Neural and cognitive substrates of omega-3 fatty acid supplementation: a voxel-based morphometry study in aged mice

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Introduction. As major components of neuronal membranes, omega-3 polyunsaturated acids (n-3 PUFA) exhibit a wide range of regulatory functions, modulating synaptic plasticity, neuroinflammation, oxidative stress and neuroprotection[1]. Recent human studies with morphological MRI-based techniques have revealed a putative neuroprotective effect of n-3 PUFA in aging, with a positive correlation between peripheral n-3 PUFA levels, and total and hippocampal gray matter (GM) volume[2]. Consistent with this, higher dietary n-3 PUFA levels have been associated with delayed or reduced age-induced cognitive decline. However, the uncontrolled impact of genetic, environmental and socio-economical status in human populations complicate the interpretation of correlational epidemiological studies[3]. As a result of this, a causal relationship between n-3 PUFA intake, cognitive function and GM morphology has yet to be unambiguously demonstrated. In an attempt to address this question, we have mapped regional GM volume using voxel-based morphometry (VBM) and recorded hippocampal-dependent cognitive performance in aged inbred mice upon 8-week treatment with n-3 PUFA or control fatty acid (olive oil). We show that n-3 PUFA treated-mice exhibit better cognitive performance and greater hippocampal and prefrontal GM volume, an effect that was strongly correlated with total brain n-3 PUFA concentration.

Methods. All experiments were carried out in accordance with the Italian law governing animal welfare and protection. **Animals:** MRI experiments were performed on aged C57Bl/6 (87-week-old). N=10 mice were treated with a mixture of fatty acids containing high levels of n-3 PUFA, including eicosapentaenoic acid (EPA), decosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). A control group (n=10) was treated with olive oil for isocaloric intake control. Before imaging all mice were submitted to a battery of behavioral tests to evaluate cognitive and emotional functions[1]. **Animal preparation:** High-resolution morpho-anatomical T2-weighted MR imaging of mouse brains was performed in paraformaldehyde fixed specimens[4]. **MRI acquisition:** All experiments were performed using a 7.0 Tesla MRI scanner using a FLASH 3D sequence with TR=17ms, TE=10ms, α=30°, 260x160x180 matrix with a 1.83x1.26x1.26 cm FOV. **VBM data analysis:** Intergroup differences in local GM volume were mapped using VBM[5]. Briefly, a study-based template was created aligning high-resolution T2W images to a common reference space using affine and diffeomorphic registrations. Individual images of the two groups were then nonlinearly registered to the study-based template using diffeomorphic registration. GM of spatially normalized subjects was then segmented, modulated and smoothed. Voxelwise statistics were performed using permutation tests and maps were thresholded at p=0.01. After imaging, the brain content of n3-PUFA was quantified using HPLC.

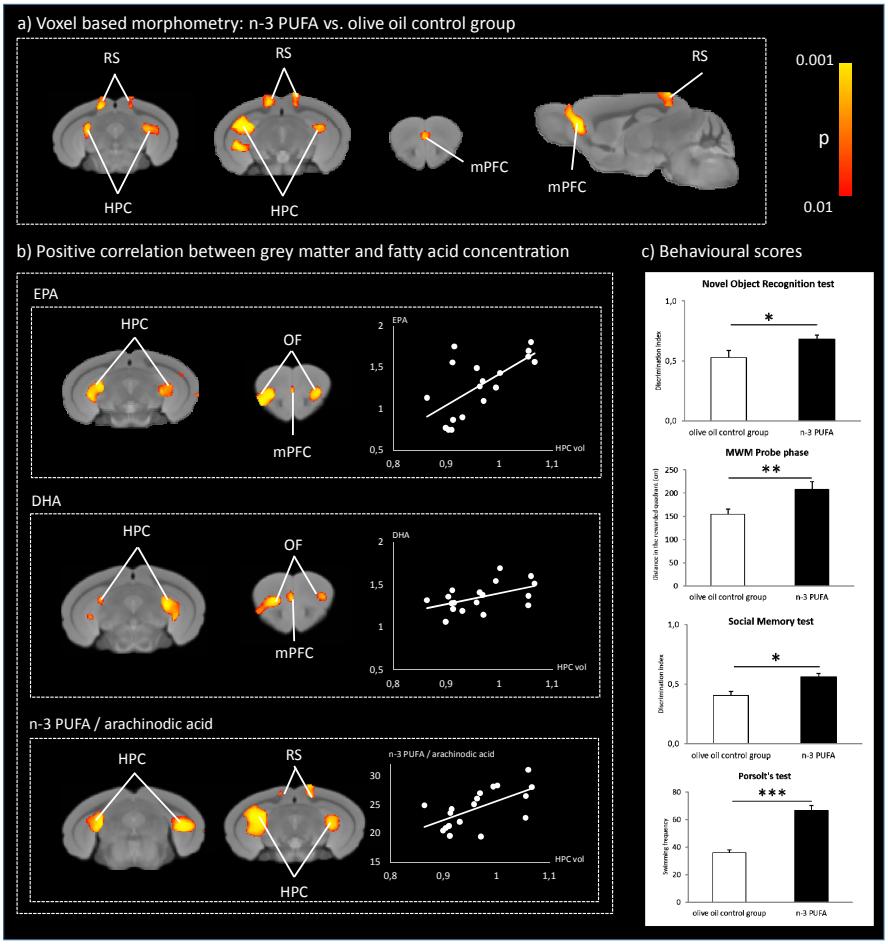

Figure 1. a) VBM revealed increased GM volumes in hippocampus, prefrontal and retrosplenial cortex of n-3 PUFA-treated animals. b) Voxelwise correlation maps between EPA, DHA, and n-3 PUFA/arachidonic acid concentration, and local GM volumes. c) Between group differences in behavioral tests. RS= retrosplenial cortex; HPC=hippocampus; mPFC=medial prefrontal cortex.

Results. VBM morphometric analysis of n-3 PUFA-treated animals revealed prominent bilateral areas of increased GM volume in the hippocampus, plus additional foci in the medial prefrontal and restrosplenial cortices (Figure 1a). No foci of significant GM volume reduction were observed throughout the brain in the n-3 PUFA-treated animals. Importantly, voxelwise correlation mapping of total brain EPA, DHA and n-3 PUFA/arachidonic acid concentration revealed foci of significant correlations encompassing all aforementioned regions, as well as in orbitofrontal areas (Figure 1b) thus corroborating a causative effect of n-3 PUFA supplementation to the GM changes mapped and the corresponding improved cognitive performance. Behavioral tests showed that n-3 PUFA treatment was associated with improved mnesic performances in the Novel Object Recognition test, Morris Water Maze and Social Memory test, and reduced depressive-like behaviors in the Porsolt's test (Figure 1c).

Discussion. These results strongly corroborate the emerging view of a pro-cognitive and protective function of dietary n-3 PUFA supplementation in the aged brain[6]. Specifically, our study demonstrates that a mixture of n-3 PUFA containing EPA, DHA and DPA can improve hippocampal-dependent cognitive function and coping responses, and results in increased GM volume in regions underlying key cognitive and emotional functions. Importantly, our study also suggests that the neuro-protective effect of n-3 PFU can take place even when the supplementation starts at late age. Collectively, these findings support the use of GM based measurements in human population as a surrogate for n-3 PUFA effects in future controlled supplementation clinical trials.

References. 1. Cutuli D et al. Front Aging Neurosci. 2014;6:220. **2.** Pottala JV et al. Neurology. 2014;82:435-42. **3.** Denis I et al. Ageing Res Rev. 2013;12:579-94. **4.** Tucci V et al. J Clin Invest. 2014;124:1468-82. **5.** Ashburner J, Friston KJ. NeuroImage. 2000;11:805-21. **6.** Ruxton et al. J Hum Nutr Diet 2004;17:449–459.