## FORCED EXERCISE INDUCES ANXIETY-LIKE BEHAVIOR ASSOCIATED WITH REDUCED ELECTROPHYSIOLOGICAL NEURONAL ACTIVITY AND GUT AND BRAIN ENDOCANNABINOIDOME CHANGES

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**Background:** Physical exercise is well known to have robust impact on health. In particular, recent studies suggest that forced and voluntary exercise may differentially affect brain functioning and behavior. Indeed, voluntary exercise is recognized as a prophylactic and curative measure for various age-related diseases, by inducing neural regeneration and plasticity in hippocampus. On the other hand, forced exercise in animals is associated with an increased anxiety-like behaviors together with an increased number of surviving bromodeoxyuridine $(BrdU)^+$ cells in the dentate gyrus in hippocampus. Because changes in hippocampal neurogenesis are linked to various mental disorders, and given the key role of endocannabinoid system (ECS) in synaptic plasticity processes, the aim of this study was to explore the effect of forced exercise on the hippocampal synaptic plasticity and on the related behavioral, electrophysiological and biochemical responses.

**Methods:** C57BL/6J male mice were assigned randomly to two main groups: sedentary (SD) and exercise trained (EX) group. The training protocol consisted in a treadmill running program based on 6weeks of walking at increasing intensity. At the end of exercise protocol, anxiety, anhedonia, and spatial memory behavioral tasks were analyzed. Moreover, hippocampal neuronal activity was examined by using electrophysiological single unit recordings in CA3. Finally, endocannabinoid levels were measured by gas chromatography-mass spectrometry (GC-MS) in the serum, cortex, hippocampus, and in the intestine of both groups of mice.

**Results:** We found that forced treadmill running induced anxiety in the Open Field test, measured as the time spent in the center vs the periphery. Moreover, the time spent in the dark box in Light-dark Box Test was increased in EX animals, as compared to SD mice. Electrophysiology recordings revealed that EX animals exhibited lower firing and burst activity of hippocampal CA3pyramidal neurons, as compared with SD group. These alterations were associated with the significant reduction of the main endocannabinoid ligands, such as Anandamide (AEA), 2-Arachidonoylglycerol (2-AG) and Palmitoylethanolamide (PEA) in the hippocampus, cortex and in specific gut segments (i.e. duodenum and jejunum).

**Discussion and conclusion:** In this study, we found that forced exercise induced stress response associated with anxiety-like behavior and lower neuronal activity in CA3, which is considered a key area involved in affective and cognitive brain functioning. Moreover, EX animals exhibited the reduction of different endocannabinoidome members in hippocampus and cortex. Interestingly, a regulation of endogenous cannabinoid ligands induced by the exercise was observed also in the different parts of the intestine, suggesting a possible interaction between gut and brain in the behavioral and functional changes mediated by exercise.