, both VN and HN lose much more spontaneous spikes than obliquely preferring neurons and the high likelihood to encounter cardinal but not oblique maps in spontaneous activity disappears. It is conceivable that we are looking on two superimposed probabilities. First, there might be a local anisotropy among connections between different populations of orientation-selective neurons within this particular part of the visual field representation, caused by the split of the visual field at its midline by the corpus callosum. This anisotropy appears only under visual stimulation because we disrupt the network at this localization unilaterally and present a midline-crossing stimulus. Second, there exists a general bias towards long-range connections between neurons preferring cardinal as

opposed to more oblique contours in the overall network, which is not necessarily restricted to the border. We eliminate that bias from otherwise unimpaired spontaneous maps because we disrupt the long-range network and herewith, spontaneous longer-range waves of activity passing through which instruct the cardinal bias in spontaneous activity.

According to a recent modeling study the patchy intrinsic network would provide “a physical encoding for statistical properties of the modality represented in an area of cortex” (Muir et al., 2011). Indeed, statistical properties of natural scenes as viewed by cats exhibit a bias for cardinal with prevalence for horizontal contours (Betsch et al., 2004). A bias for cardinal orientations is also found in human natural scenes (Nasr and Tootell, 2012).

Thus, the cortical cardinal bias may be an adaptive result of natural scene properties being implemented into the network of long-range lateral connections. Adaptation can have occurred already during evolution and/or is reinforced by postnatal development when the system is exposed to visual experience (Berkes et al. 2011). The latter hypothesis is based on evidence that long-range intrinsic connections develop in an experience-dependent manner (Löwel and Singer, 1992; Singer, 1995; Schmidt et al., 1997b).

We conclude that inter- and intrahemispheric long-range connections serve as a grid preparing primary visual cortex for likely junctions in a larger visual environment encompassing the two hemifields.

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Figure captions

Figure. 1. A: Experimental setup with cooling loop on the right (R) and recording chamber on the left (L) hemisphere. B: Vector sum of eight correlation coefficients between one evoked single frame and eight average reference maps. Light gray arrows, correlation coefficient vectors; black arrow, resultant vector.

Figure. 2. A: *Evoked and spontaneous VSD maps*. Single frames acquired before (upper row), during (middle row), and after (lower row) reversible cooling deactivation of VIC from the topographically corresponding contralateral transition zone and central area 18 of one example session. Black and white crosses mark corresponding areas on the cortex in all images. VSD response after first frame correction and subtraction of cocktail-blank. Signals normalized to the whole trial average pixel value (F_{av}). All images underwent the same clipping. Note that although overall contrast decreases both evoked and spontaneous map layouts are widely preserved during VIC deactivation. CC indicates correlation coefficient values the single frame and the single condition map for that recording session (either baseline, cooling or recovery). B, left: Surface image of blood vessels from the recorded area. Middle: random control frame. Right: Difference image obtained by intrinsic signal imaging with two different spatial frequencies (0.1.cyc/deg, 16 deg/sec and 0.5 cyc/deg, 4 deg/sec) in order to identify the 17/18 border, dark region, area 18, lighter modulated region, area 17. White continuous line, analyzed region-of-interest (ROI); white dotted line, approximate 17/18 border from difference imaging.

Figure. 3. *Evoked and spontaneous VSD map layout and selectivity are preserved when VIC are absent*. A: Distributions of correlation coefficients between reference maps (averaged over trials) and single evoked (red) and spontaneous (blue) frames are very similar during all three states, baseline, cooling and recovery, and both include much higher correlation values than those obtained with control frames (black). B: Distributions of tuning indices. Note that curves are very similar for all states and for both evoked and

spontaneous frames, but different from tuning indices obtained with control frames (black). These control tuning indices are useful as a reference of what can be considered a high value. For subsequent analysis we concentrated on frames with a tuning index over 90% of the control distribution (indicated by the dotted lines). C: Average response to the stimulus after first frame correction. F is the raw signal, F_0 is the first frame, F_{CB} is the cocktail-blank. The signal is normalized to the whole trial average pixel value (F_{av}) D: Average correlation coefficient values a single condition map and its averaged reference map. In both C and D dotted lines mark stimuli on- and offset.

Figure. 4. *VIC deactivation removes the cardinal bias from spontaneous but not evoked VSD maps.* Frequency of evoked (A) and spontaneous (B) frames (y-axis) significantly correlated with average maps of a certain preference angle (x-axis) in a representative dataset. Calculation of the preference angle is based on the sum vector of the eight single correlation coefficients (see Figure 1B). This procedure delivers a continuous distribution of angles. C: Median modulation index (MI) for the probability of that sum vector to fall in one of the three angle compartments for all 12 datasets. Dispersions are median absolute deviations. Frames associated with both horizontal (hor) and vertical (vert) preference angles dominate in both evoked (A) and spontaneous (B) maps. Note that this cardinal bias decreases in the pool of spontaneous but not evoked frames at the expense of frames assigned to oblique preference as indicated by MI decrease for the cardinal groups (horizontal: $157,5^\circ < x \leq 22,5^\circ$, or vertical: $67,5^\circ < x \leq 112,5^\circ$) rather than oblique ($112,5^\circ < x \leq 157,5$ and $22,5^\circ < x \leq 67,5^\circ$) and MI increase for the oblique group (Wilcoxon signed-rank, * p

=0.0137, 0.002 and 0.0039 for horizontal, oblique and vertical spontaneous modulation, respectively, $n = 12$ data sets). D: Spontaneous frame sequence in 10 trials of the dataset in B color-coded according to the angle of the average evoked orientation preference map with the highest correlation. All frames are counted, both above and below tuning index threshold. During cooling deactivation phases of yellow greenish and blue (cardinal orientations) get shorter at the expense of green and red (oblique orientations).

Figure. 5. *VSD tuning remains unchanged by VIC deactivation.* Average tuning index (y-axis) of single frames at identified preference angles for one dataset during evoked (A) and spontaneous (B) recordings. C: Median modulation index (MI) for the tuning magnitude at identified angles for 12 datasets. Dispersions are median absolute deviations. Note the stability of VSD tuning indices during VIC deactivation.

Figure. 6. Self-organizing frames, i.e. nodes trained by a Kohonen algorithm, confirm a cardinal bias, which is dependent on intact VIC for spontaneous activity. Self-organizing frames were trained either by evoked (A) or spontaneous (B) frames as input (see Material and Methods). Same dataset as in Figure 4. Y-axis: Number of trained frames (total of 100 trained frames) at identified preference angles (X-axis) obtained from the vector summation of the correlation values as described in Figure 1B. Note the gap between baseline and cooling curves for oblique orientations in B and how a similar gap is absent in A. C: Median MI for the number of trained frames at identified angles for 12

datasets used as input for network training. Dispersions are the median absolute deviations. Distributions marked by * are different from zero by the Wilcoxon signed rank test ($p = 0.0039$ and 0.0059 for horizontal and oblique spontaneous modulations respectively). Modulation for vertical spontaneous frames (+) was close to significance ($p = 0.0547$) and to a lesser degree so was modulation for evoked oblique frames ($p = 0.0645$)

Figure. 7. Peristimulus time histograms of 20 evoked trials and 70 spontaneous high-level spontaneous activity windows (see methods) for three different single-units during baseline (black curves) and cooling (gray curves). Polar plots depict the preference direction of motion. A: Multi-unit preferring vertically oriented contours, in contrast to spontaneous, the evoked activity was only slightly modulated by deactivation of VIC. B: Obliquely preferring multi-unit, differences between evoked and spontaneous activity changes are not obvious. C: Horizontally preferring multi-unit, pronounced rate decrease for both evoked and spontaneous activity when VIC is deactivated. Y-axis was scaled to the maximum.

Figure. 8. Firing rate changes during VIC deactivation depend on orientation preference. Averaged single-unit activity ($n = 163$) in the transition zone and central area 18 for 8 differently oriented gratings stimuli and three spontaneous states. A, B and C are based on the same data, but presented with different degrees of detail. A: Modulation indices for spontaneous activity were significantly lower than those for evoked activity (Mann-Whitney-U, $n = 469$

spontaneous and 1280 evoked responses, $p = 0.0104$). B: Spontaneous but not evoked preferred spike rates of neurons preferring horizontal or vertical contours ($n = 251$ spontaneous and 682 evoked responses of HN and VN) decreased significantly more than those of neurons preferring oblique contours ($n = 218$ spontaneous and 598 evoked responses) as indicated by more negative MIs (Mann-Whitney-U, $p = 0.0304$). C: When separating responses according to the neurons' orientation preference, it can be observed that horizontally preferring neurons decrease both evoked and spontaneous activity. In contrast, vertically preferring neurons decrease predominantly their spontaneous rates (Mann-Whitney-U, $p = 0.0002$ between 111 spontaneous and 314 evoked responses).

Highlights

- Generation of spontaneous modular visual maps does not depend on intact callosum
- Cardinal bias in spontaneous maps depends on intact horizontal connectivity
- Spontaneous spiking is more susceptible for cardinally than obliquely driven neurons