Section:- Neurobiology of Disease (Brief Communication)
Senior Editor:- Karl Herrup

Title:- Voluntary exercise decreases amyloid load in a transgenic model of Alzheimer's disease.
Abbreviated title:- Exercise effects in a transgenic model of AD

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Supplemental Methods

Solutions for Aß ELISA
Blocking buffer - 12g BSA + 0.2g NaN_3 in PBS, pH 7.4
Antigen capture buffer was applied to all wells - 50ml of 0.1M NaH_2PO_4, 150 ml of 0.1M Na_2HPO_4, 0.5g NaN_3, 0.744g EDTA, 23.3g NaCl, 10g BSA, 0.5g CHAPS, pH 7.0

RT-PCR
C_1THF was chosen as a control gene as its expression did not change in central nervous system (CNS) tissue in a number of different microarray experiments (Aimone et al., 2004; Perreau et al., 2004; Tong et al., 2001). In addition, the low expression level of C_1THF in the CNS allowed stoichiometric use of oligonucleotides for both the gene of interest and the internal control gene.

Oligonucleotides for RT-PCR
C_1THF synthase ^{5} AAGGAAAGTCGTGGGTGATG,
C_1THF synthase $^{3'}$CCGATCGTGGTGGTACTCTT,
IDE ^{5} CGGCCATCCAGAGAATAGAA,
IDE ^{3} TTTGGAGGGTCTGACAGTGA,
Neprilysin ^{5} TGCAGAAAGCAAAAACCTTG,
Neprilysin ^{3} TGTGCAATGGATTTCTCAGC.

RT-PCR conditions
Reverse transcription was performed by incubation at 50°C for 30min, then 95°C for 15min, followed by 25 to 27 cycles of: 94°C for 1 min, 54°C for 1 min, and 72°C for 1 min

Solutions for Western blotting
Extraction buffer, ph = 6.8 - 100mM Tris, 1% SDS, 1 protease inhibitor cocktail tablet from MP Biomedicals
loading buffer, pH=6.8 - 2.5mM Tris, 2% SDS, 0.007% bromophenol blue, 4% BME, 10% glycerol

Western blotting method

For each sample 16μg of protein was electrophoresed through a 4-20% Tris-HCl Criterion gel (Biorad), followed by transfer to a Sequi-blot PVDF membrane (Biorad). Following a 1 hour incubation in blocking buffer (5% milk/TTBS), the membranes were incubated overnight with the appropriate primary antibodies (in 5% milk/ TTBS) and detected with HRP-conjugated IgG secondary antibodies (in 5% milk/TTBS) (Biorad). Bound secondaries were visualized with Supersignal Chemiluminscent Substrate (Pierce) and exposed on Hyperfilm ECL (Amersham).

Supplemental References

Aimone JB et al (2004) Spatial and temporal gene expression profiling of the contused rat spinal cord. Exp Neurol 189:204-221.

Perreau VM et al (2004) Exercise induced gene expression changes in the rat spinal cord. Gene Expression in press.

Tong L et al (2001) Effects of exercise on gene-expression profile in the rat hippocampus. Neurobiol Dis 8:1046-1056.