Brief Communications

11β-Hydroxysteroid Dehydrogenase Type 1 Expression Is Increased in the Aged Mouse Hippocampus and Parietal Cortex and Causes Memory Impairments

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Increased neuronal glucocorticoid exposure may underlie interindividual variation in cognitive function with aging in rodents and humans. 11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) catalyzes the regeneration of active glucocorticoids within cells (in brain and other tissues), thus amplifying steroid action. We examined whether 11β-HSD1 plays a role in the pathogenesis of cognitive deficits associated with aging in male C57BL/6J mice. We show that 11β-HSD1 levels increase with age in CA3 hippocampus and parietal cortex, correlating with impaired cognitive performance in the water maze. In contrast, neither circulating corticosterone levels nor tissue corticosteroid receptor expression correlates with cognition. 11β-HSD1 elevation appears causal, since aging (18 months) male transgenic mice with forebrain-specific 11β-HSD1 overexpression (~50% in hippocampus) exhibit premature age-associated cognitive decline in the absence of altered circulating glucocorticoid levels or other behavioral (affective) deficits. Thus, excess 11β-HSD1 in forebrain is a cause of as well as a therapeutic target in memory impairments with aging.

Introduction

Chronically elevated glucocorticoid (GC) levels are detrimental to the brain, especially to the hippocampus. In the adult hippocampus, GC excess potentiates excitatory neurotransmission, disrupts electrophysiological functions such as long-term potentiation (LTP) thought to underlie memory, interferes with learning and recall, promotes dendritic atrophy, and may potentiate neurotoxicity (McEwen et al., 1999). Interindividual differences in plasma GC levels may underpin variation in cognitive function with aging (Meaney et al., 1995) and higher steroid levels associating with and even predicting subsequent cognitive deficits and hippocampal atrophy in rodents (Yau et al., 2002) and humans (Lupien et al., 1998). Elevated GC levels appear causal of cognitive decline with aging, since manipulations that maintain low GC levels from mid-life, such as adrenalectomy and low-dose GC replacement (Landfield et al., 1981) and antidepressant drug therapy (Yau et al., 2002), prevent the emergence of cognitive deficits with subsequent aging, at least in rodents.

GC action on target cells is not dependent merely upon hormone levels in the circulation and the density of intracellular glucocorticoid (GR) and mineralocorticoid (MR) receptors in target tissues, but also on prereceptor metabolism by 11β-hydroxysteroid dehydrogenases (11β-HSDs) (Holmes and Seckl, 2006). The adult rodent and human brains highly express only the type 1 isozyme (11β-HSD1), which catalyzes the regeneration of active glucocorticoids (cortisol, corticosterone) from inert 11-keto forms (cortisone, 11-dehydrocorticosterone) in neurons (Rajan et al., 1996), thus amplifying cellular GC action. Importantly, aged mice deficient in 11β-HSD1 (11β-HSD1$^{-/-}$ mice) have reduced intrahippocampal levels of corticosterone despite normal circulating concentrations (Yau et al., 2001). 11β-HSD1$^{-/-}$ mice are protected from the normal decline in memory and hippocampal LTP seen with aging (Yau et al., 2001, 2007), suggesting that 11β-HSD1 is an important control of intraneuronal GC action *in vivo*. Hence, 11β-HSD1 inhibition is a target for therapy of age-associated cognitive disorders (Wamil and Seckl, 2007). Indeed, in two small randomized, double-blind, placebo-controlled trials, an 11β-HSD inhibitor improved cognitive function in elderly men and patients with type 2 diabetes (Sandeep et al., 2004). However, these genetic and therapeutic manipulations affect the whole body, and 11β-HSD1 is highly expressed in peripheral organs, notably in liver and adipose tissue (Stewart et al., 1999). The primary role of 11β-HSD1 in the brain per se is not clear cut. For example, changes in peripheral (hepatic) 11β-HSD1 alone alter brain function, at least at the level of the hypothalamic-pituitary-adrenal (HPA) axis (Paterson et al., 2007).

While 11β-HSD1 is elevated selectively in adipose tissue in human and rodent obesity (Livingstone et al., 2000; Rask et al., 2002) and appears a plausible cause of metabolic syndrome

Received Feb. 10, 2010; revised March 24, 2010; accepted April 2, 2010.

This work was supported by a Wellcome Trust project grant (WT070207; M.C.H., J.L.P., J.J.M., J.R.S.), a Medical Research Council project grant (G0501596; J.L.W.Y., J.R.S.), and a Wellcome Trust program grant (WT083184; J.R.S., J.J.M.). J.L.W.Y. holds a Research Councils UK Academic Fellowship. M.C.H., J.L.W.Y., and J.R.S. are members of The University of Edinburgh Centre for Cognitive Aging and Cognitive Epidemiology, part of the cross council Lifelong Health and Wellbeing Initiative (G0700704/84698). Funding from the Biotechnology and Biological Sciences Research Council, Engineering and Physical Sciences Research Council, Economic and Social Research Council, and Medical Research Council is gratefully acknowledged.

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DOI:10.1523/JNEUROSCI.0731-10.2010

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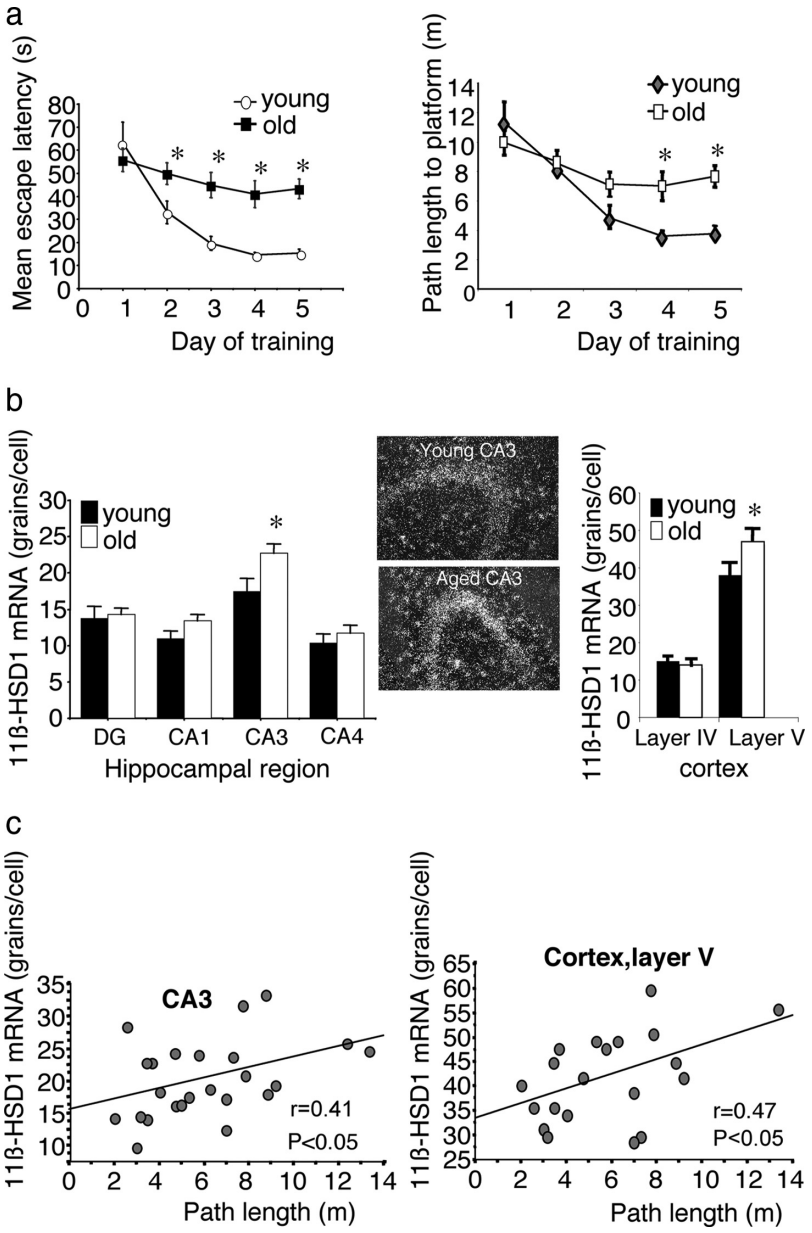

Figure 1. Cognitively impaired aged mice have increased 11β-HSD1 mRNA in hippocampus and cortex. ***a***, Aged 24–27 month C57BL/6J mice ($n = 14$) show impaired spatial learning in the water maze [escape latency and path length (mean ± SEM) to find submerged platform]; *$p < 0.001$, compared with young 6 m controls ($n = 10$). ***b***, Aged mice have increased 11β-HSD1 mRNA in CA3 hippocampal and cortical layer V cells; *$p < 0.05$ compared with young. ***c***, 11β-HSD1 mRNA expression in CA3 hippocampus and layer V cortex correlates ($p < 0.05$), with mean path length (day 5) to find the hidden platform.

(Masuzaki et al., 2001, 2003), any primary role of 11β-HSD1 in the CNS in causation of cognitive variation with aging is unexplored. Here, we examined the critical issues of whether endogenous 11β-HSD1 levels vary with cognitive function in the hippocampus of aged mice, and whether specific elevation of 11β-HSD1 in the forebrain (including the hippocampus) has cognitive consequences with aging.

Materials and Methods

Control C57BL/6J mice

Male C57BL/6J mice were purchased at 8–10 weeks old (Harlan) and maintained in cages housing 3–4 mice on standard chow (product 801190, Special Diet Services) and tap water *ad libitum* (lights on 0700–1900) in our animal facilities until they were ready for experimentation at 6 months (young) and 24–27 months (aged). All procedures were performed in strict accordance with the United Kingdom Animals (Scientific Procedures) Act (1986).

Basal (0800 h) tail venesection blood samples were taken for corticosterone assay. At least 1 week later, animals were tested in the reference memory water maze task, first learning to escape to a visible platform (submerged but marked with visible tower block protruding 10 cm on top) over four consecutive days of nonspatial training (three trials per day with curtains around the pool to hide visuospatial cues) and then a hidden platform over five consecutive days of spatial training (four trials per day with no curtains), essentially as described by Yau et al. (2007). One hour after the last spatial training trial, a 60 s probe trial was performed with the platform removed. Swim paths and measures of performance were analyzed by WaterMaze software (Actimetrics). Aged mice showing signs of motor or visual impairments were excluded (2 aged mice were excluded of 16 tested). Mice were killed by decapitation and the brains were removed and snap-frozen on soft dry ice for cryostat sectioning and *in situ* hybridization using ^{35}S-UTP-labeled cRNA antisense probes as described (Mattsson et al., 2003) for 11β-HSD1, MR, and GR mRNAs in the anterior hippocampus. Slides were dipped in photographic emulsion (NTB-2, Kodak) and exposed at 4°C for 14–21 d.

Transgenic mice with forebrain overexpression of 11β-HSD1

Generation of construct for forebrain overexpression of 11β-HSD1 and preparation of DNA fragment for mouse embryo microinjection. The rat 11β-HSD1 cDNA-based minigene, previously used to overexpress 11β-HSD1 in adipose tissue and liver (Masuzaki et al., 2001; Paterson et al., 2004), was fused in-frame at the C terminus to the influenza virus-derived hemagglutinin (HA) epitope tag by PCR-mediated, site-directed mutagenesis. This was inserted downstream of the CamIIK promoter directing transgene expression to the forebrain to yield CamIIK-HSD1, a 10.5 kb DNA fragment, was prepared for microinjection by agarose gel electrophoresis, electroelution, and dialysis against 10 mM Tris.HCl/0.1 mM EDTA (pH7.4) before dilution of DNA to a concentration of 1 ng/μl. Construct expression was confirmed by transfection into Cos7 cells, and 11β-HSD1 activity was measured.

Generation of transgenic animals and genotyping of experimental animals. Microinjection into the pronuclei of fertilized C57BL/6 × CBA/C3H F1 embryos was performed using standard techniques. G_0 offspring were screened by Southern blot hybridization analysis of tail biopsy genomic DNA digested with BamHI and probed with [α-^{32}P]dCTP-labeled rat 11β-HSD1 cDNA to reveal diagnostic restriction fragments. Transgenic lines 3615 and 3621 carrying high and low copies of the transgene, respectively, were propagated from independent founder animals. F7 or greater C57BL/6J backcross male mice were studied throughout. Mice hemizygous for the transgene [referred to as CamIIK-HSD1 or transgenic (Tg) mice] were compared with nontransgenic [wild-type (wt)] littermate controls. Animals were fed standard chow and water *ad libitum*.

Localization and expression of the 11β-HSD1 transgene. The expression pattern of the transgene was determined by immunohistochemistry, using an antibody directed against the HA tag. Mice (terminally anesthe-

tized with sodium pentabarbitone) were transcardially perfused with 4% paraformaldehyde, and the brains were postfixed and frozen. Transgene protein was localized on 60 μM coronal cryostat sections using a rabbit anti-HA antibody (catalog #71-5500, Invitrogen, Zymed Laboratories) in conjunction with the streptavidin-biotin-based peroxidase staining system (Vector Laboratories). Controls included wild-type littermates. To determine the effect of the transgene on 11β-HSD1 activity, various brain regions were homogenized and assayed as described (Sandeep et al., 2004). After this basic characterization, studies focused on the highest expressing line (3615), which exhibited hippocampal 11β-HSD1 activity 50% above that of wild type. Key data were confirmed in a separate line (3621).

HPA axis function. Male CamIIK-HSD1 Tg mice and their wt littermates aged 3–6 months were housed singly for 1 week before study. Basal blood samples were taken by tail nick at 0800–0900 h for circadian nadir and 1900–2000 h for peak corticosterone levels. At least 1 week later, animals were subjected to restraint stress (10 min), with samples taken before and 10 and 90 min after stress. Plasma corticosterone was determined by radioimmunoassay as described previously (Paterson et al., 2007).

Behavior. Male Tg mice and wild-type mice, young (6–9 months) and aged (18 months), were accustomed to the behavioral room to minimize stress. Mice were tested for affective behaviors in the open field and elevated plus maze (Holmes et al., 2006) and for learning and spatial memory retention (probe test) in the water maze as described above for C57BL/6J mice. Data were accumulated by automated video recording and quantified blind to genotype. In addition, conditioned passive avoidance was assessed in a light/dark box (step-through passive avoidance apparatus; Ugo Basile) using the following protocol: day 1: 5 min free exploration of the apparatus; day 2: latency to enter the dark compartment, followed by a mild 0.3 mA or moderate 0.5 mA 3 s footshock in the dark compartment, then retest 5 h later for latency to enter the dark compartment. Latencies are measured automatically by the integral software following the opening of the door separating the light and dark components. Old (18 months) mice (wt or Tg) failed to respond to the same magnitude of shock (0.3 mA) as young mice with an increased latency to re-enter the dark compartment, suggesting insensitivity to mild shock, and they were thus given a more intense footshock (0.5 mA).

Statistics

Differences by genotype were analyzed by Student's *t* test or one- or two-way ANOVA with *post hoc* Tukey's honestly significant difference tests, as appropriate. Significance was set at $p < 0.05$. Data are means ± SEMs.

Results

Young (6 months, $n = 10$) and aged (24–27 months, $n = 14$) male C57BL/6J mice were tested in the reference memory water maze task (Fig. 1*a*). Aged mice had slower average swim speeds (young 0.25 ± 0.01 m/s; old 0.18 ± 0.01 m/s; ANOVA, age effect, $F_{(1,22)} = 17.9$, $p = 0.0003$) but showed similar visible platform learning over 4 d of trials (decrease in escape latency: young, 18.58 ± 1.20 s; old, 21.84 ± 3.44 s; $F_{(1,22)} = 0.6$, $p = 0.4$), indicating no difference in perception (vision) or motivation with aging. While young mice were able to acquire the spatial memory task with 5 d of training, aged mice were impaired in their spatial learning performance [escape latency, ANOVA, age effect: $F_{(1,22)} = 14.8$, $p = 0.0009$; path length: $F_{(1,22)} = 4.2$, $p = 0.05$], failing to acquire the task as confirmed in the probe trial [percentage time in target quadrant of probe test: young 47.46 ± 2.77; aged 25.4 ± 2.7; ANOVA, age effect: $F_{(1,22)} = 30.8$, $p < 0.001$] (Fig. 1*a*).

Hippocampal 11β-HSD1 mRNA levels were significantly increased with aging selectively in CA3 pyramidal cells ($F_{(1,22)} = 5.6$, $p < 0.05$) (Fig. 1*b*). Examination of individual animals revealed that 11β-HSD1 mRNA in CA3 cells correlated with spatial learning and spatial memory retention [path length to platform on day 5: $r = 0.41$, $F_{(1,22)} = 4.3$, $p < 0.05$; probe test: $r = 0.44$, $F_{(1,22)} = 5.2$, $p < 0.05$] (Fig. 1*c*), such that higher 11β-HSD1 mRNA associated with poorer learning and memory. Neither MR nor GR mRNA expression was altered with age in any hippocampal subregion (supplemental Fig.S1, available at www.jneurosci.org as supplemental material). In the parietal cortex, 11β-HSD1 mRNA 11β-HSD1 mRNA expression was significantly higher in layer V of aged compared with young mice [$F_{(1,22)} = 4.4$, $p < 0.05$] and correlated negatively with spatial learning [path lengths on day 5: $r = 0.47$, $F_{(1,18)} = 5.2$, $p < 0.05$)] (Fig. 1*c*) but not spatial memory retention (probe test) ($p = 0.3$). Plasma corticosterone levels were significantly increased in aged C57BL/6J mice [young: 3.3 ± 12.5 nM; aged: 147.2 ± 18.8 nM, $F_{(1,22)} = 6.9$, $p < 0.05$] but did not correlate with water maze performance (path length on day 5: $p = 0.6$; probe: $p = 0.3$).

To determine whether the increased brain 11β-HSD1 causes learning and memory deficits, forebrain-specific CamIIK-HSD1 Tg mice (three lines) and wt littermates were generated. Tg mice exhibited transgene protein in the forebrain, notably in cortex and hippocampus, showing an increase in 11β-HSD1 activity of 125 and 50% in these regions, respectively (Fig. 2*a*).

Young (6–9 months) Tg mice learned to find and escape onto the hidden water maze platform as well as wt (Fig. 2*b*) [genotype effect: $F_{(1,15)} = 1.46$, $p = 0.37$]. While old (18 months) Tg mice

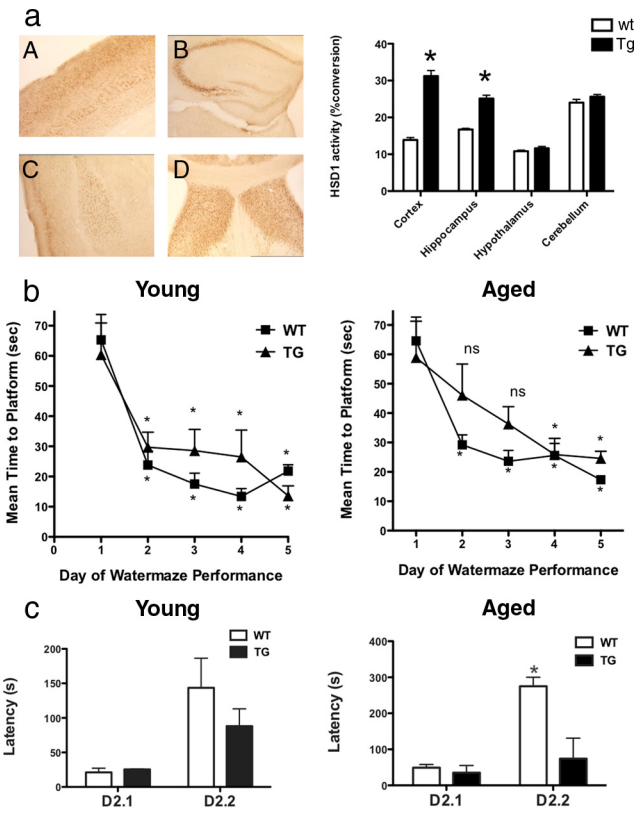

Figure 2. Forebrain overexpression of 11β-HSD1 causes cognitive decline with aging. ***a***, Left, Immunohistochemical localization of CamIIK-HSD1 transgene using HA-tag antibodies in cerebral cortex (***A***), hippocampus (***B***), amygdala (***C***), and lateral septum (***D***). Right, 11β-HSD1 activity (percentage conversion corticosterone-11-dehydrocorticosterone) was increased (*$p < 0.05$) in cerebral cortex and hippocampus of CamIIK-HSD1 (Tg) mice compared with wt, $n = 7$ per group. ***b***, Aging (18 months), but not young (6–9 months) ($n = 8$/group), Tg mice display delayed learning of water maze task compared with age-matched wt littermates; *$p < 0.05$, compared with respective day 1 value. ***c***, Attenuated retention of conditioned behavior in aging Tg mice. Young and aging Tg and wt mice were tested for latency to move from light to dark compartments on day 2 shock trial (D2.1) and 5 h postshock (D2.2); *$p < 0.05$ compared with D2.1 value.

performed the cued version of the water maze as well as wt with comparable swim speeds (wt: 0.18 ± 0.01 m/s; Tg: 0.18 ± 0.02 m/s), they were slower to learn the hidden platform location; specifically, wt mice, but not Tg mice, significantly improved between the first and second day of training [$F_{(1,14)} = 3.8$, $p < 0.01$] (Fig. 2*b*).

In the conditioned passive avoidance test, young Tg and wt mice had similarly increased latencies to re-enter the dark compartment at 5 h re test following footshock [genotype effect: $F_{(1,14)} = 0.29$, $p = 0.08$] (Fig. 2*b*). While old wt mice responded with an increased latency to enter the dark compartment, old Tg mice failed to respond to this conditioning stimulus [genotype effect: $F_{(1,14)} = 9.8$, $p = 0.01$] (Fig. 2*c*). This impaired learning was not due to anxiety-related behaviors (supplemental Table S1, available at www.jneurosci.org as supplemental material) or to altered basal, circadian, or stress-induced glucocorticoid levels (supplemental Table S2, available at www.jneurosci.org as supplemental material), mirroring normal HPA axis function in 11β-HSD1$^{-/-}$ mice on this background (Yau et al., 2007).

Discussion

The key findings in this study are that 11β-HSD1 expression is increased in the hippocampus and cortex with aging in mice and that levels of 11β-HSD1 mRNA in CA3 and cortical layer V pyramidal cells correlate with cognitive function in the water maze. Modest transgenic overexpression of 11β-HSD1 in the forebrain produces accelerated cognitive dysfunction with aging, providing the first evidence that forebrain 11β-HSD1 levels alone are sufficient to alter cognitive behavior in aging mice, an effect not due to affective dysfunction or altered plasma glucocorticoid levels.

Increased 11β-HSD1 mRNA expression with age in C57BL/6J mice was confined to hippocampal CA3 and cortical layer V pyramidal cells; interestingly, both regions are particularly sensitive to age-related damage (Casu et al., 2002; Mueller et al., 2008). In CA3 specifically, excess glucocorticoids and chronic restraint stress cause dendritic atrophy, a structural deterioration that makes neurons more vulnerable to excitotoxins (Conrad et al., 2007) and impairs spatial memory (Luine et al., 1994; Conrad et al., 1996; Sunanda et al., 2000). Moreover, spatial memory in the water maze depends critically on the integrity of the hippocampal CA3 subfield (Steffenach et al., 2002) and on plasticity-related mRNA transcripts within the hippocampal CA3 subregion (Haberman et al., 2008). Parietal cortex is also involved in spatial learning (Save and Poucet, 2009).

While the selective increase in hippocampal 11β-HSD1 mRNA expression was modest (with no changes to MR or GR expression), two lines of evidence suggest this may be sufficient to influence hippocampal functional deficits with aging. First, 11β-HSD1 mRNA in CA3 cells correlated with age-associated cognitive function. Neither plasma corticosterone levels, which increased with age, nor the hippocampal MR and GR expression correlated with spatial learning and memory. Although correlation does not prove causation, modest transgenic overexpression of 11β-HSD1 in forebrain, including hippocampus, produced hippocampus-associated cognitive deficits in the water maze and inhibitory avoidance tasks. Indeed, in this model 11β-HSD1 expression is modified solely in the forebrain to generate an accelerated cognitive decline observed at a time when the wild-type controls have unimpaired cognition; this excludes the possibility that other factors modified in aging may be causal in generating this phenotype. Since we found no evidence for either of the elevated plasma corticosterone levels (diurnal or with stress) in the transgenic mice, we infer that the likely cause of the cognitive deficits is intraneuronal glucocorticoid excess, the reverse of the situation in 11β-HSD1$^{-/-}$ mice, but in this case clearly reflecting direct effects upon the CNS rather than effects secondary to the metabolic and neuroendocrine changes seen in generalized 11β-HSD1 knock-out mice (Kotelevtsev et al., 1997; Harris et al., 2001; Morton et al., 2001).

The mechanisms leading to elevated 11β-HSD1 in specific neurons are uncertain. However, the 11β-HSD1 gene promoter is predominantly regulated directly by CCAAT/enhancer binding protein (C/EBP) transcription factors, with induction by C/EBPα and constraint by C/EBPβ, at least in liver (Chapman and Seckl, 2008). C/EBPβ is also important for memory consolidation (Taubenfeld et al., 2001) and intriguingly shows deficient induction in the aging hippocampus (Monti et al., 2005).

The cognitive deficits were only manifest with aging in Tg mice, thus excluding developmental effects of the transgenic manipulation, which are also unlikely given that expression from the CAMIIK promoter occurs postnatally, when much brain development is complete. There may be several reasons why we did not observe cognitive deficits in young Tg mice: (1) intraneuronal corticosterone concentrations (via 11β-HSD1 action and free corticosterone from blood) may not be high enough in young Tg mice, with lower plasma corticosterone levels than aged mice, to have an impact on cognitive function; (2) small cognitive impairments may indeed exist in young Tg mice, but the tasks used were not sufficiently demanding for the detection of such changes; and (3) aging per se may be necessary for the emergence of deficits. Indeed, aged mice are more sensitive to stress-induced spatial memory impairments than young mice (Buchanan et al., 2008); the time-related accumulation of myriad deficits that underpin "allostatic load" through life may also be pertinent (McEwen, 2007), interacting with increased intracellular GC exposure to initiate premature cognitive deficits. Taken alongside the rise of 11β-HSD1 in the hippocampus and layer V of the cortex of aged, cognitively impaired mice, these data suggest that cognitive decline with aging may reflect intraneuronal "Cushing's disease of the brain," tipping the balance from the beneficial adaptive effects of acute rises in glucocorticoids to the deleterious consequences of chronically elevated levels. This forms intriguing parallels with obesity and metabolic syndrome, which associate with excess 11β-HSD1 selectively in adipose tissue (Chapman and Seckl, 2008). The data also afford a rationale for developing 11β-HSD1 inhibitors for therapy of age-related cognitive disorders (Webster et al., 2007). Indeed early proof-of-concept studies suggest efficacy in humans (Sandeep et al., 2004).

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