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# Density of SMI-32 immunopositive neurons in eye-specific layers of lateral geniculate nucleus in kittens reared with monocular deprivation and unilateral convergent squint. †

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**Abstract:** To reveal the dynamics of the development of morphological changes in lateral geniculate nucleus caused by binocular vision impairment we study the changes in density of of SMI-32 immunopositive neurons in frontal sections of LGNd of both hemispheres of 2- and 3- month kittens reared with monocular deprivation or unilateral convergent squint. We had developed the custom software to divide the binocular part of A-layers into 10 consecutive sectors and to calculate the number of SMI-32 immunopositive neurons in each of them. The neuronal density was calculated and compared between groups in sectors with the same eccentricity. In monocularly deprived animals, decline of the neuronal density relative to the control group was found in layers, innervated from the deprived eye in both age groups, regardless of eccentricity. However in strabismic kittens the decrease in neuronal density was revealed only in the peripheral sectors of layer A1, driven by deviated eye. The width of this area of reduced Y-neuron density was larger in 3 months kittens, indicating that the development of the disorder has not yet stabilized. The results may be interpreted as morpho-physiological correlates of different types of human amblyopia.

**Keywords:** lateral geniculate nucleus; neurofilaments; monocular deprivation; squint; cat

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## 1. Introduction

The non-phosphorylated heavy neurofilament proteins, which can be labelled by SMI-32 antibodies, are characteristic for large, fast-conducting neurons with high-myelinated axones [1-3]. In the visual system, such properties are typical for Y-neurons – a population, devoted to motion analysis [4-6]. Early physiological experiments have shown that the number of Y-neurons and their functional properties can be changed by alteration of the visual experience, as monocular deprivation or squint [7-9]. The loss of SMI-32 positive neurons in A-layers of in lateral geniculate nucleus (LGNd), connected with a deprived/squinting eye, confirmed the morphological base of these changes [10-13]. However, whether this loss depends on eccentricity of the visual field projection is still not known. To study this, as well as the time course of the developmental changes, we have evaluated the density of SMI-32 immunopositive neurons in LGNd on experimental models of_monocular deprivation and unilateral convergent squint. Density measurements were carried out on kittens of 60 days - the age of onset of binocular vision [14-15], and 90 days, when the critical period for visual capacities ends [16] and the ratio of excitatory and inhibitory synapses in the LGN already corresponds to the level of adults [17].

## 2. Materials and Methods

The experimental procedures were approved by the Ethics Commission of the Pavlov Institute of Physiology, St. Petersburg, Russia (Protocol #30/03/2014), and performed in accordance with the requirements of European Community Council Directive (2010/63EU) on the protection of animals used in experimental and other scientific purposes.

The study was carried out on kittens which underwent a surgery at postnatal day 7-10 (before the natural eye opening). The kittens of MD groups were monocularly deprived by eyelid sutures. In the kittens of Strab groups the lateral rectus muscle and the upper and lower lateral leaflets of the eye retractor were removed to receive unilateral convergent squint. Then they were grown up until 60 or 90 days. Intact kittens of the same age groups were used as controls. The number of animals in each group is presented in Table 1.

**Table 1.** The number of animals in experimental groups.

| Group name | Age, days | Number of animals |
|---|---|---|
| Norm-60 | 60 | 4 |
| Norm-90 | 90 | 4 |
| MD-60 | 60 | 4 |
| MD-90 | 90 | 2 |
| Strab-60 | 60 | 4 |
| Strab-90 | 90 | 3 |

At the end of this period the animals were deeply anesthetized (Zoletil, 20 mg/kg and xylazine, 2 mg/kg, intramuscularly) and transcardially perfused with 4% paraformaldehyde. The frozen 50 μm thick frontal sections of LGNd of both hemispheres were prepared and ones corresponding to AP 5.5-7.0 according to Horsley-Clark coordinates were then selected for histological processing and analysis. The SMI-32 primary antibodies were used to detect the heavy neurofilament protein with DAB-Ni as a chromogen.

After the images of LGNd sections were acquired, the upper and lower boundaries of layers A and A1 and the location of SMI-32 immunopositive neurons within these boundaries were marked manually (Fig 1A). To reveal changes in the density of immunopositive neurons in layers A along the projection of visual horizontal meridian, these layers were divided into 10 consecutive sectors (Fig 1B). This was done using custom software which allowed to divide the curves of upper boundary of layer A and the lower boundary of layer A1 into segments of equal length.

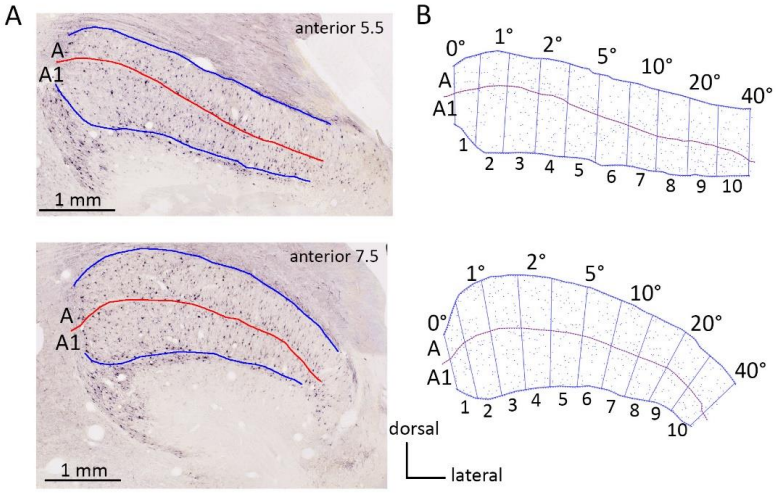


**Figure 1.** The method for image of LGN section. A – drawing of the layers A and A1 borders and marking the positions of SMI-32 immunopositive neurons. B – dividing the LGN layers onto 10 sectors.

LGNd layers A and A1 are innervated from different eyes (contralateral and ipsilateral, correspondingly). The differences between these layers in the density of immunopositive neurons (the number of neurons per $mm^2$) were calculated in kittens of all groups in all consecutive LGNd sectors using the formula of Michelson contrast:

$$D(i)=(N_A(i)-N_{A1}(i))/(N_A(i)+N_{A1}(i)), \quad (1)$$

where $N_A$ - the density of neurons in layer A, $N_{A1}$ - the density of neurons in layer A1, i = 1..10 - the sector number.

To assess the strength of the deprivation effect in hemispheres contralateral and ipsilateral to the deprived eye we compared D-values between normal and MD group as

$$Diff(i)_{MD} = |D(i)_{MD}-D(i)_{norm}| \quad (2)$$

where i = 1..10 - the sector number.

The significance of differences between groups of parameter values was determined at the $p < 0.01$ level using the method of hierarchical linear models [18]. The significance of differences in distributions was determined at the level of $p < 0.01$ using the Kolmogorov-Smirnov test. Statistical data processing was carried out in the Matlab R2016b computing platform (Matworks Inc., USA). All data are presented as mean ± standard deviation.

**3. Results and discussion**

In intact animals of both age groups (Norm-60, Norm-90) the normalized density of SMI-32 immunopositive neurons in the layer A1 exceeded that in the A layer in all sectors of the right and left hemispheres. Therefore, the relative differences in density (D) between layers A and A1, on average, had negative values. The distributions of mean D values did not differ across sectors for the left and right hemispheres (Kolmogorov-Smirnov test, $p > 0.01$); thus, data from the right and left hemispheres of all animals of the same age has been combined and used for comparisons with other groups.

The results of comparison of the D values for all groups are presented in Fig .2

In the MD-60 group, the D values significantly differed from the D values in the Norm-60 group, in all 10 sectors in both hemispheres ($p < 0.01$). In the MD-90 group, D values did not differ from the MD-60 group ($p > 0.01$). Note, that D values were positive in the hemisphere ipsilateral to the deprived eye, and negative in the hemisphere contralateral deprived eye. This indicates a decrease in the density of SMI-32 immunopositive neurons in the layers innervated from the deprived eye, in both hemispheres (in layer A of the hemisphere, contralateral to the deprived eye and layer A1 of the hemisphere ipsilateral to the deprived eye).

The strength of the deprivation effect in the hemispheres was different in the kittens of MD-60 and MD-90 groups. At the age 60 (MD-60) the strength of the deprivation effect, $Diff_{MD,}$ was higher in the LGN ipsilateral to deprived eye, where nasal hemifield of deprived eye is presented in the A1 layer. Hemispheric differences in this group of animals were found in 7/10 LGN sectors (namely, 2, 4, 5, 7-10). However, at the age 90 (MD-90) such difference was revealed in only one sector (number 2). It means the increase of deprivation effect in the LGN contralateral to deprived eye, where temporal hemifield of deprived eye is presented in the A layer. These data also indicates a delay of deprivation effect development in the temporal visual hemifield in relation to the nasal hemifield. This phenomenon was described earlier for cortical and geniculate neurons [8,19] and probably connected with later development of ipsilateral projection pathways compared to contralateral ones [20,21].

In kittens with unilateral squint, D values did not differ from the normal D values in both age groups and in all sectors in the hemisphere, contralateral to the deviated eye. But in the hemisphere ipsilateral to the deviated eye, the D values were reduced in the peripheral part of the visual field projection. Moreover, such a statistically significant decrease

relative to the Norm-60 group was observed in sectors 8-10 in the Strab-60 group and in sectors 4-10 in the Strab-90 group. Hence this decrease of cell density that can be physiological correlates of strabismic amblyopia takes place in the area that receives inputs from the temporal retina and represents projection of the nasal visual hemifield. This effect develops from periphery to center and may display not only the difference between the developmental time courses of ipsilateral and contralateral visual pathways [20,21] and later maturation of visual periphery [22], but also narrowing of the visual field of the deviated eye due to partial deprivation of temporal retina hidden behind the bridge of the nose, supposed by Ikeda [23].

Received results may be interpreted as morpho-physiological correlates of different types of human amblyopia.

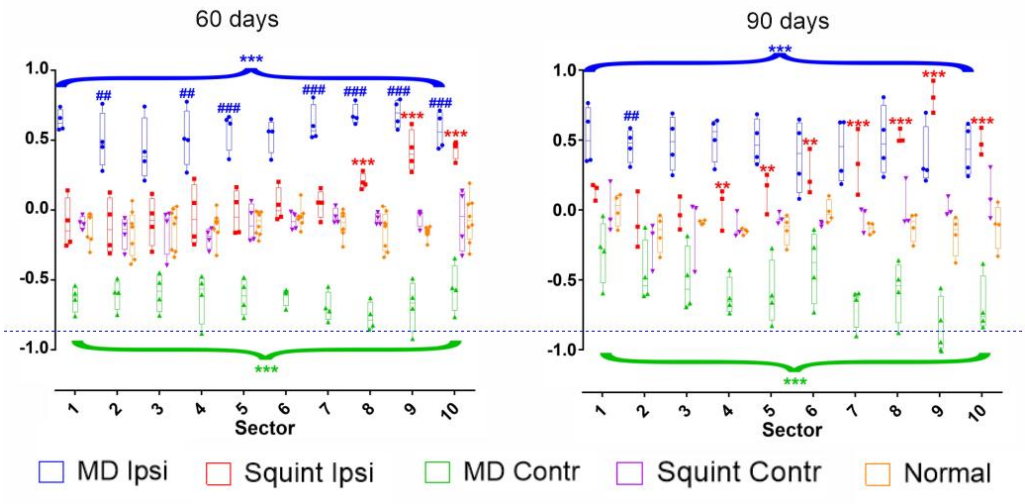


**Figure 2.** D values in kitten groups 60 and 90 days.* - Significance level with respect to Norm groups. # - Significance level with respect to other hemisphere.

## References

1. Lawson SN, Waddell PJ. Soma neurofilament immunoreactivity is related to cell size and fibre conduction velocity in rat primary sensory neurons. J Physiol. 1991;435:41-63. doi:10.1113/jphysiol.1991.sp018497
2. Tsang YM, Chiong F, Kuznetsov D, Kasarskis E, Geula C. Motor neurons are rich in non-phosphorylated neurofilaments: cross-species comparison and alterations in ALS. Brain Res. 2000;861(1):45-58. doi:10.1016/s0006-8993(00)01954-5
3. Kirkcaldie MT, Dickson TC, King CE, Grasby D, Riederer BM, Vickers JC. Neurofilament triplet proteins are restricted to a subset of neurons in the rat neocortex. J Chem Neuroanat. 2002;24(3):163-171. doi:10.1016/s0891-0618(02)00043-1
4. Rowa MH, Stone J. Conduction velocity groupings among axons of cat retinal ganglion cells, and their relationship to retinal topography. Exp Brain Res. 1976;25(4):339-357. doi:10.1007/BF00241726
5. Hsiao CF, Watanabe M, Fukuda Y. The relation between axon diameter and axonal conduction velocity of Y, X and W cells in the cat retina. Brain Res. 1984;309(2):357-361. doi:10.1016/0006-8993(84)90604-8
6. Zeck G, Lambacher A, Fromherz P. Axonal transmission in the retina introduces a small dispersion of relative timing in the ganglion cell population response. PLoS One. 2011;6(6):e20810. doi:10.1371/journal.pone.0020810
7. Sherman SM, Spear PD. Organization of visual pathways in normal and visually deprived cats. *Physiol Rev*. 1982;62(2):738-855. doi:10.1152/physrev.1982.62.2.738
8. Mower GD, Burchfiel JL, Duffy FH. Animal models of strabismic amblyopia: physiological studies of visual cortex and the lateral geniculate nucleus. *Brain Res*. 1982;281(3):311-327. doi:10.1016/0165-3806(82)90130-4
9. Mangel SC, Wilson JR, Sherman SM. Development of neuronal response properties in the cat dorsal lateral geniculate nucleus during monocular deprivation. *J Neurophysiol*. 1983;50(1):240-264. doi:10.1152/jn.1983.50.1.240
10. Bickford ME, Guido W, Godwin DW. Neurofilament proteins in Y-cells of the cat lateral geniculate nucleus: normal expression and alteration with visual deprivation. *J Neurosci*. 1998;18(16):6549-6557. doi:10.1523/JNEUROSCI.18-16-06549.1998
11. Kutcher MR, Duffy KR. Cytoskeleton alteration correlates with gross structural plasticity in the cat lateral geniculate nucleus. *Vis Neurosci*. 2007;24(6):775-785. doi:10.1017/S095252380707068X
12. Fennell, H.M. Strabismus-Induced Cytoskeletal Change in the Feline Lateral Geniculate Nucleus. Master of Science Thesis, Dalhousie University, Halifax, Canada, 2007

13. O'Leary TP, Kutcher MR, Mitchell DE, Duffy KR. Recovery of neurofilament following early monocular deprivation. *Front Syst Neurosci*. 2012;6:22. Published 2012 Apr 9. doi:10.3389/fnsys.2012.00022
14. Timney B. Development of binocular depth perception in kittens. *Invest Ophthalmol Vis Sci*. 1981;21(3):493-496.
15. Pettigrew JD. The effect of visual experience on the development of stimulus specificity by kitten cortical neurones. *J Physiol*. 1974;237(1):49-74. doi:10.1113/jphysiol.1974.sp010469
16. Hubel DH, Wiesel TN. The period of susceptibility to the physiological effects of unilateral eye closure in kittens. *J Physiol*. 1970;206(2):419-436. doi:10.1113/jphysiol.1970.sp009022
17. Winfield DA, Headon MP, Powell TP. Postnatal development of the synaptic organisation of the lateral geniculate nucleus in the kitten with unilateral eyelid closure. *Nature*. 1976;263(5578):591-594. doi:10.1038/263591a0
18. Aarts E, Verhage M, Veenvliet JV, Dolan CV, van der Sluis S. A solution to dependency: using multilevel analysis to accommodate nested data. *Nat Neurosci*. 2014;17(4):491-496. doi:10.1038/nn.3648
19. Schmidt KE, Stephan M, Singer W, Löwel S. Spatial analysis of ocular dominance patterns in monocularly deprived cats. *Cereb Cortex*. 2002;12(8):783-796. doi:10.1093/cercor/12.8.783
20. Crair MC, Horton JC, Antonini A, Stryker MP. Emergence of ocular dominance columns in cat visual cortex by 2 weeks of age. *J Comp Neurol*. 2001;430(2):235-249. doi:10.1002/1096-9861(20010205)430:2<235::aid-cne1028>3.0.co;2-p
21. Blakemore C, Van Sluyters RC. Innate and environmental factors in the development of the kitten's visual cortex. *J Physiol*. 1975;248(3):663-716. doi:10.1113/jphysiol.1975.sp010995
22. Burnat K. Are visual peripheries forever young? *Neural Plast*. 2015;2015:307929. doi:10.1155/2015/307929
23. Ikeda H, Plant GT, Tremain KE. Nasal field loss in kittens reared with convergent squint: neurophysiological and morphological studies of the lateral geniculate nucleus. *J Physiol*. 1977;270(2):345-366. doi:10.1113/jphysiol.1977.sp011956