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Midlife stress alters memory and mood-related behaviors in old age: Role of locally activated glucocorticoids

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ABSTRACT

Chronic exposure to stress during midlife associates with subsequent age-related cognitive decline and may increase the vulnerability to develop psychiatric conditions. Increased hypothalamic-pituitary-adrenal (HPA) axis activity has been implicated in pathogenesis though any causative role for glucocorticoids is unestablished. This study investigated the contribution of local glucocorticoid regeneration by the intracellular enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), in persisting midlife stress-induced behavioral effects in mice. Middle-aged (10 months old) 11β-HSD1-deficient mice and wild-type congenic controls were randomly assigned to 28 days of chronic unpredictable stress or left undisturbed (non-stressed). All mice underwent behavioral testing at the end of the stress/non-stress period and again 6–7 months later. Chronic stress impaired spatial memory in middle-aged wild-type mice. The effects, involving a wide spectrum of behavioral modalities, persisted for 6–7 months after cessation of stress into early senescence. Enduring effects after midlife stress included impaired spatial memory, enhanced contextual fear memory, impaired fear extinction, heightened anxiety, depressive-like behavior, as well as reduced hippocampal glucocorticoid receptor mRNA expression. In contrast, 11β-HSD1 deficient mice resisted both immediate and enduring effects of chronic stress, despite similar stress-induced increases in systemic glucocorticoid activity during midlife stress. In conclusion, chronic stress in midlife exerts persisting effects leading to cognitive and affective dysfunction in old age via mechanisms that depend, at least in part, on brain glucocorticoids generated locally by 11β-HSD1. This finding supports selective 11β-HSD1 inhibition as a novel therapeutic target to ameliorate the long-term consequences of stress-related psychiatric disorders in midlife.

1. Introduction

Chronic stress, which activates the HPA axis and thus increases blood levels of glucocorticoid (GC) stress hormones, causes contemporary cognitive dysfunction and may contribute to the development of depression and anxiety-related disorders (Lupien et al., 2009; Pryce and Fuchs, 2017). Stress or GC overexposure in early life (prenatal, early postnatal, juvenile) can "programme" lifetime alterations in cognitive and affective function (Meaney et al., 1988; Patchev et al., 2014). Whether an episode of chronic stress in adulthood has persisting effects or interacts with natural aging processes to advance cognitive decline and alter affective behaviors is unclear.

Recent human population-based studies have shown an association between levels of stress and accelerated cognitive decline in adults at 65 years and older (Aggarwal et al., 2014). A longitudinal study found that midlife stress in women was associated decades later with a 60% greater risk of developing dementia (Johansson et al., 2010). Animal studies show that vulnerability to GC or stress-induced cognitive impairment is greater in middle and old age compared with young adults (Bodnoff et al., 1995; Sandi and Touyarot, 2006).

Although mechanisms leading to the development of stress-related cognitive and anxiety-type disorders are not fully understood, GCs have an important role. The hippocampus, with its high expression of corticosteroid receptors [mineralocorticoid receptor (MR) and glucocorticoid receptor (GR)], is particularly vulnerable to chronic stress or high GC levels which reduce dendritic complexity (Magarinos and McEwen, 1995), and alter synaptic plasticity (Kim et al., 2007). GC activation of MR and GR modulates HPA axis and cognitive function (Harris et al., 2013). Moreover, elevated circulating GC levels as a consequence of impaired HPA axis negative feedback correlate with impaired

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hippocampal dependent memory performance in aged humans and animals (Issa et al., 1990; Lupien et al., 1998).

It is now clear that active GCs (corticosterone in rodents) within specific brain regions are derived not only from the circulation (only ~5% of circulating active GCs are unbound and thus available to penetrate the blood brain barrier), but also by local regeneration from inert and largely unbound circulating 11-keto forms (11-dehydrocorticosterone). This local regeneration is catalysed by the intracellular enzyme, 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) which is abundantly expressed in brain regions involved in cognition, anxiety and HPA axis regulation including the hippocampus, cortex and amygdala (Wyrwoll et al., 2011). Importantly, increased brain GC levels derived locally from 11β-HSD1 activity have emerged as key determinants of age-related cognitive decline (Yau and Seckl, 2012; Yau et al., 2015a). In wild type mice, 11β-HSD1 mRNA expression in the hippocampus and cortex increases with aging and correlates with impaired spatial memory (Holmes et al., 2010). Young adult 11β-HSD1 knockout mice show normal performances in spatial memory and anxiety behaviors (Yau et al., 2007; Yau et al., 2001). However, a protective cognitive phenotype becomes apparent with aging in 11β-HSD1 knockout mice (Yau and Seckl, 2012). Thus, 11β-HSD1 knockout mice resists age-related cognitive deficits found in aged matched wild type mice (Yau et al., 2007; Yau et al., 2001). Short-term administration of selective 11β-HSD1 inhibitors, which lower intra-hippocampal GC levels without affecting circulating GC levels, improves spatial memory in already aged mice (Sooy et al., 2010; Wheelan et al., 2015; Yau et al., 2015a). Any role of 11β-HSD1 deficiency to modulate the impact of chronic stress on cognitive and affective functions is unexplored.

We investigated whether a period of chronic stress in midlife has long lasting detrimental effects on cognition, anxiety and mood-related behaviors into early old age in mice. Psychological 'rat predator' stressors were included in a chronic unpredictable stress paradigm that models the uncontrollable nature of adverse life events (Herman et al., 1995). We also examined if these effects are prevented or attenuated by 11β-HSD1 deficiency.

2. Material and methods

2.1. Animals

Mice homozygous for targeted disruption of the *Hsd11b1* gene that encodes 11β-HSD1 (*Hsd11b1*$^{-/-}$, KO), congenic on the C57BL/6J genetic background (Carter et al., 2009), and age-matched C57BL/6J wild type (*Hsd11b1*$^{+/+}$, WT) controls were bred in house. The C57BL/6J genetic background chosen is the most widely used inbred mouse strain for behavioral testing. All mice were housed under standard conditions (7:00 am to 7:00 pm light/dark cycle, 21° C) with food and water available *ad libitum* until experimentation at ~10 months of age. Animal procedures were carried out under the auspices of the UK Animals (Scientific Procedures) Act 1986 and the European Communities Council Directive of 22 September 2010 (Directive 2010/63/EU).

2.2. Midlife chronic variable stress

Male WT and KO mice at ~10 months of age (midlife) were randomly assigned to non-stress or stress groups (n = 10/group/genotype). The chronic stress paradigm was identical for WT and KO mice, with stressors applied to the two groups concurrently, and consisted of exposure to one or two alternating stressors daily 6 days per week for 4 weeks. Stressors applied included (1) restraint in small rodent plastic restraint tubes for 2 min, 5 min, 10 min or 15 min; (2) forced warm swim (25° C or 30° C) for 1 min, 2 min or 3 min; (3) forced cold swim (15° C) for 1 min or 2 min; (4) non-contact predator exposure (rat) for 4 h or overnight; (5) predator odour exposure (rat soiled bedding material) overnight; (6) overnight lighting; (7) removal of nesting material overnight; (8) novel object placement within home cages overnight and (9) isolated housing for 4 h. The timing and duration of the morning or afternoon stressors and the day of the week with no stress exposure varied in an unpredictable manner. Non-stressed mice were transported to/from procedure rooms in their home cages but were not handled.

2.3. Behavioral testing

All mice underwent behavioral testing in a longitudinal manner starting the day after the last stressor in midlife with spatial recognition memory (Y-maze) followed by contextual fear memory. After a rest period of 6 months, the following tests were carried out in the order listed: spatial memory (Y-maze), contextual fear memory, anxiety-related behaviors (open field and elevated plus maze), spatial working memory (Y-maze) and depressive-like behavior (tail suspension test). For a detailed timeline of stress exposure and behavioral testing from midlife to early aging (18 months) see supplementary information (Fig. S1). To minimize the impact of each behavioral task affecting the outcome of later testing with carryover effects, the order of testing was from least stressful (Y-maze) to more stressful (tail suspension) with a rest period of 2–7 days between different tasks. The affective behavior tasks were carried out only in early old age (and not midlife) as these are highly influenced by earlier testing.

Mice were transported to the behavior testing room in their home cages and left to acclimatize for at least 30 min prior to behavioral assessment. The movement of each mouse in the test apparatus (Y-maze, elevated-plus maze and open field) was tracked using a ceiling mounted camera and analysed using ANYMAZE software (Stoelting, Dublin, Ireland).

2.3.1. Y-maze

Spatial recognition and working memories were tested in a Y-maze surrounded with external cues to aid navigation as described previously (Yau et al., 2007). Y-maze navigation is based on the animal's innate curiosity to explore novel areas and is not considered stressful. For spatial recognition memory, mice were placed into one of the arms of the maze (start arm) and allowed to explore the maze for 5 min with the entrance of one arm blocked off (training trial). After a 2 h inter-trial interval (ITI), mice were returned into the start arm of the maze for the test phase with all three arms available for exploration (5 min) including the novel (previously unvisited) arm. Spatial memory retention was measured as time spent in the novel arm calculated as a percentage of the total time spent in all three arms. The external cues surrounding the Y-maze were changed for re-testing 6 months later in aged mice. For spatial working memory (spontaneous alternation) mice were placed in the centre of the Y-maze and allowed to explore all three arms for 5 min. The number of arm entries and the sequence of arms entered were recorded. The percentage alternation was calculated as number of alternations (entries into three different arms consecutively) divided by the total possible alternations (i.e. number of arms entered minus 2) and multiplied by 100.

2.3.2. Open-Field test

The apparatus and experimental conditions for open-field testing were as previously described (Yau et al., 2007). Each mouse was placed in the centre of the open field arena and allowed to explore for 5 min. Times spent in the centre and at the sides of the apparatus were scored.

2.3.3. Elevated plus-maze (EPM)

The apparatus and experimental conditions used for EPM were as described (Yau et al., 2007). Each mouse was placed in the central platform of the maze facing one of the open arms and allowed to explore freely for 5 min. Times spent in the open and enclosed arms were scored.

2.3.4. Tail suspension test (TST)

Each mouse was suspended 30 cm off the floor by the base of the tail taped to the edge of a table with its body dangling in the air. The TST