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Analysis of mitochondrial supply of neuronal processes during the postnatal brain maturation in OXYS rats – a model of sporadic Alzheimer's disease

Yukhtanov D.[1, 2]*, Muraleva N.[1], Tyumentsev M.[1]

[1] *Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia*

[2] *Novosibirsk State University, Novosibirsk, Russia*

** d.yukhtanov@g.nsu.ru*

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Motivation and Aim: Age is the most significant risk factor for the sporadic form of Alzheimer's disease (AD, sporadic form accounts for > 95 % of all cases) – the most common form of senile dementia, with one in nine people above 65 and one-third over 85 suffering AD. In recent years, convincing evidence of the validity of the "mitochondrial cascade" hypothesis [1] has been obtained; with said hypothesis positing that age-related dysfunction of mitochondria underlies pathogenesis of the sporadic form of AD. Previously using senescence-accelerated OXYS rats as model of the sporadic form of AD, we have, firstly, confirmed the validity of the mitochondrial hypothesis [2], and, secondly, raised the issue of genetically determined structural and functional changes in mitochondria potentially influencing the process of brain maturation in the early postnatal period and thus determining predisposition to the development of AD. Indeed, recently researchers have turned their attention to the fact that the risk factors of eventual cognitive impairment and accelerated brain aging (a major risk factor of AD development) could be laid down already in the early postnatal period of life, during completion of brain development. Evidently, the basis of successful neuronal differentiation and formation of neural circuits during final stages of brain maturation might be connected to the mitochondrial biogenesis and supply needed to meet brain energy demand. The goal of this study was to compare the parameters of OXYS rats' brain development with the state of neuronal mitochondria – their distribution across cellular compartments in neurons of the hippocampus and brain cortex of OXYS rats, energy metabolism efficiency, mitochondrial dynamics, and traffic.

Methods: We examined prefrontal cortex and CA1 region of the hippocampus (brain regions most vulnerable to AD) of OXYS and Wistar (control) rat strains aged 7, 14 and 20 postnatal days. These ages are associated with the end of migration of neurons (7 days), the peak of synaptogenesis and apoptosis (14 days), and the end of nervous system maturation (20 days). Using electron microscopy, we have assessed such ultrastructural parameters as spatial density of mitochondria in the cortical and hippocampal neuropil and neuronal somata, their distribution to neuronal processes and synaptic terminals, as well as the intensity of mitochondrial dynamics (fission and fusion). Principal proteins governing mitochondrial traffic: TRAK1/2 (motor protein adapters), Miro1/2 (mitochondrial cargo adapters) and syntaphilin (synaptic docking protein) were measured using Western blotting. Functionally we have measured the mitochondrial transmembrane potential (a parameter reflecting mitochondrial ATP-synthesizing capacity), which was accomplished using confocal microscopy of hippocampal acute slices stained with potential-selective dye – this technique owing to the unique hippocampal architecture allows for selective assessment of the transmembrane potential in somata and neuropil.

Results: In the OXYS brain cortex even though during the peak synaptogenesis (P14) there is a tendency towards OXYS axonal terminals having increased mitochondrial supply compared to Wistar, by the end of postnatal brain development (P20), less mitochondria were found to reside in axonal terminals. At the same time, total mitochondrial content averaged across neuropil (mitochondria docked in terminals together with mitochondria in transit in neuronal processes between terminals and soma) was increasing in both strains from P7 to P20 but this increase was more pronounced in OXYS rats. In cortical neuropil we have also detected signs of time-dependent intensification of the activity of mitochondrial dynamics from P14 to P20. Neuropil of the CA1 region of the hippocampus mirrors changes found in the cortex in terms of elevated mitochondrial content in the neuropil. However, unlike in cortex, OXYS hippocampal mitochondria were found to localize more to the dendritic terminals. Additionally, while no interstrain differences in the mitochondrial supply to the axonal terminals were revealed, we have found that following the peak of synaptogenesis in Wistar rats the number of axonal mitochondria decreases but in OXYS rats this number remains unchanged. Interestingly we did not find signs of ATP deficiency in hippocampal neurons of OXYS rats – an assessment of the transmembrane potential of mitochondria of the CA1 field of the hippocampus at the ages P7, P14, and P20 using hippocampal acute slices did not reveal significant interstrain differences. Neither have we found marked changes in the ultrastructural parameters in the somata signifying that in the studied ages mitochondria still retain sufficient levels of ATP-synthesizing capacity and mitochondrial biogenesis. Regarding mitochondrial traffic proteins in the studied period, in the cortex of OXYS rats, only the levels of Miro2 at the P7 and P14 ages was higher than in Wistar, and by the age of P20 the interstrain differences were no longer present due to a sharp decrease in the protein level in OXYS rats. In the hippocampus of OXYS rats only the TRAK2 level increased by the end of brain maturation, but its content did not exceed the levels of the age-matched Wistar rats. Syntaphilin is present in both brain structures at a very low level, with no difference between OXYS and Wistar rats.

Conclusion: Our data indicate that in OXYS rats the period of brain maturation is marked by alterations of mitochondrial distribution to the neuropil of the brain structures most vulnerable to the AD, these alterations primarily concerning mitochondrial supply of synaptic terminals. Optimal mitochondrial biogenesis and distribution corresponding to the brain energy demands is the basis for the successful differentiation and maturation of neurons, synaptogenesis, formation of neural networks and the completion of the formation of the brain as a whole – processes which were shown to be impaired in OXYS rats [3]. Failure to perform this task in a tightly-regulated, time-sensitive manner might interfere with the synaptogenesis. Following that, the decreased ability of neurons to establish proper contacts in due time might ultimately lead to the formation of aberrant neural networks and exacerbation of energy insufficiency, pointing to the improper mitochondrial distribution as an important underlying factor of neurodegeneration.

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