Supporting Information

Creer et al. 10.1073/pnas.0911725107

Training and testing of aged mice on the intermediate pattern separation task

Subjects. Eight 22-month-old male C57/BL6 mice (Jackson Laboratories) were individually housed in a temperature-controlled (22 °C) room with a 12-h light/dark cycle (lights on 7:00 AM to 7:00 PM). Mice were given ad libitum access to water and were food-deprived to 85% free feeding weight throughout the study.

Similar to the adult mice, the aged animals completed Pavlovian training conditioning in which a white square stimulus was presented randomly in one of five possible locations on the screen (all locations were on the bottom of the screen and were aligned horizontally). Each pellet delivery was combined with a tone. The ITI was 30,000 ms. The screen did not need to be touched, but if the mouse touched any part of the screen, including a nonlit area, a tone was generated and a pellet was given. Mice were given 30 automated Pavlovian training trials for 1 day.

Once Pavlovian training was complete, animals moved on to the "must touch" stage. Each trial began with the presentation of one white square stimulus displayed randomly in one of five possible locations. The mouse was rewarded for touching the white stimulus part of the screen; in such case, a tone was presented and a reward was given. A touch to a location that did not contain a square was incorrect; no tone was generated, no reward was given, and the stimulus remained on the screen until it was touched. The next trial began after the food pellet was eaten (i.e., when an exit from the pellet receptacle was detected). The ITI was 5,000 ms. Mice continued on to the next stage of training when they accomplished a criterion of completing 30 trials in 1 day.

Subsequently, in the "must initiate" stage, stimuli were presented in the same manner as in the previous stage. A reward was only given for correct stimulus touches. After reward collection, the animal was required to initiate the next trial by an additional nose poke to the pellet receptacle. Mice were given this training for 3 weeks and scored 43.03% (±1.01%). Given that the mice did not achieve the 70% correct criterion in this task, from this time on, they were tested in a modified version of the experiment. Specifically, mice were trained on the intermediate pattern separation task (Fig. 1*A*) for 10 days. Thereafter, mice were housed with running wheels, given a series of five daily BrdU injections, and then tested again on the same task for another 10 days as described below.

Spatial Reversal Task: Block 1, Before the Running. Animals were placed in the operant boxes with six-hole masks (Fig. 1*A*). White squares (the stimuli) were presented in two of the six possible locations, aligned in a row along the bottom of the screen. The two stimuli were in positions 2 and 5 if position 1 was the furthest left, and each position was numbered consecutively from left to right. Only one of the squares was reinforced with the tone and pellet, and the correct side was counterbalanced between mice. When the mouse had achieved the criterion of seven of eight trials correct (i.e., it had completed the acquisition phase), the reinforced stimulus was reversed (i.e., the previously incorrect location became the correct location, and vice versa). When the mouse completed this first reversal (seven of eight trials correct on the new side), the reward contingency was again reversed. This procedure continued for 60 trials or a maximum of eight reversals. On the next day, the mouse was presented with the same stimuli, and the correct side was the same as the side that was last correct in the previous session; that is, if a mouse started with position 5 correct and achieved acquisition and completed two reversals to criterion (the session therefore ending during its third reversal), it would start with position 2 being the correct stimulus on the next day. Mice were given one block of 10 days of the spatial reversal task before starting voluntary exercise.

Exercise and BrdU Injections. On completion of the first block of 10 days of the spatial reversal task, animals were divided into two groups: control (CON, $n = 4$) and exercise (RUN, $n = 4$) and running wheels were added to the cages of the exercise group. One day thereafter, mice were given daily i.p. injections of BrdU (50 mg/kg) over 5 consecutive days. After the injections, mice were given 2 days before further testing.

Spatial Reversal Task: Block 2. Mice were given the same task as described above for the spatial reversal task, with intermediate separation between stimuli, for 10 days. Mice were perfused 17 days after the onset of running.

Histology/Immunohistochemistry

Animals were deeply anesthetized with sodium pentobarbital (Merial) and perfused transcardially with 0.9% NaCl solution, followed by 4% (wt/vol) paraformaldehyde (PFA) in 0.1 M phosphate buffer, pH 7.4. Brains were postfixed in 4% PFA for 3 days, followed by equilibration in 30% (wt/vol) sucrose. Tissue was sectioned coronally (40 μm) on a freezing microtome (ThermoFisher) and stored at −20 °C in cryoprotectant solution.

Quantity and Phenotype of Newly Born Cells

Immunohistochemistry for BrdU and immunofluorescent double-labeling for BrdU and NeuN were performed on free-floating 40-μm coronal sections. The antibodies used were rat anti-BrdU (Accurate; Harlan Sera-Lab) at a 1:100 ratio and mouse anti-NeuN (Millipore) at a 1:100 ratio. To determine the number of BrdU-labeled cells, staining for BrdU with the peroxidase method was used (ABC system, with biotinylated donkey anti-mouse antibodies and diaminobenzidine as a chromogen; Vector Laboratories). The fluorescent secondary antibodies used were donkey anti-mouse Cy3 (Jackson ImmunoResearch) and anti-rat Alexa-Fluor 488 (Invitrogen) at a concentration of 4 μL/mL.

A one-in-six series of adjacent sections was stained with DAPI [1 μL per 10 mL of Tris-buffered saline (TBS) for 10 min] to visualize the nuclei and to measure granule cell layer volume. The granule cell area was traced using a semiautomatic stereology system (StereoInvestigator; MicroBrightfield) and a 20× objective (BX51; Olympus). The granule cell reference volume was determined by summing the traced granule cell areas for each section multiplied by the distance between sections sampled. The number of BrdU-labeled cells was then related to granule cell layer sectional volume and multiplied by the reference volume to estimate total number and density of BrdU-positive cells.

Vasculature

Lectin staining (*Lycopersicon esculentum*; Vector) was used to visualize hippocampal blood vessels. A 1-in-12 series of sections was rinsed with TBS and then blocked for 30 min at room temperature in a solution of TBS containing 5% (vol/vol) preimmune donkey serum and 0.3% Triton-X100 (TBS^{++}). Sections were incubated at 4 °C for 72 h with biotinylated lectin diluted at a ratio of 1:4,000 in TBS^{++}. Thereafter, sections were rinsed with TBS and incubated for 3 h in streptavidin Cy3 (1:500 ratio), followed by DAPI for 10 min.

Statistical Analysis

The number of days it took for the sedentary and running mice to reach the training criterion and histological data were analyzed with a two-tailed *t* test comparison of two independent samples. In the adult mice, probe trial data for acquisition were compared between groups using repeated-measures ANOVA (group × separation). Reversal data were analyzed using ANOVA with repeated measures at each separation (group × session). In aged animals, both acquisition and reversal data were analyzed over blocks of 10 days before and after the onset of running (group × block). Fisher's posthoc two-tailed independent sample *t* tests were used to compare performance within sessions or blocks. Regression analysis was applied to determine whether there was a correlation between newly born neuron number and behavioral performance. All statistical analyses were carried out using Statview (Abacus Corporation).

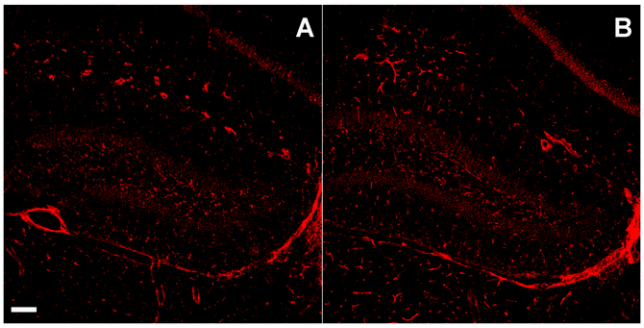

Fig. S1. In adult mice, lectin-stained vessel density was quantified using imaging software (Slidebook; Intelligent Imaging Innovations Inc.). Dentate gyrus angiogenesis, as measured by the area covered with lectin stained vessels in the dentate gyrus including the molecular layer, was significantly increased ($P < 0.05$) in running mice (*B*) compared with controls (*A*). (Scale bar: 100 μm.)