N°Anonymat:

The candidate will provide (i) a title to the article, (ii) a short description of the biological context and general problematics and (iii) an analysis of the the data (for each panel, describe the aim of the experiment, the general methodology, the results and conclude). Finally the candidate will formulate 2 to 4 questions raised by the study (iv).

LETTER

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(i) TITLE?????

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INTRODUCTION

Adult neurogenesis occurs in local microenvironments, or neurogenic niches, in the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the hippocampus. Permissive cues within the neurogenic niche are thought to drive the production of new neurons and their subsequent integration into the neurocircuitry of the brain, directly contributing to cognitive processes including learning and memory. Importantly, the neurogenic niche is localized around blood vessels, allowing for potential communication with the systemic environment.

(ii)- Specific problematics of the article

RESULTS

(iii)- Data analysis

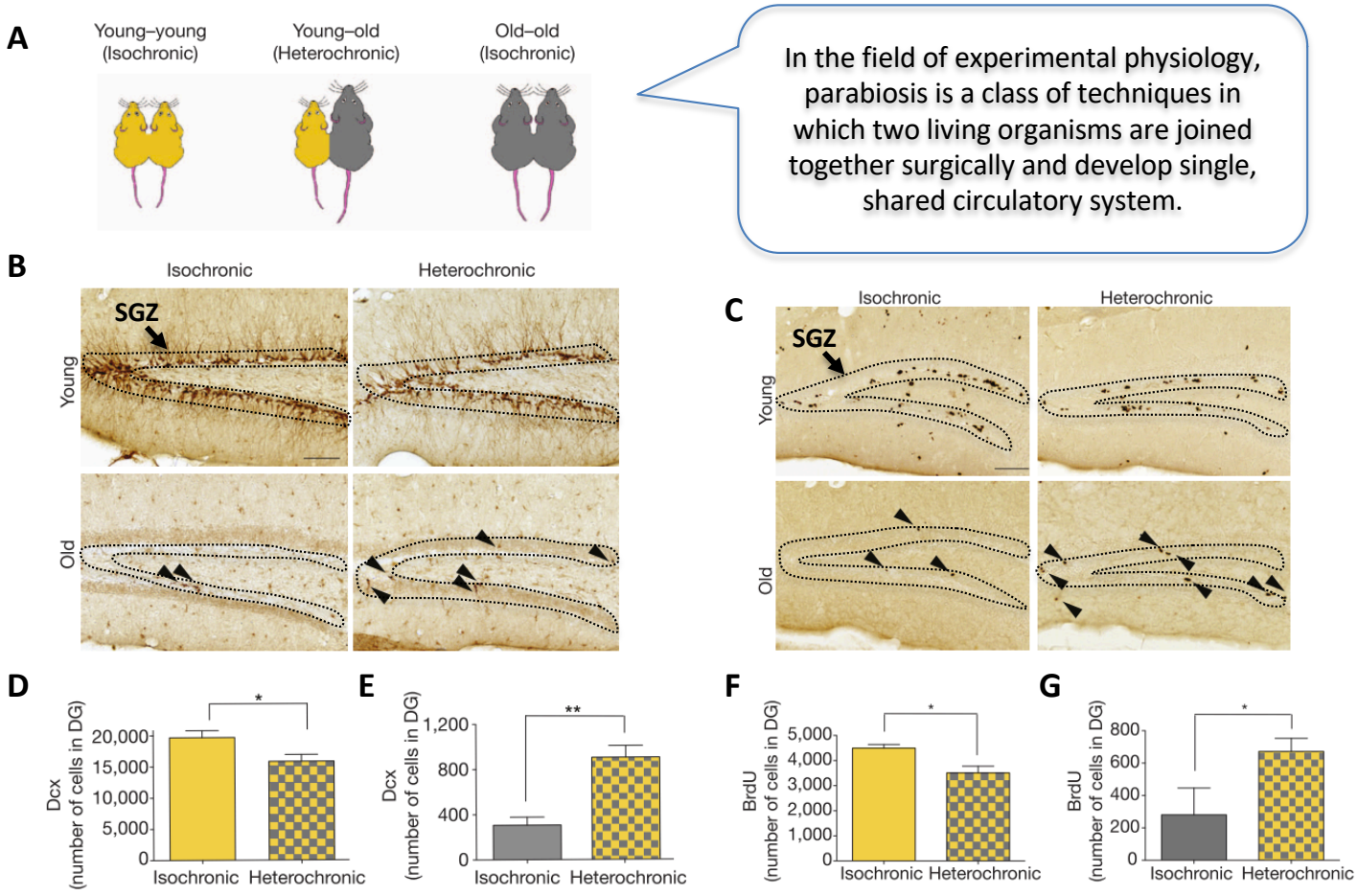

Fig1: Heterochronic parabiosis effects on adult dentage gyrus neurogenesis. (A) Schematic showing parabiotic pairings. (B, C) Representative fields of Dcx (B; neuronal marker) and BrdU (C) immunostaining of young (3–4 months; yellow) and old (18–20 months; grey) dentate gyrus 5 weeks after isochronic or heterochronic parabiosis (arrowheads point to individual cells; scale bars, 100mm). (D-G) Quantification of neurogenesis (D, E) and proliferating cells (F, G) in the young (D, F) and old (E, G) subgranular zone (SGZ; delineated by dotted lines) of the dentate gyrus (DG) after parabiosis. No difference were observed between unpaired age-matched animals and isochronic animals (data not shown). All are data are presented as mean+/- s.e.m.; *P,0.05; **P,0.01, t-test.

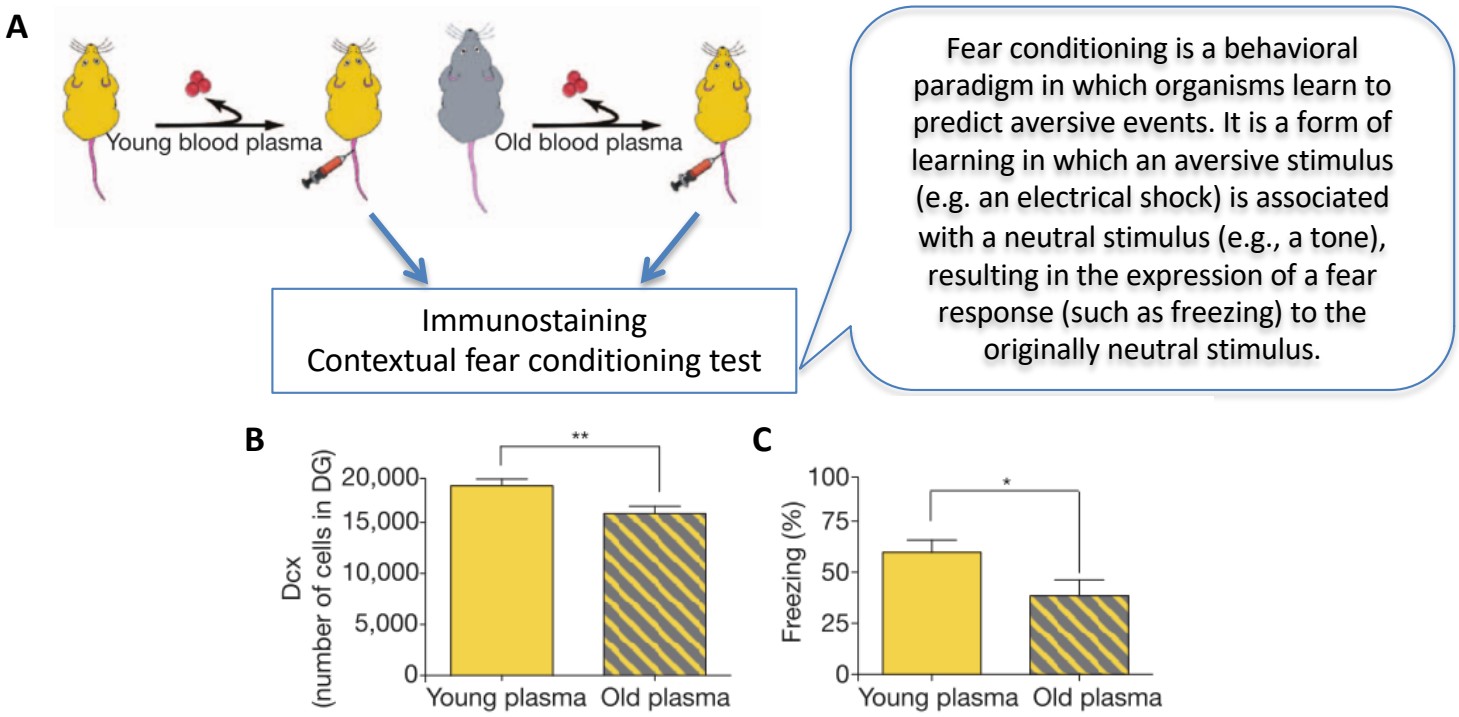

Fig2: Effects of old blood tranfusion on adult neurogenenesis and learning. (A) Schematic of young (3–4 months) or old (18–22 months) plasma extraction and intravenous (i.v.) injection into young (3 months) adult mice. (B) Quantification of neurogenesis in the young dentate gyrus after plasma injection. (C) Hippocampal learning assessed by contextual fear conditioning in young adult mice after young or old plasma injections nine times over 24 days. All data are presented as mean+/- s.e.m.; *P,0.05; **P,0.01, t-test.

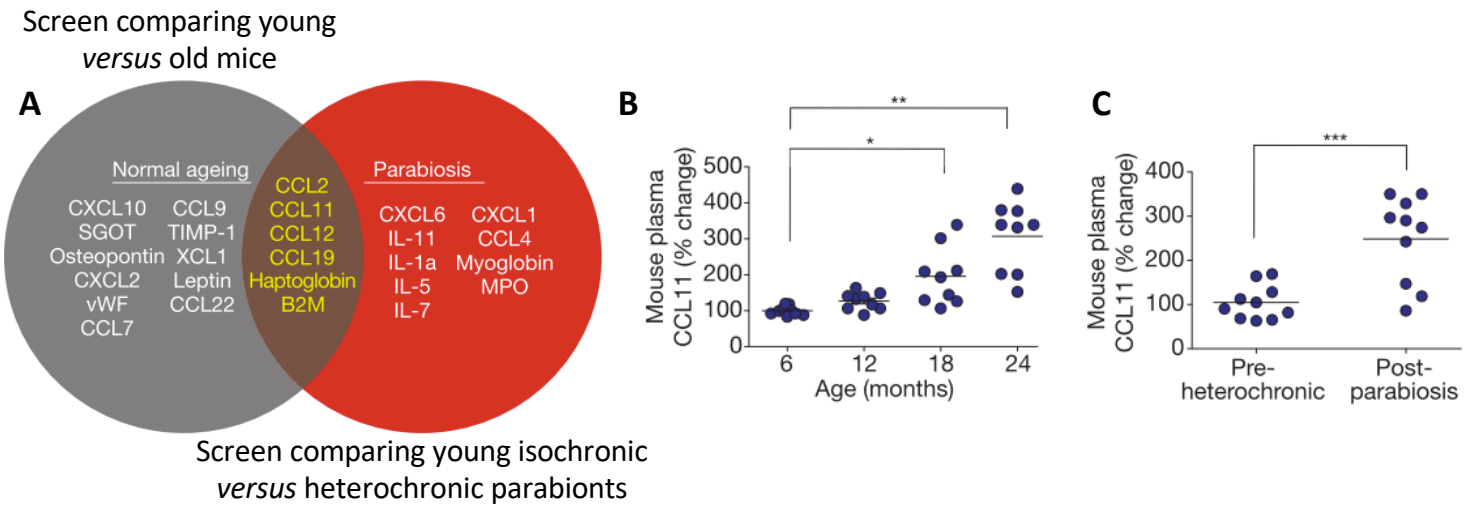

Fig3: Systemic chemokine levels during ageing and heterochronic parabiosis. (A) Venn diagram of results from ageing and parabiosis proteomic screens. In grey are shown the seventeen age-related plasma factors that were found to increase with normal ageing, in red are shown the fifteen plasma factors that were found to increase in young heterochronic parabionts compared to young isochronic ones, and in the brown intersection are the six factors elevated in both screens. (B, C) Changes in plasma concentrations of the CCL11 chemokine as a function of age (B) and in young (6 month old) heterochronic parabionts before (pre-heterochronic) and after (post) parabiotic pairing (D). All data are presented as dot plots with mean; *P,0.05; **P,0.01; ***P,0.001, ANOVA, Tukey's post-hoc test (B), t-test (C).

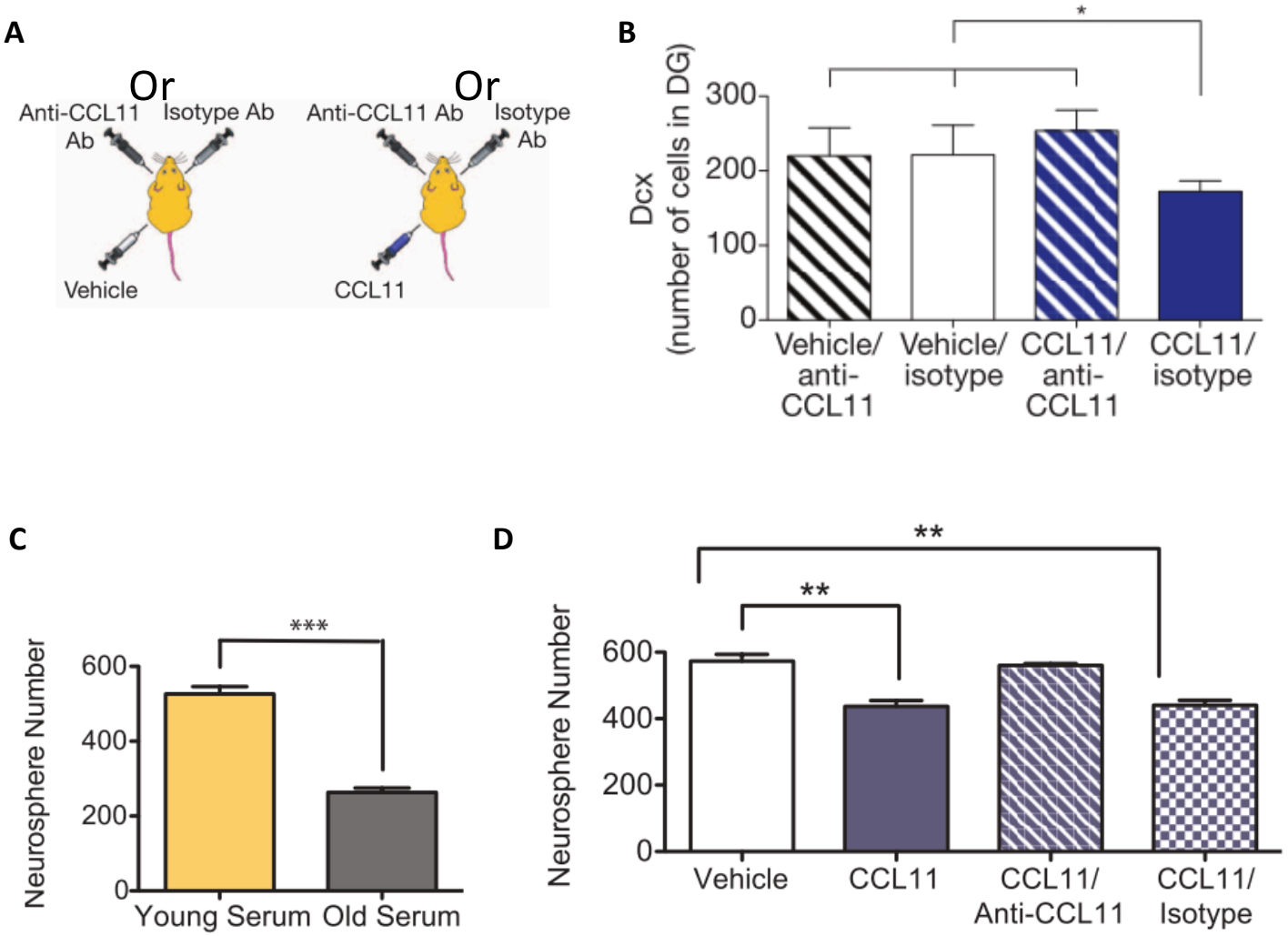

Fig4: Analysis of CCL11 effects on dentate gyrus neurogenesis
(A) Schematic of young adult mice given unilateral stereotaxic injections of anti-CCL11 neutralizing or isotype control antibody (Ab) followed by systemic injections with either recombinant CCL11 or PBS (vehicle). (B) Quantification of neurogenesis in the dentate gyrus after systemic and stereotaxic treatment. Bars represent mean number of cells in each section. All data are presented as mean +/- 6s.e.m.; *P,0.05; **P,0.01; ANOVA, Dunnet's or Tukey's post-hoc test. (C, D) Neurosphere assay on primary neural stem stem cells isolated from the dentate gyrus. Cells were grown under self-renewal conditions and exposed for four days either to serum isolated from young (2-3 months) or old (18-22 months) mice (C) or to PBS (vehicle), CCL11, CCL11 + anti-CCL11 neutralizing antibody or to CCL11 + a non-specific isotype control antibody (D).

CONCLUSION

(iv)- Ask questions

METHODS

Mice. The following mouse lines were used: C57BL/6 (The Jackson Laboratory), C57BL/6 aged mice (National Institutes of Ageing), Dcx-Luc mice[20] and C57BL/6J-Act-GFP (Jackson Laboratory). For parabiosis experiments male and female C57BL/6 mouse cohorts were used. For all other *in vivo* pharmacological and behavioural studies young (2–3 months) wild-type C57BL/6 male mice were used. Mice were housed under specific pathogen-free conditions under a 12 h light–dark cycle and all animal handling and use was in accordance with institutional guidelines approved by the Veterans Affairs Palo Alto Committee on Animal Research.

Immunohistochemistry. Tissue processing and immunohistochemistry was performed on free-floating sections following standard published techniques[24]. Briefly, mice were anaesthetized with 400 mg kg^{-1} chloral hydrate (Sigma-Aldrich) and transcardially perfused with 0.9% saline. Brains were removed and fixed in phosphate-buffered 4% paraformaldehyde, pH 7.4, at 4 °C for 48 h before they were sunk through 30% sucrose for cryoprotection. Brains were then sectioned coronally at 40 µm with a cryomicrotome (Leica Camera) and stored in cryoprotective medium. Primary antibodies were: goat anti-Dcx (1:500; Santa Cruz Biotechnology), rat anti-BrdU (1:5,000; Accurate Chemical and Scientific Corp.), goat anti-Sox2 (1:200; Santa Cruz), mouse anti-NeuN (1:1,000; Chemicon), mouse anti-GFAP (1:1,500; DAKO) and mouse anti-CD68 (1:50; Serotec). After overnight incubation, primary antibody staining was revealed using biotinylated secondary antibodies and the ABC kit (Vector) with diaminobenzidine (DAB; Sigma-Aldrich) or fluorescence-conjugated secondary antibodies. For BrdU labelling, brain sections were pre-treated with 2 N HCl at 37 °C for 30 min before incubation with primary antibody. For double-label immunofluorescence of BrdU/NeuN or BrdU/GFAP, sections were incubated overnight with rat anti-BrdU, rinsed and incubated for 1 h with donkey anti-rat antibody (2.5 µg ml^{-1}; Vector) before they were stained with mouse anti-NeuN antibody. To estimate the total number of Dcx- or Sox2-positive cells per dentate gyrus immunopositive cells in the granule cell and subgranular cell layer of the dentate gyrus were counted in every sixth coronal hemibrain section through the hippocampus and multiplied by 12.

BrdU administration and quantification of BrdU-positive cells. 50 mg kg^{-1} of BrdU was injected intraperitoneally into mice once a day for 6 days, and mice were killed 28 days later or injected daily for 3 days before being killed. To estimate the total number of BrdU-positive cells in the brain, we performed DAB staining for BrdU on every sixth hemibrain section. The number of BrdU-positive cells in the granule cell and subgranular cell layer of the dentate gyrus were counted and multiplied by 12 to estimate the total number of BrdU-positive cells in the entire dentate gyrus. To determine the fate of dividing cells a total of 200 BrdU-positive cells across 4–6 sections per mouse were analysed by confocal microscopy for co-expression with NeuN and GFAP. The number of double-positive cells was expressed as a percentage of BrdU-positive cells.

Parabiosis and flow cytometry. Parabiosis surgery followed previously described procedures[19]. Pairs of mice were anaesthetized and prepared for surgery. Mirror-image incisions at the left and right flanks, respectively, were made through the skin. Shorter incisions were made through the abdominal wall. The peritoneal openings of the adjacent parabionts were sutured together. Elbow and knee joints from each parabiont were sutured together and the skin of each mouse was stapled (9-mm autoclip, Clay Adams) to the skin of the adjacent parabiont. Each mouse was injected subcutaneously with Baytril antibiotic and Buprenex as directed for pain and monitored during recovery. Flow cytometric analysis was done on fixed and permeabilized blood plasma cells from GFP and non-GFP parabionts. Approximately 40–60% of cells in the blood of either parabiont were GFP-positive 2 weeks after parabiosis surgery. We observed 70–80% survival rate in parabionts 5 weeks after parabiosis surgery.

Extracellular electrophysiology. Acute hippocampal slices (400-µm thick) were prepared from unpaired and young parabionts. Slices were maintained in artificial cerebrospinal fluid (ACSF) continuously oxygenated with 5% CO_2/95% O_2. ACSF composition was as follows: (in mM): NaCl 124.0; KCl 2.5; KH_2PO_4 1.2; $CaCl_2$ 2.4; $MgSO_4$ 1.3; $NaHCO_3$ 26.0; glucose 10.0 (pH 7.4). Recordings were performed with an Axopatch- 2B amplifier and pClamp 10.2 software (Axon Instruments). Submerged slices were continuously perfused with oxygenated ACSF at a flow rate of 2 ml min^{-1} from a reservoir by gravity feeding. Field potential (population spikes and EPSPs) was recorded using glass microelectrodes filled with ACSF (resistance: 4–8 MΩ). Biphasic current pulses (0.2 ms duration for one phase, 0.4 ms in total) were delivered in 10-s intervals through a concentric bipolar stimulating electrode (FHC). No obvious synaptic depression or facilitation was observed with this frequency stimulation. To record field population spikes in the dentate gyrus, the recording electrode was placed in the lateral or medial side of the dorsal part of the dentate gyrus. The stimulating electrode was placed right above the hippocampal fissure to stimulate the perforant pathway fibres. Signals were filtered at 1 KHz and digitized at 10 KHz. Tetanic stimulation consisted of 2 trains of 100 pulses (0.4 ms pulse duration, 100 Hz) delivered with an inter-train interval of 5 s. The amplitude of the population spike was measured from the initial phase of the negative wave. Up to five consecutive traces were averaged for each measurement. Synaptic transmission was assessed by generating input–output curves, with stimulus strength adjusted to be ~30% of the maximum. LTP was calculated as mean percentage change in the amplitude of the population spike following high-frequency stimulation relative to its basal amplitude.

Contextual fear conditioning. The paradigm was done following previously published techniques[27]. In this task, mice learned to associate the environmental context (fear-conditioning chamber) with an aversive stimulus (mild foot shock; unconditioned stimulus (US)), enabling testing for hippocampal-dependent contextual fear conditioning. As contextual fear conditioning is hippocampus and amygdala dependent, the mild foot shock was paired with a light and tone cue (conditioned stimulus (CS)) in order to also assess amygdala-dependent cued fear conditioning. Conditioned fear was displayed as freezing behaviour. Specific training parameters are as follows: tone duration is 30 s; level is 70 dB, 2 kHz; shock duration is 2 s; intensity is 0.6 mA. This intensity is not painful and can easily be tolerated but will generate an unpleasant feeling. More specifically, on day 1 each mouse was placed in a fear-conditioning chamber and allowed to explore for 2 min before delivery of a 30 s tone (70 dB) ending with a 2 s foot shock (0.6 mA). Two minutes later, a second CS–US pair was delivered. On day 2 each mouse was first place in the fear-conditioning chamber containing the same exact context, but with no adminstration of a CS or foot shock. Freezing was analysed for 1–3 min. One hour later, the mice were placed in a new context containing a different odour, cleaning solution, floor texture, chamber walls and shape. Animals were allowed to explore for 2 min before being re-exposed to the CS. Freezing was analysed for 1–3 min. Freezing was measured using a FreezeScan video tracking system and software (Cleversys).

RAWM. Spatial learning and memory was assessed using the RAWM paradigm following the exact protocol described previously[26]. The goal arm location containing a platform remains constant throughout the training and testing phase, whereas the start arm is changed during each trial. On day 1 during the training phase, mice are trained for 15 trials, with trials alternating between a visible and hidden platform. On day 2 during the testing phase, mice are tested for 15 trials with a hidden platform. Entry into an incorrect arm is scored as an error, and errors are averaged over training blocks (three consecutive trials).

Cranial irradiation. Adult mice (8–12 weeks) were sham irradiated (controls) or irradiated at 5 Gy three times over 8 days using the Mark I gamma irradiator and killed at 8–10 weeks after irradiation to collect brains for immunohistochemical analyses. Each mouse was placed in a restrainer that was fitted into a slot in the lead brick shield so that the back of the skull was facing the source of radiation when positioned in the radiation chamber. The shield is constructed of lead bricks such that only the hippocampal/midbrain area was exposed to radiation. Calibration for 5 Gy radiation was done using nanoDot. Shielded areas were protected with an exposure rate ten times lower than the exposed area. RAWM studies were done on irradiated mice at least 6 weeks after the radiation procedure. This time frame ensured adequate recovery of the animals. All data were from 8 irradiated and 10 sham-irradiated mice.

Plasma collection and proteomic analysis. Mouse blood was collected from 400–500 young (2–3 months) and old (18–22 months) animals into EDTA-coated tubes via tail vein bleed, mandibular vein bleed, or intracardial bleed at time of death. EDTA plasma was generated by centrifugation of freshly collected blood and aliquots were stored at −80 °C until use. Human plasma and CSF samples were obtained from academic centres and subjects were chosen based on standardized inclusion and exclusion criteria as previously described[29,30]. The relative plasma concentrations of cytokines and signalling molecules were measured in human and mouse plasma samples using standard antibody-based multiplex immunoassays (Luminex) by either Rules Based Medicine, a fee-for-service provider, or by the Human Immune Monitoring Center at Stanford University. All Luminex measurements where obtained in a blinded fashion. All assays were developed and validated to Clinical Laboratory Standards Institute (formerly NCCLS) guidelines based upon the principles of immunoassay as described by the manufacturers.

CCL11, MSCF, antibody, or plasma administration. Carrier-free recombinant murine CCL11 dissolved in PBS (10 µg kg^{-1}; R&D Systems), carrier-free recombinant MCSF dissolved in PBS (10 µg kg^{-1}; Biogen), rat IgG2a neutralizing antibody against mouse CCL11 (50 µg kg^{-1}; R&D Systems, clone 42285), and isotype-matched control rat IgG2a recommended by the manufacturer (R&D Systems, clone 54447) were administered systemically via intraperitoneal injection over ten days on day 1, 4, 7 and 10. The same reagents (0.50 µl; 0.1 µg µl^{-1}) were also administered stereotaxically into the dentate gyrus of the hippocampus in some experiments (coordinates from bregma: $A = -2.0$ mm and $L = -1.8$ mm; from brain surface: $H = -2.0$ mm). Pooled mouse serum or plasma was collected from young (2–3 months) mice and old (18–20 months) mice by intracardial bleed at time of death. Serum was prepared from clotted blood collected without anticoagulants;