Edinburgh Research Explorer

Forebrain-specific transgene rescue of 11-HSD1 associates with impaired spatial memory and reduced hippocampal BDNF mRNA levels in aged 11-HSD1 deficient mice

Forebrain-specific transgene rescue of 11β-HSD1 associates with impaired spatial memory and reduced hippocampal BDNF mRNA levels in aged 11β-HSD1 deficient mice

Sarah Caughey[1, 2], Anjanette P. Harris[1, 2], Jonathan R. Seckl[1, 2], Megan C. Holmes[1, 2] and Joyce L.W. Yau[1, 2]

[1]UoE/BHF Centre for Cardiovascular Science and [2]Centre for Cognitive Ageing and Cognitive Epidemiology, University of Edinburgh, UK

Corresponding author:

Dr Joyce LW Yau,

UoE/BHF Centre for Cardiovascular Science,

Queen's Medical Research Institute,

47 Little France Crescent,

Edinburgh EH16 4TJ, UK.

Tel: +44 (0) 131 242 6760

Fax: +44 (0) 131 242 6779

E-mail: Joyce.Yau@ed.ac.uk

Running title: Brain 'rescue' of 11β-HSD1 in global knockout mice

Abstract

Mice lacking the intracellular glucocorticoid-regenerating enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) are protected from age-related spatial memory deficits. 11β-HSD1 is expressed predominantly in brain, liver and adipose tissue. Reduced glucocorticoid levels in brain in the absence of 11β-HSD1 may underlie the improved memory in aged 11β-HSD1 deficient mice. However, the improved glucose tolerance, insulin sensitization and cardio-protective lipid profile associated with reduced peripheral glucocorticoid regeneration may potentially contribute to the cognitive phenotype of aged 11β-HSD1 deficient mice. In this study, transgenic mice with forebrain-specific overexpression of 11β-HSD1 (Tg) were inter-crossed with global 11β-HSD1 knockout mice (HSD1KO) to examine the influence of forebrain and peripheral 11β-HSD1 activity on spatial memory in aged mice. Transgene-mediated delivery of 11β-HSD1 to the hippocampus and cortex of aged HSD1KO mice reversed the improved spatial memory retention in the Y-maze but not spatial learning in the watermaze. Brain-derived neurotrophic factor (BDNF) mRNA levels in the hippocampus of aged HSD1KO mice were increased compared to aged wild type mice. Rescue of forebrain 11β-HSD1 reduced BDNF mRNA in aged HSD1KO mice to levels comparable to aged wild type mice. These findings indicate that 11β-HSD1 regenerated glucocorticoids in forebrain and decreased levels of BDNF mRNA in the hippocampus plays a role in spatial memory deficits in aged wild type mice but 11β-HSD1 activity in peripheral tissues may also contribute to spatial learning impairments in aged mice.

Introduction

Chronically elevated circulating glucocorticoids (GC; cortisol in humans and corticosterone in rodents) as a consequence of impaired hypothalamic-pituitary-adrenal (HPA) negative feedback regulation often accompanies ageing and cognitive decline (1-3). Prolonged exposure of the brain to stress levels of GCs is thought to contribute to the cognitive deficits in aged individuals (4, 5). Indeed, animal studies have shown chronic stress or high GC

levels cause hippocampal dendritic atrophy, impaired synaptic plasticity, decreased neurogenesis and impaired memory (6, 7). Maintenance of low corticosterone (CORT) levels throughout life in rats prevents age-associated hippocampal morphology changes and impairs cognition supporting high CORT levels as a cause rather than a consequence of hippocampal damage (8).

Glucocorticoids bind to two types of intracellular corticosteroid receptors in brain, the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR), to modulate learning and memory (9). Prior to receptor binding, GC levels can be modulated by the action of 11β-hydroxysteroid dehydrogenases which interconvert active and inactive GCs. 11β-HSD1 is highly expressed in liver, adipose tissue and brain where it acts predominantly as a 11β-reductase *in vivo* to regenerate active GCs (cortisol, corticosterone) within cells from inert 11-keto forms (cortisone, 11-dehydrocorticosterone) thus effectively amplifying local GC levels (10). The other isozyme 11β-HSD2, acts as an exclusive 11β-dehydrogenase inactivating GCs and is mostly expressed in the distal nephron thus protecting otherwise non-selective MRs from GCs *in vivo* (11). In the adult brain, 11β-HSD1 is the sole isozyme expressed in regions important for cognition such as hippocampus and cortex (10).

11β-HSD1 generated GCs play a crucial role in hippocampus-dependent memory deficits associated with ageing (12). Aged mice with life-long deficiency of 11β-HSD1, despite similarly elevated plasma CORT levels to aged wild type mice, exhibit lower intra-hippocampal CORT levels and similar spatial memory performance to young adult mice (13).

11β-HSD1 deficient mice also exhibit a protective metabolic phenotype as noted by reduced glucose and insulin levels when on high fat diets (14, 15). Increased glucose levels over time associate with cognitive decline in non-diabetic non-demented elderly subjects (16). Furthermore, animal models of hyperglycaemia exhibit impaired spatial learning (17, 18). It is therefore possible that the deficiency of 11β-HSD1 in peripheral tissues may contribute to

the improved cognitive phenotype of aged 11β-HSD1 deficient mice. If the cognitive effects are solely a consequence of a lack of 11β-HSD1 in brain, then we predict that forebrain specific transgene rescue of 11β-HSD1 would reverse the improved cognition and any associated changes in hippocampal mRNA expression in aged 11β-HSD1 deficient mice. To test this we inter-crossed transgenic forebrain-specific 11β-HSD1 overexpression mice (19) with the global 11β-HSD1-deficient mice (14). In addition to MR and GR, we examined brain derived neurotrophic factor (BDNF) mRNA levels in the hippocampus given its important role in regulating synaptic plasticity mechanisms that underlie learning and memory (20) and its modulation by GCs (21).

Materials and methods

Animals: Transgenic mice with forebrain-specific overexpression of 11β-HSD1 under the CamIIK promoter were generated as described (19). Mice hemizygous for the transgene (referred to as transgenic (Tg) mice) were intercrossed with global 11β-HSD1 deficient mice (*hsd11b1$^{-/-}$*; referred to as HSD1KO) (22) to generate *hsd11b1$^{-/-}$* mice with forebrain-specific expression of *hsd11b1* (referred to as Tg+HSD1KO) and without the transgene (referred to as HSD1KO). Both Tg and *hsd11b1$^{-/-}$* mice were congenic on C57BL/6J strain. Non-transgenic (wild-type (WT)) littermates were used as *hsd11b1$^{+/+}$* controls. Male mice were used in the studies and maintained under standard conditions (12h light:dark cycle, lights on at 7:00AM, *ad libitum* access to standard chow and water) until experimentation. All animal procedures were approved by the University of Edinburgh Ethical Review committee and were carried out to the highest standards under the United Kingdom Animals Scientific Procedures Act, 1986 and the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Cognitive behavioural tests (Y-maze and watermaze)

Basal tail venesection blood samples were taken from all mice between 0830h and 1030h one week prior to behavioural testing. Aged (24m) HSD1KO, Tg+HSD1KO and WT mice were first tested in the Y-maze to assess their spatial recognition memory retention as previously described (13). The Y-maze apparatus consisted of three enclosed Plexiglas arms (50 cm long, 11cm wide and 10 cm high) surrounded by visible extra maze spatial cues. On the day of Y-maze testing, all mice were transported to the behavioural testing room in their home cages and left to acclimatise to the environment for 30 min. A handful of bedding material from each cage was added to the Y-maze and mixed evenly to distribute the combined scents of the mice covering the floor of the maze. During trial 1, each mouse was allowed to freely explore two arms of the Y-maze with one arm blocked ('novel' arm) for 5 min before returning to their home cage. Following a 1 min inter-trial interval (ITI) each mouse was returned to the Y maze with all arms now open for another 5 min exploration. The time spent in the novel arm was calculated as a percentage of the total time in all three arms. The 1min ITI test ensured the aged mice were engaging in the novelty of the task and had no visual problems seeing the spatial cues around the maze. All mice were re-tested in the Y-maze (with new cues around maze) 3 days later with a 2h ITI to measure spatial memory. The mice movements in the arms of the maze were tracked and analysed using AnyMaze software (Stoelting, Dublin, Ireland).

At least 1 week after Y-maze testing, aged mice were trained in the watermaze as described (13) to measure spatial learning and memory. Young 6m WT mice were included to confirm impaired spatial memory with ageing in *hsd11b1*$^{+/+}$ controls. Mice were first given 3 days of non-spatial training (four 90 s trials per day with curtains around pool to obscure external cues) to find the submerged platform marked with a visible Lego block protruding on top. This tests for visual, motivational or motor deficits that might influence their performance in the spatial learning task. Mice unable to find the platform within 20 s on day 3 were excluded. For spatial training, mice were given 20 trials (20 min ITI) over 5 consecutive days

with no curtains around pool so they could navigate towards the hidden platform using the spatial cues around the room. Each trial starts with the mouse facing the wall of pool at one of 4 randomly chosen locations and ends when they find the platform or when 90s has elapsed in which case they are guided to the platform by hand. After 30s on the platform, the mice are returned to their home cage. Twenty-four hours after the last training trial, each mouse was given a probe test where they are placed in the pool (with platform removed) and left to swim freely for 60s. Swim paths were tracked using a video camera mounted in the ceiling and data analysis were analysed using the Watermaze software (Actimetrics, Evanston, IL). Twenty-four hours later, mice were weighed and culled by cervical dislocation. The brains were removed with one half hemisphere frozen on powdered dry ice for *in situ* hybridization and the remaining half hemisphere dissected with hippocampus and cortex frozen on dry ice and stored at -80°C. Livers were collected, frozen on dry ice and stored at -80°C whilst the adrenals were stored in formalin at 4°C. Excess fat was dissected from each adrenal prior to weighing on a microbalance.

11β-HSD1 enzyme activity

Liver, cortex and hippocampal samples were homogenised in 500µl of homogenising buffer (5mM Tris, 1mM EDTA, 20% glycerol) and assayed for 11β-dehydrogenase activity as described (23). Samples were diluted in C buffer (10% glycerol, 50 mM sodium acetate, 1 mM EDTA; pH 6.0) to give 50µg/ml protein concentration for liver, 200µg/ml protein concentration for cortex and 400µg/ml protein concentration for hippocampus. The assay, containing 10mM 3H-corticosterone as a tracer and 2mM NADP as a co-factor, was incubated at 37°C for 20 min for liver samples, 4h for hippocampus and cortex samples. The steroids were extracted with ethyl acetate and the percentage conversion of [³H]-corticosterone to [³H]-11-dehydrocorticosterone was analysed by thin-layer chromatography.

Corticosterone radioimmunoassay

Plasma corticosterone levels were determined using an in-house radioimmunoassay as described (24) with corticosterone antiserum kindly donated by Dr C. Kenyon (University of Edinburgh, Edinburgh, UK).

In situ hybridisation

Antisense RNA probes for MR and GR were generated by *in vitro* transcription from plasmid vectors containing the appropriate cDNA insert, in the presence of [^{35}S]-UTP with SP6- and T7-RNA polymerases respectively as previously described (25). For detection of BDNF mRNA, mouse brain cDNA was used to generate 'floppy' PCR template for probe synthesis. Primers (see Table S1) with 5' extensions containing phage polymerase consensus sites were used to generate the BDNF cDNA template corresponding to bases 843-1362 of the mouse BDNF mRNA (GenBank accession number AY057908) (26). cDNA was pooled from several reactions and purified with High Pure PCR product Purification Kit (Roche, UK). Nucleotide sequencing revealed 100% homology between the amplied fragment and previously reported sequence. Antisense and sense cRNA probes (520bp length) were prepared from the cDNA template by *in vitro* transcription in the presence of [^{35}S]-UTP (PerkElmer, MA, USA) using T3 or T7 RNA polymerase, respectively.

Cryostat (10μm) coronal brain sections at the level of the dorsal hippocampus from the behaviourally tested animals thaw-mounted onto microscope slides were hybridised with the [^{35}S]-UTP labelled cRNA antisense probes for MR, GR and BDNF essentially as described (25). The specificity of the BDNF antisense cRNA probe was first tested prior to the main experiment in control brain sections along with the sense BDNF cRNA probe. Slides were dipped in photographic emulsion (NTB-2, Kodak) and exposed at 4°C for 20 days before being developed (Kodak D19) followed by counterstaining with pyronin (1%). Silver grains were counted within a fixed circular area over individual cells under x40 light microscope objective using the Zeiss KS300 image analysis system. The analysis was carried out blind

to genotype, 15-18 cells/subregion were assessed for each animal and background (determined over areas of white matter) subtracted.

Statistics

Y-maze data were analysed by two-way analysis of variance (ANOVA) with % time in novel arm (1min ITI and 2h ITI) and genotype as the independent variables. Within animal comparisons of % time in arms of Y-maze were analysed by multiple paired t-tests with Bonferroni adjusted $P<0.025$. Watermaze data (visible platform and spatial learning) were analysed by two-way repeated measures ANOVA with escape latency over days of training and genotype as the independent variables. Probe test data were analysed by two-way ANOVA with % time in the different quadrants and genotype as independent variables. In situ hybridization mRNA data were analysed by two-way ANOVA with subregions and genotype or age as independent variables. Enzyme bioactivity, plasma corticosterone and adrenal weight data were analysed using one-way ANOVA for effects of genotype. All data were analysed using GraphPad Prism 6 software and statistical differences were determined with *post-hoc* Bonferroni's multiple comparisons test (adjusted P values shown in figure legends). All data are presented as the mean ± S.E.M.

Results

Rescue of forebrain 11β-HSD1 activity in 11β-HSD1-deficient mice by CamIIK-HSD1 transgene

11β-HSD1 activity (measured as 11β-dehydrogenase conversion of [3H]-CORT to [3H]-11DHC in tissue homogenates) was present in liver, hippocampus and cortex of WT controls but absent from all tissues tested in HSD1KO mice (Fig. 1). When HSD1KO mice were bred with transgenic mice with forebrain-specific overexpression of 11β-HSD1 (Tg mice) to generate Tg+HSD1KO mice, 11β-HSD1 activity was recovered in hippocampus and cortex to levels similar to WT controls but remained absent in liver (Fig. 1).

Improved Y-maze spatial memory in aged 11β-HSD1 deficient mice reversed by CamIIK-HSD1 transgene rescue

All groups of aged mice, WT, HSD1KO and Tg+HSD1KO, responded to novelty in the Y-maze with no effect of genotype, spending significantly more time in novel arm than previously visited arms (P<0.02) after a 1 min inter-trial interval (ITI) (Fig. 2A). The % time spent in the novel arm after a 2h ITI was significantly different from 1 min ITI (F $_{1, 54}$ = 18, P<0.0001) with a genotype x time interaction effect (F $_{2, 54}$ = 5.4, P<0.01) (Fig. 2B). Aged HSD1KO mice spent more time in the novel arm (P<0.05) than WT and Tg+HSD1KO after 2h ITI, confirming the improved cognitive phenotype as previously described (13). Both aged WT and Tg+HSD1KO mice showed impaired spatial memory, spending less time in the novel arm after the 2h ITI than 1min ITI (P<0.01) (Fig. 2B) and not more time in novel arm than previously visited arms (Fig. 2A).

Improved watermaze spatial learning in aged 11β-HSD1-deficient mice attenuated by CamIIK-HSD1 transgene rescue

WT mice showed a decline in escape latencies over the 5 days of training trials (F $_{4, 40}$ = 15.63, P<0.0001, Fig. 3A) indicating learning but aged mice displayed longer escape latencies to find the hidden platform compared to young controls (age effect, F $_{1, 10}$ = 10.28, P<0.01, Fig. 3A) thus confirming impaired spatial learning. Although aged WT, HSD1KO and Tg+HSD1KO mice all showed a decrease in escape latency effect over days of training (2-way repeated measures ANOVA, F $_{4, 68}$ = 27.62, P <0.0001, Fig. 3B), there was a significant genotype effect (F $_{2, 17}$ = 3.9, P<0.05, Fig. 3B). Aged HSD1 KO mice showed improved spatial learning (reduced escape latencies) compared to WT controls (F$_{1,11}$= 5.8, P<0.05, Fig. 3B). Spatial learning in Tg+HSD1KO mice however did not differ significantly from HSD1KO or WT mice although there was a trend for better learning versus WT mice (F$_{1, 11}$= 4.5, P=0.06, Fig. 3B). These differences were not a consequence of vision or motor deficits in the aged mice since average daily swim speeds across the days of training did not differ significantly between genotypes and all mice were able to find the visible platform equally

after 3 days of training (Fig. S1). Interestingly, the escape latency on the first day of visible platform training was significantly lower in aged HSD1KO mice compared to both WT and Tg+HSD1KO mice. This quicker escape latency appears to reflect brain 11β-HSD1 deficiency since this was not observed in the Tg+HSD1KO mice. However, any impact on overall performance of the HSD1KO mice is not clear as all groups learnt the visible platform position equally on the other two days.

Aged WT mice showed impaired spatial memory (24h after the last training trial) during the probe trial (minus platform), failing to spend more time in the platform quadrant of the pool than other quadrants (Fig. 3C). Aged HSD1KO but not Tg+HSD1KO mice displayed a preference for the target platform quadrant ($F_{3, 68}$ = 5.9, P=0.001, Fig. 3C) during the first 30 seconds of the probe trial indicating intact spatial memory of the platform location but the % time in the target quadrant was not significantly greater than in aged WT mice. When the full 60 seconds of the probe test was analysed, aged HSD1KO mice no longer showed a significant preference for the target quadrant over all the other quadrants (Fig.3C). This is consistent with a decrease in target quadrant preference after the first 30 seconds (27).

Increased BDNF mRNA levels in the hippocampus of aged 11β-HSD1-deficient but not Tg+HSD1 KO mice.

BDNF mRNA was highly expressed in all hippocampal regions, particularly CA3 and in cortex of control WT mice (Fig. 4A,B). When compared to young WT mice, BDNF mRNA levels in the aged WT mice was significantly decreased with age in hippocampal CA3 and CA4 (approximately 41% and 48% decrease, respectively; $F_{1, 32}$ = 25.88, P<0.0001, Fig. 4B) and in parietal and piriform cortex (approximately 42% and 39% decrease respectively; $F_{1, 16}$ = 54.9, P<0.0001, Fig.4B). In the aged (24m) WT, HSD1KO and TG+HSD1KO mice, BDNF mRNA expression was significantly altered by genotype ($F_{2, 68}$ = 15.5, P<0.0001) and hippocampal region ($F_{3, 68}$ =25.13, P<0.0001) (Fig. 4C). BDNF mRNA levels were increased selectively in CA3 hippocampal cells (approximately 70% increase, P<0.001) in aged

HSD1KO compared to WT mice (Fig. 4C, D). Rescue of 11β-HSD1 expression in forebrain reduced BDNF mRNA in CA3 of Tg+HSD1KO mice (P<0.05, compared to HSD1KO mice) to levels not different from aged WT controls (Fig. 4C, D). BDNF mRNA expression was not affected by genotype in cortex but there was a significant genotype x region interaction ($F_{2, 34}$ = 3.54, P<0.05, Fig. 4C). Tg+HSD1KO mice showed a small increase in BDNF mRNA levels compared to WT controls in the piriform cortex (Fig. 4C). Hippocampal MR and GR mRNA levels in contrast were not altered in either aged HSD1KO or Tg+HSD1KO mice (Fig. S2).

Hyperplasia of adrenal glands in aged 11β-HSD1 deficient mice not rescued by CamIIK-HSD1 transgene

The left adrenals were significantly enlarged by approximately 30% ($F_{2,31}$=4.7, P<0.05) in aged HSD1KO compared to aged WT controls (Table S2). Aged 11β-HSD1-deficient mice with the CamIIK-HSD1 transgene rescue of 11β-HSD1 activity in forebrain (Tg+HSD1KO) showed a non-significant trend for heavier adrenals (Table S2). Total adrenal weights (left and right) when expressed relative to body weight were significantly increased by approximately 33% ($F_{2, 30}$= 4.67, P<0.05) in both aged HSD1KO and Tg+HSD1KO compared to WT mice (Table S2). Basal plasma CORT levels were not significantly different between aged HSD1KO, Tg+HSD1KO and WT mice (Table S2).

Discussion

The main finding from this study is that CamIIK-HSD1 transgene rescue of 11β-HSD1 activity in the forebrain reversed both increased hippocampal BDNF mRNA and improved spatial memory in aged 11β-HSD1 deficient mice to aged WT mice levels. This implicates 11β-HSD1 activity in forebrain as a key contributor to the mechanisms that underlie impaired spatial memory in aged WT mice. Furthermore, 11β-HSD1 activity outwith the brain appears to make some contribution towards the cognitive effects of aged mice, at least under stress conditions as in the watermaze.

11β-HSD1-deficient mice have been shown to resist hyperglycaemia provoked by obesity or stress reflecting reduced intracellular GC levels in adipose and liver (14, 23). Since, hyperglycaemia and hyperinsulinaemia associates with cognitive decline in humans and some animal models (16, 28-30), a deficiency of 11β-HSD1 in liver and adipose tissues could potentially contribute to the cognitive phenotype of aged 11β-HSD1-deficient mice. We previously reported no change in glucose tolerance but increased insulin sensitivity in aged 11β-HSD1-deficient mice suggesting a possible influence on cognitive function (13). Aged 11β-HSD1 deficient mice with CamIIK-HSD1 transgene rescue of 11β-HSD1 activity in forebrain no longer showed enhanced spatial memory in the Y-maze with similar performance to aged WT mice. These data suggest that peripheral 11β-HSD1 activity has little effect on spatial memory in aged mice under basal conditions in the Y-maze. However, when tested in the more stressful watermaze task, the improved spatial learning in aged 11β-HSD1 deficient mice (13) was not fully reversed with CamIIK-HSD1 transgene rescue of 11β-HSD1 in the forebrain. Instead, aged Tg+HSD1KO mice show spatial learning performances that were between aged WT mice and aged 11β-HSD1 deficient mice with no significant difference between the genotypes. Whereas hyperglycaemia and increased CORT levels associated with stress impairs hippocampal neurogenesis, synaptic plasticity and learning (31), 11β-HSD1-deficient mice has been shown to resist hyperglycaemia induced by stress or obesity (14, 15). Thus the beneficial cognitive effects of peripheral deficiency of 11β-HSD1 in the aged Tg+HSD1KO mice may only become apparent under conditions of stress such as during the watermaze.

Adrenal weights are increased in aged 11β-HSD1-deficient mice compared to aged WT controls (13) as would be anticipated for increased CORT production to compensate for reduced local production at brain feedback sites (22). Here, the increased adrenal size was not associated with increased basal plasma CORT levels as observed in 11β-HSD1-deficient mice on the 129 strain (22, 24, 32) thus confirming a lack of effect on the C57BL/6J strain (13). CamIIK-HSD1 transgene rescue of 11β-HSD1 activity in forebrain however did

not reverse the increased adrenal size relative to body weight in the aged 11β-HSD1-deficient mice. This may reflect reduced bulk CORT synthesis due to lack of 11β-HSD1 in tissues outwith the forebrain notably the liver of aged Tg+HSD1KO mice. Indeed, transgene rescue of 11β-HSD1 activity in liver restores hyperplasia of the adrenal gland in 11β-HSD1-deficient mice supporting a major influence of hepatic GC metabolism on HPA function (33).

BDNF is well established to play an essential role as a mediator of neural plasticity and consequently memory formation, particularly in hippocampus-dependent tasks (34, 35). BDNF levels in the hippocampus have been shown to decline with age in rats (36, 37) and in humans (38). Our observed decrease in BDNF mRNA expression in the hippocampus of aged WT mice relative to young controls may contribute to their impaired spatial memory. In support, BDNF mRNA expression in the hippocampus of non-impaired and impaired aged rats correlates with their spatial memory performance in the watermaze (39). Moreover, chronic deficiency of BDNF in mice leads to impaired spatial memory in an age-dependent manner (40). Several studies have also shown increases in hippocampal BDNF mRNA expression following a learning task in animals with improved memory but no change in BDNF mRNA levels in those with impaired memory (39, 41, 42). In line with these studies, the increased BDNF mRNA in the CA3 sub-region of the hippocampus in aged 11β-HSD1-deficient mice associated with improved spatial memory whereas aged WT mice with lower BDNF mRNA were impaired in both Y-maze and watermaze. Although hippocampal BDNF mRNA levels in aged 11β-HSD1-deficient mice were similar to young WT mice, a lack of effect of ageing remains to be confirmed with the inclusion of young 11β-HSD1-deficient mice.

The regulation of the BDNF gene is complex with at least eight differentially regulated promoters each giving rise to mRNA transcripts with a distinct 5' exon spliced to a common 3' coding exon but with all transcripts encoding an identical BDNF protein (43). As with most studies we investigated the effect of age on total BDNF mRNA in the WT mice so any change in one or more specific BDNF mRNA splice transcripts is not known. However, the

decrease in total BDNF mRNA in the CA3 hippocampus in the aged WT mice may reflect a decrease in all the four predominant 5' exon-specific transcripts with age (37).

The neuronal-specific transcription factor NPAS4 is a key regulator of brain plasticity and cognitive function (44). We have previously found decreased Npas4 mRNA in the hippocampus of aged memory impaired WT mice but maintained levels in aged 11β-HSD1-deficient mice (45). Since BDNF is one of the target genes of Npas4, the reduced BDNF mRNA in the hippocampus of aged WT mice may be a consequence of reduced Npas4 levels. Both Npas4 and BDNF mRNA in the hippocampus have been shown to decrease with age in rats (46). Moreover, both stress and elevated levels of CORT down regulate hippocampal BDNF mRNA expression (47-49). Thus the lower BDNF mRNA levels in CA3 hippocampus of aged WT and aged Tg+HSD1KO mice when compared to aged 11β-HSD1-deficient mice may be the result of increased intracellular CORT levels by 11β-HSD1 activity in brain.

In conclusion, rescue of 11β-HSD1 in the forebrain of global 11β-HSD1-deficient mice appears sufficient to re-instate the age-associated spatial memory deficits in the Y-maze, an effect associated with lower BDNF but not MR or GR mRNA expression in the hippocampus. Interestingly, the improved spatial learning in the watermaze was not fully reversed in the aged Tg+HSD1KO mice suggesting a contribution of peripheral 11β-HSD1 deficiency to cognitive function under stress conditions such as during the watermaze task.

Acknowledgements

References

1.   Lupien SJ, de Leon M, de Santi S, Convit A, Tarshish C, Nair NPV, Thakur M, McEwen BS, Hauger RL, Meaney MJ. Cortisol levels during human aging predict hippocampal atrophy and memory deficits. *Nature Neurosci*. 1998; **1**69-73.

2.   Issa AM, Rowe W, Gauthier S, Meaney MJ. Hypothalamic-pituitary-adrenal activity in aged, cognitively impaired and cognitively unimpaired rats. *J Neurosci*. 1990; **10**(10): 3247-54.

3.   Yau JL, Olsson T, Morris RG, Meaney MJ, Seckl JR. Glucocorticoids, hippocampal corticosteroid receptor gene expression and antidepressant treatment: relationship with spatial learning in young and aged rats. *Neuroscience*. 1995; **66**(3): 571-81.

4.   Lupien SJ, McEwen BS, Gunnar MR, Heim C. Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature reviews*. 2009; **10**(6): 434-45.

5.   Sapolsky RM. Stress, glucocorticoids, and damage to the nervous system: The current state of confusion. *Stress*. 1996; **1**1-19.

6.   McEwen BS, de Leon MJ, Lupien SJ, Meaney MJ. Corticosteroids, the Aging Brain and Cognition. *Trends Endocrinol Metab*. 1999; **10**(3): 92-6.

7.   McEwen BS. Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. *Eur J Pharmacol*. 2008; **583**(2-3): 174-85.

8.   Landfield PW, Baskin RK, Pitler TA. Brain aging correlates: Retardation by hormonal-pharmacological treatments. *Science* 1981; **214**581-4.

9.   McEwen BS, DeKloet ER, Rostene W. Adrenal steroid receptors and action in the nervous system. *Physiol Rev*. 1986; **66**1121-88.

10.   Wyrwoll CS, Holmes MC, Seckl JR. 11beta-hydroxysteroid dehydrogenases and the brain: from zero to hero, a decade of progress. *Front Neuroendocrinol*. 2011; **32**(3): 265-86.

11.   Chapman K, Holmes M, Seckl J. 11beta-hydroxysteroid dehydrogenases: intracellular gate-keepers of tissue glucocorticoid action. *Physiological reviews*. 2013; **93**(3): 1139-206.

12.   Yau JL, Seckl JR. Local amplification of glucocorticoids in the aging brain and impaired spatial memory. *Front Aging Neurosci*. 2012; **4**24.

13.   Yau JL, McNair KM, Noble J, Brownstein D, Hibberd C, Morton N, Mullins JJ, Morris RG, Cobb S, Seckl JR. Enhanced hippocampal long-term potentiation and spatial learning in aged 11beta-hydroxysteroid dehydrogenase type 1 knock-out mice. *J Neurosci*. 2007; **27**(39): 10487-96.

14.   Kotelevtsev Y, Holmes MC, Burchell A, Houston PM, Schmoll D, Jamieson P, Best R, Brown R, Edwards CRW, Seckl JR, Mullins JJ. 11ß-Hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid-inducible responses and resist hyperglycemia on obesity or stress. *Proc Natl Acad Sci USA*. 1997; **94**14924-9.

15.   Morton NM, Paterson JM, Masuzaki H, Holmes MC, Staels B, Fievet C, Walker BR, Flier JS, Mullins JJ, Seckl JR. Novel adipose tissue-mediated resistance to diet-induced

visceral obesity in 11 beta-hydroxysteroid dehydrogenase type 1-deficient mice. *Diabetes*. 2004; **53**(4): 931-8.

16. Ravona-Springer R, Moshier E, Schmeidler J, Godbold J, Akrivos J, Rapp M, Grossman HT, Wysocki M, Silverman JM, Haroutunian V, Beeri MS. Changes in glycemic control are associated with changes in cognition in non-diabetic elderly. *J Alzheimer's Disease : JAD*. 2012; **30**(2): 299-309.

17. Biessels GJ, Kamal A, Urban IJ, Spruijt BM, Erkelens DW, Gispen WH. Water maze learning and hippocampal synaptic plasticity in streptozotocin-diabetic rats: effects of insulin treatment. *Brain Res*. 1998; **800**(1): 125-35.

18. Biessels GJ, Gispen WH. The impact of diabetes on cognition: what can be learned from rodent models? *Neurobiol Aging*. 2005; **26 Suppl 1**36-41.

19. Holmes MC, Carter RN, Noble J, Chitnis S, Dutia A, Paterson JM, Mullins JJ, Seckl JR, Yau JL. 11beta-hydroxysteroid dehydrogenase type 1 expression is increased in the aged mouse hippocampus and parietal cortex and causes memory impairments. *J Neurosci*. 2010; **30**(20): 6916-20.

20. Cunha C, Brambilla R, Thomas KL. A simple role for BDNF in learning and memory? *Front Mol Neurosci*. 2010; **3**1.

21. Schaaf MJ, De Kloet ER, Vreugdenhil E. Corticosterone effects on BDNF expression in the hippocampus. Implications for memory formation. *Stress*. 2000; **3**(3): 201-8.

22. Carter RN, Paterson JM, Tworowska U, Stenvers DJ, Mullins JJ, Seckl JR, Holmes MC. Hypothalamic-pituitary-adrenal axis abnormalities in response to deletion of 11beta-HSD1 is strain-dependent. *J Neuroendocrinol*. 2009; **21**(11): 879-87.

23. Morton NM, Holmes MC, Fievet C, Staels B, Mullins JJ, Seckl JR. Improved lipid and lipoprotein profile, hepatic insulin sensitivity and glucose tolerance in 11beta-hydroxysteroid dehydrogenase type 1 null mice. *J Biol Chem*. 2001; **276:** 41293-300.

24. Harris HJ, Kotelevtsev Y, Mullins JJ, Seckl JR, Holmes MC. Intracellular regeneration of glucocorticoids by 11ß-HSD-1 plays a key role in regulation of the HPA axis: analysis of 11ß-HSD-1 deficient mice. *Endocrinology*. 2001; **142:** 114 - 20.

25. Yau JL, Noble J, Seckl JR. Site-specific regulation of corticosteroid and serotonin receptor subtype gene expression in the rat hippocampus following 3,4-methylenedioxymethamphetamine: role of corticosterone and serotonin. *Neuroscience*. 1997; **78**(1): 111-21.

26. Hashimoto T, Bergen SE, Nguyen QL, Xu B, Monteggia LM, Pierri JN, Sun Z, Sampson AR, Lewis DA. Relationship of brain-derived neurotrophic factor and its receptor TrkB to altered inhibitory prefrontal circuitry in schizophrenia. *J Neurosci* 2005; **25**(2): 372-83.

27. Blokland A, Geraerts E, Been M. A detailed analysis of rats' spatial memory in a probe trial of a Morris task. *Behav Brain Res*. 2004; **154**(1): 71-5.

28. Convit A. Links between cognitive impairment in insulin resistance: an explanatory model. *Neurobiol Aging*. 2005; **26 Suppl 1**31-5.

29.   Convit A, Wolf OT, Tarshish C, de Leon MJ. Reduced glucose tolerance is associated with poor memory performance and hippocampal atrophy among normal elderly. *Proc Natl Acad Sci U S A*. 2003; **100**(4): 2019-22.

30.   McCall AL. Altered glycemia and brain-update and potential relevance to the aging brain. *Neurobiol Aging*. 2005; **26 Suppl 1**70-5.

31.   Stranahan AM, Arumugam TV, Cutler RG, Lee K, Egan JM, Mattson MP. Diabetes impairs hippocampal function through glucocorticoid-mediated effects on new and mature neurons. *Nature Neurosci*. 2008; **11**(3): 309-17.

32.   Yau JL, Noble J, Kenyon CJ, Hibberd C, Kotelevtsev Y, Mullins JJ, Seckl JR. Lack of tissue glucocorticoid reactivation in 11beta -hydroxysteroid dehydrogenase type 1 knockout mice ameliorates age-related learning impairments. *Proc Natl Acad Sci U S A*. 2001; **98**(8): 4716-21.

33.   Paterson JM, Holmes MC, Kenyon CJ, Carter R, Mullins JJ, Seckl JR. Liver-selective transgene rescue of hypothalamic-pituitary-adrenal axis dysfunction in 11beta-hydroxysteroid dehydrogenase type 1-deficient mice. *Endocrinology*. 2007; **148**(3): 961-6.

34.   Korte M, Carroll P, Wolf E, Brem G, Thoenen H, Bonhoeffer T. Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. *Proc Natl Acad Sci U S A*. 1995; **92**(19): 8856-60.

35.   Lu B, Nagappan G, Lu Y. BDNF and synaptic plasticity, cognitive function, and dysfunction. *Handb Exp Pharmacol*. 2014; **220**223-50.

36.   Hattiangady B, Rao MS, Shetty GA, Shetty AK. Brain-derived neurotrophic factor, phosphorylated cyclic AMP response element binding protein and neuropeptide Y decline as early as middle age in the dentate gyrus and CA1 and CA3 subfields of the hippocampus. *Exp Neurol*. 2005; **195**(2): 353-71.

37.   Chapman TR, Barrientos RM, Ahrendsen JT, Hoover JM, Maier SF, Patterson SL. Aging and infection reduce expression of specific brain-derived neurotrophic factor mRNAs in hippocampus. *Neurobiology of Aging*. 2012; **33**(4): 832 e1-14.

38.   Murer MG, Yan Q, Raisman-Vozari R. Brain-derived neurotrophic factor in the control human brain, and in Alzheimer's disease and Parkinson's disease. *Progress in neurobiology*. 2001; **63**(1): 71-124.

39.   Schaaf MJ, Workel JO, Lesscher HM, Vreugdenhil E, Oitzl MS, de Kloet ER. Correlation between hippocampal BDNF mRNA expression and memory performance in senescent rats. *Brain Res*. 2001; **915**(2): 227-33.

40.   Petzold A, Psotta L, Brigadski T, Endres T, Lessmann V. Chronic BDNF deficiency leads to an age-dependent impairment in spatial learning. *Neurobiol Learn Mem*. 2015; **120**52-60.

41.   Hall J, Thomas KL, Everitt BJ. Rapid and selective induction of BDNF expression in the hippocampus during contextual learning. *Nature Neurosci*. 2000; **3**(6): 533-5.

42.   Schaaf MJ, Sibug RM, Duurland R, Fluttert MF, Oitzl MS, De Kloet ER, Vreugdenhil E. Corticosterone effects on BDNF mRNA expression in the rat hippocampus during morris water maze training. *Stress*. 1999; **3**(2): 173-83.

43.   Aid T, Kazantseva A, Piirsoo M, Palm K, Timmusk T. Mouse and rat BDNF gene structure and expression revisited. *J Neurosci Res.* 2007; **85**(3): 525-35.

44.   Maya-Vetencourt JF. Activity-dependent NPAS4 expression and the regulation of gene programs underlying plasticity in the central nervous system. *Neural plasticity.* 2013; **2013:** 683909.

45.   Qiu J, Dunbar DR, Noble J, Cairns C, Carter R, Kelly V, Chapman KE, Seckl JR, Yau JL. Decreased Npas4 and Arc mRNA Levels in the Hippocampus of Aged Memory-Impaired Wild-Type But Not Memory Preserved 11beta-HSD1 Deficient Mice. *J Neuroendocrinol.* 2016; **28**(1).

46.   Calabrese F, Guidotti G, Racagni G, Riva MA. Reduced neuroplasticity in aged rats: a role for the neurotrophin brain-derived neurotrophic factor. *Neurobiology of Aging.* 2013; **34**(12): 2768-76.

47.   Smith MA, Makino S, Kvetnansky R, Post RM. Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *J Neurosci* 1995; **15**(3 Pt 1): 1768-77.

48.   Murakami S, Imbe H, Morikawa Y, Kubo C, Senba E. Chronic stress, as well as acute stress, reduces BDNF mRNA expression in the rat hippocampus but less robustly. *Neurosci Res.* 2005; **53**(2): 129-39.

49.   Jacobsen JP, Mork A. Chronic corticosterone decreases brain-derived neurotrophic factor (BDNF) mRNA and protein in the hippocampus, but not in the frontal cortex, of the rat. *Brain Res.* 2006; **1110**(1): 221-5.

Figure legends

Figure 1. Effect of forebrain-specific 'rescue' of 11β-HSD1 in aged *hsd11b1*^{-/-} mice on 11β-HSD1 activity in brain and liver

11β-HSD1 enzyme activity measured ex-vivo as percentage of [^{3}H]-corticosterone (CORT) conversion to [^{3}H]-11-dehydrocorticosterone (11-DHC) in hippocampus, cortex and liver homogenates from aged 24 month-old wild type (WT), *hsd11b1*^{-/-} (HSD1KO) and Tg+HSD1KO mice (HSD1KO mice with 11β-HSD1 rescued in the forebrain). § P<0.0001 compared to WT; *** P<0.0001 compared to WT and Tg+HSD1KO. N= 10-14 per genotype. Data are means ± SEM.

Figure 2. Forebrain specific expression of 11β-HSD1 in aged *hsd11b1*^{-/-} mice impairs spatial memory in the Y-maze.

(A) Data shows within animal comparisons of % time spent in the novel, other and start arms following a 1 min and 2h inter-trial interval (ITI) for 24 month-old aged wild type (WT),

hsd11b1$^{-/-}$ (HSD1KO), and Tg+HSD1KO mice. * P<0.02, **P<0.01, ***, P<0.0001 compared to the other 2 arms. (B) Comparison of % time spent in novel arm during the 1 min and 2h ITI between genotypes. Spatial memory significantly impaired in WT and Tg+HSD1KO mice (*post-hoc* Bonferroni's multiple comparisons test adjusted P values: ***P=0.0006, **P=0.0012 compared to corresponding 2h ITI). N= 8-13 per genotype. Data are means ± SEM.
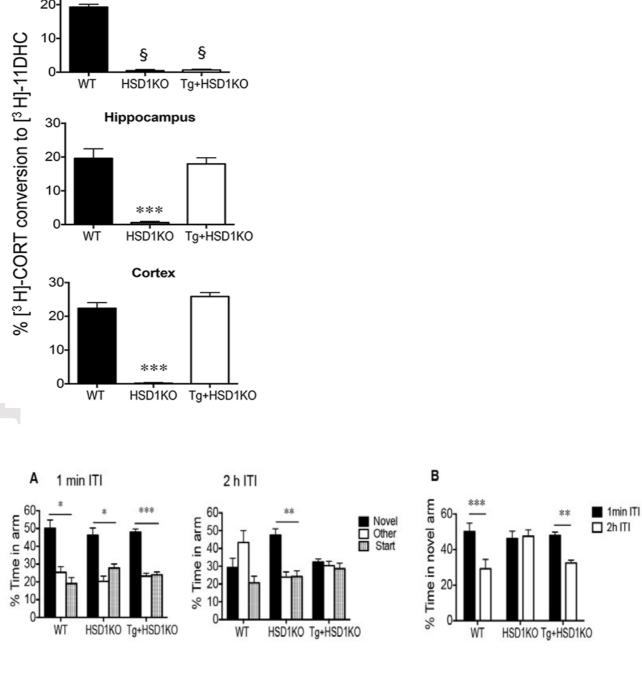
Figure 3. Effect of forebrain specific 'rescue' of 11β-HSD1 in aged *hsd11b1*$^{-/-}$ mice on spatial learning in the watermaze.
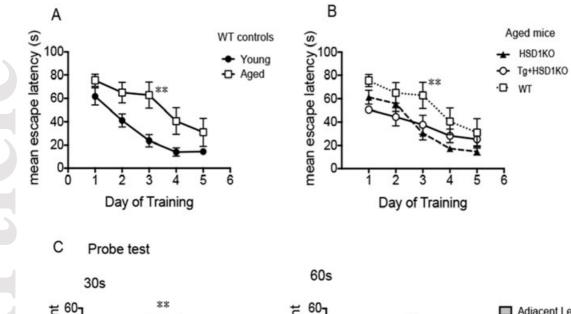
(A) Impaired spatial learning in aged (24 month-old) wild type (WT) mice (versus young (6 month-old) WT mice). ∗∗P<0.01 compared to corresponding time point of young WT mice. (B) Spatial learning of aged (24 month-old) wild type (WT), *hsd11b1*$^{-/-}$ (HSD1KO), and Tg+HSD1KO mice. ∗P<0.05 compared to corresponding time point of aged HSD1KO mice. (C) Spatial memory retention (24h after last training trial) of aged (24 month-old) wild type (WT), *hsd11b1*$^{-/-}$ (HSD1KO), and Tg+HSD1KO mice. The % time in target quadrant was not significantly affected by genotype but within the groups there was an effect of quadrant (*post-hoc* Bonferroni's multiple comparisons test adjusted P values: ∗P=0.01, ∗∗P<0.006 compared to the different quadrants of the pool as indicated). N=6-7 per genotype. Data are means ± SEM.
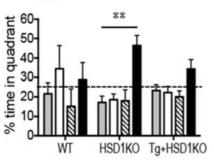
Figure 4. Effect of forebrain-specific 'rescue' of 11β-HSD1 in aged *hsd11b1*$^{-/-}$ mice on BDNF mRNA levels in the hippocampus and cortex. (A) Autoradiograph showing distribution of BDNF mRNA in coronal section at the level of the dorsal hippocampus from a control adult C57BL6/J brain. (B) BDNF mRNA levels in hippocampal subregions and cortex of young 6 month-old and aged 24 month-old C57BL6/J control mice. (C) BDNF mRNA levels in hippocampal subregions and cortex of aged 24 month-old wild type (WT), *hsd11b1*$^{-/-}$ (HSD1KO), and Tg+HSD1KO mice. N=6-7 per genotype. Data are means ± SEM. (D) Representative dark-field photomicrographs of *in situ* hybridization showing BDNF mRNA in
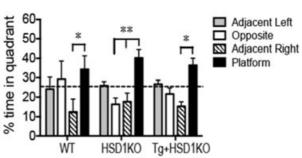
the CA3 subregion of the hippocampus from wild type (WT; young 6 month-old and aged 24 month-old) aged HSD1KO and aged Tg+HSD1KO mice. Autoradiographic silver grains appear white. *Post-hoc* Bonferroni's multiple comparisons test adjusted P values: *** P<0.0001, **P<0.008, *P<0.03 compared to corresponding aged mice indicated in figure.
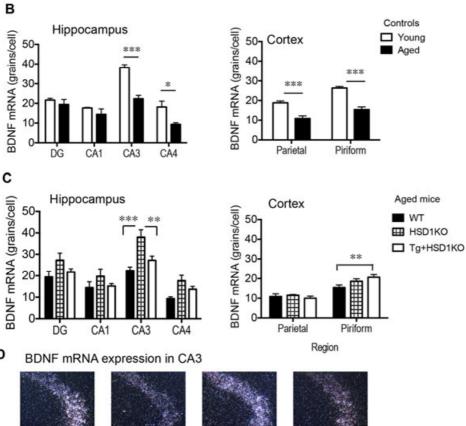
A

B

C Probe test

30s

60s

BDNF mRNA

A

B

Hippocampus

Cortex

Controls
- Young
- Aged

C

Hippocampus

Cortex

Aged mice
- WT
- HSD1KO
- Tg+HSD1KO

Region

D BDNF mRNA expression in CA3

Young WT   Aged WT   Aged HSD1KO   Aged Tg+HSD1KO