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The daily cycle of glutamate in the retina of OXYS rats during aging and the development of retinopathy

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Motivation and Aim: Glutamate performs many important functions in the retina, such as visual signal transmission and regulation of the circadian rhythm [1]. Glutamate contributes to the synchronization of the activity of many retinal cells and changes in the expression of their genes. Information on daytime and nighttime fluctuations in glutamate is limited and inconsistent. However, the idea is put forward that biochemical changes and circadian rhythms of glutamate may be risk factors for the development of neurodegenerative processes in the retina, in particular, the development of age-related macular degeneration (AMD) [2], which remains the top cause of blindness and irreversible visual impairment in industrialized countries. Nowadays, there is some information about deviations in neurotransmitter systems in the retina in AMD. Nonetheless, there is little information on retinal changes in the glutamatergic systems during aging and even less at different stages of AMD development. It is also not clear whether these changes are a cause or consequence of the development of the neurodegenerative process. Expectedly, the manifestation of clinical signs of diseases is preceded and accompanied by pathological changes at the molecular level, which are difficult to study in humans.
Methods and Algorithms: In this study, we used OXYS and Wistar rats as a control. OXYS rats are a unique model of premature aging whose manifestation is early development of retinopathy similar to AMD. According to our data, even at a young age, the retinopathy that develops in OXYS rats is similar to the dry atrophic AMD type in humans. Male OXYS rats at a preclinical stage (20 days), early stage (3 months), and late stage (18 months) of the pathology and normal controls (age-matched male Wistar rats) were used in this work. To investigete daily cycle of glutamatergic systems in retina the sampling took place during the day (10 a.m.) and at night (2 a.m.). The concentration of unbound glutamate in rat retina samples was measured by colorimetric method. Western blot analysis was used to assess the levels of key enzymes of glutamate metabolism (glutaminase and glutamine synthetase), the ionotropic glutamate receptor (NMDA(R1)) and the glutamate transporter (EAAT1/GLAST). To study the localization of the above proteins, immunohistochemical staining with fluorescent antibodies was used.
Results: In Wistar rats with normal aging, there was a characteristic daily difference of glutamate concentrations in all age: increased in daytime and decreased in nighttime. In OXYS rats, the difference in daily concentration disappeared with age. Glutamate is synthesized *de novo* from glutamine in glial cells (astrocytes and Müller cells) by glutaminase. Glutamine synthetase catalyzes the amidation of glutamate to glutamine. Western blotting revealed that the daytime and nighttime levels of glutaminase did not change with age in the retina of OXYS and Wistar rats. But ANOVA showed that the protein level of glutaminase was higher in OXYS rats than in Wistar rats in both daytime

and nighttime at the all ages. The significant differences were found between in 18-months-old OXYS and Wistar rats at the daytime ($p < 0,05$) and between 20-days-old and 3-month-old OXYS and Wistar rats at the nighttime ($p < 0.05$).

At the daytime the protein level of glutamine synthetase increased by the age of 18 months in Wistar rats but did not change with age in the retina of OXYS. By age 18 months, we detected significant upregulation of the glutamine synthetase protein in OXYS rats as compared with Wistar rats ($p < 0.05$). At the nighttime the protein level of the glutamine synthetase increased from the age of 20 days to 3 months in Wistar rats, while no significant differences were found in OXYS rats. No significant interstrain differences were found in the rat retina in night.

In summary, the level of glutaminase was higher and level of glutamine synthetase was lower in old OXYS rats than in old Wistar rats. These data indicated that the glutamine/glutamate ratio was shifting in favor of glutamate in the retina of OXYS rats during development of retinopathy.

GLAST (glutamate aspartate transporter 1, also is known as EAAT1) removes glutamate from the extracellular space and is needed to maintain low nontoxic extracellular glutamate concentrations. At the daytime, Western blotting uncovered a significant increase in GLAST protein levels with age in the retina of OXYS and Wistar rats ($p < 0.05$). Also it was showed that the level of GLAST was lower in OXYS rats than in Wistar rats. By age 3 and 18 months, we detected a significant decline of the GLAST protein level in OXYS rats as compared with Wistar rats ($p < 0.05$).

At night, the level of GLAST protein increased with age in Wistar rats ($p < 0.05$), but did not change in OXYS rats either. During the period of active disease progression (18 months), the level of the GLAST transporter in OXYS rats was significantly lower than in Wistar rats ($p < 0.05$).

Ionotropic NMDA glutamate receptors are responsible for fast neurotransmission. In this work, we assayed glutamate ionotropic receptor N-methyl-D-aspartate (NMDA) receptor subunit NR1 (NMDAR1). The study of the NMDAR1 protein level in the daytime by Western blot did not show significant differences between ages and lines. At the nighttime we detected increasing NMDAR1 protein level in OXYS rats with age and no age-dependent alterations in Wistar rats. The significant upregulated of NMDAR1 protein level in OXYS as compared Wistar rats was detected at the age of 3 and 18 months ($p < 0.05$).

Conclusion: It has been shown that similar changes occur in the retina of rats during healthy aging and the development of retinopathy. So the level of the glutamate transporter increases with age in the retina of rats. The development of retinopathy in the retina of OXYS rats is accompanied by a smoothing of the daily rhythm of glutamate, an increase in the level of the glutaminase enzyme and a decrease in glutamine synthetase, as well as a decrease in the level of the glutamate transporter. These alterations are leading to disruption of the glutamatergic system in the OXYS rats retina and an increase in degenerative changes.

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