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

Shkorbatova P.Y., Lyakhovetskii V.A., Alexeenko S.V.
Pavlov Institute of Physiology RAS, Saint Petersburg

The non-phosphorylated heavy neurofilament proteins, which can be labelled by SMI-32 antibodies, are characteristic for large, fast-conducting neurons.

In the visual system, such properties are typical for Y-neurons – a population, devoted to motion analysis

It is known that in monocularly deprived or squinting animals the Y-neurons population declines in A-layers of lateral geniculate nucleus (LGN), connected with a deprived/squinting eye.

However, whether this loss depends on eccentricity of the visual field projection is still not known.

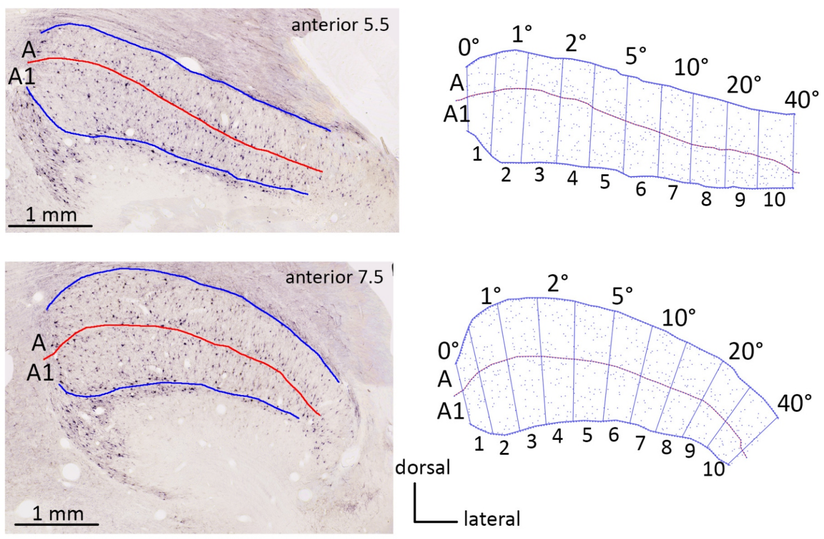
The purpose of our work was to evaluate such changes of Y-neurons density in LGN of experimental models of monocular deprivation and unilateral convergent strabismus.

Materials and Methods

- The study was carried out on kittens which underwent a surgery at postnatal day 7-10 before the natural eye opening (monocular deprivation - by unilateral eyelid sutures, unilateral convergent strabismus - by myotomy of lateral rectus muscle and upper and lower lateral leaflets of retractor muscle).
- After transcardial perfusion fixation the frozen 50 μm thick frontal sections of LGN 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.
- The upper and lower boundaries of layers A and A1 and the location of SMI-32 immunopositive neurons within these boundaries were marked manually on the images of the sections .
- 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. 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.
- 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:

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

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.

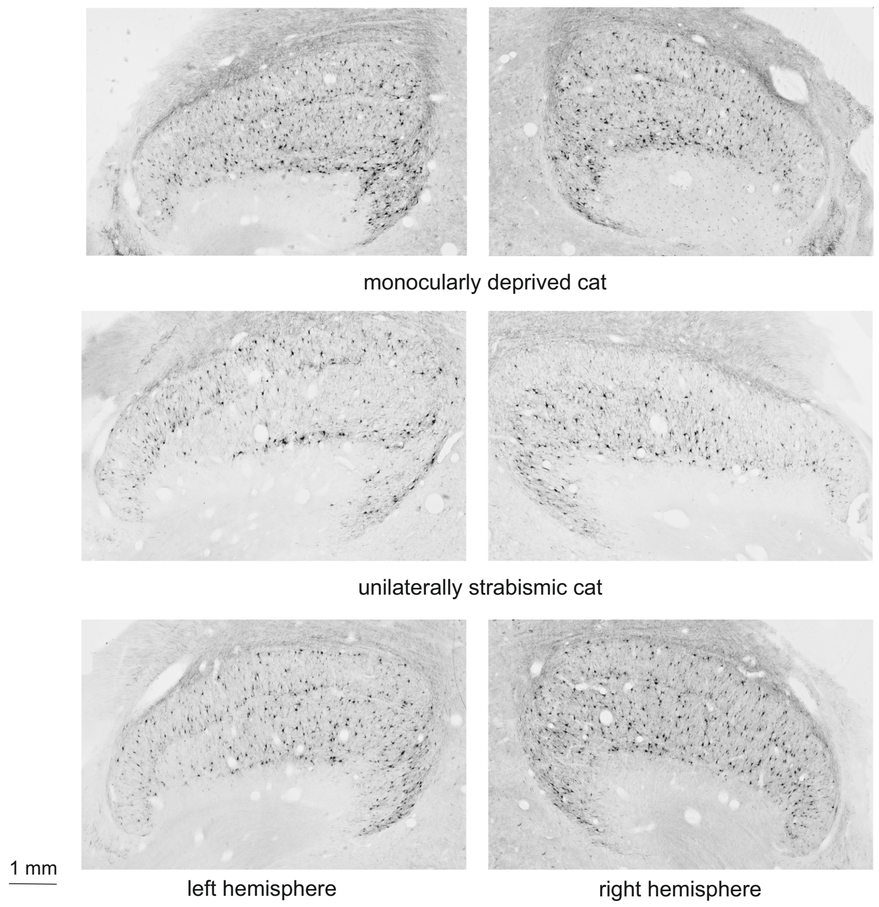


- To assess the strength of the deprivation effect in hemispheres contralateral and ipsilateral to the deprived eye we compared D-values between normal and MD group as

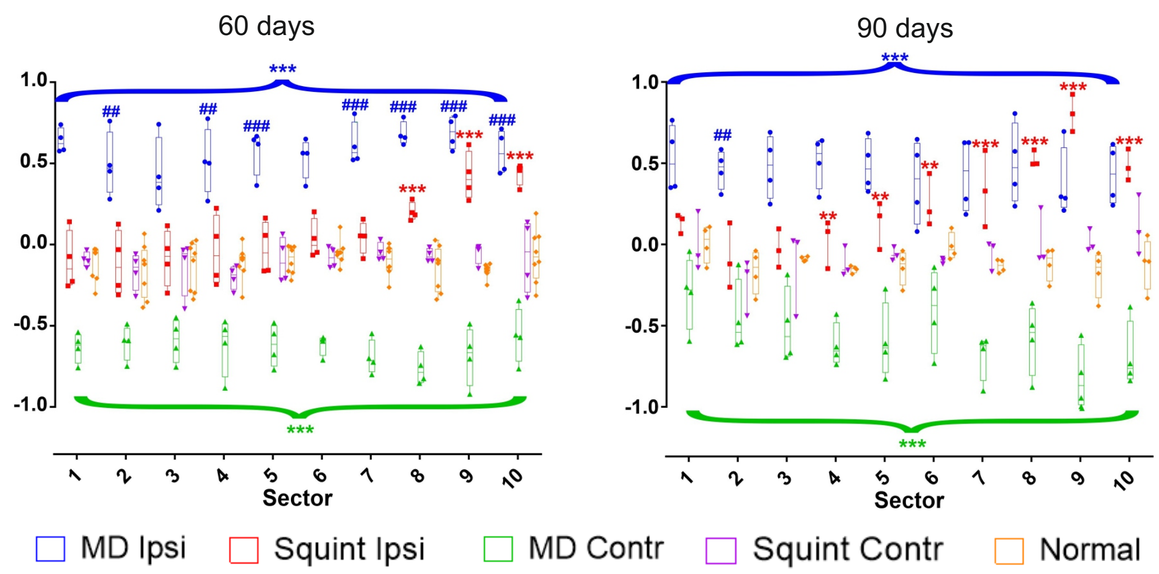
$$Diff(i)_{MD} = |D(i)_{MD}-D(i)_{norm}|$$

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

LGNd frontal sections



D values in kittens of 60 and 90 days



** - Significance level with respect to Norm groups. # - Significance level with respect to other hemisphere.*

- In intact animals of both age groups the relative differences in density (D) between layers A and A1, on average, had negative values because 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
- In the MD-60 and MD-90 groups the D values did not differ from each other and significantly differed from the D values in the Norm-60 group, in all 10 sectors in both hemispheres ($p < 0.01$). 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
- The strength of the deprivation effect, $Diff_{MD}$ in MD-60 group 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 (2, 4, 5, 7-10). However, in MD-60 group such difference was revealed in only one sector (2). This phenomenon is probably connected with later development of ipsilateral projection pathways compared to contralateral ones.
- In kittens with unilateral squint, D values did not differ from the normal D values in both age groups and in any sector 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 takes place in the area that receives inputs from the peripheral temporal retina. 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, but also partial deprivation of temporal retina hidden behind the bridge of the nose.
- Received results may be interpreted as morpho-physiological correlates of different types of amblyopia.