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 Aβ ELISA
Blocking buffer - 12g BSA + 0.2g NaN₃ in PBS, pH 7.4
Antigen capture buffer was applied to all wells - 50ml of 0.1M NaH₂PO₄, 150 ml of 0.1M Na₂HPO₄, 0.5g NaN₃, 0.744g EDTA, 23.3g NaCl, 10g BSA, 0.5g CHAPS, pH 7.0

RT-PCR
C₁,THF 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 C₁,THF in the CNS allowed stoichiometric use of oligonucleotides for both the gene of interest and the internal control gene.

Oligonucleotides for RT-PCR
C₁,THF synthase 5' AAGGAAAGTCGTGGGTGATG, 3' CCGATCGTGGTGTACTCTT, 5' CGGCCATCCAGAGAATAGAA, 3' TTTGGAGGGTCTGACAGTGA, 5' TGCAGAAAGCAGAAAAACCTTG, 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 Chemiluminescent Substrate (Pierce) and exposed on Hyperfilm ECL (Amersham).

Supplemental References
