Supporting Information

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SI Materials and Methods

Animals. Inbred male 3-mo-old C57BL/6 mice were supplied by the Animal Breeding Center of The Weizmann Institute of Science. Inbred male 17- to 24-mo-old C57BL/6 mice were supplied by the National Institute on Aging (NIA). IFN-γ receptor knockout animals (IFN-γR1-KO) mice on the B6 background were purchased from The Jackson Laboratory. Aged mice were allowed a 1-mo adaptation period following shipment from the NIA to our laboratory. The cages were placed in a light- and temperature-controlled room, and all behavioral tests were conducted during the dark hours. All animals were handled according to regulations formulated by The Weizmann Institute's Animal Care and Use Committee and maintained in a pathogen-free environment.

Flow Cytometric Cell-Sample Preparation and Analysis. Before tissue collection, blood was collected into heparin-containing tubes, and mice were intracardially perfused with PBS. Spleens were mashed with the plunger of a syringe and treated with ammonium-chloride-potassium (ACK) lysing buffer to remove erythrocytes. Choroid plexus (CP) tissues were isolated from the lateral, third, and fourth ventricles of the brain, incubated at 37 °C for 45 min in PBS (with Ca^{2+}/Mg^{2+}) containing 400 U/mL collagenase type IV (Worthington Biochemical), and then manually homogenized by pipetation. Lymph nodes were mashed with the plunger of a syringe. All samples were stained according to the antibody's protocols. Antibodies used included Alexa-700–conjugated anti-CD45.2, PercpCy5.5-conjugated anti–T-cell receptor (TCR)β, PE-conjugated anti-CD4, FITC-conjugated anti-CD44, and antigen-presenting cell–conjugated anti-CD62L (BD Pharmingen and eBioscience). Cells were analyzed on an LSRII cytometer (BD Biosciences) using FACSDiva (BD Biosciences) and FlowJo (Tree Star) software. In each experiment, relevant negative-control groups and single-stained samples for each tissue were used to identify the populations of interest and to exclude others.

Active Immunization and Cell Isolation. Mice were immunized s.c. at their flanks with either spinal cord homogenate (SCH) or ovalbumin (OVA), each emulsified in an equal volume of CFA containing 2 mg/mL *Mycobacterium tuberculosis*. SCH was prepared by manually homogenizing the spinal cord of young C57BL/6 mice in PBS. Mice were intracardially perfused with PBS 7 d after immunization, and their spleens, bone marrow, and CPs were extracted and processed to single-cell suspensions as described above. $CD4^+$ cells were isolated by magnetic depletion of $CD4^+$ T cells on a MACS column (Miltenyi Biotec) according to the manufacturer's protocol.

cDNA Library Preparation for TCRβ Sequencing. Total RNA was extracted from $CD4^+$ T cells derived from naïve spleen and CP tissues of C57BL/6 mice as well as from spleens of OVA-immunized and SCH-immunized mice. RNA was extracted using an RNeasy Mini Kit (Qiagen) and reverse-transcribed with SuperScript II reverse transcriptase (Invitrogen) using a primer specific for the TCRβ constant region linked to the Solexa 3′ adapter (Cβ-3′adp). cDNA was then used as a template for high-fidelity PCR amplification (Phusion; Finnzymes) using a pool of 23 Vβ-specific primers, divided into five primer groups, to minimize potential for cross-hybridization. Each Vβ primer was linked to a restriction-site sequence for the ACUI restriction enzyme (New England BioLabs). PCR reactions were performed in duplicate, and PCR products were then pooled and cleaned using the QIAquick PCR Purification Kit (Qiagen), followed by enzymatic digestion, in accordance with the ACUI protocol (New England BioLabs). The ACUI enzyme was used to cleave the 14 bp downstream of its binding site, enabling positioning of the Illumina sequencing primer in close proximity to the junction region and assuring sequencing of the entire variable CDR3 region. Digestion produced a 2-bp overhang for the ligation of the Illumina 5′ adapter, which was linked to a 3-bp barcode sequence at its 3′ end. Overnight ligation was performed using T4 ligase (Fermentas) at 16 °C in accordance with the manufacturer's protocol. A second round of PCR amplification was performed (24 cycles), using primers for the 5′ and 3′ Illumina adapters. Final PCR products were run on an agarose gel (2%) and purified using the Wizard SV Gel and PCR Clean-Up System (Promega). Final library concentrations were measured using a NanoDrop spectrophotometer. The libraries were sequenced using an Illumina Genome Analyzer II.

Computational Analysis of TCRβ Sequencing Data. For preprocessing of the data, we used the Smith–Waterman alignment algorithm (1) to assign to each sequencing read its variable (Vβ) and joining (Jβ) gene, using germ-line Vβ/Jβ gene segment sequences downloaded from the IMGT database (2). Reads that were not assigned either a Vβ or Jβ and other erroneous reads were eliminated. We then clustered the library-derived reads using a version of the quality threshold clustering algorithm (3) to correct for nucleotide copying errors (up to two errors for each read). The clustering procedure identified unique CDR3β clonotypes, defined as the most prevalent read found in each cluster. The clonotype sequences were then translated, and those clonotypes that lacked a stop codon in-frame with the V/D/J sequences were considered for further analysis. This analysis computed statistics of V/D/J use, statistical properties of the number of deletions and insertions of nucleotides at both VD and DJ junctions, as well as distributions of CDR3 lengths. The analysis was done using the R statistical software package (R Development Core Team; www.r-project.org). Frequencies of Vβ and Jβ segment use were measured for all samples in each treatment group. Correlation coefficients were calculated, based on the sample mean of combined Vβ and Jβ use in each group, using MATLAB (Mathworks). Hierarchical clustering was performed, based on combined Vβ and Jβ use, in all groups (clustergram; MATLAB).

Primary Culture of Choroid Plexus Cells. Mice were perfused from the left ventricle of the heart with PBS, and their CPs were removed under a dissecting microscope (Stemi DV4; Zeiss) in PBS into tubes containing 0.25% trypsin and kept on ice. The tubes were shaken for 20 min at 37 °C and the tissue was dissociated by pipetting. The cell suspension was washed in culture medium for epithelial cells (DMEM/HAM's F12; Invitrogen) supplemented with 10% (vol/vol) FCS (Sigma-Aldrich), 1 mM l-glutamine, 1 mM sodium pyruvate, 100 U/mL penicillin, 100 mg/mL streptomycin, 5 μg/mL insulin, 20 μM Ara-C, 5 ng/mL sodium selenite, and 10 ng/mL EGF, and cultured (~250,000 cells per well) at 37 °C, 5% CO_2 in 24-well plates (Corning) coated with poly-L-lysine (PLL; Sigma-Aldrich). After 24 h, the medium was changed and the cells were either left untreated or treated with either 100 ng/mL IFN-γ (Peprotec), serial dilution or 10 ng/mL IL-4 (Peprotec), or a combination of 100 ng/mL IFN-γ and 10 ng/mL IL-4 for 24 h. RNA isolation was done with an RNA MicroPrep Kit (Zymo Research) according to the manufacturer's protocol.

Immunohistochemistry and Immunocytochemistry. For whole-mount staining of the choroid plexus, isolated tissues were fixed with 2.5% paraformaldehyde for several hours and subsequently transferred to PBS containing 0.05% sodium azide. Before staining, the dissected tissues were washed and blocked (20% horse serum, 0.3% Triton X-100, and PBS) for 1 h at room temperature with shaking. Whole-mount staining with primary (in PBS containing 2% horse serum and 0.3% Triton X-100) and secondary antibodies (in PBS) was performed for 1 h at room temperature with shaking. Each step was followed by three washes in PBS. The tissues were mounted onto slides, using Immu-Mount (9990402; Thermo Scientific), and sealed with coverslips. For staining of sectioned brains, two different tissue preparation protocols (paraffin-embedded and microtomed frozen sections) were applied, as previously described (4). For immunocytochemistry, CP cells were isolated as described above and grown on PLL-coated coverslips for 7 d, replacing the medium every 3 d. Cytokines were added for the last 24 h of culture. Following 7 d of culture, the wells were washed with PBS and the cells were fixed either with 2.5% paraformaldehyde for 20 min or methanol:acetone (1:1) for 10 min at −20 °C, following by two washing steps with PBS. For CCL11 staining, cells were treated with Brefeldin A 2 h before fixation to arrest protein secretion. The coverslips of the cultured CP cells were incubated with the indicated antibodies and mounted with GVA mounting solution (Invitrogen). The following primary antibodies were used: rat anti-CD3 (Abcam); mouse anti-MHCII (Abcam); mouse anti-arginase 1 (BD Biosciences); mouse anti-cytokeratin (Covance); rat anti-CCL11 (R&D Systems); rabbit anti-BDNF (Alomone Labs); rabbit or mouse anti–ZO-1 (Invitrogen); and rabbit anti-Claudin 1 (Invitrogen). Secondary antibodies included Cy2/Cy3-conjugated donkey anti-rat, -rabbit, or -mouse antibody and Cy3-conjugated donkey anti-goat (1:200; all from Jackson ImmunoResearch). The slides were exposed to Hoechst stain (1:2,000; Invitrogen) for 1 min. Two negative controls were routinely used in the immunostaining procedures: staining with an isotype control antibody followed by a secondary antibody, and staining with a secondary antibody alone. For morphological quantification, the average cross-sectional area of epithelial cells was measured following Nissl staining. Cell borders (20–25 cells per picture; 20 pictures per group) were marked, and the area was quantified using Image-Pro Plus software (Media Cybernetics, Inc.) in a blinded fashion.

Multiplex Cytokine Analysis System. CPs were isolated from lateral, third, and fourth ventricles and pooled in groups of four, due to the limited amount of protein extracted from a single CP. The excised tissues were homogenized in PBS containing protease inhibitors (1:100; P8340; Sigma). Four freeze–thaw cycles (3 min each) were performed to break the cell membranes. Homogenates were then centrifuged for 10 min at $500 \times g$, and the total protein quantities in supernatants were determined by Bradford reagent. Frozen supernatants were assayed in duplicate using a multiplex bead-based Luminex assay (MILLIPLEX Mouse Cytokine/Chemokine Panel; Millipore), performed by outsourcing (American Medical Laboratories) according to the manufacturer's instructions. Results are expressed as picograms of protein per milligram of total tissue protein.

RNA Purification, cDNA Synthesis, and Real-Time Quantitative PCR. Total RNA of the hippocampus was extracted with TRI Reagent (Molecular Research Center) and purified from the lysates using an RNeasy Kit (Qiagen). Total RNA of the choroid plexus was extracted using an RNA MicroPrep Kit (Zymo Research). mRNA (1 μg) was converted to cDNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The expression of specific mRNAs was assayed using fluorescence-based real-time quantitative PCR (qPCR). Quantitative PCR reactions were performed using Power SYBR Green PCR Master Mix (Applied Biosystems). Quantification reactions were performed in triplicate for each sample using the standard curve method. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), peptidylprolyl isomerase A (PPIA), and hypoxanthine guanine phosphoribosyltransferase (HPRT) were chosen as reference genes according to their stability in the target tissue. The amplification cycles were 95 °C for 5 s, 60 °C for 20 s, and 72 °C for 15 s. At the end of the assay, a melting curve was constructed to evaluate the specificity of the reaction. For some genes, the cDNA was preamplified in 14 PCR cycles with nonrandom PCR primers, thus increasing the sensitivity of the subsequent real-time PCR analysis (PreAmp Master Mix Kit; Applied Biosystems). For these genes, expression was determined using TaqMan Real-Time PCR, according to the manufacturer's instructions (Applied Biosystems). All quantitative real-time PCR reactions were performed and analyzed using a 7500 Real-Time PCR System (Applied Biosystems). The following TaqMan probes were used: Mm02342430_g1 (*ppia*), Mm00446968_m1 (*hprt*), Mm00445260_m1 (*il-4*), Mm01168134_m1 (*ifn-γ*), Mm00441238_m1 (*ccl11*), and Mm00475988_m1 (*arg-1*). In addition, the following primers were used: *gapdh* forward 5′-AATGTGTCCGTCGTGGATCTGA-3′ and reverse 5′-GATGCCTGCTTCACCACCTTCT-3′; *ppia* forward 5′-AGCATACAGGTCCTGGCATCTTGT-3′ and reverse 5′-CAAAGACCACATGCTTGCCATCCA-3′; *ccl11* forward 5′-CATGACCAGTAAGAAGATCCC-3′ and reverse 5′-CTTGAAGACTATGGCTTTCAGG-3′; *arg-1* forward 5′-AAGACAGGGCTCCTTTCAG-3′ and reverse 5′-TGTTCACAGTACTCTTCACCT-3′; *bdnf1* forward 5′-CCTGCATCTGTTGGGGAGAC-3′ and reverse 5′-GCCTTGTCCGTGGACGTTTA-3′; *ho1* forward 5′-AGATGACACCTGAGGTCAAGCACA-3′ and reverse 5′-GCAGCTCCTCAAACAGCTCAATGT-3′; and *pgc1α* forward 5′-GCCAAACCAACAACTTTATCTC-3′ and reverse 5′-GTTCGCTCAATAGTCTTGTTCTC-3′.

Irradiation and Bone Marrow Transplantation. Aged C57BL/6 wild-type mice were subjected to total-body γ-irradiation from a cobalt source (a single dose of 950 rad), while their heads were protected to avoid radiation-induced brain damage. Transplanted bone marrow (BM) was composed of either whole BM or BM depleted of T cells, which was prepared by magnetic depletion of $CD3^+$ cells on a MACS column (Miltenyi Biotec) according to the manufacturer's protocol. BM transplantation was performed on the following day by i.v. injection of 5×10^6 bone marrow cells suspended in PBS (total volume 0.15 mL). For detection of BM cells following transplantation, cells were prelabeled with carboxyfluorescein succinimidyl ester (CFSE; Molecular Probes) before transplantation. For labeling, 5×10^6 BM cells (20×10^6 cells/mL) were incubated in PBS (without Ca^{2+}/Mg^{2+}) supplemented with 5 μM CFSE (Molecular Probes) for 8 min at 25 °C. Following incubation, the cells were washed with RPMI containing 8% FBS.

Morris Water Maze. Acquisition and probe trials were performed as previously described (5). Following the probe trial, mice were given three additional trials without the platform to extinguish their initial memory of the platform's position. In the reversal phase, the platform was placed in a new location in the pool (opposite to where it was located in the acquisition phase) and the mice were given three trials/d on 2 consecutive days, conducted in a similar manner to the initial acquisition. Position and movement of the mice were recorded using an EthoVision automated tracking system (Noldus).

Statistical Analysis. The specific tests used to analyze each set of experiments are indicated in the figure legends. Data are expressed as mean ± SEM. In the graphs, y axis error bars represent the SEM. Statistical analysis was performed using Prism 5.0 software (GraphPad Software). Means between two groups were compared by two-tailed, unpaired Student's t test. One-way ANOVA was used to compare several groups, and the Newman–Keuls test for

pairwise comparisons was used for follow-up post hoc comparison of groups. Data from behavioral tests were analyzed using two-way repeated-measures ANOVA with group treatment or age and number of days as between-subject factors. All histology and behavioral experiments were conducted in a randomized and blinded fashion.

1. Smith TF, Waterman MS (1981) Identification of common molecular subsequences. *J Mol Biol* 147(1):195–197.
2. Lefranc MP, et al. (2009) IMGT, the international ImMunoGeneTics information system. *Nucleic Acids Res* 37(Database issue):D1006–D1012.
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4. Ziv Y, Avidan H, Pluchino S, Martino G, Schwartz M (2006) Synergy between immune cells and adult neural stem/progenitor cells promotes functional recovery from spinal cord injury. *Proc Natl Acad Sci USA* 103(35):13174–13179.
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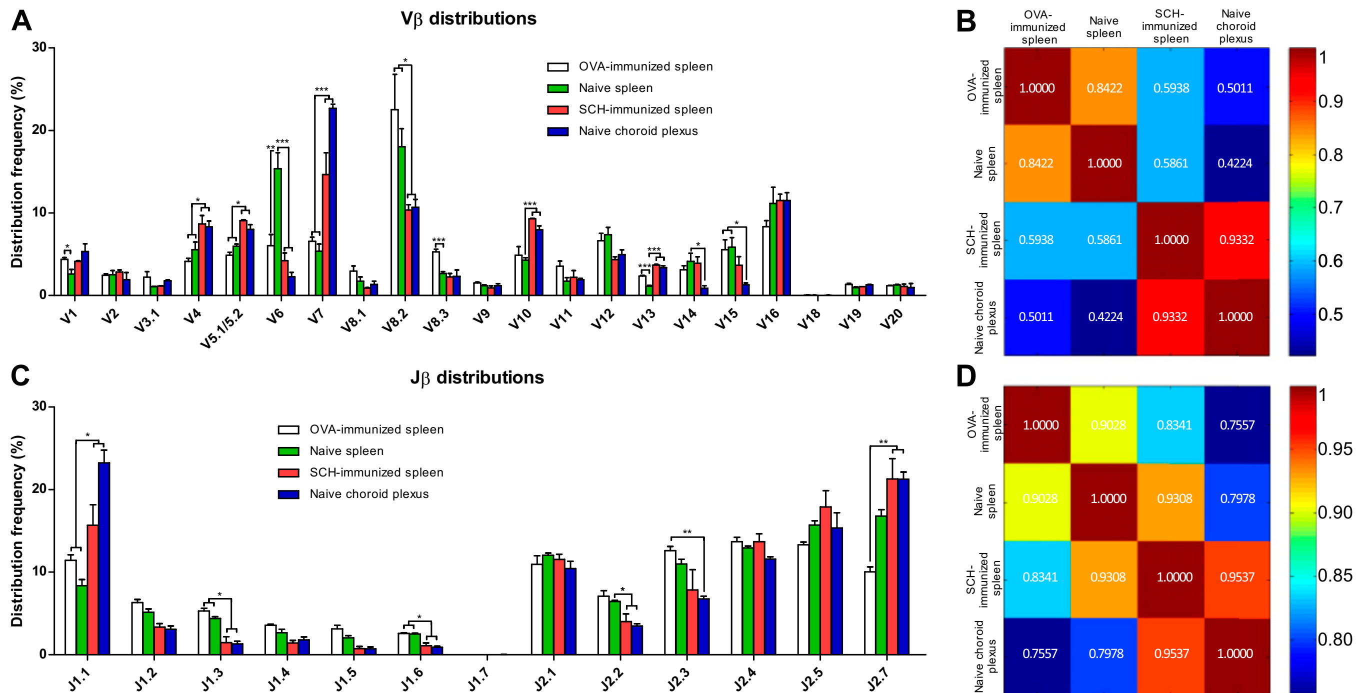

Fig. S1. The choroid plexus CD4+ TCR repertoire is enriched with CNS-specific clones. (*A* and *B*) Vβ use (*A*) and correlation coefficients (*B*) for spleens of OVA-immunized mice, SCH-immunized mice, naïve spleen, and naïve choroid plexus ($n = 3$ per group; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; one-way ANOVA, Newman–Keuls post hoc). (*C* and *D*) Jβ use (*C*) and correlation coefficients (*D*) for the same groups as in *A* and *B*.

Fig. S2. Comparison of the clonal expansion induced by OVA and SCH immunization. A Venn diagram showing the intersection between libraries of clones that were significantly up-regulated in OVA- or SCH-immunized spleens ($n = 3$ per group) compared with spleens of naïve nonimmunized mice ($n = 3$). A small overlap is observed between these two libraries.

Fig. S3. Th2-mediated inflammation of the CP epithelium during the aging process. (A) Representative micrographs showing morphological Nissl staining in young (3-mo-old) and old (20-mo-old) mouse CP. (Scale bars, 50 μm.) (B) Nissl staining was used to measure the average cross-sectional area of epithelial cells in μm^2 (***$P < 0.001$; Student's t test) and to assess epithelial hypertrophy.

Fig. S4. Changes in *arg1* mRNA levels, measured by real-time qPCR, in the CP of young wild-type (3-mo-old) and young IFN-γR-KO mice (3-mo-old) ($n = 5$–6 per group; *$P < 0.05$; Student's t test).

Fig. S5. IL-1β, IL-6, and TNF-α protein levels (pg/mg tissue) in the CP of young (3-mo-old) and old (20- to 22-mo-old) wild-type mice ($n = 16$ per group; *$P < 0.05$; Student's t test).

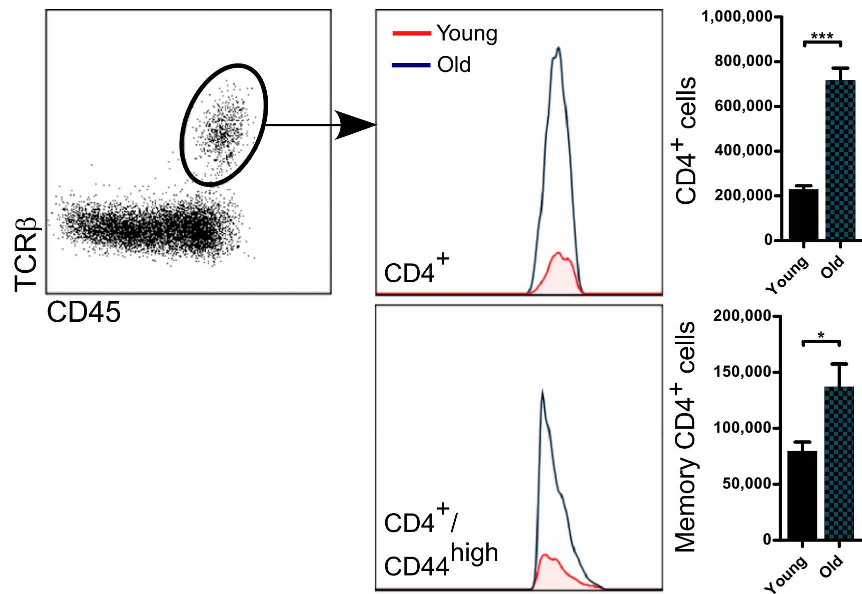

Fig. S6. Representative FACS histograms and quantitative analysis for CD4$^+$ T cells and CD4$^+$/CD44^{high} memory T cells in the bone marrow of young (3-mo-old) and old (22-mo-old) mice. Cells were pregated by CD45$^+$ and TCRβ (n = 4–5 per group; *$P < 0.05$; ***$P < 0.001$; Student's t test).

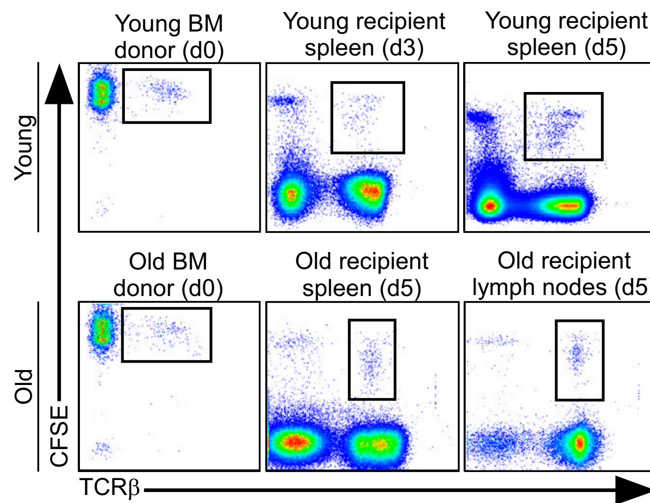

Fig. S7. Representative flow cytometry plots demonstrating resting (CFSE^{high}) and dividing (CFSE^{low}) T cells (TCRβ$^+$) in donor BM on the day of transplantation (d0), in the spleen of young recipients on days 3 and 5 posttransplantation (*Upper*), and in the spleen and cervical lymph nodes of aged recipients on day 5 posttransplantation (*Lower*).

Fig. S8. Cognitive ability induced by homeostatis-driven proliferation of pseudoautologous BM is T cell-dependent. Aged mice with memory impairments were transplanted with either complete BM, derived from donor mice of identical age to the recipients, or with T cell-depleted BM from the same donors. The mice were tested in a Morris water maze 8 wk following transplantation, together with nontreated young mice of the same genetic background. In both the acquisition and the reversal phases of the test, the performance of aged mice transplanted with aged BM depleted of T cells (aged-BMT-T) was significantly impaired compared with young mice, whereas the performance of aged mice transplanted with whole BM (aged-BMT) was similar to that of the young mice [acquisition: repeated-measures ANOVA: groups: $P = 0.005$, days: $P = 0.0005$, groups x days: $P = 0.02$; reversal: analysis of covariance, comparing groups regarding differences between the performance of each mouse in the 2 d of the test (day 2 − day 1), with the day 1 measure as the covariant: $P = 0.0002$, $^{*}P < 0.05$ (young vs. aged-BMT-T)]. Reversal: performance in the reversal phase according to the swimming path length to the platform [$P < 0.0001$, $^{\#}P < 0.05$ (aged-BMT-T vs. aged-BMT), $^{*}P < 0.05$ (aged-BMT-T vs. young)].