

# Influence of rhythmic light stimulation on orientation signal within visual cortex columns in the cat

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The present study used optical imaging to investigate the development of the optical signal within orientational columns in primary visual cortex of cats reared under conditions of rhythmic light stimulation. Results showed that, although inter-columnar spacing was unchanged, a 3-5-fold decrement in optical signal from orientation columns and a drastic decline in contrast sensitivity was observed in both areas 18 and 17. These data suggest the modification of cortical columnar functioning under artificially correlated synchronization of retinal input.

Key words: primary visual cortex, orientation modules, rhythmic light stimulation, postnatal development, cat

A characteristic feature of the primary visual cortex of some mammalian species is the tangential packing of neurons with similar functional and biochemical properties. Neurons form groups that are in accordance with properties such as ocular dominance, processing of stimuli orientation, preferences to movement direction, and level of activity of the cytochrome oxidase enzyme. Such functional domains are referred to as ‘cortical columns’ or ‘modules’ (Hubel and Wiesel, 1962; Hübener et al., 1997). The development of columnar systems during ontogenesis and their function remains a matter of debate. Numerous studies that are historically related to the works of Wiesel and Hubel (1963) show that the formation of the functional structure in the visual cortex depends on the individual’s visual experience during the critical period of early postnatal development. For cats, the critical period for the formation of the normal visual function begins during the 3<sup>rd</sup> week of postnatal development and lasts until the 18–24<sup>th</sup> week (Daw 2006). The final columnar structure and function of the visual cortex itself may strongly depend on the visual experience (Sengpiel et al., 1999).

Several authors have suggested that the development of the cortical columns is sensitive to a tempo-

rary pattern of excitation of retinal input (Stryker and Strickland, 1984; Katz and Crowley, 2002). The nature of the stimulation of the retina can be experimentally modified via electrical stimulation and rhythmic light stimulation (RLS). This stimulation can lead to an artificially correlated activity of axons of ganglion cells at the output from the eye. To-date, there is no consensus on whether such stimulation contributes to the separation (i.e., segregation) of the columns or, on the contrary, prevents it. Indeed, both effects have been reported in previous studies (Stryker and Strickland, 1984; Weliky and Katz, 1997; Schmidt et al., 2008; Merkulyeva et al., 2015). Data showing the effect of RLS on specific characteristics of the population of neurons in functional columns of the visual cortex remains scarce. Optical imaging is a reliable method for visualizing and evaluating activity of cortical columns in real time (Grinvald et al., 1999; Bondar et al., 2011). Based on intrinsic signals, functional optical mapping allows for detection of an increase in deoxyhemoglobin level after appropriate sensory stimulation, due to an increase in oxygen consumption by the activated cortical tissue (Grinvald et al., 1999). Modifications of this method make it possible to quickly obtain functional

maps for different stimulation conditions and groups of experimental animals (Kalatsky and Stryker, 2003). The present study examined, using optical imaging, the development of the system of cortical columns in animals reared under conditions of prolonged RLS.

Experiments were carried out with 14 normal pigmented kittens of both sex (ages 3 - 4 months). All procedures were conducted in accordance with a protocol approved by the Animal Care Committee of the Pavlov Institute of Physiology and followed the European Community Council Directive (2010/63EU) and guidelines of the National Institute of Health Guide for the Care and Use of Laboratory Animals. All animals were reared in a 12 dark/light cycle. Seven kittens were reared from the moment of eye opening under conditions of binocular RLS performed daily for 12 hours ('RLS'). Parameters of RLS stimulations were 15 Hz flash frequency, 40 ms light duration, and 15 cd/m<sup>2</sup> brightness. Seven animals were used as age-matched controls ('Control'). Optical recordings were made from visual areas 18 and 17 in anesthetized and paralyzed animals. All rearing, surgical procedures, and the optical imaging protocol, have been detailed in previous work (Merkul'yeva et al., 2015; Shumikhina et al., 2018).

Briefly, in experiments with intrinsic optical imaging, animals were monocularly stimulated by continuously drifting and rotating (6°/s) square-wave grating (Kalatsky and Stryker, 2003). Contrast was 50, 12.5, 6.25, and 1.56% using the display (mean luminance 40 cd/m<sup>2</sup>) with a refresh rate of 70 Hz placed 57 cm from the cat's eyes. Contrast was calculated with the following formula:  $[(B_{max}-B_{min})/(B_{max}+B_{min})] \times 100\%$ , wherein  $B_{max}$ =brightness of the light stripes of the grating, and  $B_{min}$ =brightness of the dark stripes of the grating. Spatial frequency of the grating was assessed as the number of pairs of "light stripe/dark stripe" (i.e., cycles) per angular degree (cycles/deg). Varying the width of these stripes allowed us to vary the spatial frequency of the grating. We used gratings with a spatial frequency of 0.2 and 0.8 cycles/deg, that allowed us to separate areas 17 and 18 (compare images in Fig.1; Issa et al., 2000) in the subsequent analysis of functional maps (Fig.1B, 1C). Obtaining raw data for a single functional map usually lasted 15 minutes, which corresponded to 15 repetitions of identical stimulation conditions. The baseline activity level of the cortex was assessed in maps obtained with the monitor turned off (background maps, BG). The intrinsic signal from the primary visual cortex was recorded using a DALSA 1M30 CCD camera (DALSA, Waterloo, Canada) controlled by custom-made software (VKImaging, USA). Images were focused on the photosensitive matrix of the camera with Pentax and Canon lenses (magnification x1). The optical signal was recorded from a cortical area 11 mm x 11 mm in size.

To obtain a surface vascular pattern (Fig. 1A) and functional maps, a halogen lamp and fiber optic illuminator was used along with green (546 nm) or red (610 nm) interference filters. Initially, the image of the cortical surface was recorded on a 1024 x 1024 pixel matrix, and then sample rate was reduced spatially (averaging 2 x 2 pixels, final size 512 x 512) and temporarily (averaging of 4 frames; 7.5 Hz) for the data stored on the computer disk. Fourier analysis was used to extract changes in cortical reflectance synchronous with the frequency of visual stimulation (Kalatsky and Stryker, 2003). This approach makes it possible to obtain high resolution functional maps that are free of physiological artifact, such as respiration and heart beat. Given the lack of *a priori* information regarding specific orientation preference, two types of optical maps were created from the single stimulation: (1) a phase map and (2) an amplitude map. The magnitude of the optical response is expressed as a fractional change in the light reflection  $\times 10^4$  (Cang et al., 2005). The functional signal from the orientation columns was analyzed using custom made scripts in Matlab 7.0.4 (MathWorks, USA). A threshold value (mean-SD) was then established. This threshold value allowed for the identification of the singularity areas (SING) and the loci of the orientation (ORI) sensitivity on the functional maps (Fig. 1D-1F). The data are presented as mean  $\pm$  SD. Statistical significance was determined using the Mann-Whitney (M-W) test, with group as a between-subjects factor and cortical region as a within-subjects factor.

For each kitten, 2 - 6 maps of orientation functional maps were analyzed separately for areas 18 and 17. The spatial distribution of the optical signal obtained for the maximum contrast stimuli is shown in Fig. 2 as three-dimensional profiles. Broad light loci with a high level of optical orientation signal marks the iso-orientation columns (ORI loci). These columns alternate with dark circular-oval loci, wherein the signal is insensitive to the orientation of the stimulus (singular points, SING loci). The pairs of ORI and SING loci are regularly distributed over the surface of the cortex. The orientational maps obtained in this study are similar to those observed in earlier experiments on cats (Grinvald et al., 1999). In areas 18 and 17, the optical maps of RLS animals resemble the optical maps of control cats (Fig. 2B, 2F), but with a lower amplitude (Fig. 2D, 2H).

To determine the dependence of the amplitude of the optical signal on contrast, we compared the average ORI signals for different stimulation conditions (Fig. 1G, 1H). For all cats, the optical signal in area 17 was weaker than in area 18. In particular, the differences were 1.6-2 times weaker for contrast values of 100% and 50%, and 3-3.6 times weaker for contrasts of 12.5% and 6.25. We did not find differences in the magnitude

of contrast reduction between control and RLS-cats. With a contrast of 1.56%, the optical maps in area 17 are

largely the same as background maps (BG). Thus, the lowest contrast used to estimate the signal in area 17 is

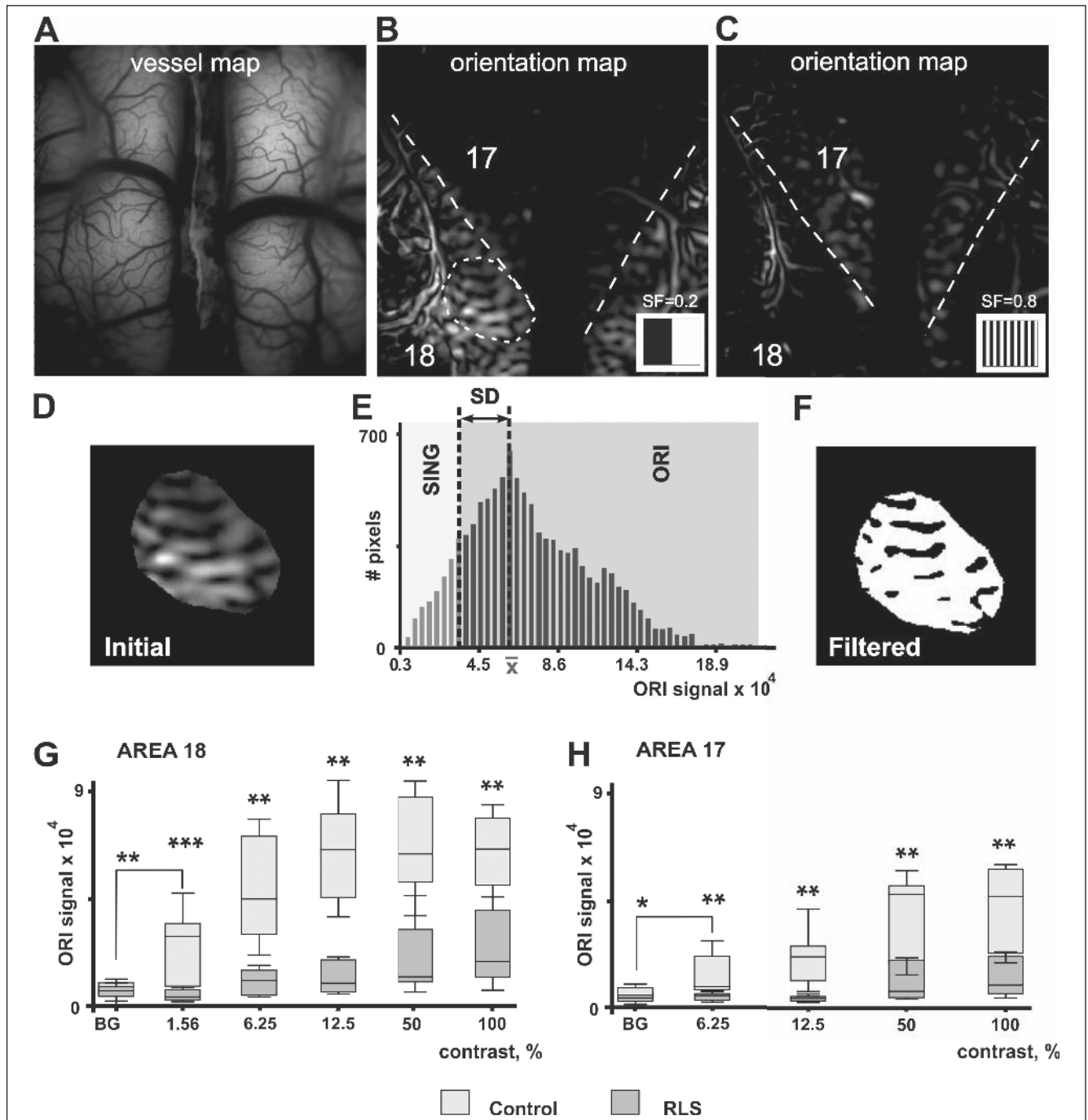


Fig. 1. Assessment and analysis of the optical orientation maps in visual areas 18 and 17 of cat. (A) vessel map. (B, C) a method for revealing a border between areas 18 and 17. (B) optical orientation map for grating with a lower spatial frequency (i.e., 0.2 cycles/deg), (C) optical orientation map for grating with a higher spatial frequency (i.e., 0.8 cycles/deg). (D-F) a method for assessing the average orientation signal from optical orientation maps. (D) manually outlined region of interest (ROI) within the optical orientation map. (E) a histogram of distribution of pixels with different optical orientation signals. ORI and SING – pixels belong to the selectivity and singularity loci.  $\bar{x}$  – a mean value of the orientation signal. SD – standard deviation. (F) ROI after filtration. ORI and SING are marked with white and black pixels, respectively. (G, H) an optical orientation for the gratings with different contrast, in visual area 18 (G) and area 17 (H) of control kittens (dark gray), and kittens reared in rhythmic light stimulation (RLS, light gray). BG – background signal. \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ .

6.25%. Different dependencies of the optical signal on contrast in, for example, orientational functional maps in areas 18 and 17, can be related to the singularities of the afferent entrances to these regions. Thus, the entry

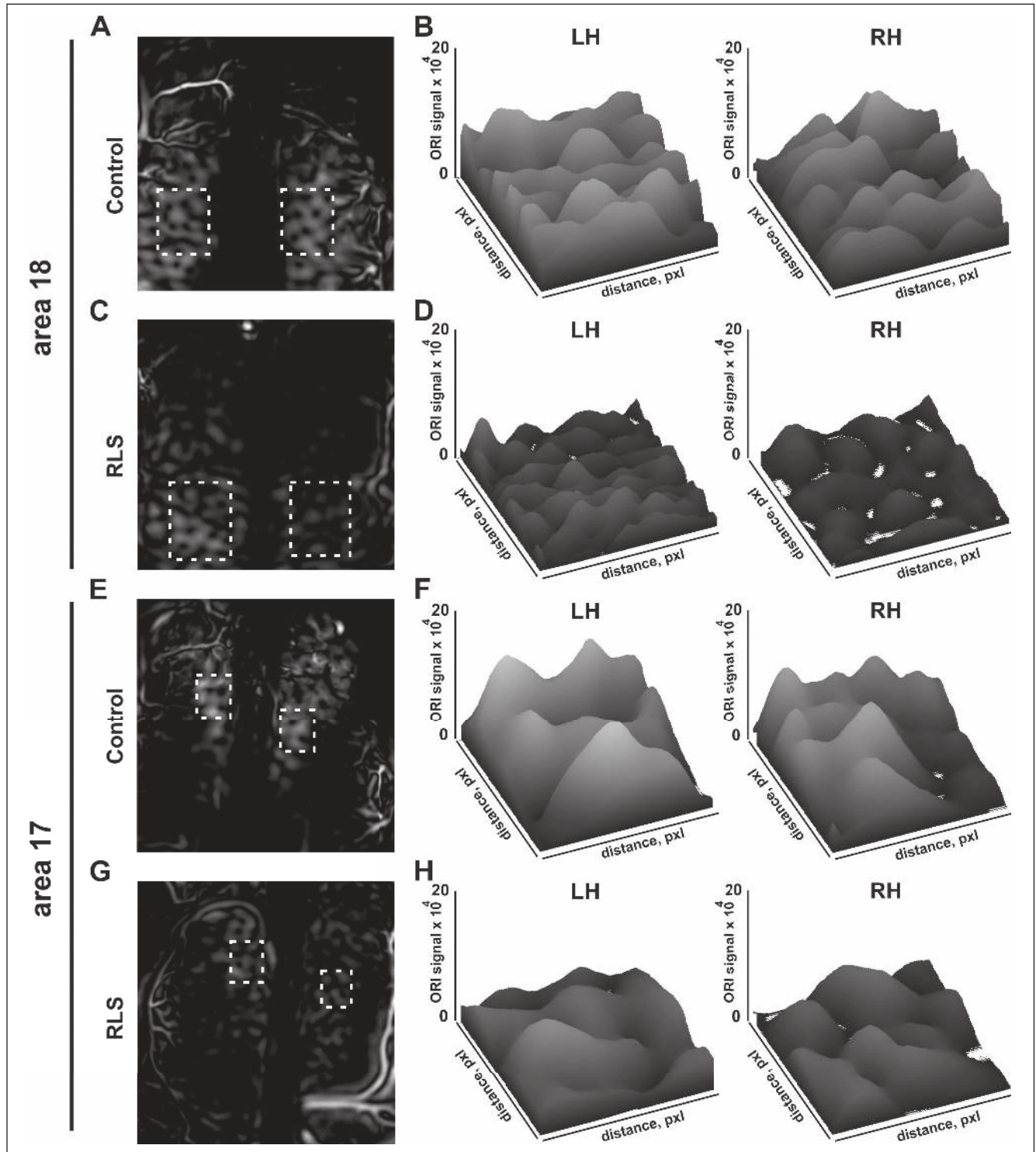


Fig. 2. Optical orientation signal in visual area 18 (A-D) and area 17 (E-H) of control kittens (A-B; E-F), and kittens reared in rhythmic light stimulation (RLS) (C-D; G-H). Stimuli of contrast 1.0 and spatial frequency 0.2 cycles/deg or 0.8 cycles/deg were used to evoke response in areas 18 and 17, respectively. (A, C, E, G) optical orientation maps; (B, D, F, H) profiles of the orientation signal within the regions of interest (ROIs) outlined in A, C, E, G by dashed lines. LH - left hemisphere, RH - right hemisphere.

of Y cells with a high contrast sensitivity predominates in region 18, whereas the inputs from lower contrast X cells dominate in region 17 (Ferster, 1990; Murphy et al., 2000).

In both visual areas of the RLS cats, a decrease in signal was observed for all contrast values (Table I). In these animals, the optical signal in response to the presentation of stimuli with low contrasts (i.e., 6.25% and 1.56% in area 18, and 12.5% and 6.25% in area 17) was so weak that, in most cases, it was impossible to identify any columnar organization. In that case, the functional maps resembled background maps (BG). In contrast, the optical maps in control cats frequently showed the presence of orientation columns, even at the lowest contrast, and significantly exceeded the background level (in area 18:  $2.5 \pm 0.6$  vs.  $0.76 \pm 0.09$ ,  $p=0.0082$ , in area 17:  $1.4 \pm 0.35$  vs.  $0.65 \pm 0.08$ ,  $p=0.035$ ). It is important to note that, in areas 17 and 18 of cats in the RLS group, we observed a linear dependence of the optical signal on the contrast level. This is in contrast to the control animals, which demonstrated a plateau on the graphical dependence at contrast levels of 12.5% – 100% for area 18, and 50% – 100% for area 17. Previous research has shown that it is possible to use intrinsic optical mapping to investigate visual functions, such as contrast sensitivity (Bondar et al., 2011). Data obtained in present study demonstrate a reduced ability of the cortex to respond to images of different contrast in cats reared during RLS.

To analyze the basic spatial pattern of cortical orientation columns on optical maps (Fig. 3A), an in-

ter-column scatter was measured. The scatter was calculated as the distance between adjacent singularity loci. In area 18, the average inter-columnar distance in the control group was  $585 \pm 42.6 \mu\text{m}$ , and in the RLS kittens,  $588 \pm 50.8 \mu\text{m}$ . In area 17, the average inter-columnar distance in the control group was  $546 \pm 37.7 \mu\text{m}$ , and in RLS kittens,  $576 \pm 42.1 \mu\text{m}$  (Fig. 3B). No difference in inter-columnar distance was observed between areas 17 and 18, in either the control (M-W=29;  $p=0.076$ ) or RLS groups (M-W=27;  $p=0.799$ ). Additionally, no significant difference in inter-columnar distance was observed between the control and experimental groups (area 18: M-W=81,  $p=0.507$ ; area 17: M-W=7,  $p=0.106$ ). A similar stability in the spatial structure of cortical columns was previously observed in studies of monocular deprivation (Schmidt et al., 2002), dark rearing (Luhmann et al., 1990), and artificially induced strabismus (Kaschube et al., 2003). Together, these results indicate the independence of the global columnar architecture over the visual experience.

The present experimental data indicate that prolonged exposure to RLS during a critical period of development affects the functioning of orientation columns. In particular, we observed a decrease in optical signal recorded from the primary visual cortex in response to stimuli of variable contrast. A disturbance in the functional structure of the orientation columns in visual cortex of ferrets was previously demonstrated using repetitive electrical stimulation of retinal ganglion cells axons (Weliky and Katz, 1997). Ferrets have partially separate ON and OFF channels that target sep-

Table I. Median values for orientational optical response in the primary visual cortex (i.e., areas 18 and 17) of cats reared in control conditions (n=7) and in rhythmical light stimulation (RLS, n=6-7). Significance of the data was assessed by Mann-Whitney (M-W) test.

| contrast | Control | RLS    | n1; n2 | Statistical data |        |
|----------|---------|--------|--------|------------------|--------|
|          |         |        |        | M-W              | p      |
| Area 18  |         |        |        |                  |        |
| 100%     | 6.762   | 1.891  | 7; 7   | 4                | 0.0037 |
| 50%      | 6.545   | 1.244  | 7; 7   | 1                | 0.0012 |
| 12.5%    | 6.701   | 0.9665 | 7; 7   | 1                | 0.0013 |
| 6.25%    | 4.551   | 1.091  | 7; 7   | 0                | 0.0003 |
| 1.56%    | 3.016   | 0.4135 | 7; 7   | 2                | 0.0047 |
| Area 17  |         |        |        |                  |        |
| 100%     | 4.739   | 0.626  | 7; 7   | 4                | 0.007  |
| 50%      | 4.858   | 0.692  | 7; 7   | 4                | 0.007  |
| 12.5%    | 2.214   | 0.393  | 7; 6   | 1                | 0.0012 |
| 6.25%    | 0.9005  | 0.322  | 7; 6   | 0                | 0.0012 |

arate ON and OFF sublayers within the lateral geniculate nucleus (LGN) (Stryker and Zahs, 1983). Previous investigators have proposed that disruption of the orientation columns in ferrets is a result of coactivation of both ON and OFF ganglion cells during stimulation (Weliky and Katz, 1997). Recently, we observed a similar stratification of the LGN A-laminae in cats rather than ferrets (Merkulyeva et al., 2018). This stratification may also relate to the ON and OFF subsystems.

Another possible explanation for the observed decrease in optical signal could be a disturbance in the development of X and Y cell systems that target the visual cortex. Indeed, pathways organized by Y cells are more strongly affected by visual deprivation than X cell pathways (Sherman and Spear, 1982; Michalski and Wróbel, 1994; Burnat et al., 2012). We have previously found that interconnections between the primary visual cortex and one of the main cortical targets of the

Y channel, the posterior-medial lateral suprasylvian area (PMLS), are altered in cats reared under 15 Hz RLS (Merkulyeva et al., 2012). Thus, based on these data, it is possible that Y channels are indeed altered after RLS.

It also should be mentioned that rhythmical neuronal activity plays an important role in sensory processing and in the development of neuronal circuits (Singer, 1999; Wróbel et al., 2007). A previous study by Chen et al. (2015) recorded neuronal activity in the developing primary visual cortex of awake mice and reported that, “from the pre-critical period to critical period, the peak frequency of spontaneous fast oscillatory activities shifts from the beta band (15–35 Hz) to the gamma band (40–70 Hz)”. The authors link these data with experience-dependent maturation of the visual cortex. Given these data, we can expect an alteration in the normal development of the mature neuronal rhythmical activity in our cats exposed to a 15 Hz flickering environment.

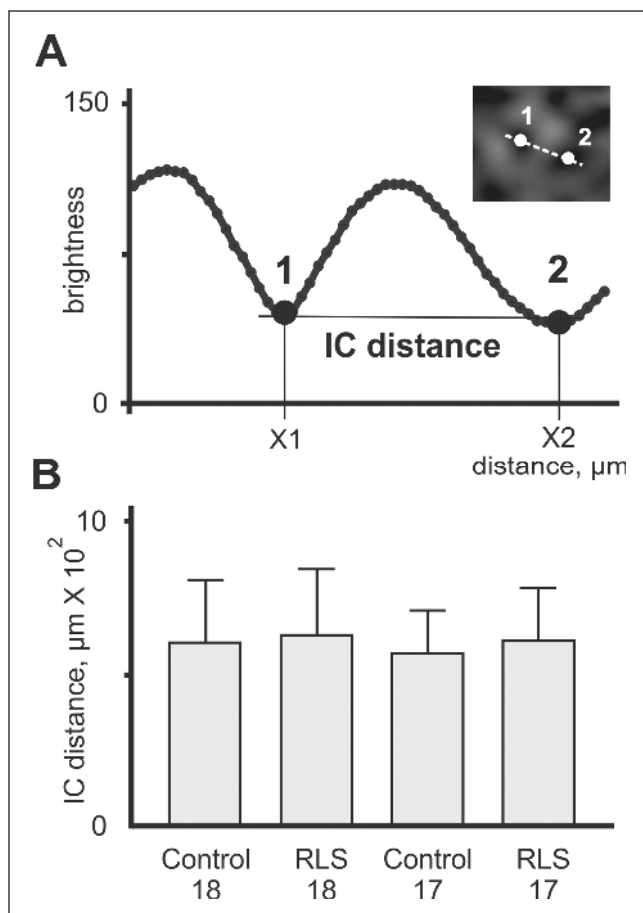


Fig. 3. Inter-columnar spacing for optical orientation maps in visual areas 18 and 17. (A) – a method for assessing inter-columnar spacing. A 2-D profile of the brightness along the white dashed line passes through the pairs of singularity loci 1-2 (in insert). X1 and X2 – values of the distance, in  $\mu\text{m}$ . (B) inter-columnar spacing for optical orientation maps of control kittens and kittens reared in rhythmic light stimulation (RLS). Mean  $\pm$  SD.

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