Enhanced Hippocampal Long-Term Potentiation and Spatial Learning in Aged 11β-Hydroxysteroid Dehydrogenase Type 1 Knock-Out Mice

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Glucocorticoids are pivotal in the maintenance of memory and cognitive functions as well as other essential physiological processes including energy metabolism, stress responses, and cell proliferation. Normal aging in both rodents and humans is often characterized by elevated glucocorticoid levels that correlate with hippocampus-dependent memory impairments. 11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) amplifies local intracellular (“intracrine”) glucocorticoid action; in the brain it is highly expressed in the hippocampus. We investigated whether the impact of 11β-HSD1 deficiency in knock-out mice (congenic on C57BL/6) strain on cognitive function with aging reflects direct CNS or indirect effects of altered peripheral insulin-glucose metabolism. Spatial learning and memory was enhanced in 12 month “middle-aged” and 24 month “aged” 11β-HSD1/−/− mice compared with age-matched congenic controls. These effects were not caused by alterations in other cognitive (working memory in a spontaneous alternation task) or affective domains (anxiety-related behaviors), to changes in plasma corticosterone or glucose levels, or to altered age-related pathologies in 11β-HSD1/−/− mice. Young 11β-HSD1/−/− mice showed significantly increased newborn cell proliferation in the dentate gyrus, but this was not maintained into aging. Long-term potentiation was significantly enhanced in subfield CA1 of hippocampal slices from aged 11β-HSD1/−/− mice. These data suggest that 11β-HSD1 deficiency enhances synaptic potentiation in the aged hippocampus and this may underlie the better maintenance of learning and memory with aging, which occurs in the absence of increased neurogenesis.

Key words: glucocorticoids; memory; ageing; neurogenesis; LTP; hippocampus

Introduction

Deficits in cognitive function are prominent although not inevitable features of normal aging. In aged animals and humans, interindividual differences in cognitive function associate with variation in plasma glucocorticoid (GC) levels (Issa et al., 1990; Yau et al., 1995; Lupien et al., 1998). Chronic GC excess is detrimental to the hippocampus, potentiating excitatory neurotransmission, disrupting key electrophysiological functions such as long-term potentiation (LTP) thought to underpin memory, causing dendritic atrophy and sometimes even neuronal death (McEwen et al., 1999). Conversely, maintaining low GC levels throughout life minimizes age-related hippocampal atrophy and cognitive impairments (Landfield et al., 1981; Meaney et al., 1988).

Previous findings suggest that GC action depends not only on circulating hormone levels and the density of intracellular mineralocorticoid (MR) and glucocorticoid (GR) receptors, but also on prereceptor metabolism by intracellular enzymes (Seckl and Walker, 2001), specifically the 11β-hydroxysteroid dehydrogenases (11β-HSDs). 11β-HSD type 2, a high-affinity 11β-dehydrogenase, catalyzes rapid inactivation of GCs, acting to exclude GCs from otherwise nonselective MR in the distal nephron. 11β-HSD2 is barely expressed in the adult CNS. In contrast, 11β-HSD type 1 is highly expressed in liver and adipose tissue and is the major isozyme in the adult brain in rodents (Moisan et al., 1990) and humans (Sandep et al., 2004). Although 11β-HSD1 is bidirectional in tissue homogenates, in intact cells, including neurons, 11β-HSD1 is a reductase, catalyzing regeneration of active GCs [cortisol in humans; corticosterone (CORT) in rodents] from inert circulating 11-keto forms [cortisone; 11-dehydrocorticosterone (11-DHC)], themselves produced by renal 11β-HSD2. Thus, 11β-HSD1 locally amplifies GC action (Seckl and Walker, 2001). The directional predominance of 11β-HSD1 is believed driven by proximity to hexose-6-phosphate...
dehydrogenase in the endoplasmic reticulum, which generates plentiful NADPH cofactor for 11β reduction (Bujalska et al., 2005). Primary hippocampal neuronal cultures express 11β-HSD1; 11-DHC (through its conversion to CORT) potentiates excitatory amino acid neurotoxicity in these cells, and inhibition of 11β-HSD attenuates such cell endangerment (Rajan et al., 1996).

In vivo, 11β-HSD1 knock-out (11β-HSD1−/−) mice resist metabolic disturbances such as hyperglycemia with dietary obesity, effects compatible with reduced intrahepatic and adipose GC metabolism disturbances such as hyperglycemia with dietary obesity. For the studies described here, the outbred 1997), were backcrossed to the outbred MF1 background for two generations. The outbred MF1 background is not perfectly isogenic, but has been shown to be a highly reproducible background for long-term studies (Kotelevtsev et al., 1997; Morton et al., 2004). 11β-HSD1−/− mice also have lowered intrahippocampal CORT levels (Yau et al., 2001). The original 11β-HSD1−/− mice on the 129P2-OlaHsd background cannot perform the conventional hidden platform water maze task, but show less age-related deficits in a simplified flag-cued version of the water maze (Yau et al., 2001). Although compatible with the notion that reduced 11β-HSD1 activity is beneficial, this finding leaves open the question as to whether this is a cognitive or perceptual effect. Intriguingly, the 11β-HSD inhibitor carbenoxolone improves aspects of cognitive function in humans (Sandep et al., 2004). Whether these effects in mice and humans reflect direct or indirect (e.g., metabolic) actions on the brain is uncertain. We measured hippocampus-dependent cognitive function in aged 11β-HSD1−/− mice reared on the C57BL/6J background and examined whether the underlying mechanisms for the improved cognitive phenotype with age was centrally mediated (LTP and neurogenesis in the aged hippocampus) and/or an indirect consequence of changes in peripheral GC levels or glucose homeostasis.

Materials and Methods

Animals

11β-HSD1−/− mice, originally generated by targeted disruption of the 11β-HSD1 gene on the 129P2/OlaHsd background (Kotelevtsev et al., 1997), were backcrossed to the outbred MF1 background for two generations. For the studies described here, the outbred 11β-HSD1−/− mice with approximately equal amounts of 129 and 129 genomes were transferred to the C57BL/6J strain (Harlan, Bicester, UK) by continuous backcross for 12 generations. After this extensive backcross, the congenic strain generated is 99.9% genetically identical to the parental strain and, therefore, age-matched C57BL/6J mice were used as controls. 11β-HSD1−/− mice were crossed every week with wild-type C57BL/6J mice to prevent genetic drift. The heterozygous mice were then bred with homozygous knock-outs for the following year’s experimental crosses. The experimental animals were obtained from homozygous knock-out matings (no more than three crosses). C57BL/6J control mice were obtained at 6–8 weeks old from Harlan and left undisturbed to age in our facility alongside the 11β-HSD1−/− mice housed under standard conditions on a 12 h light/dark cycle (lights on at 7:00 A.M.), with food and water ad libitum. The C57BL/6J genetic background was chosen because this strain is recommended for water maze testing (Crawley, 1999). All procedures were performed in strict accordance with the U.K. Animals (Scientific Procedures) Act, 1986. Young mice were 3–5 months of age, middle-aged mice were 12 months of age, and aged mice were 24–26 months of age.

Behavioral studies

Water maze. Young (5 months) and aged (24–25 months) 11β-HSD1−/− mice and C57BL/6J controls were tested in the Morris water maze (120 cm diameter) filled with opaque water (25 ± 1°C). Mice were trained to swim to a 15 cm diameter hidden platform located in the center of one of four quadrants of the pool 1 cm below the water surface. This task is one of the most widely used tests of spatial memory in mice, despite the fact that interpretation can sometimes be difficult as a consequence of nonspatial factors (e.g., wall hugging, floating, jumping off platforms) found in many mouse strains (Wahlsten et al., 2005). However, C57BL/6J mice are among the best performers in the water maze and many mice can be tested in a day in contrast to other spatial memory tasks, such as the radial arm maze. First, the mice were given 2 d of nonspatial training (three trials per day, with curtains around pool) to find the submerged platform marked with a visible tower block protruding 10 cm on top to test for visual, motivational, or motor deficits that may influence their performance in spatial learning. Mice unable to reach the platform within 30 s on day 2 were excluded. Spatial learning was tested next with no curtains around the pool during which mice had to navigate toward the hidden platform using the available spatial cues in the room. The mice were given 16 trials (20 min intertrial interval) over four consecutive days. Trials started with the mouse placed into the pool facing the wall at one of four start locations chosen randomly across trials and allowed to swim until they found the platform. Any mouse that failed to find the platform within 90 s was guided onto the platform by the experimenter. The animal is then left on the platform for 30 s before being returned to its home cage and dried under a warming lamp. One hour after the last training trial, a probe test was performed with the platform removed from the pool and the mice allowed to swim freely for 60 s. Swim paths were monitored by a video camera mounted in the ceiling. Measures of latency, swim speed, and percentage time in each quadrant of the pool during the probe trial were analyzed by Watermaze software (Actimetriks, Evanston, IL).

Y-maze. A new group of young (3 months), middle-aged (12 months), and aged (24 months) 11β-HSD1−/− mice and C57BL/6J controls were tested in the Y-maze (apparatus consisted of three enclosed arms, 50 cm long, 1 cm wide, and 10 cm high made of black Plexiglas, set at an angle of 120° to each other, in the shape of a Y). The floor of the maze was covered in coarse-soiled sawdust mixed between trials to eliminate olfactory cues. Visual cues were placed around the maze in the testing room and kept constant throughout the testing sessions. The test consisted of two trials separated by a time interval (intertrial interval (ITI)). Briefly, in the first trial (acquisition trial), mice were placed at the end of a pseudo-randomly chosen arm (start arm) and allowed to explore the maze for 5 min with one of the arms closed. Mice were returned to their home cage located away from the test room during the intertrial interval. In the second trial (retention trial), the mice were allowed to explore freely all three arms of the maze for 5 min. The time spent in each arm was registered from video recordings by an observer blind to the genotype of the mice. The time spent in the novel arm (previously closed in the first trial) was calculated as a percentage of the total time in all three arms during the first 2 min of the retention trial. This time corresponds to the maximal exploratory activity in the novel arm, which declines thereafter (Dellu et al., 1992; Conrad et al., 1999). Values were compared with a random level (chance level) for time spent exploring the three arms (i.e., 33%/13%13%13% for a novel vs. a familiar arm). The mice were given 3 min ITI before the beginning of the second trial to allow for a complete exploration of the maze and also to test that the mice (particularly the aged mice) are able to see the spatial cues. Mice were retested 7 d later to measure spatial memory performance with a 2 h ITI. Middle-aged mice were retested 1 year later, when 24 months of age, in the same maze but in a different environment.

Working memory: spontaneous alternation. All three arms of the Y-maze were left open and the animal was introduced at the center of the maze with the facing direction randomized. The number and sequence of arms entered were recorded for 5 min. The percent alternation was calculated as the number of alternations (entries into three different arms consecutively) divided by the total possible alternations (i.e., the number of arms entered minus 2) and multiplied by 100.

Elevated plus maze and open field. After the Y-maze, the same 11β-HSD1−/− and control mice (24–25 months) were tested in the elevated plus maze and then in the open field. All mice were handled briefly in the testing room daily 5 d before testing in the apparatus. The plus maze consisted of two open arms (25 × 5 cm) and two closed arms (25 × 5 cm, surrounded by 15 cm high walls made of pearl translucent acrylic). The base of the maze was made of black opaque acrylic and the two pairs of identical arms, which emerged from a central platform (5 × 5 cm), positioned opposite each other; the maze was supported 1 m above the ground. The test was initiated by placing the mouse on the central platform of the maze facing one of the open arms and letting it explore freely.
for 5 min. The maze floor and walls were cleaned with alcohol between tests. A video camera above the apparatus recorded the behavior of each mouse.

The open-field apparatus was a square open box constructed from black acrylonitrile butadiene styrene (50 × 50 × 15 cm) and the floor was separated into 36 equal squares with adhesive tape. At the start of the test, a mouse was put in one corner of the open field and allowed to explore freely for 5 min, during which its behavior was monitored with a video camera. After each session, the open-field box was wiped with ethanol to remove any olfactory cues. Parameters measured from the video were: time spent grooming, rearing, and defecation.

Fasted plasma glucose and insulin levels
Naïve young (5 months) and aged (26 months) 11ß-HSD1−/− and C57BL/6J control mice were fasted overnight (16 h). Blood samples were taken by tail venesection into EDTA microtubes (Sarstedt, Leicester, UK) after the mice were awake. The open-field box was wiped with ethanol to remove any olfactory cues. Plasma glucose was measured with an Infinity Glucose kit (Thermo Scientific, Bucks, UK) at 0 min (just before glucose injection) and at 5, 15, 30, 60, 120, 180, and 240 min after glucose injection. Blood samples were taken by tail venesection into EDTA microtubes (Sarstedt, Leicester, UK) at 0 min (just before glucose injection) and at 5, 15, 30, 60, and 120 min intervals after the glucose load. Plasma glucose was measured with an Infinity Glucose kit (Thermo Scientific, Bucks, UK) at 5, 15, 30, 60, and 120 min after glucose injection.

Glucose tolerance test
In a separate experiment, naïve 11ß-HSD1−/− and C57BL/6J mice aged 26 months were fasted overnight and injected intraperitoneally with 2 mg/g body weight d-glucose (25% stock solution in saline). Blood samples were taken by tail venesection into EDTA microtubes (Sarstedt, Leicester, UK) at 0 min (just before glucose injection) and at 5, 15, 30, 60, and 120 min intervals after the glucose load. Plasma glucose was measured with an Infinity Glucose kit (Thermo Scientific, Bucks, UK) at 5, 15, 30, 60, and 120 min after glucose injection.

CORT response to an acute restraint stress
Basal morning tail venesection blood samples were taken from naïve aged (26 months) 11ß-HSD1−/− mice and age-matched C57BL/6J controls. On the following morning, the mice were put in a restraint tube for 10 min each and blood sample was taken by tail venesection immediately after restraint, 45 min and 90 min later.

Basal plasma CORT measurements
Blood samples were taken by tail venesection (≤50 μl) from the aged (26 months) and young (5 months) C57BL/6J controls and 11ß-HSD1−/− mice in the morning. Plasma CORT was measured using an in-house specific radioimmunoassay (AI Dujaili et al., 1981) modified for microtiter plate scintillation proximity assay (GE Healthcare UK, Little Chalfont, UK). The intra-assay and interassay coefficients of variation were <10%. CORT antiserum was kindly donated by Dr C. Kenyon (Edinburgh, UK).

Electrophysiology
11ß-HSD1−/− mice and age-matched C57BL/6J controls were 26 months of age when electrophysiological recordings were performed. After cervical dislocation between 10:00 A.M. and 12:00 P.M., slices of mouse hippocampus (400 μm thick) cut in the parasagittal axis using a Campden vibriscallicer were maintained at 32°C in an interface-type recording chamber containing artificial CSF, pH 7.4, containing (in mM) 124 NaCl, 3 KCl, 26 NaHCO3, 1.25 NaH2PO4, 1 MgSO4, 2 CaCl2, and 10 glucose, and allowed to equilibrate for 60–90 min. The solution was bubbled with 95% O2 and 5% CO2 and perfused at a flow rate of 1.5–2 ml/min. Two bipolar stimulation electrodes were placed in the stratum radiatum on either side of the recording electrode in area CA1. After stimulation of fibers in the Schaffer collateral–commissural pathway, field EPSPs (fEPSPs) were recorded using a tungsten recording electrode. Two pathways were stimulated alternately every 60 s and the strength of presynaptic fiber stimulation every 60 s for each pathway was adjusted to evoke fEPSPs that were 50% of the maximal fEPSPs slope. After a stable baseline (20–30 min), the first pathway was stimulated with a single 100 Hz train for 1 s for the induction of LTP. Synaptic responsiveness was recorded every 30 s for a duration of 30 min after tetanus. The second unstimulated pathway was used as a control for baseline EPSP recordings. Custom software (written by Patrick Spooner, Edinburgh University, UK) was used to control stimulation, record signals, and for analysis. The system used National Instruments (Austin, TX) acquisition hardware (M or E series) combined with Digitimer (Welwyn Garden City, UK) Neurolog equipment and the software was written in Labview.

Bromodeoxyuridine injections and immunohistochemistry
To assess levels of cell proliferation in the dentate gyrus, a separate cohort of young (5 months) and aged (22–25 months) 11ß-HSD1−/− mice and age-matched C57BL/6J controls received intraperitoneal injections of bromodeoxyuridine (BrdU; Sigma, St. Louis, MO) at 50 mg/kg body weight, one per day for 8 d. On the final day, 2 h after the last injection, mice were perfused transcardially with 4% paraformaldehyde in phosphate buffer. The brains were removed, stored in paraformaldehyde overnight at 4°C, and then transferred into 20% sucrose. The cryoprotected brains were sectioned serially at 40 μm through the hippocampus (~1.22 to −2.46 mm from bregma) in the coronal plane using a freezing microtome and each section was collected and stored individually in 24-well tissue culture plates (Corning, Corning, NY) containing cryoprotectant (30% ethylene glycol, 20% glycerol, 50% sodium phosphate buffer, pH 7.4) at −20°C. The immunohistochemical detection of BrdU-positive cells was performed using an avidin–biotin peroxidase method with sections being mounted every sixth section (240 μm apart) starting at level of the hippocampus, where the two horns of the dentate gyrus separate (~1.34 mm from bregma). Free-floating sections were equilibrated in Tris-buffered saline (TBS), pH 7.6, for 5 min, incubated in citrate buffer, pH 6, for 15 min at 80°C, and treated with 3% hydrogen peroxide for 10 min to block endogenous peroxidases. The sections were then equilibrated in borate buffer, pH 8.5, for 15 min followed by 2 × 5 min washes in TBS, and incubated in 2% hydrogenic chloride for 30 min at 37°C to denature DNA. Non-specific binding sites were blocked in 20% normal rabbit serum (Vector Laboratories, Burlingame, CA) and 5% bovine serum albumen in TBS for 1 h at room temperature (RT). This was followed by incubation overnight at 4°C in primary antibody (sheep anti-BrdU, 1:3000; Europa Bioproducts, Cambridge, UK). Sections were then incubated at RT for 1 h in secondary biotinated antibody (anti-sheep IgG, 1:500; Vector Laboratories). Immunopositive cells were visualized by incubation in the avidin biotinylated horseradish peroxidase solution (Vectastain ABC Elite kit) for 30 min at RT and developed in diaminobenzidine liquid (Dako, High Wycombe, UK). Sections were mounted onto microscope slides, counterstained with hematoxylin (Vector Laboratories), dehydrated through graded alcohols, cleared in xylene, and mounted in dioxyrene plasticizer xylene mountant. Nonspecific staining was assessed in the absence of primary antibody. A section from each brain containing the subventricular zone of the lateral ventricles was included as a positive control.

Quantification of BrdU-immunoreactive cells
All slides were randomized and coded before quantitative analysis. BrdU-positive cells were counted bilaterally on every sixth section through the dentate gyrus (nine sections per animal) through a 40× objective using a light microscope. Only cells on the border of the subgranular zone (SGZ) and hilus (cells located two or more cells away from the SGZ) were counted. The total number of BrdU-positive cells was estimated according to the optical dissector method (West, 1993). BrdU-positive cells in all focal planes through the 40 μm section (except the uppermost focal plane) were included. To estimate the total number of BrdU-labeled cells in the hippocampus, the number of BrdU-positive cells counted in the SGZ and hilus was multiplied by a factor of 6.

Survival curve and pathology
Cohorts of 11ß-HSD1−/− mice (n = 134) and C57BL/6J controls (n = 142) were monitored on a daily basis from birth up to 127 weeks and any were deaths noted. The survival was not monitored further and remaining mice were used for experimentation. For the detailed pathology examination, the tissues had to be processed quickly, so mice that were found dead were not used. To overcome this, any mice that deviated from normal health were culled by CO2 euthanasia. The alimentary canal from the esophagus to rectum was dissected in one piece and then perfused with formalin by injecting at equal distances down the tract. The skull was opened and the brain left in situ. Then, the whole mouse was stored in 10% formalin at 4°C until enough mice were collected for histology. A total of four aged male 11ß-HSD1−/− (20–27 months) and 6 C57BL/6J

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control mice (23–28 months) were examined. Organs and tissues examined histologically were the stomach, small intestine, large intestine and caecum, adrenal glands, thyroid gland, mesentry, head including eyes, pituitary, trigeminal ganglion, olfactory and nasal, middle and inner ear, brain (three levels), lung, liver and gallbladder, kidneys, heart, spleen, pancreas, seminal vesicles, prostate gland, urinary bladder, testis, lymphosilvicial complex, dorsal skin, femur, and tibia (serial sections).

Data analysis
All data where appropriate were analyzed using two-factor ANOVA with genotype and age as the independent variables. Where ANOVA revealed a significant interaction between factors, post hoc tests were conducted. Comparison of synaptic responses was performed with ANOVA for repeated measures with time as the main variable. Significance was set up p < 0.05. Values are shown as the mean ± SEM.

Results
Aged 11β-HSD1−/− mice show better spatial learning in the water maze
Both young (5 months) and aged (24–25 months) 11β-HSD1−/− mice, congenic on the C57BL/6J background, and C57BL/6J controls learned the location of the hidden platform as shown by a decrease in escape latencies over days of training (F(3,102) = 29.2; p < 0.001) (Fig. 1A). The number of times the mice failed to find the platform within the 90 s over the 4 d of training did not differ between genotype, but did increase with age [F(1,31) = 22.9; p < 0.001; C57BL/6J mice, 0.6 ± 0.2 (young) and 3.5 ± 0.5 (aged); 11β-HSD1−/− mice, 0.6 ± 0.3 (young) and 2.2 ± 0.6 (aged), data are the number of times guided to the platform]. Young 11β-HSD1−/− and control mice had similar escape latencies despite the 11β-HSD1−/− mice displaying faster average swim speeds (F(1,14) = 12; p < 0.01) (Fig. 1B). Aged controls were significantly impaired (compared with young controls) in spatial learning, showing longer escape latencies for each day of testing (F(1,12) = 47; p < 0.001) (Fig. 1A). Aged 11β-HSD1−/− mice were better at finding the hidden platform than aged controls across the days of training (F(1,12) = 4.6; p < 0.05) with shorter mean latencies, which reached significance on day 4 (F(1,18) = 8.5; p < 0.01). At this time, aged 11β-HSD1−/− mice did not differ from young mice of either genotype. The improved learning in aged 11β-HSD1−/− mice did not reflect differences in swim speed (Fig. 1B). Anxiety-related traits such as thigmotaxis or wall hugging in the water maze can also affect learning, but the extent of thigmotaxis in these mice was small and only apparent during the first day of training. Interestingly, 11β-HSD1−/− mice spent a greater percentage of time near the side walls than aged-matched controls during the first trial (F(1,11) = 6.0; p < 0.05) and there was an age effect [F(1,11) = 4.4; p < 0.05; C57BL/6J mice, 0.4 ± 0.3 (young) and 2.1 ± 0.6 (aged); 11β-HSD1−/− mice, 2.7 ± 1.0 (young) and 7.5 ± 3.0 (aged); data are the percentage of time near side walls during trial 1]. Consistent with the mean latencies, the latency for the initial trial of each day, which reflects recall after 24 h from the previous day, also showed better learning (shorter latencies) in aged 11β-HSD1−/− mice than aged controls (F(1,11) = 5.9; p < 0.05) (Fig. 1C). Because, aged controls and 11β-HSD1−/− mice had significantly slower swim speeds than young mice, the path lengths to the platform were analyzed to determine any influence of swim speeds on escape latency. The path lengths on the first and last days of testing showed a significant group effect (F(3,32) = 13.1; p < 0.001) and groups by days interaction (F(3,32) = 3.0; p < 0.05). Consistent with the mean latency and first trial latency findings, aged control mice showed impaired spatial learning with longer path lengths to the platform compared with young controls (F(1,17) = 16.9; p < 0.001) (Fig. 1D). In contrast, aged 11β-HSD1−/− mice showed similar path lengths to young mice of either genotype. To test the retention of the task, the platform was removed for a 60 s probe test 1 h after the last trial; there were no significant effects by age or genotype in the probe test (percentage of time in target quadrant; young control, 38 ± 4; young 11β-HSD1−/−, 38.9 ± 2.5; aged control, 36 ± 4.5; aged 11β-HSD1−/−, 32.5 ± 2.7). Thus, a lack of 11β-HSD1 in aged C57BL/6J mice enhances acquisition in the water maze task.

Spatial memory in the Y-maze is impaired with age in C57BL/6J controls but not in 11β-HSD1−/− mice
To determine whether the age-associated cognitive improvement in 11β-HSD1−/− mice in the water maze, which involves a component of stress, also pertains to a less-stressful cognitive modal-
ity, mice were tested in the Y-maze, a simple two-trial spatial memory task that involves spontaneous exploratory behavior of novel areas and presents little or no intrinsic stress and yet is sensitive to exogenous stress (Conrad et al., 1996) and chronic GC manipulations (Coburn-Litvak et al., 2003). To test for any group motivational differences, young (3 months), middle-aged (12 months), and aged (26 months) mice of both genotypes were tested using a short 1 min intertrial interval; this immediate Y-maze task controls the spontaneous novelty exploration of the mice when no retention is required and is not dependent on hippocampal function (Sarnyai et al., 2000). All groups spent more time in the novel arm than the previously visited arms (Fig. 2A). There was no effect of age or genotype, indicating a similar exploratory drive in all groups. The delayed version of the Y-maze, with a 2 h intertrial interval, was used to measure hippocampal-dependent memory (Sarnyai et al., 2000). Two-factor ANOVA revealed a significant genotype (F(1,60) = 12.6; p < 0.001) effect and interaction between age and genotype (F(2,60) = 3.6; p < 0.05). Both middle-aged and aged control mice showed impaired spatial memory, spending less time in the novel arm than young controls (Fig. 2B). In contrast, middle-aged and aged 11β-HSD1−/− mice...
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Figure 2. A, Similar performances of C57BL/6J controls (n = 10–14) and 11β-HSD1−/− mice (n = 10–12) in the Y maze 1 min after the first encounter with the maze. B, Impaired hippocampal learning and memory in old C57BL/6J (12 and 24 months) and young (3 months) 11β-HSD1−/− mice. *p < 0.05 compared with C57BL/6J (3 months) controls. **p < 0.01 compared with age-matched C57BL/6J controls.

maintained novel arm exploration at similar levels to young mice of either genotype (Fig. 2B). Thus 11β-HSD1−/− mice resist age-related decline in spatial memory retention in another test and the genotype difference is evident by middle-age.

Working memory was studied by measuring spontaneous alternation in the Y-maze. There was no significant effect of age or genotype on the percentage of alternation (data not shown). This suggests that although hippocampus-dependent spatial memory is impaired with age in control mice, nonhippocampal working memory is intact with age in both control and 11β-HSD1−/− mice.

Basal and post-stress plasma CORT levels in aged C57BL/6J control and 11β-HSD1−/− mice

Aged 11β-HSD1−/− mice had heavier adrenals than age-matched C57BL/6J controls (11β-HSD1−/−, 2.6 ± 0.2 mg, n = 8; control, 1.47 ± 0.08 mg, n = 10; F(1,17) = 42.2; p < 0.001), presumably reflecting the increased adrenal synthesis necessary to replace the substantial peripheral regeneration usually effected by 11β-HSD1 (Andrew et al., 2005). Basal morning plasma CORT levels were elevated with age (F(1,26) = 53.6; p < 0.001) (Fig. 3A). There was no difference by genotype in basal plasma CORT levels in the young mice, but the aged 11β-HSD1−/− mice showed a trend for increased CORT levels compared with aged C57BL/6J controls [p = 0.08 (Fig. 3A); F(1,7) = 8.1, p < 0.05, at time 0 before restraint stress (Fig. 3B)]. Although both genotypes exhibited a plasma CORT rise to 10 min restraint stress, levels did not differ at any time point after stress (Fig. 3B). Interestingly, whereas the CORT levels in aged C57BL/6J controls remained elevated at 90 min (p < 0.05 compared with 0 min, paired t test), suggesting impaired hypothalamic–pituitary–adrenal (HPA) feedback, levels were back to baseline (0 min) in the aged 11β-HSD1−/− mice by 45 min (p = 0.6, paired t test) (Fig. 3B).

Anxiety-related behaviors in aged C57BL/6J controls and 11β-HSD1−/− mice

Another potential confounding explanation for the differences with genotype and age in spatial memory tasks is anxiolytic inhibition of cognitive performance reminiscent of the reversal with antidepressant treatment of pseudodemnetia seen in elderly humans with severe affective disorders (Lantz and Buchalter, 2001). To explore this possibility, anxiety-related behaviors were examined in the elevated plus maze and open field. No significant differences were found in the time spent or entries into the arms of the elevated plus maze between the aged control and 11β-HSD1−/− mice, although both groups were more anxious than their young counterparts (70 and 63% less time in the open arms than young C57BL/6J, p < 0.05, and 11β-HSD1−/− mice, p < 0.01, respectively) (data not shown). Similarly, in the open field test, locomotion, rearing and grooming behavior, periods spent immobile, and defecation did not differ between aged 11β-HSD1−/− and control mice (data not shown). Thus, major anxiolytic effects are unlikely to explain the protection against cognitive decline with aging in 11β-HSD1−/− mice.

Lack of effect of 11β-HSD1 deficiency on glucose tolerance in aged C57BL/6J mice

Impaired glucose tolerance is linked with cognitive decline with aging (Convit et al., 2003). Moreover, variations in long-term glycaemic control within the normal range correlate with cognitive function in elderly humans (MallaUCullich et al., 2004), and young 11β-HSD1−/− mice resist hyperglycemia with obesity or stress (Kotelevtsev et al., 1997). However, neither fasting nor post
glucose load glucose levels differed between aged C57BL/6J controls and 11β-HSD1$^{-/-}$ mice (Fig. 4), and both genotypes of aged mice showed maintained glucose tolerance within the normal range for young adult C57BL/6J mice (Morton et al., 2004). Insulin resistance without glucose intolerance might also relate to cognitive changes with aging, although findings are discordant (Pagano et al., 1996; Convit, 2005). Fasting insulin levels increased with age ($F_{1,114} = 5.4; p < 0.05$) in control C57BL/6J mice but not in 11β-HSD1$^{-/-}$ mice (Fig. 4B). Aged 11β-HSD1$^{-/-}$ mice showed lower fasting insulin levels compared with aged C57BL/6J controls ($F_{1,11} = 6.8; p < 0.05$), and the ratio of fasting insulin/glucose, a measure of insulin sensitivity, suggests that 11β-HSD1$^{-/-}$ but not control C57BL/6J mice have significantly increased insulin sensitivity with aging ($F_{1,11} = 4.9; p = 0.05$) (Fig. 4C). Thus, differences in glucose homeostasis are also unlikely to account for the cognitive protection with aging in 11β-HSD1$^{-/-}$ mice, although their insulin-sensitized phenotype might be pertinent.

Survival and pathology of the aged 11β-HSD1$^{-/-}$ mice do not differ from C57BL/6J controls

Another possibility is that 11β-HSD1 deficiency might extend the lifespan or dramatically reduce disease occurrence such that aged 11β-HSD1$^{-/-}$ mice are functionally "younger" than chronologically similarly aged controls. To examine this, we allowed 142 C57BL/6J and 134 11β-HSD1$^{-/-}$ mice to age. Kaplan–Meier analyses suggested that loss of 11β-HSD1 did not affect the age-specific survival or longevity ($p = 0.2$ by log rank test), with both control and 11β-HSD1$^{-/-}$ mice [knock-out (KO)] showing 60% survival at 30 months of age (data not shown). Some mice (20–30 months) deviated from normal health and were therefore killed electively and analyzed by detailed histopathology. One control mouse had mild suppurative arthritis. All mice had perivascular lymphoplasmacytic infiltrates in the submandibular salivary gland. 11β-HSD1 deficiency did not appear to influence the severity or distribution of these infiltrates in the salivary glands. Other age-dependent lesions that were more variable and that did not appear to be influenced by 11β-HSD1 deficiency were liver atrophy (two control, one KO), fatty liver (one KO), hydronephrosis (one KO), hydrocephalus (one control), and mild polycystic liver (one control). GC excess causes osteoporosis and 11β-HSD1 has been proposed to influence bone density (Cooper et al., 2003). However, serial sections of decalcified femur, stifle joint, and tibia/fibula failed to reveal differences between aged control and 11β-HSD1$^{-/-}$ mice in cortical thickness, trabecular density, trabecular thickness, distribution of fat in distal tibia, or qualitative differences in stifle articular surfaces, broadly reflecting findings in young 11β-HSD1$^{-/-}$ mice (Justesen et al., 2004). Thus, there was no indication that 11β-HSD1 deficiency altered the general rate of senescence, mortality, or the rates of age-associated pathologies such as neoplasia, inflammation, or osteoporosis. There was also no indication that 11β-HSD1 deficiency altered the prevalence or distribution of age-associated neoplasms (e.g., follicular B-cell lymphomas or plasmacytoma) or altered the course or severity of age-dependent glomerulosclerosis.

LTP is enhanced in aged 11β-HSD1$^{-/-}$ mice

Given the foregoing, it is conceivable that there is a specific CNS effect of 11β-HSD1 deficiency on hippocampal function with aging. To test this, LTP was induced in the area CA1 of hippocampal slices of aging mice by a single 100 Hz tetanus stimulation (1 s duration), which mimics the physiological bursts of neuronal activity. Hippocampal slices from both genotypes developed significant LTP that was maintained over the 1 h time course of the experiment. The mean level of potentiation seen at 30 min in the aged C57BL/6J mice (~110% of the pretetanization baseline level) (Fig. 5) was much lower than typically observed in area CA1 from young adult C57BL/6J slices using the same induction protocol [144 ± 10%, reported by Nguyen et al. (2000)]. In contrast, aged 11β-HSD1$^{-/-}$ mice showed significantly increased LTP in CA1 compared with aged C57BL/6J control mice ($F_{1,23} = 4.79; p < 0.05$) (Fig. 5). The stable baseline EPSP in the control pathway indicates that the potentiation was input specific (Fig. 5).

Cell proliferation in the dentate gyrus of the hippocampus is increased in young but not aged 11β-HSD1$^{-/-}$ mice

Another possible explanation of maintenance of cognitive function in aged 11β-HSD1$^{-/-}$ mice might be increased neurogenesis itself under inhibitory control by GCs (Gould et al., 1990; Cameron and Gould, 1994). Young and aged C57BL/6J and 11β-HSD1$^{-/-}$ mice were given BrdU (50 mg/kg, i.p.) daily for 8 d to label dividing cells. There was a significant increase in cell proliferation in the SGZ, but not the hilus of young 11β-HSD1$^{-/-}$ mice compared with young C57BL/6J controls ($F_{1,13} = 9.4; p < 0.05$) (Fig. 6). With aging, the total number of BrdU-positive cells was drastically reduced in both genotypes in the SGZ ($F_{1,22} = 57.6; p < 0.001$) and hilus ($F_{1,22} = 33.2; p < 0.001$) of the dentate gyrus (Fig. 6). Although there was a significant age by genotype interaction in the SGZ ($F_{1,22} = 10.6; p < 0.01$), cell...
proliferation did not differ significantly in the aged mice by genotype in SGZ or hilus.

Discussion

Aged C57BL/6J mice developed cognitive deficits in the water maze and Y-maze, the latter apparent by middle age. In contrast, nonhippocampal tasks such as the immediate version of Y-maze and spontaneous alternation, a simple working memory task, were unaffected by aging. The age-related cognitive decline was markedly reduced in 11β-HSD1−/− mice, which also performed nonhippocampal tasks as well as controls. These findings build on our previous report of cognitive protection in aged humans with 11β-HSD inhibition (Sandeep et al., 2004) and studies in 11β-HSD1−/− mice on the 129 background, a strain not optimal for cognitive testing, which could only be trained to learn the proximally cued version of the water maze (Yau et al., 2001). Thus, 11β-HSD1 deficiency/inhibition protects against the "normal" hippocampus-associated cognitive decline with aging in mice and humans. The key question is how and specifically whether this is mediated in the CNS or via peripheral effects?

Aged 11β-HSD1−/− mice showed significant enhancement in LTP in CA1 compared with age-matched C57BL/6J controls. This indicates an important effect of 11β-HSD1 likely directly within the brain on hippocampal function (synaptic plasticity) and may reasonably be inferred to underlie, at least in part, the improved learning and memory in the aged 11β-HSD1−/− mice. The level of hippocampal LTP induced in the CA1 of the 26-month-old C57BL/6J mice in the present study was much lower than typically observed in young adult C57BL/6J mice (Nguyen et
al., 2000) suggesting a likely effect of age. Although some studies have reported a decrease in hippocampal LTP with age (Shankar et al., 1998; Riesenweig and Barnes, 2003), this has not been a ubiquitous finding (Deupree et al., 1993; Bach et al., 1999). Strain and/or age differences may contribute toward the discrepancies between studies. Whatever, the data show enhanced LTP in CA1 in slices from age-matched mice.

Hippocampal-associated cognitive functions (Issa et al., 1990; Bodnoff et al., 1995) and LTP (Pavlidis et al., 1993; Alfarez et al., 2003) are inhibited by chronically elevated plasma CORT levels. The enhanced LTP in aged 11β-HSD1−/− mice might be attributable to reduced intracellular CORT levels, as shown in hippocampus on the 129 background (Yau et al., 2001) and in peripheral tissues on C57BL/6J (Morton et al., 2004). It does not appear to be attributable to reduced peripheral CORT levels, indeed these tended to be raised basally in aged 11β-HSD1−/− mice, echoing previous findings in young animals on 129 and 129-MF1 strain backgrounds (Kotelevtsev et al., 1997; Harris et al., 2001; Yau and Sekel, 2001). In contrast, young 11β-HSD1−/− mice on C57BL/6J have “normal” basal CORT levels, presumably because of plastic changes in GR in feedback sites (R. Carter, J. R. Sekel, and M. C. Holmes, unpublished observation). The trend for increased basal CORT levels in the aged 11β-HSD1−/− mice suggest that this adaptation is not fully maintained with aging. Clearly reduced circulating CORT levels are not responsible for the cognitive and LTP effects of 11β-HSD1−/−. However, the greater recovery of CORT levels after stress in the aged 11β-HSD1−/− mice may contribute to their improved cognitive performance (Issa et al., 1990; Bizen et al., 2001).

Previous studies have highlighted the possible contribution of newborn neurons in the dentate gyrus, one of two regions in the adult mammalian brain that continues to produce new neurons in adulthood, to cognitive processes (Shors et al., 2002). Because stress and GCs inhibit the production of hippocampal granule cells (Cameron and Gould, 1994; Gould and Tanapat, 1999), and 11β-HSD1 is expressed in the dentate gyrus, the presumed reduction in intrahippocampal CORT levels throughout life in 11β-HSD1−/− mice might plausibly protect against the documented age-related decline in neurogenesis (Cameron and McKay, 1999; Drapeau et al., 2003). Indeed, young 11β-HSD1−/− mice had markedly increased hippocampal neurogenesis, compatible with lower intrahippocampal CORT levels. This might have long-lasting effects on hippocampal function because prenatal stress reduces hippocampal neurogenesis to old age (Lemaire et al., 2000). Although 11β-HSD1 is little expressed in the hippocampus until birth (Diaz et al., 1998), it is highly expressed postnatally (Moisan et al., 1992) and it is therefore conceivable that 11β-HSD1 deficiency associates with increased neurogenesis from early postnatal life. However, both genotypes showed markedly reduced newborn cell proliferation with age in the subgranular zone of the dentate gyrus, consistent with previous reports (Cameron and McKay, 1999; Heine et al., 2004), although the total number of newborn cells in aged 11β-HSD1−/− mice tended to be greater than aged controls. Approximately 50% of adult-born granule cells die within 1 month, the remaining mature granule neurons survive ~5 months (Dayer et al., 2003). Thus, the marked increase in cell proliferation in the dentate gyrus in young 11β-HSD1−/− mice is unlikely to have a significant impact on cell number in old age, although any effects in middle-age might contribute. But whether the newly born cells actually mature into functional neurons requires additional investigation by examination of their survival and differentiation. It is noteworthy that although previous studies indicate that newborn cells can help
pears to make a physiologically significant impact on CORT actions within the hippocampus in vivo, most notably with aging. Note, however, that our results do not control for possible maternal effects because the 11β-HSD1+/− mice and C57BL/6J controls were not littermates. Previous studies have shown that differences in maternal care in rats can influence the development of behavior and endocrine responses to stress in the adult offspring (Liu et al., 1997; Francis et al., 1999; Liu et al., 2000). Whether the 11β-HSD1 mutation affects maternal behavior is not known. Although we cannot exclude potential effects of differences in maternal behavior, our data would suggest a predominant genetic effect because (1) the improved cognitive phenotype has been shown previously on a different genetic background (Yau et al., 2001); (2) although maternal effects tend to influence the adult offspring behavior (Liu et al., 2000; Francis et al., 2003) and this may persist to old age (Meany et al., 1988), in our study we only see the improved cognition in aged 11β-HSD1+/− mice and not in young 11β-HSD1+/− mice; and (3) at least in our hands, in 11β-HSD2−/− mice similarly congenic on C57BL/6J and which lack the fetoplacental “barrier” enzyme to maternal GCs during prenatal development, we see persisting (programming) effects on the offspring’s affective but not cognitive function (Holmes et al., 2006) (Holmes and Seckl, unpublished observation), effects similar, although not identical, in both littersmates and the separately mated inbred lines. Why 11β-HSD1 should regenerate potentially damaging GCs in the hippocampus is obscure. Perhaps 11β-reductase activity acts to help maintain constitutive GC-sensitive cellular functions when circulating levels are low, such as during the circadian nadir, or as an extra “booster” on stress. Interestingly, 11β-HSD1 is increased in the hippocampus in late gestation, perhaps increasing GCs for neuronal and glial maturational events (Wan et al., 2002). The damaging effects may only emerge when there is a chronic increase in 11β-reductase activity and poor HPA feedback, such as might occur during aging or disease, presumably beyond an age when selectional pressures act. Whatever the evolutionary context, selective 11β-HSD1 inhibitors may be therapeutically useful in age-related cognitive impairments.

References
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