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11 β -Hydroxysteroid dehydrogenase type 1, brain atrophy and cognitive decline

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Abstract

Excess cortisol levels are linked with brain atrophy and cognitive decline in older people. 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) potently amplifies intracellular glucocorticoid action by converting inert cortisone to active cortisol, but any causal importance in brain ageing is unexplored. We tested the hypotheses that higher systemic 11 β -HSD1 activity predicts brain atrophy and cognitive decline in older men.

In a longitudinal study of 41 men (65-70y at baseline) we measured baseline systemic 11 β -HSD1 activity, the urinary 5 α - and 5 β -tetrahydrocortisol to tetrahydrocortisone ratio (THFs/THE), and assessed change in brain atrophy, white matter lesions and cognitive function over six years.

Baseline THFs/THE correlated negatively with baseline hippocampal volumes (left: $r=-0.37$; right: $r=-0.34$; $p<0.05$) and positively with ventricular volumes ($r=0.43$, $p=0.006$) and periventricular white matter lesions ($\rho=0.31$, $p=0.047$). Importantly, baseline THFs/THE but not cortisol predicted increase in ventricular volumes ($r=0.33$, $p=0.037$) and decline in processing speed ($r=-0.55$, $p=0.0002$) over six years.

The predictive link between systemic 11 β -HSD1 activity and progressive brain atrophy and cognitive decline suggests 11 β -HSD1 inhibition as a plausible therapy for brain ageing.

Keywords

Cognition; glucocorticoids; cortisol; cerebral atrophy; white matter lesions; dementia; aging

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INTRODUCTION

The mechanisms underlying inter-individual variation in cognitive decline with ageing are poorly understood and are a major priority for medical research. The issue has become more pressing with recent findings that the density of plaques and tangles, pathological hallmarks of Alzheimer's disease, fails to associate with cognitive function in the very elderly (Savva et al., 2009). There therefore remains a substantial explanatory gap in the mechanisms of brain ageing leading to cognitive dysfunction. Around one third of ageing animals and humans show elevated baseline levels of glucocorticoids which is associated with the development of atrophy of brain regions rich in glucocorticoid receptors and with deficits in cognitive function (Elgh et al., 2006; Issa et al., 1990; Lupien et al., 1998; MacLulich et al., 2005). Chronically elevated glucocorticoid levels adversely affect cognitive processes, inhibit hippocampal long-term potentiation, the putative synaptic electrophysiological basis for memory, and cause neuronal damage and perhaps even death (de Kloet, 2004; McEwen, 2002; Starkman et al., 2001). These adverse effects occur directly and also by potentiation of other neuronal insults, for example excitotoxicity (Armanini et al., 1990; Schubert et al., 2008), reactive oxygen species (Patel et al., 2000), metabolic stress (Lee et al., 2009) and effects of beta-amyloid (Catania et al., 2007). Elevated glucocorticoids also promote atherogenesis and thus may link to ischemic damage to the brain (van Rossum et al., 2008; Walker, 2007). Tissue sensitivity to glucocorticoids is also important. A common polymorphism of the intracellular glucocorticoid receptor which reduces sensitivity to steroids is associated with a lower risk of dementia (van Rossum et al., 2008).

Tissue glucocorticoid action is determined not only by circulating cortisol levels and target cell density of intracellular receptors but also by intracellular enzymes that metabolize glucocorticoids and thus 'gate' their access to receptors. Key to this is the microsomal enzyme 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1). This enzyme predominantly functions as a reductase, catalyzing conversion of inert cortisone to active cortisol, thus amplifying local glucocorticoid action within cells. Though oxidative activity can be observed in tissue homogenates and purified enzyme preparations, in vivo and in intact hippocampal cells 11 β -HSD1 functions almost exclusively as a reductase (Holmes and Seckl, 2006; Rajan et al., 1996). 11 β -HSD1 is present in peripheral tissues, notably liver and adipose tissue, and in multiple regions of the brain (Holmes and Seckl, 2006). In contrast, 5 α - and 5 β -reductases (also present in multiple peripheral tissues and the CNS), as well as 11 β -HSD type 2 (present mainly in the kidney and in discrete areas of the brain involved in salt regulation), catalyze conversion of cortisol into inactive metabolites, thus reducing local glucocorticoid action (Andrew et al., 1998). These enzymes have local effects on target cell receptor exposure to active glucocorticoids. Additionally, there may be effects on hypothalamic-pituitary-adrenal (HPA) axis functioning. Compensatory changes in the HPA axis may occur to maintain plasma glucocorticoid levels in the face of altered peripheral clearance of cortisol, and glucocorticoid metabolizing enzymes are expressed in brain/pituitary cells involved in HPA axis feedback control, potentially altering the negative feedback signal (Rasmuson et al., 2001).

Recent studies have implicated 11 β -HSD1 activity in cognitive decline. Mice homozygous for deleterious mutations of the *hsd11b1* gene encoding 11 β -HSD1 are resistant to ageing-related cognitive decline (Yau et al., 2001), have lower intracerebral glucocorticoid levels despite normal or even slightly elevated plasma levels, and show enhanced hippocampal long-term potentiation (Yau et al., 2007). This is not a general retardation of ageing because the mice have the same lifespan as congenic wild-type controls. 11 β -HSD1 mRNA expression is increased in aged mouse brain, and higher expression within older animals is associated with worse cognitive function. Moreover, genetically-altered mice with increased forebrain 11 β -HSD1 activity show worse cognitive functioning, despite having unaltered

systemic corticosterone levels (Holmes et al., 2010). Whether or not 11 β -HSD1 or any other glucocorticoid metabolizing enzyme is causally related to cognitive decline in humans is unknown, although in healthy elderly men and subjects with type 2 diabetes, inhibition of 11 β -HSD for 4–6 weeks improved aspects of cognitive functioning (Sandeep et al., 2004).

An *in vivo* estimate of the systemic activity of 11 β -HSD1 is provided by the ratio of total steroid ring A-reduced metabolites of cortisol [5 β -tetrahydrocortisol (THF) and 5 α -tetrahydrocortisol (5 α -THF)] and cortisone [tetrahydrocortisone (THE)] in urine (Best and Walker, 1997; Courtney et al., 2008), abbreviated as THFs/THE. Thus higher THFs/THE indicates higher systemic 11 β -HSD1 activity and hence glucocorticoid regeneration. We performed a longitudinal study in initially healthy, non-demented men aged 65–70 to test the hypothesis that higher 11 β -HSD1 activity, but not activity of other glucocorticoid metabolizing enzymes or total glucocorticoid production, predicts brain atrophy (indexed by change in lateral ventricular volume and hippocampal volume), white matter lesions (as an indirect index of central vascular dysfunction) and cognitive decline during follow-up over six years.

METHOD

Subjects

The study was approved by the Lothian Health Ethics Committee. Subjects were healthy male volunteers aged 65–70 years (at the time of first recruitment in 1998–2000 [wave 1]) who were living in Edinburgh, Scotland. Six years later the same subjects were invited back for repeat analysis (2004–2006 [wave 2]). After complete description of the study to the subjects, written informed consent was obtained (at each wave). For wave 1 (N=76) subjects were recruited through an invitation letter and interview and gave informed consent. In wave 1 each subject was free of significant illness, including dementia, stroke, ischemic heart disease and depressive illness, excessive alcohol intake (> 30 units per week) and none was taking regular medication. In wave 2 the subjects cognitive and neuroimaging assessments were repeated. Those who had developed a history of stroke, dementia, cancer, or depression, or those taking psychotropic medication, were excluded (N=14). For both waves, these disorders were excluded by a combination of measures: questionnaire to the general practitioner, inspection of the medical records including the drug history, clinical interview, formal cognitive testing, and the Geriatric Depression Scale. Other reasons for exclusion (N=21) were that subjects had moved away, declined to participate, or had died. In the present study, data from subjects who participated in both waves and who had full glucocorticoid (wave 1), neuroimaging and cognitive data (waves 1 and 2) were analyzed (N=41). Those subjects who did not complete wave 2 did not differ from those who did in age, systolic blood pressure, diastolic blood pressure, body mass index, right or left hippocampal volume, total ventricular volume, deep or periventricular white matter lesions, cognitive test performance, or urinary glucocorticoid measures ($p > 0.05$ by t-test or Mann-Whitney U test).

Blood samples

In wave 1, venous blood was analyzed for urea and electrolytes, calcium, liver function tests, thyroid function tests, glucose, hemoglobin, white cell count, platelet count, B12 and folate levels by Clinical Biochemistry, Lothian NHS, and any subject with abnormalities according to standard clinical criteria was excluded. These tests were repeated in wave 2 and similar criteria for exclusion were applied, though subjects with fasting glucose levels above 7.0 mmol/l were not excluded. Blood pressure, weight and height were measured at both waves.

Magnetic resonance imaging and analysis

In wave 1, brain imaging was performed in an Elscint Prestige magnetic resonance (MR) scanner operating at 1.9T. Structural image acquisition followed a three-view localizer and consisted of a coronal T₁-weighted three-dimensional gradient-echo sequence with slices perpendicular to the long axis of the hippocampus covering the entire brain and skull (TE 9.254, TR 28.5, tip angle = 25°, field of view (FOV) 18cm, matrix 180 × 180, slice thickness 1.5 mm (no interslice gap)). Subjects also underwent fast spin echo T₂ axial imaging of the whole brain (TR 4000, TE 96, tip angle 160°, FOV 23 cm, matrix 256×256, slice thickness 5 mm, 1 mm slice gap,) and a FLAIR sequence (TE 6000, TI 2000, FOV 24cm, matrix 252×256, 5mm slice thickness, 2.5mm slice gap).

In wave 2, imaging was performed on a GE Signa LX 1.5T (General Electric) MR scanner, equipped with a self-shielding gradient set (22mT/m maximum gradient strength and 120 T/m/s slew rate) and manufacturer supplied “bird-cage” quadrature head coil. Participants received a sagittal T₁-weighted spin echo sequence covering the whole head (TR 450, TE 9, FOV 24cm, matrix 256×224, slice thickness 5 mm (no gap)) and a volume scan consisting of a T₁-weighted 3D inversion recovery prepared sequence (3D IR_PREP) acquired in the coronal plane with slices perpendicular to the long axis of the hippocampus and covering the whole head (TI 600 with TE set to minimum, FOV 22cm, matrix 256×192, slice thickness 1.7mm (no gap)). They also received axial fast-spin echo T₂ (TE 102, TR 6300, FOV 24cm, matrix 256×256 slice thickness 5.6mm (no gap)) and FLAIR (TE 140, TR 9000, TI 2200, FOV 22cm, matrix 256×128, slice thickness 5.6mm (no gap)) sequences covering the whole brain.

Image analysis—For wave 1 image analysis was carried out on Sun workstations (Sun Microsystems, Mountain View, CA, USA) using Analyze software v7.5 (Mayo Clinic, Rochester, MN). For wave 2, image analysis was carried out on a PC using Analyze v7.0 (for Windows). Volumetric methods were the same for both waves. Hippocampal and ventricular volumes, and intracranial area (a validated estimate of intracranial volume) were obtained as previously described using validated methods (Ferguson et al., 2005; MacLulich et al., 2002). Volumetric analysis was carried out for both waves by the same experienced research fellow who was blind to all other cognitive and biochemical data. Wave 2 data were anonymized. White matter lesions in the periventricular and deep white matter were assessed on hard copies of the T₂ and FLAIR images using the Fazekas scale, a widely-used four-point visual rating tool that evaluates periventricular hyperintensities (PVH) and deep white matter hyperintensities (DWMH) separately (Fazekas et al., 1993).

Cognitive Testing

We used the following standard, reliable and validated tests as previously described (MacLulich et al., 2002): Raven’s Standard Progressive Matrices (non-verbal reasoning) (Raven et al., 1977), Logical Memory (paragraph recall: immediate and delayed) and Visual Reproduction (visuospatial memory) from the Wechsler Memory Scale-Revised (Wechsler, 1987), the Rey Auditory-Verbal Learning Test (word list-learning) (Lezak, 1995), the Benton Visual Retention Test (Sivan, 1992), the Controlled Word Association Test (verbal fluency, using letters C, F, and S) (Lezak, 1995), the Digit-Symbol Substitution Test (attention and processing speed) from the Wechsler Adult Intelligence Scale (Wechsler, 1981), and the National Adult Reading Test (estimation of prior general cognitive ability) (Nelson and Willison, 1991).

Urinary glucocorticoid measurements

Glucocorticoid metabolites were quantified in 24 urine collection (sampled at wave 1) by gas chromatography electron impact mass spectrometry following solid phase extraction,

hydrolysis of conjugates and formation of their methoxime-trimethylsilyl derivatives, as described previously (Best and Walker, 1997). The following two main measures were analyzed in this study: (1) the ratio of (5beta-THF + 5alpha-THF) to those of cortisone (THE) as an indirect indicator of systemic 11 β -HSD1 activity (THFs/THE), and (2) the sum of total cortisol metabolites [THFs, THE, alpha-cortolone, cortisone, cortisol, beta-cortolone, beta-cortol, alpha-cortol] as a measure of total cortisol production. To determine if any results relating to apparent variations in estimated systemic 11 β -HSD1 activity could be explained by alterations in other pathways we also examined further sets of measures, specifically, the ratio of urinary cortisol (F) to cortisone (E), an indicator of renal 11 β -HSD2 activity, and absolute values and ratios indexing 5alpha- (5alpha-THF, 5alpha-THF/F) and 5beta-reductase activity: 5beta-THF, 5beta-THE, 5beta-THF/F, and 5beta-THE/E (Andrew et al., 1998).

Statistical analysis

(1) Cross-sectional analyses (wave 1)—To give an estimate of brain atrophy, hippocampal and ventricular volumes were adjusted for estimated intracranial capacity by using the saved residuals from a linear regression with intracranial area as the independent variable. This is a validated method (Ferguson et al., 2005). The adjusted volumes were then correlated with the glucocorticoid variables. Scores on cognitive ability tests (at wave 1) were adjusted using linear regression for the National Adult Reading Test (NART; a validated test of premorbid IQ (McGurn et al., 2004)), to give an estimate of long-term ageing-related cognitive decline. These adjusted scores were correlated with urinary glucocorticoids, metabolites and ratios. White matter lesion scores for PVH and DWMH were correlated with glucocorticoid data.

(2) Longitudinal analyses—Linear regression was used to adjust second wave neuroimaging and cognitive variables for first wave neuroimaging and cognitive variables. This method gives estimates of change of these variables between wave 1 to wave 2 of the study. The adjusted neuroimaging and cognitive variables were then correlated with the glucocorticoid variables. Correlations with wave 2 white matter lesion scores are reported but these scores were not adjusted for wave 1 scores because different scanners and sequences were used at each wave and may have influenced white matter lesion scoring. Pearson correlations were used except for white matter lesion scores, when Spearman's non-parametric correlations were used.

RESULTS

At wave 1, subjects were aged 65-70 years (mean 67.3, SD 1.3), and at the second wave 71-76 years (mean 73, SD = 1.3). Descriptive data for neuroimaging and cognitive measures are shown in Table 1. Means (standard deviations), in units of micrograms per day, for urinary glucocorticoids and metabolites (measured only in wave 1) were: total glucocorticoid metabolites 6887 (3340), cortisol 92 (33), cortisone 141 (75), 5alpha-THF 1815 (1029), 5beta-THF 1739 (830), and 5beta-THE 1862 (1100). Mean systolic blood pressures in waves 1 and 2 were 145 (SD 19) and 150 (SD 18), respectively. Mean diastolic blood pressures in waves 1 and 2 were 82 (SD 10) and 83 (9), respectively. Mean body mass index in wave 1 was 27 (SD 3) and in wave 2, 27 (3). Four participants had developed fasting glucose levels above 7.0 mmol/l in wave 2; these participants were referred for further evaluation for type II diabetes mellitus.

Mean hippocampal volumes did not decline between wave 1 and wave 2, whereas mean ventricular volumes were significantly larger in wave 2, indicating progressive general brain atrophy over the six-year study period (Table 1). Of the cognitive tests, the Digit-Symbol

Substitution Test, the Benton Visual Retention Test, Visual Reproduction and Raven's Standard Progressive Matrices showed statistically significant declines between wave 1 and wave 2. Logical Memory scores showed improved scores (Table 1).

Glucocorticoids, and neuroimaging and cognitive measures

Wave 1—Estimated systemic 11β -HSD1 activity (THFs/THE) significantly and negatively correlated with wave 1 hippocampal volumes (left: $r=-0.37$, $p=0.016$; right: $r=-0.34$, $p=0.031$), and positively correlated with ventricular volumes ($r=0.43$, $p=0.006$). Further, THFs/THE was significantly and positively correlated with periventricular white matter lesions (PVH ratings; $\rho=0.31$, $p=0.047$) (Table 2). Thus, wave 1 THFs/THE correlated significantly in the predicted directions with four of the five baseline neuroimaging variables. THFs/THE did not correlate with wave 1 cognitive ability. Total glucocorticoid production did not correlate with neuroimaging variables or cognitive ability at wave 1.

Wave 2—THFs/THE at wave 1 was positively and significantly correlated with wave 2 ventricular volumes (which were adjusted for wave 1 ventricular volumes) ($r=0.33$, $p=0.037$). Thus, THFs/THE predicted *additional* ventricular enlargement, indicative of further brain atrophy, between wave 1 and wave 2. Wave 2 hippocampal volumes (adjusted for wave 1) were not associated with THFs/THE, but as stated above, there was no significant change in hippocampal volumes in the six years between the two waves of testing. Baseline THFs/THE also showed a trend in the predicted direction to correlate with wave 2 periventricular white matter lesions (PVH ratings) ($\rho=0.29$, $p=0.071$) (Table 3).

THFs/THE was negatively and significantly correlated with adjusted wave 2 processing speed (digit symbol substitution test) at $r=-0.55$ ($p=0.0002$). This indicates that wave 1 THFs/THE predicted a highly significant decline in processing speed between wave 1 and wave 2. THFs/THE was not associated with significant change in the other cognitive tests. Total glucocorticoid production did not correlate with change in hippocampal or ventricular volumes, or change in cognitive function (Table 3).

To test whether variations in enzymes other than 11β -HSD1 contribute to associations with THFs/THE ratio, we explored associations with other urinary metabolite ratios. Cortisol/cortisone (F/E) ratios, reflecting renal 11β -HSD2 activity, did not correlate with neuroimaging or cognitive variables at either wave. There were some significant correlations among the absolute levels and ratios reflecting hepatic reductase activity. 5β -THF/F was negatively correlated with wave 1 PVH ratings ($r=-0.32$, $p=0.043$) and wave 2 PVH ratings ($\rho=-0.33$, $p=0.036$), and positively correlated with wave 2 (adjusted) Digit-Symbol Substitution Test ($r=0.35$, $p=0.025$).

Given that THFs/THE was associated with both increased ventricular volume and decline in processing speed, we conducted mediation analysis to explore the possibility that the processing speed decline was mediated by the impact of THFs/THE on ventricular volume. The Sobel Test statistic was -1.72 , with a two-tailed probability of $p=0.085$. This does not support ventricular volume as a major mediator of the effect of THFs/THE on cognition.

Body mass index did not correlate significantly with glucocorticoid variables, neuroimaging variables, or cognitive function. Controlling for age, body mass index or blood pressure made no difference to the overall pattern of results.

DISCUSSION

The main novel finding in this study was that higher baseline systemic 11β -HSD1 activity (THFs/THE), but not total glucocorticoid production rates or other glucocorticoid metabolic

indices, prospectively associated with progressive general brain atrophy and decline in processing speed over a six year period in older men. Higher THFs/THE was also associated with lower baseline hippocampal volumes and greater white matter lesion load, though not with baseline cognitive ability.

In this initially very healthy sample of older men, hippocampal volume did not decline significantly over the six year period. This is consistent with other studies showing that hippocampal volume shows little atrophy in non-demented young-elderly individuals (Ferguson et al., 2009), in contrast to ventricular volume, which shows a mean progressive increase from late middle-age and which is an early predictor of mild cognitive impairment and dementia (Carlson et al., 2008; Carmichael et al., 2007). Additionally, performance in three out of the seven cognitive tests did not decline significantly over the six year gap between assessments. Of the three tests that showed decline, only processing speed, as measured by the Digit Symbol Substitution Test, was associated with baseline THFs/THE ratios. However, processing speed is the earliest cognitive domain to show decline with ageing, shows large declines, accounts for substantial part of the variance in ageing-related decline in other cognitive domains, and is an early predictor of dementia (Finkel et al., 2007; Salthouse, 2000). Decline in processing speed is closely associated with ventricular enlargement (Longstreth et al., 2000). Therefore, in early ageing in relatively healthy individuals the THFs/THE ratio may be related to early loss of cerebral hemispheric white matter volume and the associated decline in processing speed, but not with hippocampal grey matter loss typically linked to declarative memory loss.

Other than THFs/THE, there were some other significant correlations between glucocorticoid metabolite levels and ratios, neuroimaging and cognitive variables. However, in contrast to the relationships with THFs/THE, no consistent pattern was evident. Importantly, the F/E ratio, indicative of renal 11 β -HSD2 activity, was not correlated with any neuroimaging or cognitive variables. Thus, our results are broadly consistent with the hypothesis that higher systemic 11 β -HSD1 activity, as indexed by THFs/THE, is specifically associated with adverse changes in the brain in older individuals. Whilst this ratio may be confounded by differential changes in 5 α - or 5 β -reductases, other indices of these enzymes were mainly not associated with ageing-related changes in cognition or brain volumes, especially changes with time. Thus it is reasonable to suggest that the component of the THFs/THE ratio that correlates with brain structure and function is, in large part, a reflection of 11 β -HSD1.

Our results are consistent with in vitro and in vivo studies (in animals and humans) suggesting that lower 11 β -HSD1 activity protects against adverse neural and cognitive outcomes with ageing (Rajan et al., 1996; Sandeep et al., 2004; Yau et al., 2001; Yau et al., 2007). Which tissues are exhibiting increased 11 β -HSD1 activity and contributing to higher THFs/THE ratios is currently unknown, though most 11 β -HSD1 activity is in the liver/splanchnic bed (Andrew et al., 2005; Basu et al., 2004). At least some aspects of the regulation of 11 β -HSD1 (i.e. control by glucocorticoids) is similar in brain, liver and other organs (Holmes and Seckl, 2006; Low et al., 1994; Pelletier et al., 2007), so peripheral 11 β -HSD1 may mirror local activity in CNS, though more studies are required to resolve this important issue. Moreover, recent data in mice suggest that hepatic 11 β -HSD1 activity may impact upon the brain since the alterations in HPA axis activity seen in mice globally deficient in 11 β -HSD1 are reversed by rescue of the enzyme in liver alone (Paterson et al., 2007). The mechanisms by which variations in overall 11 β -HSD1 activity might affect the brain are unclear. If increased THFs/THE values do reflect increased *central* 11 β -HSD1 activity, then brain exposure to glucocorticoids would likely be greater, with well-studied deleterious consequences (Yau et al., 2001). Alternatively, higher systemic 11 β -HSD1

activity might give rise to subtle metabolic disturbance, such as increased insulin resistance and higher glucose in the non-diabetic range (MacLulich et al., 2004).

We did not find that total urinary glucocorticoids were associated with neuroimaging or cognitive variables. These results differ from a recently published study of 538 people aged 70-79, which found that higher urinary cortisol predicted cognitive decline (Karlman et al., 2005); however, the duration of collection of cortisol was different (12hr, versus 24hr in the present study), the participants in the present study were younger and healthier at the time of investigation and the smaller sample size here may have precluded finding a modest association.

There were some limitations which should be noted. This is an observational study and therefore cannot demonstrate that THFs/THE activity plays a causal role in brain atrophy and cognitive decline. It is possible that subclinical neurodegenerative processes already occurring in wave 1 could be responsible for increased 11 β -HSD1 activity through as yet unknown mechanisms, or that the subclinical declines in processing speed were associated with a degree of stress which in turn led to activation of the HPA axis and associated higher THFs/THE activation. Interventional studies would help to resolve this issue. THFs/THE is an indirect measure of systemic 11 β -HSD1 activity and the functioning of other enzymes could alter this ratio. However, to some extent this possibility can be excluded by examining F/E and the levels and metabolite ratios reflecting activity of 5 α - and 5 β -reductases, as we have done in the present study. The length of time between the two waves of assessment was relatively short, and this might mean that more subtle changes could have been missed. The sample size was relatively small, and subjects were healthy at baseline. All subjects were male; this was because of sex differences in glucocorticoid levels (Otte et al., 2005) and because we wished to avoid any confounding effects of hormone replacement therapy (commonly prescribed in our population at the time of initial sampling). We examined relationships among multiple variables and this give rise to the possibility of type I statistical errors. However, we tested pre-specified hypotheses concerning potential adverse associations between higher estimated systemic 11 β -HSD1 activity and overall glucocorticoid production, and adverse neurocognitive outcomes which were firmly based on prior work with animal models and also rational theoretical predictions. We used different scanners at the two time-points (the original scanner had to be replaced because of an unanticipated change in ownership of the manufacturers of the original scanner). This affects the analysis of change in white matter lesions or other measures and future studies should aim to avoid this methodological issue. However, at high resolution and with similar image acquisition protocols and careful standardization of measured volumes against intracranial volume, there is a very low percentage error rate between different scanners (Reig et al., 2007).

In conclusion, these results are consistent with the possibility that increased estimated systemic 11 β -HSD1 activity is associated with detrimental effects on the CNS and cognitive decline. Further work will help to dissect the pathways through which such associations may operate. These findings support the possibility that inhibition of 11 β -HSD1 may have beneficial effects in the CNS to prevent some detrimental effects of ageing (Sandeep et al., 2004).

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TABLE 1
Neuroimaging and cognitive test data at waves 1 (aged 65-70) and 2 (aged 71 to 76)

	Wave 1 (SD)	Wave 2 (SD)	^a p-value
Left hippocampal volume (mm ³)	3439 (468)	3495(402)	0.373
Right hippocampal volume (mm ³)	3514(485)	3523(435)	0.886
Total ventricular volume (mm ³)	30980(20776)	38930 (26460)	< 0.001
Periventricular WMH number	1.04(0.80)	1.11(0.80)	N/A
Deep WMH number	0.88(0.73)	0.89(0.77)	N/A
Verbal fluency	42.3 (12.9)	40.1 (11.4)	0.080
Digit-Symbol Substitution Test	49.9 (10.7)	43.1 (10.9)	< 0.001
Auditory-Verbal Learning Test	48.6 (6.7)	50.0 (9.0)	0.116
Benton Visual Retention Test ^b	4.9 (3.0)	6.0 (3.8)	0.016
Logical Memory	46.2 (12.1)	50.7 (15.1)	0.006
Visual Reproduction	60.2 (12.8)	46.3 (15.1)	< 0.001
Raven's Matrices	41.9 (8.1)	39.0 (9.1)	< 0.001

^a paired t-test

^b Error score, ie. higher scores indicate worse performance.

ABBREVIATION WMH: white matter hyperintensities.

TABLE 2
Correlations between indices of 11 β -HSD1 (THFs/THE) and cortisol production (TUGM), and neuroimaging and cognitive variables in wave 1

	THFs/THE	TUGM
WAVE 1		
Left hippocampal volume	-.37 **	.13
Right hippocampal volume	-.34 *	.18
Total ventricular volume	.43 *	-.22
Periventricular WMH	.32 *	-.07
Deep WMH	.30	-.15
Verbal fluency	-.03	.23
Digit-Symbol Substitution Test	.01	.28
Auditory-Verbal Learning Test	-.15	.10
Benton Visual Retention Test ^a	.10	.07
Logical Memory	-.15	.02
Visual Reproduction	-.07	.25
Raven's Matrices	-.24	.30

* p<0.05;

** p<0.01

Hippocampal and ventricular volumes are adjusted for estimated intracranial capacity. Wave 1 cognitive tests are adjusted for estimated premorbid IQ.

^a Higher scores indicate worse performance.

ABBREVIATIONS TUGM: total urinary glucocorticoid metabolites; THFs/THE: ratio of tetrahydrometabolites of cortisol (5 β -THF + 5 α -THF) to those of cortisone (THE); WMH: white matter hyperintensities.

TABLE 3
Correlations between indices of 11 β -HSD1 (THFs/THE) and cortisol production (TUGM), and adjusted neuroimaging and cognitive variables in wave 2

	THFs/THE	TUGM
WAVE 2		
Left hippocampal volume	.11	.08
Right hippocampal volume	.05	-.06
Total ventricular volume	.33 *	-.20
Periventricular WMH	.26	.05
Deep WMH	.05	-.07
Verbal fluency	-.07	.06
Digit-Symbol Substitution Test	-.55 **	.18
Auditory-Verbal Learning Test	.05	-.05
Benton Visual Retention Test ^a	-.18	-.12
Logical Memory	-.10	-.18
Visual Reproduction	.02	.07
Raven's Matrices	-.18	.16

* p<0.05;

** p<0.01

Wave 2 hippocampal and ventricular volumes are adjusted for their Wave 1 counterparts.

Wave 2 cognitive tests are adjusted for Wave 1 test scores.

^aHigher scores indicate worse performance.

ABBREVIATIONS TUGM: total urinary glucocorticoid metabolites; THFs/THE: ratio of tetrahydrometabolites of cortisol (5 β -THF + 5 α -THF) to those of cortisone (THE); WMH: white matter hyperintensities