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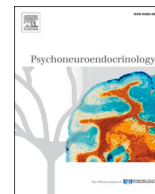
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11 β -Hydroxysteroid dehydrogenase 1 deficiency prevents PTSD-like memory in young adult mice

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ABSTRACT

Post-traumatic stress disorder (PTSD) is characterized by the co-existence of a persistent strong memory of the traumatic experience and amnesia for the peritraumatic context. Most animal models, however, fail to account for the contextual amnesia which is considered to play a critical role in the etiology of PTSD intrusive memories. It is also unclear how aging affects PTSD-like memory. Glucocorticoids alter the formation and retention of fear-associated memory. Here, we investigated whether a deficiency of 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) (an intracellular glucocorticoid generating enzyme) and aging modulates fear conditioning and PTSD-like memory in mice. We first measured memory in 6 months and 24 months old 11 β -HSD1 deficient (HSD1 KO) and wildtype (WT) mice following *paired* tone-shock fear conditioning. Then, separate groups of mice were exposed to restraint stress immediately after *unpaired* tone-shock contextual fear conditioning. Compared with young controls, aged WT mice exhibited enhanced auditory cued fear memory, but contextual fear memory was not different. Contextual fear memory retention was attenuated in both young and aged HSD1 KO mice. In contrast, auditory cued fear memory was reduced 24 h after training only in aged HSD1 KO mice. When fear conditioned with stress, WT mice displayed PTSD-like memory (i.e., increased fear to tone not predictive of shock and reduced fear to 'aversive' conditioning context); this was unchanged with aging. In contrast, young HSD1 KO mice fear conditioned with stress showed normal fear memory (i.e., increased fear response to conditioning context), as observed in WT mice fear conditioned alone. While aged HSD1 KO mice fear conditioned with stress also displayed normal contextual fear memory, the fear response to the 'safe' tone remained. Thus, a deficiency of 11 β -HSD1 protects against both amnesia for the conditioning context and hypermnnesia for a salient tone in young adult mice but only contextual amnesia is prevented in aged mice. These results suggest that brain 11 β -HSD1 generated glucocorticoids make a significant contribution to fear conditioning and PTSD-like memory. 11 β -HSD1 inhibition may be useful in prevention and/or treatment of PTSD.

1. Introduction

Traumatic or extreme stress experiences are better remembered than ordinary everyday events. This has an adaptive value allowing the individual to avoid repetition by responding to cues associated with danger. Normally, intrusive fear memory retrieval declines over time but in anxiety disorders such as post-traumatic stress disorder (PTSD), fear memories are exaggerated, do not extinguish, and are inappropriately and vividly recalled by a trauma-associated cue resulting in persistent re-experiencing of the trauma (Yehuda et al., 1998). PTSD patients experience two paradoxical memory problems, involuntary *hypermnnesia* for the core traumatic event and declarative *amnesia* for the

context surrounding the trauma (Brewin, 2011; Desmedt et al., 2015; Layton and Krikorian, 2002). The contextual amnesia impairs the subject's ability to restrict fear to the traumatic place and cues, and is considered a critical factor in the development of intrusive fear memory recollections (Al Abed et al., 2020; Brewin, 2011).

Considerable evidence from human and animal studies have implicated glucocorticoid (GC) hormones (corticosterone in rodents, cortisol in humans) in the development of abnormal fear responses in PTSD (Kaouane et al., 2012; Yehuda, 2009; Zoladz and Diamond, 2013). Whereas low to moderate levels of stress or arousal promotes strong adaptive memories, high stress levels typically impair spatial/explicit information processing while enhancing emotional/traumatic memories

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(Sandi and Pinelo-Nava, 2007). This inverted U curve describing performance under stress is most notable for hippocampus-dependent memories and can be mimicked by GCs (Kim and Diamond, 2002). Simple associative learning such as fear conditioning (or amygdala-dependent tasks) exhibits proportional increases in memory with increasing stress levels (Cordero et al., 1998; Dos Santos Correa et al., 2019). GCs acting via glucocorticoid receptors (GR) in the hippocampus and basolateral amygdala enhances the consolidation of fear memories (Abrari et al., 2009; Roozendaal and McGaugh, 1997).

Unusually for a stress-related disorder, PTSD is not characterized by high circulating cortisol levels but instead some studies report no differences while others show lower basal cortisol levels compared to healthy individuals, suggesting a hypersensitive hypothalamic-pituitary-adrenal axis (Yehuda, 2009; Zoladz and Diamond, 2013). Peripheral GC measurements, however, do not accurately reflect the crucial brain GC levels which can be quite different. Brain GCs are derived not only from the circulation (~5 % of GCs are freely available to penetrate the blood brain barrier) but also from intracellular GC metabolism by 11β -hydroxysteroid dehydrogenase type 1 (11β -HSD1), an enzyme that intracellularly regenerates active GCs from inactive 11-keto derivatives (Wyrwoll et al., 2011). 11β -HSD1 is highly expressed in brain regions important for cognition including hippocampus, amygdala, and occipital cortex (Bhatt et al., 2020; Wyrwoll et al., 2011).

GCs locally generated by 11β -HSD1 make a significant contribution to age-related hippocampus-dependent memory impairments. Thus, deficiency or inhibition of 11β -HSD1, which associates with lower intrahippocampal corticosterone levels during cognitive testing, improves spatial memory in aged mice (Sooy et al., 2010; Wheelan et al., 2018; Yau and Seckl, 2012; Yau et al., 2015a). 11β -HSD1 deficiency also protects against the effects of chronic stress on cognitive behaviors in aging mice (Wheelan et al., 2018). Since exposure to extreme stress is well established as the primary cause of PTSD, 11β -HSD1 generated GCs may be centrally involved in mechanisms leading to the pathophysiology. While the majority of animal models of PTSD have focused exclusively on the persistence of strong fear memories (Desmedt et al., 2015), the first mouse model of PTSD that recapitulated both cue-related hypermnnesia and contextual amnesia was reported following contextual fear conditioning with post-conditioning corticosterone (Kaouane et al., 2012). Whether 11β -HSD1 deficiency affects PTSD-like fear memory in mice has not been examined. It is also unclear how aging, which associates with elevated circulating GCs, modulates PTSD-like memory. PTSD in the elderly is less well characterized (Lapp et al., 2011; Spitzer et al., 2008). We therefore investigated whether 11β -HSD1 deficiency and aging alter fear conditioning and stress-induced PTSD-like memory in mice.

2. Materials and methods

2.1. Animals

Mice homozygous for a targeted disruption of the *Hsd11b1* gene that encodes 11β -HSD1 (*Hsd11b1*^{-/-}, HSD1 KO) congenic on C57BL/6J genetic background (Carter et al., 2009) and age-matched C57BL/6J wild type (*Hsd11b1*^{+/+}, WT) controls were bred in-house. Male mice were housed in groups of 3–5 per cage under standard controlled conditions on a 12 h light/dark cycle (lights on at 07:00 A.M.) with food and water *ad libitum* until experimentation. All experiments took place during the light phase and were conducted under Home Office project licence PPL No. 70/7870 in accordance with the UK Scientific Procedures (Animals) Act, 1986, amended in 2012 to comply with the European Directive 2010/63/EU, and were approved by the University of Edinburgh Animal Welfare and Ethical Review Body.

2.2. Plasma corticosterone levels

One week prior to fear conditioning, morning (8–9 am) tail venesection blood samples were collected from the experiment 1 mice into Microvette EDTA coated tubes (Sarstedt Inc). Plasma corticosterone levels were measured in duplicate using an in-house radioimmunoassay (Al Dujaili et al., 1981) modified for microtiter plate scintillation proximity assay (GE Healthcare, UK). The assay sensitivity was 0.1 ng/ml and intra-assay and inter-assay coefficients of variation were both < 10%.

2.3. Fear conditioning

Animals were trained in a fear conditioning chamber (25 cm × 25 cm × 38 cm) which consisted of two inter-changeable aluminum side walls and Plexiglas rear and front walls, and a removable grid floor of stainless-steel rods (3.2 mm diameter, 4.7 mm apart). The grid floor was connected to a precision-regulated shocker and speaker located on the sidewall, 15 cm from the floor, for auditory cue (tone) delivery. The fear conditioning apparatus was housed within a ventilated and sound-attenuated box (Coulbourn Instruments, USA). Both foot-shock and auditory cue delivery was controlled by FreezeFrame software (Actimetrics). A camera on top of the chamber continuously recorded the animal's activity and freezing behavior (complete absence of movement except those necessary for respiration) as a video file. The automatic detection of freezing was analyzed offline by an observer blind to experimental groups using FreezeFrame which monitors the animals at up to 15 times per second and detects movements as small as 1 mm. FreezeFrame measures the variance in pixel intensity across successive video frames (taken at 1 Hz) and generates a percentage freezing score. The chamber was cleaned with 70 % ethanol before each trial.

2.3.1. Experiment 1: effect of aging and 11β -HSD1 deficiency on fear conditioning

Male WT and HSD1 KO mice were tested in a contextual and auditory-cued fear conditioning paradigm as shown in Fig. 3A. Mice (6 and 24 months of age) were allowed to freely explore and acclimatize to the chamber within a neutral contextual background (silver aluminum wall tiles with no visual spatial cues or odor but with the grid floor present) for 4 min on two consecutive days before fear conditioning. This pre-exposure allows the mice to become familiar to the chamber later used for the tone memory test. On day 1 (training), each mouse was given 3 min of free exploration in the chamber within a specific context A (black and white patterned tiled walls of chamber, objects placed in front of transparent wall and odor (vanilla essence) acting as the static environmental cues) before exposure to an auditory tone (30 s, 2.6 kHz, 80 dB) that was co-terminated with the delivery of a single foot-shock (2

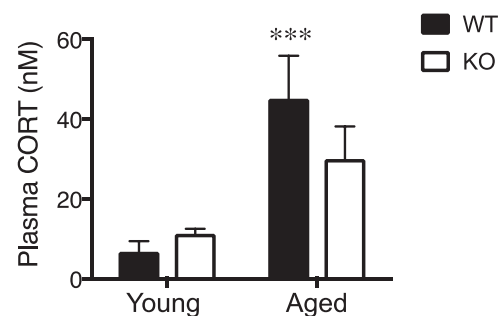


Fig. 1. Plasma corticosterone levels increased with age. Basal morning tail venesection plasma corticosterone (CORT) levels in young and aged wild type (WT) and 11β -HSD1 deficient (KO) mice. Bonferroni post-hoc tests, *** $P < 0.001$ vs. corresponding young WT mice; $P = 0.07$ for KO aged vs young. Young mice: $n = 6$ per genotype; aged mice: $n = 7$ per genotype. Data shown are means \pm SEM.

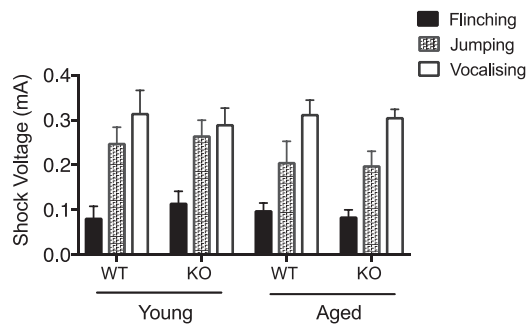


Fig. 2. Pain thresholds in wild type and 11 β -HSD1 deficient mice. Young (6 months) and aged (24 months) wild type (WT) and 11 β -HSD1 deficient (KO) mice individually placed in the fear conditioning chamber show no difference in foot shock intensity when they start to display flinching, jumping and vocalization. Young mice: $n = 6$ per genotype; aged mice: $n = 7$ per genotype. Data shown are means \pm SEM.

s, 0.6 mA). After the shock, the mouse remained in the test chamber for an additional 30 s. In this basic (and hence adaptive) tone-shock pairing conditioning paradigm, the static contextual cues stand in the “background” compared to the salient tone which is the main predictor of the shock. Animals learn to associate the occurrence of the aversive unconditional stimulus (US: foot-shock) with the conditioned stimulus (CS: discrete tone or specific context). Contextual fear memory was assessed 24 h later when the mouse was exposed to context A in the absence of any tones or shocks and freezing responses measured with FreezeFrame for the duration of 240 s. Cued fear memory was tested one hour later when mice were exposed to context B (neutral with silver aluminum wall tiles; no grid floor) for 3 min free exploration before one tone was presented for 30 s in absence of shock (Fig. 3A). Freezing behavior was measured using FreezeFrame software during exploration (pre-tone), tone exposure and 30 s after tone exposure.

2.3.2. Pain response thresholds in WT and HSD1KO mice

To test the effect of genotype and age on foot-shock sensitivity, pain response thresholds were assessed 24 h after the final extinction trial. Individual mice were placed in the chamber within a neutral context and allowed to acclimatize for 2 min before electric foot-shocks (2 s, 0.05 mA) of increasing intensity in 0.05 mA increments every 30 s were delivered until behavioral responses of flinching, jumping, and vocalizing were identified by two observers.

2.3.3. Experiment 2: Effect of aging and 11 β -HSD1 deficiency on PTSD-like memory

A separate cohort of male WT and HSD1 KO mice (6 and 24 months of age) were fear conditioned (FC) using a paradigm in which the tone presentation is never followed immediately by the foot-shock delivery (tone unpaired to shock) as previously described (Kaouane et al., 2012). This means that the tone, even though salient, is not directly associated with the shock. Animals should identify the conditioning context A which stands in the foreground, and not the salient tone, as the main predictor of the foot-shock. Prior to training, all mice were acclimatized to the chamber within a neutral context for 4 min over two consecutive days as in experiment 1. On day 1 of training, each mouse was placed in the chamber and allowed to explore freely for 120 s before delivery of a pseudo-random distribution of two tones (30 s, 2.6 kHz, 80 dB) and two shocks (2 s, 0.6 mA) (Fig. 4A). After 20 s, animals were either returned to their home cage (FC alone) or placed in a restraint tube for 20 min (FC + stress) to mimic a “high intensity threat” (Fig. 4A). Cued and contextual fear memory were tested 24 h and 26 h after training, respectively when mice were re-exposed to the tone in the neutral “safe” context B or training context A alone.

2.4. Statistical analysis

All data were analyzed with GraphPad Prism 7 software using analysis of variance (ANOVA) followed by Bonferroni’s multiple comparisons post hoc test (adjusted p-values) when appropriate. Plasma CORT concentrations were log transformed to meet assumptions of normality and analyzed by two-way ANOVA with age and genotype as independent variables. For the contextual and cued fear conditioning data, two-way repeated measures (RM) ANOVA was performed separately in the young and aged mice using genotype (WT vs HSD1 KO) as the between-subject factors and freezing behavior to context or auditory cue over the days as the within-subject factor. The effect of age on contextual or cued memory was analyzed for each genotype. Significance was set at $p < 0.05$. All data are presented as mean \pm standard error of mean (SEM).

3. Results

3.1. Plasma corticosterone levels in WT and HSD1 KO mice

Basal morning plasma corticosterone (CORT) levels were differentially elevated with age as a function of genotype (two-way ANOVA; age: $F_{1,22} = 28.46$, $P < 0.0001$; genotype: $F_{1,22} = 0.62$, $P = 0.43$; age \times genotype interaction: $F_{1,22} = 4.78$, $P < 0.05$; Fig. 1). Post-hoc analysis revealed increased ($P < 0.0001$) plasma CORT levels in aged vs young WT mice, but this increase was not significant in HSD1 KO mice ($P = 0.07$). In our previous studies, plasma CORT levels were significantly elevated with age (5–6 months vs 24–26 months) in both WT and HSD1 KO mice with no genotype effect (Yau et al., 2007, 2011, 2015a).

3.2. Pain response thresholds in WT and HSD1 KO mice

Pain threshold sensitivity to the fear conditioning foot-shock was measured in all young and aged WT and HSD1KO mice the day after the last memory test. The shock voltage that initiated flinching, jumping and vocalizing behaviors was not significantly affected by age or 11 β -HSD1 deficiency [two-way ANOVA; flinching (age: $F_{1,22} = 0.13$, $P = 0.72$; genotype: $F_{1,22} = 0.23$, $P = 0.63$); jumping (age: $F_{1,22} = 2.18$, $P = 0.15$; genotype: $F_{1,22} = 0.02$, $P = 0.89$); vocalizing (age: $F_{1,22} = 0.04$, $P = 0.85$; genotype: $F_{1,22} = 0.23$, $P = 0.64$), Fig. 2]. Thus, altered fear memory responses in WT and HSD1KO mice are not caused by differences in pain sensitivity.

3.3. 11 β -HSD1 deficiency impairs normal/adaptive fear memory in young mice

Young 6 months old WT and HSD1 KO mice were trained in a fear conditioning chamber with the delivery of a tone paired with single 0.6 mA foot-shock. Contextual and auditory cued fear memory from 1 to 6 days post-training (Fig. 3A). In this basic (and hence adaptive) single tone-shock pairing schedule, both the salient tone and the background conditioning context A are predictors of the shock. No detectable freezing behavior was measured during the 3 min free exploration in the conditioning context prior to shock delivery. Post-shock freezing levels during training in context A were not significantly affected by genotype ($F_{1,21} = 0.72$, $P = 0.40$) in young 6 months old mice (Fig. 3B).

Young WT mice displayed a conditioned fear to the context 24 h after training, and contextual memory extinction with decreasing levels of freezing across the days of re-exposure to context A (two-way RM ANOVA; day: $F_{3,30} = 51.06$, $P < 0.0001$; Fig. 3B). In contrast, this adaptive fear memory to the context, which stands in the background to the tone (main predictor of shock), was impaired in young HSD1 KO mice as shown by negligible freezing behavior in comparison to young WT mice when re-exposed to context A (two-way RM ANOVA; genotype: $F_{1,10} = 44.46$, $P < 0.0001$; genotype \times day: $F_{3,30} = 35.44$, $P < 0.0001$; Fig. 3B).

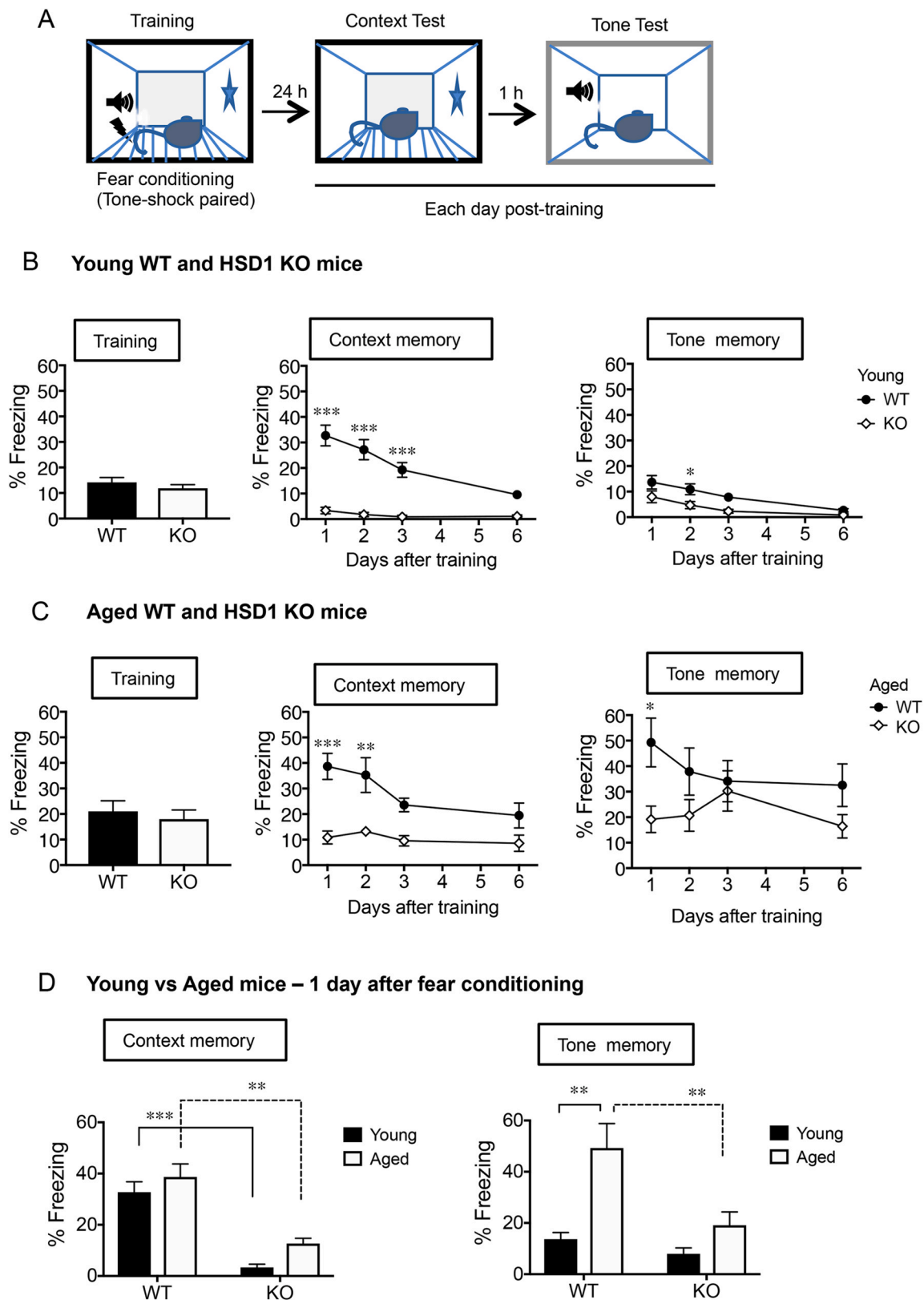


Fig. 3. 11 β -HSD1 deficiency attenuates normal/adaptive contextual and cued fear conditioning in young and aged mice. (A) Schematic diagram showing fear conditioning schedule to a tone (30 s, 2.6 kHz, 80 dB) co-terminated with single foot-shock (2 s, 0.6 mA) and memory tests in young (6 months) and aged (24 months) wild type (WT) and 11 β -HSD1 deficient (KO) mice. The conditioning context A is shown in black and context B in gray outline. (B, C) Freezing responses during fear conditioning training and memory tests over days of re-exposure to context A and auditory cue (in context B) in young and aged mice. Bonferroni post-hoc tests, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; vs. age-matched KO mice. (D) Contextual and cued fear memory in young versus aged WT and KO mice one day post-training. ** $P < 0.01$, *** $P < 0.001$ vs young or aged groups as shown. Young mice: $n = 6$ per genotype; aged mice: $n = 7$ per genotype. Data shown are means \pm SEM.

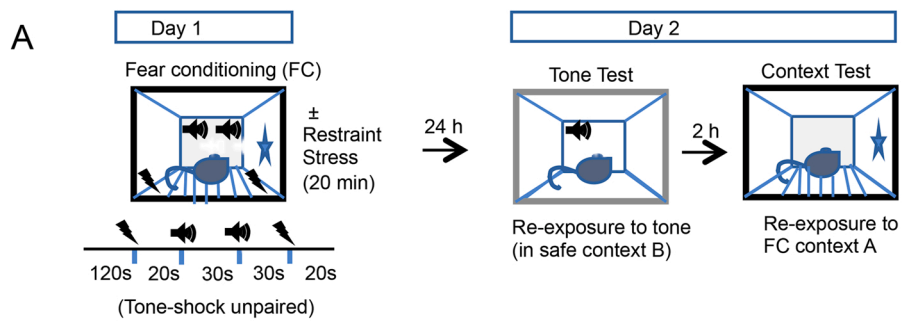
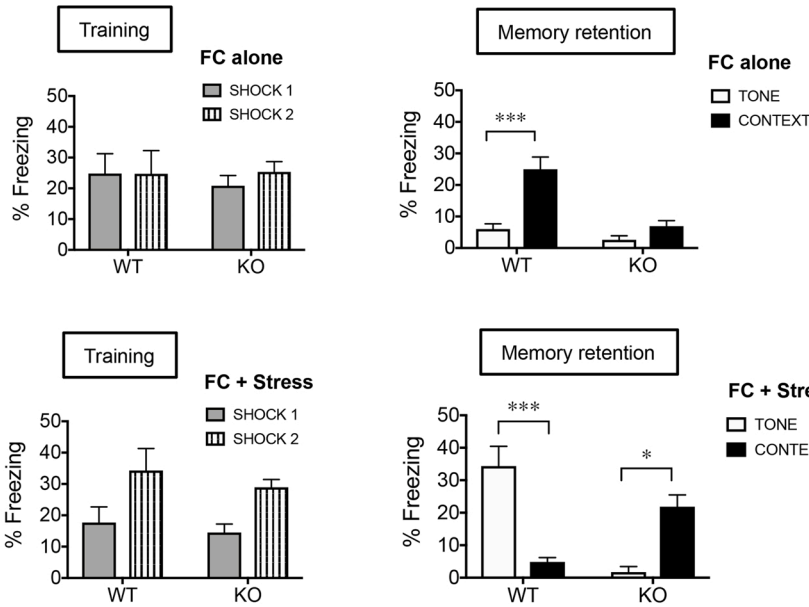
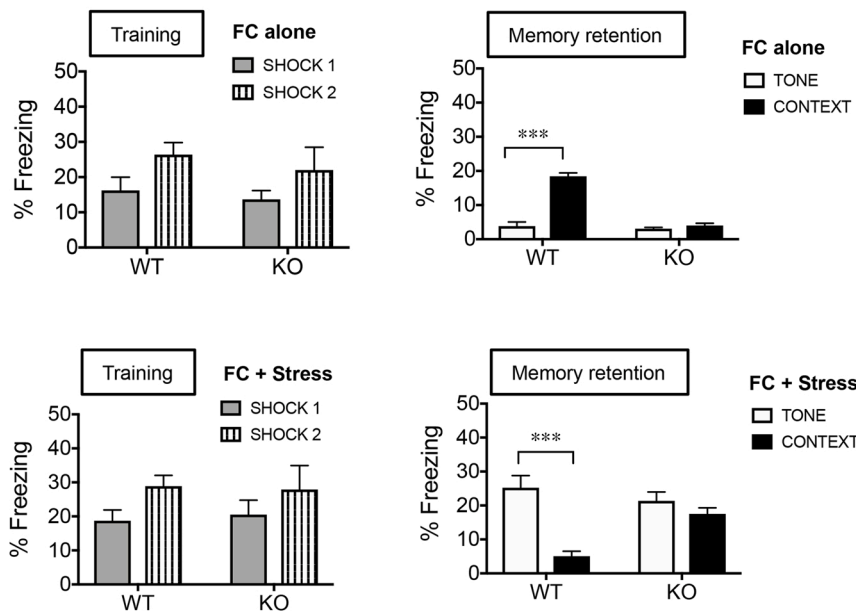


Fig. 4. Fear conditioning with restraint stress induces abnormal/maladaptive PTSD-like memory in wild type but not 11 β -HSD1 deficient mice. (A) Experimental design: mice were submitted either to contextual fear conditioning (FC) alone (shocks delivered *unpaired* with tones) or FC with 20 min restraint stress. The conditioning context A is shown in black and context B in gray outline. In this tone-shock unpairing paradigm, context A (and not tone) predicts the shock. Two memory tests followed 24 h later (re-exposure to tone (cue) in familiar ‘safe’ context B, then to conditioning context 2 h later). (B) Young mice [6 months: wildtype (WT); 11 β -HSD1 deficient (KO)]. Freezing responses (% of time) after exposure to foot-shocks during FC training in young mice assigned to FC alone and FC + Stress groups. During the memory tests young WT mice of the FC alone group expressed ‘normal’ contextual fear memory (i.e., high freezing responses to context A) whereas fear responses were low to both context and tone in FC + Stress groups. In contrast, young WT mice of the FC + Stress group displayed PTSD-like memory (i.e., high freezing responses to tone not predictive of shock) whereas young KO mice expressed ‘normal’ contextual fear memory (high freezing to context predictive of shock). Bonferroni post-hoc tests, ** $P < 0.01$, *** $P < 0.001$ vs corresponding response to tone or context, FC alone groups: WT ($n = 9$), KO ($n = 7$); FC + Stress groups: WT ($n = 9$), KO ($n = 6$). (C) Aged mice [24 months: wildtype (WT); 11 β -HSD1 deficient (KO)]. Freezing responses (% of time) after exposure to foot-shocks during FC training in aged mice assigned to FC alone and FC + Stress groups. During the memory tests aged WT mice of the FC alone group expressed normal/adaptive contextual fear memory (i.e., high freezing responses to context) whereas fear responses were low to both context and tone in aged KO mice. In contrast, aged WT mice of the FC + Stress group displayed PTSD-like memory (i.e., high freezing responses to tone) whereas in aged KO mice freezing responses increased to both context and tone. Bonferroni post-hoc tests, *** $P < 0.001$ vs corresponding response to tone or context, FC alone groups: $n = 6$ (WT), $n = 5$ (KO); FC + Stress groups: $n = 6$ (WT), $n = 5$ (KO). Data shown are means \pm SEM.

B Young WT and HSD1 KO mice



C Aged WT and HSD1 KO mice



Cued-fear memory was assessed 1 h following the context memory test (Fig. 3A). Young WT mice displayed low levels of freezing behavior when re-exposed to the tone in context B 25 h after training. No detectable freezing behavior was measured during the 3 min free exploration in context B before and after the tone presentation, thus confirming the specificity of the conditioned fear response to the tone (Fig. 3B). The lower than anticipated conditioned fear to the tone may, in part, be a consequence of the order of testing with context test one hour before the cue test. Thus, it is possible that some extinction process occurred following re-exposure to the FC chamber context A that contributed to reduced freezing to the tone in context B. Young HSD1 KO mice showed overall reduced conditioned fear (% freezing) to the tone compared to young WT mice (two-way RM ANOVA; genotype: $F_{1,10} = 6.24$, $P < 0.05$; day: $F_{3,30} = 26.51$, $P < 0.0001$; Fig. 3B).

3.4. Effect of aging on normal/adaptive fear memory in WT and HSD1 KO mice

Aged 24 months old WT and HSD1 KO mice underwent the same fear conditioning training (tone paired with single 0.6 mA foot-shock) as for the young mice. Contextual and auditory cued fear memory were examined from 1 to 6 days post-training (Fig. 3A). No detectable freezing behavior was measured during the 3 min free exploration in the conditioning context prior to shock delivery. Post-shock fear responses during training were similar in aged WT and HSD1 KO mice, however, there was a trend for increased freezing in aged mice compared to young mice ($F_{1,21} = 4.12$, $P = 0.05$; Figs. 3B and 3C).

Contextual fear memory retention (% freezing) was not significantly altered with aging in 24 months old WT mice (compared to young 6 months WT mice) but there was an effect of day of testing (two-way RM ANOVA; age: $F_{1,11} = 1.94$, $P = 0.19$; day: $F_{3,33} = 20.33$, $P < 0.0001$; Figs. 3B and 3C). Aged WT mice displayed less decline in freezing levels from day 3 to day 6 (17.4% decrease, $P = 0.43$, paired t-test, day 3 vs day 6) compared to young WT mice (50.4% decrease, $P < 0.01$, paired t-test, day 3 vs day 6) (Figs. 3B and 3C). Aged HSD1 KO mice showed significantly reduced contextual fear memory retention compared to aged WT mice across the days of testing (2-way RM ANOVA; $F_{1,12} = 19.35$, $P < 0.001$; day: $F_{3,36} = 6.52$, $P < 0.01$; genotype x day: $F_{3,36} = 3.29$, $P < 0.05$; Fig. 3C). The low freezing levels displayed by aged HSD1 KO mice during the context tests was significantly greater than in young HSD1 KO mice and did not differ across the days of testing [2-way RM ANOVA; age: $F_{1,11} = 21.79$, $P < 0.001$; day: $F_{3,33} = 1.33$, $P = 0.28$; day x age: $F_{3,33} = 0.65$, $P = 0.59$; Figs. 3B and 3C].

We also assessed cued-fear memory in the aged mice 1 h following the context memory test (Fig. 3A). No detectable freezing behavior was measured during the 3 min free exploration in context B before and after the tone presentation, thus confirming the specificity of the conditioned fear response to the tone. Cued fear memory (% freezing) was increased with ageing in both WT ($F_{1,11} = 32.18$, $P < 0.001$) and HSD1KO mice ($F_{1,11} = 21.09$, $P < 0.001$) (Figs. 3B and 3C). Aged HSD1 KO mice showed significantly lower levels of freezing to the tone compared to aged WT mice during the cue (tone) memory test (RM ANOVA; genotype: $F_{1,12} = 8.56$, $P < 0.05$; Fig. 3C). The fear response to tone re-exposure failed to decline over the days of repeated memory tests in both aged WT and HSD1 KO mice (day: $F_{3,36} = 0.66$, $P = 0.58$; genotype x day: $F_{3,36} = 1.06$, $P = 0.38$; Figs. 3B and 3C).

Contextual fear memory 24 h after training was not significantly altered by aging (6 m vs 24 m) ($F_{1,21} = 4.32$, $P = 0.05$) but there was an effect of genotype ($F_{1,21} = 56.96$, $P < 0.0001$) with lower conditioned fear to the context in HSD1 KO than age-matched WT mice (Fig. 3D). In contrast, cued fear memory 25 h after training showed an effect of age ($F_{1,22} = 14.67$, $P < 0.001$), and genotype ($F_{1,22} = 8.59$, $P < 0.01$) (Fig. 3D). Conditioned fear to the tone was significantly increased ($P < 0.01$) with aging in WT but not KO mice (Fig. 3D). 11β -HSD1 deficiency reduced ($P < 0.01$) cued fear memory in aged but not young mice (Fig. 3D).

3.5. High intensity threat (FC + Stress) induces abnormal (PTSD-like) fear memory in young WT but not HSD1 KO mice

To determine whether 11β -HSD1 deficiency affects the formation of abnormal (PTSD-like) fear memory, we randomly assigned young (6 months) mice to either fear conditioning (FC) alone (basic stressful threat) or FC with addition of restraint stress ("traumatic" threat) groups before undergoing a predicting-context fear conditioning training as described (Kaouane et al., 2012) (Fig. 4A). In this FC paradigm, young mice with 'normal' adaptive fear learning and memory are anticipated to identify the conditioning context (paired with shock) and not the salient tone cue (unpaired with shock) as the correct predictor of the shock. Whereas in experiment 1 (CS-US pairing conditioning), the mice are processing a background context compared to the tone (main predictor of shock), here in this CS-US unpairing procedure, the contextual cues stand in the foreground to the tone (salient cue not predictive of shock).

Young WT and HSD1 KO mice assigned to the low intensity threat (FC alone) and high intensity threat (FC + Stress) experimental groups displayed similar levels of freezing during training with no effect of genotype but there was an effect of the two shocks [FC + Stress mice: $F_{1,26} = 7.85$, $P < 0.01$] (Fig. 4B). All mice (FC alone and FC + Stress) displayed no detectable freezing behavior in the conditioning context prior to the shocks during training or in the "safe" context B before and after the tone during the memory tests.

Fear memory responses in young WT and HSD1 KO mice 24 h following FC alone showed a main effect of genotype [$F_{1,28} = 17.1$, $P < 0.001$]; conditioning stimulus (CS: tone or context) [$F_{1,28} = 20.1$, $P = 0.0001$] and genotype x CS interaction [$F_{1,28} = 7.94$, $P < 0.01$] (Fig. 4B). Young WT (FC alone) mice displayed adaptive contextual fear memory as indicated by significantly greater freezing ($P < 0.001$) to the 'aversive' conditioning context A (predictive of shock) than to the tone (salient cue not predictive of shock in 'safe' context B) (Fig. 4B). In contrast, young HSD1 KO (FC alone) mice showed negligible freezing to the tone and low freezing levels to context A (predictor of shock) which was significantly lower than in young WT (FC alone) mice (Bonferroni *post-hoc* tests, $P < 0.001$, Fig. 4B).

Addition of a heterogeneous stress (restraint) after fear conditioning altered fear memory responses in young WT and HSD1 KO mice with a significant genotype x CS interaction [$F_{1,25} = 32.12$, $P < 0.0001$] but no main effect of genotype [$F_{1,25} = 3.21$, $P = 0.08$] or CS (tone or context) [$F_{1,25} = 1.15$, $P = 0.29$] (Fig. 4B). PTSD-like fear memory was induced in the young WT (FC + stress) mice as shown by the abnormal increased freezing response to the tone (not predictive of shock) in 'safe' context B [$P < 0.0001$ vs young WT (FC alone) mice] and decreased freezing to the conditioning context A (predictor of shock), [$P < 0.01$ vs young WT (FC alone) mice] (Fig. 4B). In contrast, young HSD1 KO (FC + stress) mice displayed adaptive increased fear memory responses to context A (predictor of shock), [$P < 0.0001$ vs young HSD1 KO (FC alone) mice] with no freezing to the tone (Fig. 4B).

3.6. 11β -HSD1 deficiency partially prevents abnormal (PTSD-like) fear memory in aged mice

We next determined whether aging modulates the protective effect of 11β -HSD1 deficiency on abnormal (PTSD-like) fear memory formation. Aged (24 months) mice were randomly assigned to either FC alone or FC + Stress (20 min restraint) groups before undergoing a predicting-context fear conditioning training (Kaouane et al., 2012) (Fig. 4A). Aged WT and HSD1 KO mice showed similar levels of freezing during training with no effect of genotype [FC alone group: $F_{1,18} = 0.67$, $P = 0.42$; FC + Stress group: $F_{1,18} = 0.01$, $P = 0.92$] but there was an effect of the two shocks [FC alone mice: $F_{1,18} = 4.82$, $P < 0.05$] (Fig. 4C). All aged mice (FC alone and FC + Stress) displayed no detectable freezing behavior in the conditioning context prior to the shocks during training or in the "safe" context B before and after the tone

during the memory tests.

Following FC alone, aged WT mice displayed adaptive fear memory 24 h after training (i.e., increased freezing to the conditioning context A (predictive of shock), $P < 0.0001$ compared to low freezing to tone in 'safe' context B, Fig. 4C), similar to young WT (FC alone) mice (Fig. 4B). In contrast, aged HSD1 KO (FC alone) mice showed little freezing during re-exposure to the tone and conditioning context A (Fig. 4C) as observed in young HSD1 KO (FC alone) mice (Fig. 4B).

FC + Stress induced abnormal (PTSD-like) fear memory in aged WT mice (i.e., increased freezing to the tone (not predictive of shock), $P < 0.0001$ compared to low freezing to context A (correct predictor of shock) (Fig. 4C), as in the young WT (FC + Stress) mice (Fig. 4B). In contrast, aged HSD1 KO mice after FC + stress showed increased freezing during re-exposure to conditioning context A (predictor of shock) and to the tone (not predictive of shock) in "safe" context B (Bonferroni *post-hoc* tests: $P < 0.001$ vs aged HSD1 KO (FC alone) mice, Fig. 4C). The percentage freezing to the tone was significantly increased with age in the HSD1 KO (FC + stress) mice (Bonferroni *post-hoc* tests: $P < 0.0001$ vs young HSD1 KO (FC + Stress) mice, Figs. 4B and 4C). Thus, while aged HSD1 KO mice avoided the contextual amnesia after FC + Stress, this did not prevent the enhanced cued fear response in 'safe' context B.

4. Discussion

In this study, we investigated the impact of 11 β -HSD1 deficiency and aging on the formation of normal/adaptive and abnormal (PTSD-like) fear memory in male mice. Our results show that in young adults, compared to wildtype mice, 11 β -HSD1 deficient (HSD1 KO) mice display an impairment of normal/adaptive fear memory (both background contextual and cued fear memory), while 11 β -HSD1 deficiency prevents PTSD-like memory formation (i.e., both the contextual amnesia and the abnormal fear response to a salient irrelevant tone). In aged mice, 11 β -HSD1 deficiency also impairs normal/adaptive fear memory, while partially preventing PTSD-like memory formation, promoting a normal contextual memory but leaving unchanged the abnormal fear response to the salient cue.

Once fear memories are formed, they undergo a process of consolidation immediately after training (Nader et al., 2000). GCs are critically involved in this process (McGaugh and Roozendaal, 2002). Post-training administration of corticosterone (CORT) enhances the consolidation of contextual fear memory in a dose-dependent manner, acting in part, via GRs in the hippocampus (Abrari et al., 2009; Kaouane et al., 2012; Revest et al., 2005). Our finding of impaired background contextual fear memory in HSD1 KO mice suggests that with 11 β -HSD1 deficiency, the single tone-shock pairing conditioning likely results in lower than optimal brain CORT levels required to activate GRs for fear memory consolidation (Conrad et al., 2004; McGaugh and Roozendaal, 2002). In the absence of intracellular 11 β -HSD1 generated CORT in HSD1 KO mice, brain CORT levels are derived only from the ~ 5 % free CORT entering from the circulation. Basal plasma CORT levels were not altered in the young or aged HSD1 KO mice consistent with our earlier studies (Yau et al., 2007, 2011, 2015a). Hence, any increase in plasma CORT (e.g., during stress or diurnal rise) would elevate brain CORT levels in HSD1 KO mice. We have previously shown by *in vivo* microdialysis a lower rise in intrahippocampal CORT levels in freely behaving young and aged HSD1 KO mice over the diurnal peak and during a swim stress compared to age-matched WT controls (Yau et al., 2015a, 2015b). Mice treated with a selective 11 β -HSD1 inhibitor, UE2316, also showed attenuated contextual fear memory (Sarabdjitsingh et al., 2014; Wheelan et al., 2015). Moreover, boosting brain CORT levels in the young HSD1 KO mice by fear conditioning with restraint stress immediately post-training, shown to increase plasma and brain CORT levels in C57BL6 mice (Kaouane et al., 2012), enhanced contextual fear memory.

GR antagonists infused directly into the basolateral amygdala impairs fear memory consolidation (Donley et al., 2005). 11 β -HSD1

deficiency had surprisingly little effect on amygdala dependent cued fear memory 25 h after training in young mice. However, the unusually low conditioned fear to the tone observed in the young WT mice, a possible result of the order of memory tests (context 1 h prior to tone), may mask a decrease in the HSD1 KO mice due to a floor effect. In a previous study that used a similar single tone-shock pairing, but tested context and cued fear memory (24 h after training) in separate groups of C57BL6J mice, cued fear memory was unaltered in mice pre-treated with a selective 11 β -HSD1 inhibitor (Sarabdjitsingh et al., 2014). The relatively low expression of 11 β -HSD1 in the amygdala in young mice (especially compared to hippocampus) (Pelletier et al., 2007) may indicate the enzyme has only a small effect on total intracellular CORT levels in this structure.

Animal models of PTSD typically involve the application of a variety of stressors which are perceived as a threat to survival (Daskalakis et al., 2013). Extreme stress due to trauma may impair hippocampal processing which in turn leads to maladaptive (PTSD-like) memories whereby encoding of the surrounding background context of the event is disrupted while affective/sensory representations (salient cues) are enhanced (Brewin, 2011). In our study, restraint stress immediately after an unpaired tone-shock contextual fear conditioning induced PTSD-like memory in WT mice i.e., increased fear response to the "safe" tone (not previously paired to shock) and reduced fear response to the conditioning context (correct predictor of shock) as previously reported (Kaouane et al., 2012). Contextual fear conditioning with intra-hippocampal or systemic injection of CORT also induces contextual amnesia and promotes a cognitive imbalance in favor of the amygdala-based tone fear conditioning in mice (Al Abed et al., 2020; Bouarab et al., 2021; Ducourneau et al., 2021; Kaouane et al., 2012). The inability to restrict fear responses to appropriate predictors is a critical feature of PTSD in humans when contextual cues occurring around the time of the trauma are often forgotten whereas irrelevant cues are strongly remembered (Elzinga and Bremner, 2002). Strikingly, young HSD1 KO mice following the same fear conditioning with restraint stress schedule failed to show PTSD-like memory, instead maintaining a normally high fear response to the (predictive) conditioning context and little freezing to the irrelevant tone (not predictive of shock). In the absence of locally generated CORT, the reduced hippocampal CORT levels in HSD1 KO mice (Yau et al., 2015a, 2015b) after FC + Stress are likely below the threshold level required for inducing PTSD-like memory as shown in a previous study with CORT-injected mice (Kaouane et al., 2012). Hence, 11 β -HSD1 deficiency can have contrasting effects on fear memory depending on the conditioning procedure (basic stressful vs "traumatic" or highly stressful). Young HSD1 KO mice display impaired fear memory after FC alone (tone-shock paired or unpaired) but adaptive fear memory after FC + Stress (tone-shock unpaired). In the presence of local 11 β -HSD1-generated CORT in young WT mice, brain CORT levels after FC + Stress will likely be elevated high enough to impair contextual fear memory and increase fear generalization to the tone (i.e., abnormal PTSD-like memory). 11 β -HSD1-generated CORT in the brains of WT mice therefore contributes to switching normal adaptive fear memory to maladaptive (PTSD-like) memory after a "traumatic" stress. A large increase in CORT levels in specific brain regions (particularly hippocampus and amygdala) is likely a key factor in the formation of normal vs PTSD-like fear memory, but previous studies have shown that these high CORT levels are not causal per se (Finsterwald et al., 2015; Kaouane et al., 2012). Additional mechanisms (yet to be identified) triggered by a highly stressful event in combination with an excess of CORT have been suggested as a potential determining factor underlying the formation of PTSD-like fear memory (Finsterwald et al., 2015).

While aging impairs spatial memory in WT mice (Yau and Seckl, 2012), background contextual fear memory, which is also dependent on the hippocampus, was unaffected by aging consistent with previous studies in rats and mice (Bergado et al., 2011; Foster et al., 2012; Gould and Feiro, 2005). In contrast, amygdala-dependent cued fear memory was enhanced with aging in WT mice as reported in aged rats (Mesches

et al., 2004) but others show no change 24 h after training or impairments at longer time intervals in aged mice (Gould and Feiro, 2005). Differences in the fear conditioning procedures (e.g., number of tone-shock pairings) and age of mice or rats tested could, in part, underlie the different results obtained for aging effects on cued fear memory. In line with our findings, aging is also associated with enhanced emotional memory in humans (Jacques et al., 2009). Notably, 11 β -HSD1 deficiency was associated with reduced cued fear memory 24 h after training in aged but not young mice, perhaps reflecting increased brain 11 β -HSD1 expression with aging as shown in WT mice (Holmes et al., 2010) and in humans (Bini et al., 2020).

Research into how aging affects PTSD is limited (Lapp et al., 2011) and most preclinical models of PTSD have been in young animals (Richter-Levin et al., 2019; Schoner et al., 2017). Despite elevated plasma and increased brain CORT levels in aged WT mice (Yau et al., 2015a,b), PTSD-like memory induced following fear conditioning with restraint stress was similar to that displayed in young WT mice. However, unlike in young HSD1 KO mice, 11 β -HSD1 deficiency did not fully protect against stress-induced PTSD-like memory in aged mice. Circulating CORT levels tend to rise with ageing. Brain CORT levels in aged HSD1 KO mice after FC + Stress may be at a moderate level resulting in equal freezing responses to both the tone and conditioning context (correct predictor of shock), but not high enough to cause PTSD-like contextual amnesia as demonstrated in mice given increasing doses of CORT after fear conditioning (Kaouane et al., 2012). Future studies could directly test this hypothesis by measuring intrahippocampal CORT in the aged WT and HSD1 KO mice after FC + Stress and manipulating intrahippocampal CORT levels in aged HSD1 KO mice immediately after fear conditioning.

Short-term stress upregulates 11 β -HSD1 in the prefrontal cortex, amygdala and hippocampus (Ergang et al., 2015). The combination of foot-shocks and restraint stress used to induce PTSD-like memory in this study could potentially increase 11 β -HSD1 in brain thus contributing to increased local CORT levels as reported in the hippocampus (Kaouane et al., 2012). Our finding that 11 β -HSD1 plays an important role in PTSD-like memory support studies that show increased GC levels enhances fear memory consolidation (Abrari et al., 2009), promotes fear memory generalization (Lesuis et al., 2021) and impairs the ability to correctly predict threat (Al Abed et al., 2020; Kaouane et al., 2012). Furthermore, each time a fear memory is retrieved or reactivated, it can become transiently labile and must be made stable again through reconsolidation (Nader and Hardt, 2009). Elevated GCs during spontaneous memory reactivations (flashbacks) in safe contexts can enhance reconsolidation of the original fear memory, thereby strengthening the reinstatement of fear and may underlie the persistence of fear memories in PTSD and anxiety disorders (Drexler et al., 2015). Interestingly, a positron emission tomography (PET) imaging in humans reported increased prefrontal-limbic (including amygdala) 11 β -HSD1 availability in a group of individuals with PTSD compared to healthy, trauma-exposed controls (Bhatt et al., 2021). However, analysis of the PTSD group revealed that higher prefrontal-limbic 11 β -HSD1 availability associated with lower PTSD threat and loss symptoms which is the opposite of what would be anticipated if we assume full extrapolation from enzyme availability to activity (Bhatt et al., 2021). Since confounders (including comorbid depression, use of psychotropic medications and cannabis) were not excluded from the small PTSD group of 16 individuals, this association may be indirect. While GCs given during exposure therapy can enhance the consolidation of a new safety memory through extinction learning, the original fear memory is unaffected and can spontaneously return (Bouton, 2002). One potential clinical translation of our findings might be to weaken the original fear memory using selective 11 β -HSD1 inhibitors to reduce GR activation and hence impair memory reconsolidation after reactivation.

The current studies have been carried out in male mice as with previous studies modeling PTSD-like fear memory in mice (Al Abed et al., 2020; Kaouane et al., 2020, 2012). Given that women are twice as

likely to develop PTSD compared to men (Duckers and Olf, 2017), it will be important to include females in future preclinical studies (Day and Stevenson, 2020). The mechanisms that underlie these sex differences is not clear but 17 β -estradiol have been implicated to play a role (Day and Stevenson, 2020). Whether female mice will show greater PTSD-like fear memory formation (i.e., greater increased % freezing to salient tone and contextual amnesia) remains to be determined.

5. Conclusions

11 β -HSD1 deficiency can have opposite effects on fear memory in young adult mice depending on the conditioning paradigm, impairing adaptive contextual fear memory (after FC alone) and preventing abnormal PTSD-like memory (after FC + Stress) displaying instead adaptive contextual fear memory. In aged mice, 11 β -HSD1 deficiency partially protects against PTSD-like fear memory formation by preventing contextual amnesia but not the strong fear response to the 'safe' tone. These results support a critical role for local 11 β -HSD1 generated GCs in fear conditioning and the switch from adaptive fear memory (after basic stressful condition) to maladaptive PTSD-like memory (after traumatic/high stress condition). Selective 11 β -HSD1 inhibitors may be of particular interest when designing novel therapeutic treatments to reduce PTSD-related fear memories and associated symptoms. Older individuals may require longer or modified treatment strategies. Future clinical trials could test selective 11 β -HSD1 inhibitors in reconsolidation-based therapies for PTSD.

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CRedit authorship contribution statement

NW: Investigation, Data curation, Formal analysis. **JLWY:** Conceptualization, Supervision, Formal analysis, Visualization, Writing – original draft. **JRS:** Writing – Review & Editing.

Declaration of Competing Interest

All authors have no declarations of interest to disclose. This is stated in the manuscript and in the cover letter.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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