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Development of Meynert Cells in the Cat Visual Cortex in Conditions of Stimulation with Flashing Light

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The effects of rhythmic light stimulation on the postnatal development of the visual system were studied in relation to the formation of Meynert neurons in field 17 and the posteromedial area of the lateral suprasylvian sulcus (PMLS) in kittens reared with exposure to flashing light (frequency 15 Hz). Neuron body cross-sectional area and cytochrome oxidase (CO) activity levels were measured in the visual cortex of control (n = 6) and stimulated (n = 6) animals. Increases in the level of CO activity were seen in Meynert cells in field 17 and the PMLS area, by about 37%. There was also a decrease in the cross-sectional area of the bodies of Meynert neurons located in the PMLS, by 20% compared with normal. The existence of functional impairments of the Y conducting channel in stimulated animals and the possibility that binocular vision is suppressed are discussed.

Keywords: visual cortex, Meynert cells, rhythmic light stimulation.

Neuronal plasticity is a key concept in the physiology of vision. For more than 50 years, studies of its mechanisms have led particularly to formation of the concept of a "critical period" – a stage in early postnatal ontogeny characterized by a maximal level of sensitivity to changes in the visual environment and, hence, maximal plasticity [5]. Among methods for experimental modification of the visual environment during development, stimulation with flashing lights (rhythmic light stimulation, RLS) has received little study. Our investigations of the effects of prolonged stimulation of young cats with flashing lights at a frequency of 15 Hz demonstrated impairments to the corticocortical connection systems between field 17 and the posteromedial area of the lateral suprasylvian sulcus (PMLS) [2]. Thus, the aim of the present work was to undertake a detailed study of the cellular composition of the primary visual cortex and the PMLS after stimulation, with particular focus on analysis of Meynert cells – separated large pyramidal neurons in layer V, which organize the descending connections of the visual cortex in the Y-conducting channel, which is one of the basic visual conducting channels responsible for processing information on movements and the spatial relationships between objects [7, 9, 10].

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Materials and Methods

Experiments were performed on 12 normally pigmented three-month-old cats from five litters in compliance with the requirements of the Directives of the European Parliament for the protection of animals used for experiments and other scientific purposes (2010/63EU) and the use of animals for experimental studies. Six animals formed the control group and six were reared for three months (from the day of eye opening) in the presence of RLS at a frequency of 15 Hz in a 12:12 regime (12 h stimulation, 12 h complete darkness). Sources of flashing light were two light-emitting diode panels (35 LEDs per panel) positioned on opposite walls flashing at a frequency of 15 Hz (flash duration 40 msec, panel brightness 40 cd/m^2). At age three months, the animals were subjected to general anesthesia (i.m. Zoletil, 1 ml/kg) and transcardiac perfusion with 0.9% NaCl solution and 4% paraformaldehyde. After perfusion, brains

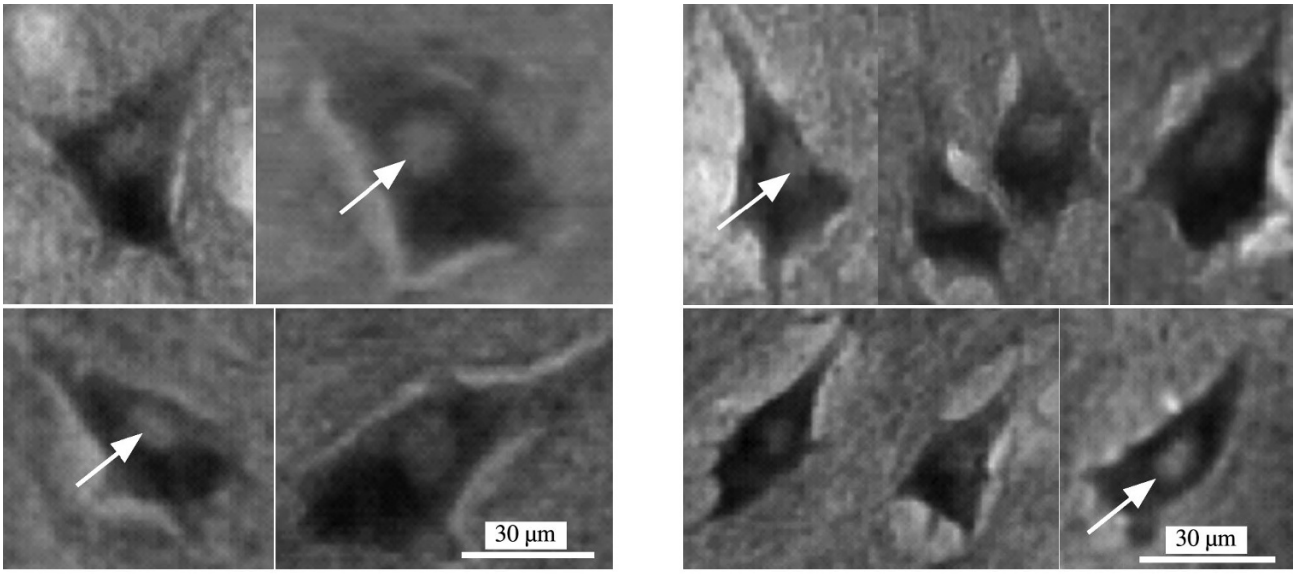

Fig. 1. Meynert cells in the posteromedial area of the lateral suprasylvian sulcus in control animals (*a*) and animals reared in conditions of rhythmic light stimulation (*b*). White arrows show light nuclei. Histochemical reaction for cytochrome oxidase.

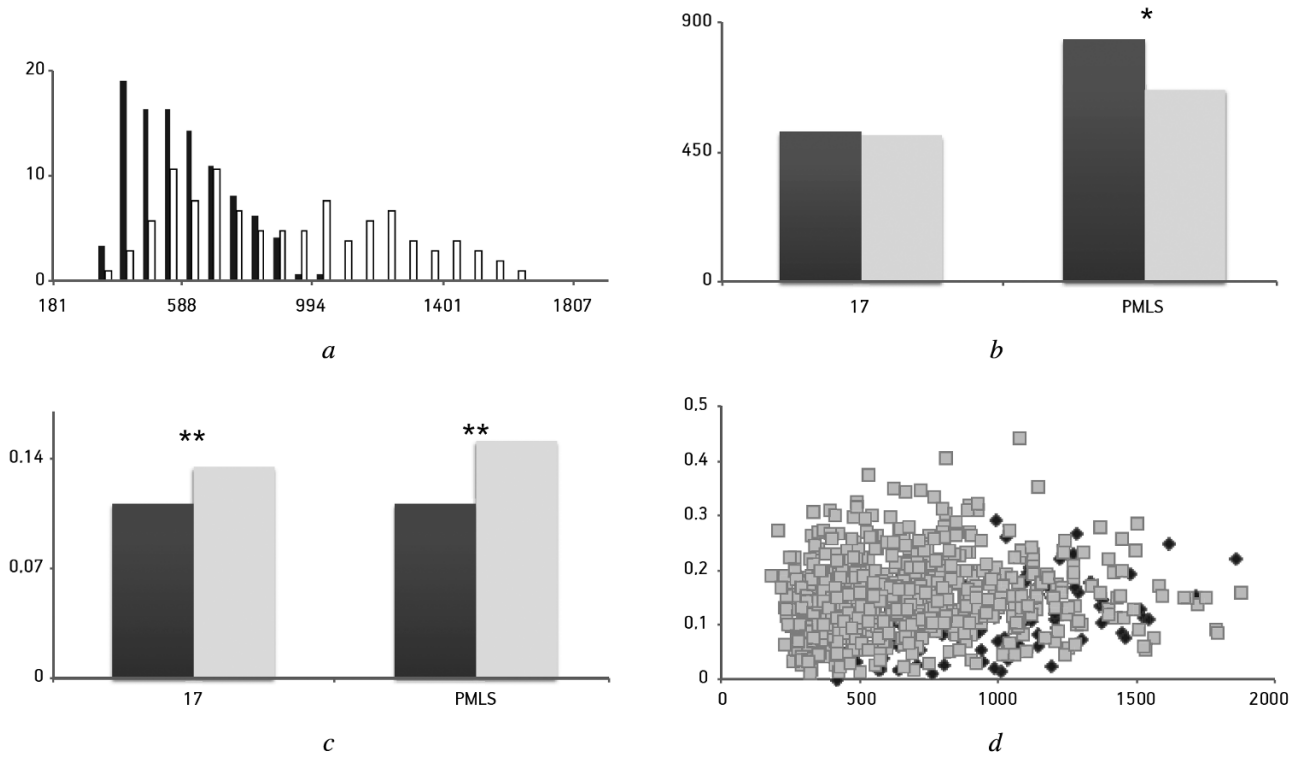

Fig. 2. Parameters of Meynert cells in field 17 and the posteromedial area of the lateral suprasylvian sulcus (PMLS) in control kittens and animals reared in conditions of rhythmic light stimulation. *a*) Histogram showing the distribution of cells by body cross-sectional area in field 17 (dark columns) and the PMLS area (light columns) in control animals. The abscissa shows body cross-sectional area (μm^2); the ordinate shows proportions of neurons with different cross-sectional areas (%); *b*, *c*) cross-sectional areas of bodies (*b*) and relative optical densities of neurons (*c*) in control kittens (dark columns) and with rhythmic light stimulation (light columns). The abscissas show brain areas; the ordinates show the values measured: *b*) μm^2; *c*) U. Significance levels: *99.0%, **99.9%; *d*) plot of relationship between neuron body cross-sectional area and relative optical density in the PMLS area in control animals (dark symbols) and with rhythmic light stimulation. The abscissa shows neuron body cross-sectional area (μm^2); the ordinate shows relative optical density (U).

were extracted and immersed in 30% sucrose (1–2 days), which was followed by preparation of series of frontal sections of thickness 50 μm. Meynert cells were visualized using a histochemical method to detect cytochrome oxidase (CO) activity, as CO contents correlate with levels of functional activity in the neuropil and individual neurons [12, 13]. Morphometric analysis of neurons in layer V was performed using a computer fitted with a Jenaval light microscope (Carl Zeiss, Germany), a Baumer Optronix camera (Baumer, GmbH, Germany), and VideoTest Master 4.0 software (VideoTest, Russia). Cross-sectional areas of neuron bodies were measured, along with the ratios of the optical density (OD_{rel}) of Meynert cells (OD_{cell}) to the optical density of the surrounding neuropil, taken as background (OD_b) calculated using the Michaelson contrast formula [(OD_{rel} = = ($OD_{cell} - OD_b$)/($OD_{cell} + OD_b$)]. Experiments addressed Meynert cells located in 40 frontal brain sections in control animals and 50 sections from stimulated kittens at Horsley–Clarke levels A4.0–A5.0. The usual size of Meynert neuron bodies is greater than 20 × 20 μm [3, 6], and the neurons studied here were consistent with this. A total of 770 neurons from the RLS group were analyzed, along with 250 from the control group. Significant differences between non-normal distributions were identified using the χ^2 test. Experiments were conducted using equipment at the Resources Center for the Development of Molecular and Cellular Technologies, St. Petersburg University.

Results

Frontal sections of field 17 and the PMLS area in animals of both groups showed dark pyramidal neurons with light nuclei, separated or in groups of 2–3 cells; levels of CO activity in the cytoplasm of these cells were much higher than in the surrounding neuropil (Fig. 1, *a*, *b*). The distances between neurons varied over a wide range: from 10 to 2000 μm. On average, these values in the control and stimulation groups were not different, at 470 ± 87 and 392 ± 3 μm.

In normal conditions, the cross-sectional area of Meynert neurons in field 17 was significantly less than that in the PMLS (520 ± 25 and 840 ± 60 μm^2, respectively, $\chi^2 = 63.55$, $p < 0.001$). This difference was determined by the wider spread in values in the PMLS where, in contrast to field 17, about 33% of neurons had cross-sectional areas of greater than 1000 μm^2 (Fig. 2, *a*). In stimulated animals, there were no differences in neuron body cross-sectional area in field 17; in the PMLS, the cross-sectional area of Meynert cells showed some decrease: from 840 ± 60 to 666 ± 20 μm^2 ($\chi^2 = 27.8$, $p = 0.05$) (see Fig. 2, *b*). It should be noted that the PMLS region in both control and stimulated animals contained particularly large neurons with cross-sectional areas exceeding 1500 μm^2, accounting for 2.9 and 2.4% of all Meynert neurons, respectively.

We used OD_{rel} as a measure of the functional activity of Meynert neurons, as this reflects the ratio between levels of CO activity in neurons and the surrounding neuropil. In control animals, we found no significant differences between OD_{rel} for field 17 and PMLS region cells – OD_{rel} values were identical and the mean in both cases was 0.10 ± 0.003. Stimulated animals showed increases in OD_{rel} in both visual areas (to 0.140 ± 0.005 in field 17 and to 0.150 ± 0.005 in the PMLS area, $p < 0.001$, see Fig. 2, *c*).

Differences between the Meynert cell populations in the PMLS region in control and stimulated animals were most apparent on comparison of the relationships be the cross-sectional areas of these neurons and their OD_{rel} values (see Fig. 2, *d*). In normal conditions, this was a single cloud of values showing a linear relationship between the parameters (correlation coefficient = 0.4, $n = 250$, $p < 0.0001$). In stimulated kittens, the relationship between the cross-sectional area of neuron bodies and their optical density remained highly significant (correlation coefficient = 0.13, $n = 770$, $p < 0.001$), though the plot of the relationship had two clouds of values: one coinciding with the cloud in normal conditions and one corresponding to a group of neurons with bodies of smaller cross-sectional area and higher levels of OD_{rel} (see Fig. 2, *d*)

Discussion

The main result of the present study was the finding of a bimodal distribution of body size and CO activity in Meynert cells in stimulated kittens. One of the clouds of values coincided with the cloud in normal conditions, while the other, absent in normal conditions, contained neurons with higher CO levels and smaller sizes. It is difficult to provide an exhaustive explanation for the occurrence of this second distribution. On the one hand, Winfield et al. noted that the visual cortex in kittens aged 2–3 weeks differed from the cortex in older kittens in that layer V contained many small and density distributed cells, hindering discrimination of Meynert cells [11]. If it is difficult for Meynert neurons to develop in stimulation conditions, it may be that the second population of these cells results from persistence of these small, immature neurons.

On the other hand, a significant increase in the functional activity of the neuropil in the neuron ensemble system has been demonstrated in the primary visual cortex, with high levels of CO activity (CO blobs) in cats stimulated with flashing lights [1], as well as narrowing of these blobs, which, in the light of data showing that blobs are located in the centers of eye dominance columns [8], suggests that increases in CO activity occur in segments of eye dominance columns containing exclusively monocular neurons. Studies in cats and primates have demonstrated that the axon terminals of Meynert cells reach neighboring eye dominance columns receiving nerve fibers from both the right and left eyes, suggesting that they have a role in binocular interactions in the deep layers of the visual cortex [4, 6]. Thus, the underdevelopment of Meynert cells may reflect impairment to binocular vision in stimulated kittens.

Both CO blobs and Meynert cells belong to the same visual conducting channel – the so-called Y system (the magnosystem in primates). It has been suggested that the main

functions of this system involve processing information relating to the movement of visual objects and analysis of the relative positions of stimuli [7, 10]. The development of the elements of this system, as previously observed [2] and seen here to be modified, can be regarded as one of the causes of changes in the pattern of corticocortical connections between field 17 and the PMLS area in stimulated animals.

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