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Supplementary Data

Anti-ATP Synthase Autoantibodies Induce Neuronal Death by Apoptosis and Impair Cognitive Performance in C57BL/6J Mice

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SUPPLEMENTARY METHODS

Control study for antibody specificity

In preliminary experiments, we tested a preparation of intravenous immonoglobulines (IVIG) at the dose of 6 μg/μl as a control of antibody effect specificity. This dose was found to have no effect (completely overlapping with vehicle injection) on general and on cognitive behavior (supplementary Figure 1). Briefly, mice were injected ICV either with vehicle (PBS) or IVIG and tested in a spatial memory task following the procedure indicated below. No difference was found in latency to reach the platform during the *acquisition phase* in the two groups ($F(1,18) = 0.003$; $p = 0.9577$) or in the time spent in the acquisition zone during the *probe trial* ($F(1,6) = 2{,}664$; $p = 0.1538$). $n = 4$ C57BL/6J male mice in each treated group.

Morris water maze procedure

Briefly, experimental subjects were introduced in a Plexiglas circular pool filled with water (24–26°C), which was rendered opaque with powdered non-toxic tempera paint, and underwent 4 acquisition trials to learn to locate the position of a platform hidden below the water surface. Each trial was spaced from the other by a 45 min interval and had 60 s duration (cut-off time) during which animals freely swam until they found the platform or until the cut-off time. Ninety minutes following the last training trial, the platform was removed from the pool and mice were tested for memory retention (probe trial).

Open field test procedure

Subjects were individually placed in an open field for 15 min and their spontaneous behavior scored (latency, frequency, and duration of squares *crossings*, *rearing*, *grooming*, and *immobility*). *Thigmotaxis* and risk assessment behavior (*stretched-attend posture*) were considered as indexes of emotionality. Videotapes were analyzed using dedicated software ("The Observer 3.0" Noldus, The Netherlands).

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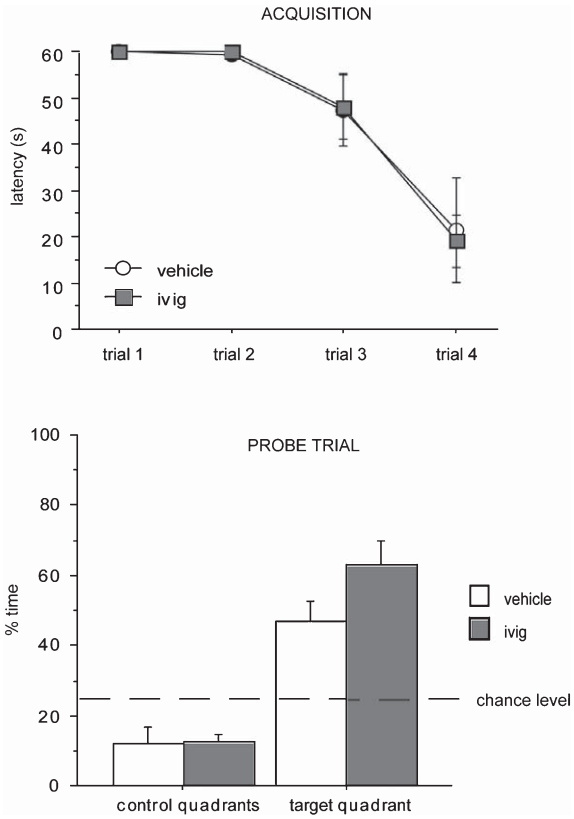

Supplementary Figure 1. Learning and memory in the MWM. No difference was found in the learning curve in the acquisition phase in mice injected with IVIG, compared to vehicle (PBS). In addition, the two groups showed comparable memory retention during the probe trial of the spatial navigation task.

Actin and nuclear staining

For actin filament (F-actin) detection: after fixation with 4% paraformaldehyde in phosphate-buffer saline (PBS), sections were permeabilized with 0.2% Triton×-100 (Sigma-Aldrich, St. Louis, MO, USA) in PBS for 15 min. Samples were then stained with fluorescein-phalloidin (Sigma-Aldrich) at 37°C for 30 min. For nuclear staining, the dye Hoechst 33258 (Sigma-Aldrich) was used.

SDS-PAGE and western blot

Cell lysate were run in 15% SDS-PAGE and nitrocellulose membranes were incubated with specific anti-caspase-3 rabbit antibodies diluted 1 : 1,000 in PBS (CellSignaling, Danvers, MA) or with anti-cytochrome c diluted 1 : 100 in PBS (Santa Cruz Biotechnology, Santa Cruz, CA). After incubation with peroxidase-conjugated, goat anti-rabbit IgG (Bio-Rad), the reactions were developed by SuperSignal West Pico chemiluminescent Substrate (Pierce, Rockford, IL, USA). The membranes were re-probed with anti-mouse anti β-actin mAb (Amersham, Gent, Belgium).