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Topic: AS07 Aging and Neurodegenerative Disorders

## FROM BDNF/ TRKB NEUROTROPHIC PATHWAY TO VESICULAR RELEASE AT AGED NEUROMUSCULAR JUNCTION

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Age-related conditions, such as sarcopenia or neurodegenerative disorders, are a burden for a huge part of the society. However, very little is known about the molecular presynaptic pathways and the neurotrophic bidirectional communication between the nervous system and muscles at the neuromuscular junctions (NMJ), which are fundamental for muscle proper function in aged individuals. Our group has focused on the molecular mechanisms behind its regulation and the key signaling pathways that control neurotransmission, which are crucial for this interaction. The muscle-derived Brain-derived neurotrophic-factor (BDNF) acting through its receptor, Tropomyosin-related-kinase-B (TrkB) is well known for its neuroprotective functions and has been demonstrated that enhances presynaptic downstream effector protein kinase C (PKC) isoforms and exocytotic proteins of synaptic vesicles (SNAP25 and Munc18-1) that lead to acetylcholine release at the NMJ. However, whether this signaling pathway is compromised in the aged neuromuscular system has not been analyzed yet. In addition, some muscles are more prone to fatigue than others, like the fast-twitch Extensor Digitorum Longus (EDL) muscle, and are more susceptible to show aging signs. The present study analyses by Western Blotting the differences in expression and activation of the key proteins above mentioned in young versus aged EDL rat muscles. Results show a change in the stoichiometry of the BDNF and NT-4 neurotrophins along with minor changes in the expression of the TrkB and $p75^{NTR}$ receptors. However, a reduction of PKCs activation, and a relevant decrease of phosphorylated forms of SNARE-SM proteins Munc18-1 and SNAP-25 (S187), points to a specific molecular drop-off of the exocytotic mechanism in aged NMJ. Considering these results, therapeutic strategies to recover this signaling pathway should improve NMJ functionality, slowing down the aging changes of the neuromuscular system and, thus, improving quality of life of aged people.

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## METABOLIC MAPPING OF THE AGING MOUSE BRAIN USING IN SITU ENZYMATIC ACTIVITIES AND TOF-SIMS APPLIED ON CRYOSECTIONS

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The main neurotransmitter glutamate is metabolically linked to the tricarboxylic acid cycle, and further energy provision to the cell, through the mitochondrial enzyme glutamate dehydrogenase (GDH). This maintains brain homeostasis through balanced glucose usage and glutamate recycling. Alterations in these connected pathways have been associated with neurodegenerative disorders favoured by aging. However, the age-related evolution of the central metabolic pathways regarding specific brain regions remains to be established, as well as the pivotal role of GDH in these processes. Here, we developed in situ enzyme-targeted nitroblue tetrazolium (NBT) assays coupled with metabolic profiling for ex vivo assessment of metabolic pathways and metabolite levels. We used wild-type control and brain-specific GDH null Cns-Glud1$^{-/-}$ mice of different ages reflecting the natural senescence: young (4 weeks), early adult (12 weeks), middle-aged (50 weeks), and old (90 weeks) animals. We applied the NBT assay on cryopreserved brain sections to study flagship enzyme activities: glyceraldehyde-3-P dehydrogenase (GAPDH) as a readout of glycolysis; lactate dehydrogenase (LDH) for astrocyte-neuron metabolite exchange, succinate dehydrogenase (SDH) for mitochondrial activity, and GDH for glutamate recycling. On the adjacent cryosections, we performed time-of-flight secondary ion mass spectrometry (ToF-SIMS) imaging to examine metabolites related to glutamate pathways. The NBT assay showed that enzymatic activities are region specific with the most robust activities in the dentate gyrus, inferior colliculus, superior colliculus, and molecular layer of the cerebellum. This enzymatic mapping did not systematically follow the pattern of the corresponding protein levels of the respective enzymes, as determined by immunohistochemistry. GDH was particularly active in astrocyte-rich regions, although absent in Cns-Glud1$^{-/-}$ brains. ToF-SIMS revealed the levels of metabolites corresponding to the metabolic pathways investigated by the NBT assay in the different brain regions. Collectively, the combined data sets allow in situ metabolic mapping of the aging brain with a pivotal role for GDH.

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