Full Length Article

FKBP5 and early life stress affect the hippocampus by an age-dependent mechanism

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ABSTRACT

Early life stress (ELS) adversely affects the brain and is commonly associated with the etiology of mental health disorders, like depression. In addition to the mood-related symptoms, patients with depression show dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, increased peripheral inflammation, and structural brain alterations. Although the underlying causes are unknown, polymorphisms in the FK506-binding protein 5 (FKBP5) gene, a regulator of glucocorticoid receptor (GR) activity, interact with childhood adversities to increase vulnerability to depressive disorders. We hypothesized that high FKBP5 protein levels combined with early life stress (ELS) would alter the HPA axis and brain, promoting depressive-like behaviors. To test this, we exposed males and females of a mouse model overexpressing FKBP5 in the brain (rTgFKBP5 mice), or littermate controls, to maternal separation for 14 days after birth. Then, we evaluated neuroendocrine, behavioral, and brain changes in young adult and aged mice. We observed lower basal corticosterone (CORT) levels in rTgFKBP5 mice, which was exacerbated in females. Aged, but not young, rTgFKBP5 mice showed increased depressive-like behaviors. Moreover, FKBP5 overexpression reduced hippocampal neuron density in aged mice, while promoting markers of microglia expression, but these effects were reversed by ELS. Together, these results demonstrate that high FKBP5 affects basal CORT levels, depressive-like symptoms, and numbers of neurons and microglia in the hippocampus in an age-dependent manner.

1. Introduction

In recent years, mood and stress-related disorders have received more attention due to their prevalence and growing incidence worldwide (Kessler et al., 2010, 2005; Lim et al., 2018; Substance Abuse and Mental Health Services Administration, 2017). Despite each disorder having distinct features, there is a high overlap of behavioral and physiological symptoms among them. For example, individuals with anxiety and depressive disorders may experience persistent feelings of anxiety, difficulty concentrating, fatigue, and sleep problems (Uher et al., 2014). Likewise, these disorders are influenced by common genetic and environmental factors shaping an individual's susceptibility, coping behavior, and neurobiological response (Blanco et al., 2014; Gillespie et al., 2009). The hypothalamic-pituitary-adrenal (HPA) axis is a key player that controls the stress response to environmental stimuli,

preserving system homeostasis. This balance is mainly mediated by negative feedback from glucocorticoids, specifically cortisol in humans and corticosterone in rodents (CORT) (Sapolsky et al., 1984). CORT binds to glucocorticoid receptors (GR) located in the hypothalamus and hippocampus to inhibit neuroendocrine responses; this inhibition protects the body from prolonged stress effects (Herman et al., 2016; Kovacs and Makara, 1988).

HPA axis dysfunction has been linked to multiple mental health disorders, including depression (Juruena et al., 2018). Patients with depression have persistently high CORT levels and an exaggerated response in the combined dexamethasone suppression/corticotrophin-releasing hormone stimulation (DEX/CRH) test (Schüle et al., 2009; Spijker and van Rossum, 2012). Depressed individuals also show higher levels of cytoplasmic GR correlating with higher levels of the 51 kDa FK506-binding protein (FKBP51, FKBP5) (Lukic et al., 2015). FKBP5 is an Hsp90 co-chaperone that regulates GR sensitivity by

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Abbreviations

ANOVA	Analysis of variance
CamKII	Ca2+/calmodulin-dependent protein kinase II
CORT	Cortisol, corticosterone
DCX	Doublecortin
ELISA	Enzyme-linked immunosorbent assay
ELS	Early life stress
FKBP5	FK506-binding protein 5/FK506-binding protein 51
GFAP	Glial fibrillary acidic protein
GLM	General linear model
GR	Glucocorticoid receptor
HPA	Hypothalamic-pituitary-adrenal
HPC	Hippocampus
Iba1	Ionized calcium binding adaptor molecule 1
IHC	Immunohistochemistry
LPS	Lipopolysaccharide
OF	Open field
P1	Postnatal Day 1
P21	Postnatal Day 21
PTSD	Post-traumatic stress disorder
SEM	Standard error of the mean
TST	Tail Suspension Test

reducing GR import into the nucleus (Davies et al., 2002; Wochnik et al., 2004). The use of antidepressants as well as FKBP5 knockdown or FKBP5 removal from the GR complex each restore the receptor's affinity for CORT, facilitating GR-nuclear transactivation (Pariante et al., 2001; Sabbagh et al., 2018; Tatro et al., 2009). Thus, various studies have suggested that CORT resistance may be attributed to malfunction of GR itself or to the inhibitory action of FKBP5 on GR.

Notably, FKBP5 polymorphisms have been linked with increased susceptibility to stress, HPA axis function, GR resistance, and major depression, as well as to antidepressant treatment response (Binder et al., 2004; Ferrer et al., 2018; Menke et al., 2013; Roberts et al., 2015; Santarelli et al., 2017; Scheuer et al., 2016; Stamm et al., 2016). Homozygous carriers of the T allele in FKBP5 rs1360780 have shown higher expression of FKBP5 protein following stress, which implies a functional role of this variant that may contribute to greater GR inhibition through high protein levels (Binder et al., 2004). More recent findings support the role of FKBP5 polymorphisms and their interactions with the environment to increase the risk for mood- and stress-related disorders (Buchmann et al., 2014; Klengel et al., 2013; Lahti et al., 2016; Zimmermann et al., 2011). In addition to gene-stress interactions triggered during early life, mental and biological effects from these stressful experiences can also manifest throughout the lifespan (Binder et al., 2008; Klengel et al., 2013; Kohrt et al., 2015; Santarelli et al., 2017). For example, FKBP5 polymorphisms interact with early life stressors, such as childhood trauma, conferring higher risk for depression in adolescence and adulthood (Appel et al., 2011; Kohrt et al., 2015; Lahti et al., 2016; Zimmermann et al., 2011). In young adult mice, early life stress (ELS) and high FKBP5 interact to induce anxiety-like behavior and changes in the molecular pathways involved in cell survival and growth in the hippocampus (Criado-Marrero et al., 2019).

The hippocampus is highly susceptible to stress due to the high concentration of GRs (Ladd et al., 2004; Magariños et al., 1996; Sapolsky, 1985; Wei et al., 2015). This area is responsible for inhibiting the HPA axis after stress and for processing emotional memories and responses (Sapolsky et al., 1984; Snyder et al., 2011). Moreover, ELS can alter hippocampal development across the lifespan (Brunson et al., 2005; Réus et al., 2019; Wei et al., 2015). Some stress-related adverse effects in this area include atrophy of dendrites, reduced synaptic density and inhibition of neurogenesis (Brunson et al., 2001; Dioli et al., 2019; Lupien et al., 2018). ELS exposure can further affect hippocampal development by altering non-neuronal cells, like astrocytes and microglia (Delpech et al., 2016; Réus et al., 2019; Roque et al., 2016). Decreased astrocyte and increased microglia cell counts have been reported in multiple studies investigating depressive-like symptoms in animals and depression in humans (Rajkowska et al., 2001; Rajkowska and Stockmeier, 2013; Réus et al., 2019; Rohan Walker et al., 2013). These cells play an essential role in stress-induced pro-inflammatory and cytotoxic responses, which affect neuronal differentiation and neurogenesis (Aarum et al., 2003; Koo et al., 2010; de Lucia et al.,

2016). Interestingly, FKBP5 mRNA and protein expression in the hippocampus are induced by chronic stress and exogenous CORT, supporting the relationship between FKBP5 and stress (Lee et al., 2010; Scharf et al., 2011; Wagner et al., 2012). High-risk FKBP5 variants have also been associated with reduced gray matter and hippocampal volume in patients with depression who were previously exposed to ELS (Mikolas et al., 2019; Tozzi et al., 2018; Zobel et al., 2010). Therefore, malfunction or imbalance of FKBP5 and stress-induced effects on the hippocampus may each contribute to cell atrophy and reduction in hippocampal volume in depression (Campbell et al., 2004; Han et al., 2017; Lucassen et al., 2001; Vythilingam et al., 2002).

We previously showed that the interaction of ELS and FKBP5 increases anxiety-like levels in young mice, inducing changes in the hippocampus (Criado-Marrero et al., 2019). Due the high comorbidity of anxiety and depression, here we investigated whether this interaction, ELS x FKBP5, promotes a depressive-like behavior and affects animals differently over time. To test this, transgenic mice overexpressing FKBP5 in the brain, rTgFKBP5, were exposed to ELS using the maternal separation paradigm. We compared behavioral, neuroendocrine and hippocampal changes across two ages, young and aged adulthood. These timepoints were chosen because of the high prevalence of depression during early and middle adulthood (Lim et al., 2018). Moreover, given the genetic association between FKBP5 variants and reduced hippocampal volume with depression (Zobel et al., 2010), we used immunohistochemical and stereological analyses to evaluate whether high FKBP5 x ELS promotes hippocampal neuronal loss and neuroinflammation.

2. Materials and methods

2.1. Animal subjects

All mice were bred and maintained in our institutional vivarium under standard conditions on a 12 h light/dark cycle (light cycle beginning at 06:00 and ending at 18:00) A maximum of 5 mice were housed per cage with free access to food and water. All animal experiments were carried out accordingly with the NIH Guide for the Care and Use of Laboratory Animals and approved by the USF Institutional Animal Care and Use Committee (IACUC).

Complete details about the construction of the rTgFKBP5 vector and generation of the rTgFKBP5 mice has been previously described (Blair et al., 2019). Experiments were carried out using 3 genotypes of mice: rTgFKBP5 (contains a single insertion of human FKBP5 gene and tTA on the CamKIIα promoter), tTA (expresses the tet-transactivator on the CamKIIα promoter), and wild-type (WT). We used tTA mice, in addition to WT, because they are a suitable control for rTgFKBP5 mice, since they express the tetracycline-off transactivator driven by the CamKIIα promoter but lack the FKBP5 insertion. Genotypes were confirmed by PCR amplifying DNA from ear tissue using Qiaxcel (Qiagen, Valencia, CA, USA) to analyze the PCR product.

A

B

Groups	Environmental factor	Genotypes	Total animals	Age group
1	Control (No stress)	WT	11 (6M, 5F)	Young
2		tTA	10 (5M, 5F)	Young
3		rTgFKBP5	9 (4M, 5F)	Young
4	Early life stress (ELS)	WT	10 (5M, 5F)	Young
5		tTA	10 (5M, 5F)	Young
6		rTgFKBP5	10 (5M, 5F)	Young
7	Control (No stress)	WT	10 (5M, 5F)	Aged
8		tTA	11 (5M, 6F)	Aged
9		rTgFKBP5	10 (4M, 6F)	Aged
10	Early life stress (ELS)	WT	10 (5M, 5F)	Aged
11		tTA	11 (5M, 6F)	Aged
12		rTgFKBP5	10 (5M, 5F)	Aged

Fig. 1. **Experimental procedure**. (**A**) Timeline for early life stress protocol, behavioral experiment, and collection of specimens. Maternal separation was used as the early life stressor, ELS, were pups were relocated in a separate room from their dams for 3 h daily for 14 consecutive days. (**B**) Total number of animals per group for each genotype, stress condition, and age period. A balanced number of males and females were used per condition. WT = wild-type, tTA = CamKIIα-tTA, rTgFKBP5 = FKBP5 overexpressing mice, M = males, F = females.

2.2. Early life stress model

We used the maternal separation paradigm as an early life stressor (ELS) to study the interaction of the FKBP5 gene and the environment in both young and adult mice, as we have done previously (Criado-Marrero et al., 2019). The protocol consisted of relocating the dams to new cages in a separate room for 3 h daily during the morning. A heating pad under the cage was used to maintain ambient temperature during the separation time. This procedure was repeated for 14 consecutive days starting on postnatal day 1 (P1). The non-stress control group remained undisturbed with their dams until they were weaned (P21). Age groups at the time of behavioral testing corresponded to young (60 days-old) and aged (210 days-old) adulthood. These mice were divided among 12 groups (Fig. 1A and B) to examine behavioral and molecular changes induced by environmental factors (stress and non-stress), genotype (WT, tTA and rTgFKBP5), and age (young and aged). The interactions among these factors were also investigated. The young group consisted of non-stressed control (WT = 11, tTA = 10, rTgFKBP5 = 9) and ELS mice (WT = 10, tTA = 10, rTgFKBP5 = 10), while the aged group consisted of non-stressed control (WT = 10, tTA = 11, rTgFKBP5 = 10) and ELS mice (WT = 10, tTA = 11, rTgFKBP5 = 10).

2.3. Open field test (OF)

Over the course of forty days, mice were put through a behavioral battery to assess cognition and affective-like symptoms. Only those implicated in depressive and fear phenotypes are described here. The OF was used to measure locomotor activity. It consisted of placing a mouse in the center of a square gray Plexiglas box. Individually, mice were allowed to freely explore the arena for 10 min while being video recorded (ANY-Maze software; www.anymaze.com). The chamber was cleaned with 70% ethanol between trials. The total distance travelled was recorded to evaluate locomotor differences.

2.4. Tail suspension test (TST)

For TST, each mouse was suspended by its tail with adhesive tape from a lever and video recorded for 6-min using the ANY-Maze software. An experimenter blinded to animal assignments measured the total time each mouse spent immobile during each session.

2.5. Associative fear conditioning

For this experiment, we focused only on the working memory component of fear conditioning by examining behavior during training. Mice were placed in a closed chamber with Plexiglas walls and a metal grid floor and allowed 2 min of exploration (adaptation period). At 2 min, an auditory cue (tone) was presented at 70 dB for 10 s. After 1 min, a total of four paired tone-foot shock pairings (10 s tone, 70 dB; 1 s shock, 0.5 mA) were administered with 60 s inter-stimulus intervals. Animals were video recorded, and a blinded experimenter calculated the time animals spent freezing (immobile) for the first 30 s after each tone-shock presentation. Percent time freezing was used as an index of associative learning performance.

2.6. Tissue collection and immunohistochemical (IHC) staining

This procedure has been previously described (Criado-Marrero et al., 2019). Briefly, after behavioral training, mice were euthanized with a Somnasol overdose at 150 (young) or 300 (aged) days of age. Following transcardial perfusion with 0.9% saline, brains were collected and fixed in 4% paraformaldehyde. Following cryopreservation, tissue was sectioned into horizontal slices at 25 and 50 μm for IHC and stereological analyses, respectively. Free-floating sections were stained as previously described (Blair et al., 2013; Dickey et al., 2009) using the following primary antibodies: anti-Ki-67 (Cell Signaling, Danvers, Massachusetts, USA, CS-9449; 1:1000), anti-DCX (Cell Signaling, Danvers,

Table 1
Summary of statistical analyses of CORT levels, behavioral tasks, and hippocampal stereology.

Figure	Measured condition	Groups analyzed	Factor	W or F statistic and p-value
2A	Serum CORT	All groups	**Genotype**	$W_{(2, 113)} =$ 33.47, p < 0.001
			ELS	$W_{(1, 114)} =$ 8.73, p = 0.003
			Sex	$W_{(1, 114)} =$ 17.02, p < 0.001
			Age	$W_{(1, 114)} =$ 6.43, p = 0.011
			Age/ELS	$W_{(1, 112)} =$ 15.29 p < 0.001
			Gen/ELS	$W_{(2, 110)} =$ 10.56, p = 0.005
			Gen/ELS/ Age	$W_{(2, 104)} =$ 0.44, p = 0.799
			Gen/ELS/ Sex	$W_{(2, 104)} =$ 9.017, p = 0.01
			Gen/ELS/ Age/Sex	$W_{(2, 92)} =$ 1.33, p = 0.514
2B	Serum CORT	Young Males	**Genotype**	$W_{(2, 27)} =$ 11.37, p = 0.003
			ELS	$W_{(1, 28)} =$ 3.96, p = 0.046
			Gen/ELS	$W_{(2, 24)} =$ 1.411, p = 0.494
		Aged Males	**Genotype**	$W_{(2, 27)} =$ 1.707, p = 0.426
			ELS	$W_{(1, 28)} =$ 4.83 p = 0.028
			Gen/ELS	$F_{(2, 24)} =$ 0.282, p = 0.869
2C		Young Females	**Genotype**	$F_{(2, 25)} =$ 41.93, p < 0.001
			ELS	$F_{(1, 26)} =$ 30.66, p < 0.001
			Gen/ELS	$F_{(2, 22)} =$ 12.24, p = 0.002
		Aged Females	**Genotype**	$F_{(2, 25)} =$ 1.613, p = 0.446
			ELS	$F_{(1, 26)} =$ 0.105, p = 0.745
			Gen/ELS	$F_{(2, 22)} =$ 6.53, p = 0.038
3A	TST: Total immobile time	All groups	**Genotype**	$F_{(2, 120)} =$ 15.5, p < 0.001
			ELS	$F_{(1, 121)} =$ 0.004, p = 0.952
			Sex	

Table 1 (*continued*)

Figure	Measured condition	Groups analyzed	Factor	W or F statistic and p-value
				$F_{(1, 121)} =$ 2.50, p = 0.117
			Age	$F_{(1, 121)} =$ 129.7, p < 0.001
			Age/ELS	$F_{(1, 119)} =$ 4.03, p = 0.047
			Gen/ELS	$F_{(2, 117)} =$ 2.54, p = 0.08
			Gen/ELS/ Age	$F_{(2, 111)} =$ 0.85, p = 0.429
			Gen/ELS/ Sex	$F_{(2, 111)} =$ 1.75, p = 0.178
			Gen/ELS/ Age/Sex	$F_{(2, 99)} =$ 0.78, p = 0.458
3B	OF: Total distance travelled	All groups	**Genotype**	$F_{(2,120)} =$ 2.59, p = 0.08
			ELS	$F_{(1, 121)} =$ 5.31, p = 0.023
			Sex	$F_{(1, 121)} =$ 11.40, p = 0.001
			Age	$F_{(1, 121)} =$ 14.07, p < 0.001
			Age/ELS	$F_{(1, 119)} =$ 2.43, p = 0.122
			Gen/ELS	$F_{(2, 117)} =$ 0.06, p = 0.934
			Gen/ELS/ Age	$F_{(2, 111)} =$ 0.19, p = 0.820
			Gen/ELS/ Sex	$F_{(2, 111)} =$ 0.08, p = 0.917
			Gen/ELS/ Age/Sex	$F_{(2, 99)} =$ 1.75 p = 0.178
Supporting data for 3B	OF: Total distance travelled	Young Males	**Genotype**	$F_{(2, 28)} =$ 0.49, p = 0.615
			ELS	$F_{(1, 29)} =$ 1.77, p = 0.196
			Gen/ELS	$F_{(2, 25)} =$ 0.13, p = 0.871
		Aged Males	**Genotype**	$F_{(2, 27)} =$ 1.28, p = 0.297
			ELS	$F_{(1, 28)} =$ 0.11, p = 0.916
			Gen/ELS	$F_{(2, 24)} =$ 0.76, p = 0.476
		Young Females	**Genotype**	$F_{(2, 28)} =$ 1.07, p = 0.359
			ELS	$F_{(1, 29)} =$ 2.83, p = 0.105
			Gen/ELS	

Table 1 (*continued*)

Figure	Measured condition	Groups analyzed	Factor	W or F statistic and p-value
				$F_{(2, 25)} = 0.55$, p = 0.583
		Aged Females	Genotype	$F_{(2, 31)} = 1.44$, p = 0.253
			ELS	$F_{(1, 22)} = 1.97$, p = 0.171
			Gen/ELS	$F_{(2, 28)} = 1.479$, p = 0.246
3C	TST: Total immobile time	Young Males	Genotype	$F_{(2, 28)} = 6.09$, p = 0.007
			ELS	$F_{(1, 29)} = 0.36$, p = 0.552
			Gen/ELS	$F_{(2, 25)} = 0.32$, p = 0.72
		Aged Males	Genotype	$F_{(2, 27)} = 3.51$, p = 0.04
			ELS	$F_{(1, 28)} = 0.84$, p = 0.36
			Gen/ELS	$F_{(2, 24)} = 1.26$, p = 0.30
3D		Young Females	Genotype	$F_{(2, 28)} = 6.43$, p = 0.006
			ELS	$F_{(1, 29)} = 3.27$, p = 0.08
			Gen/ELS	$F_{(2, 25)} = 2.98$, p = 0.07
		Aged Females	Genotype	$F_{(2, 31)} = 5.32$, p = 0.01
			ELS	$F_{(1, 32)} = 0.86$, p = 0.35
			Gen/ELS	$F_{(2, 28)} = 1.02$, p = 0.37
4A-D	Fear expression (freezing), 4 conditioning tones	Repeated measures, All groups	Genotype	$F_{(2, 120)} = 15.44$, p < 0.001
			ELS	$F_{(1, 121)} = 0.021$, p = 0.886
			Sex	$F_{(1, 121)} = 5.61$, p = 0.02
			Age	$F_{(1, 121)} = 24.63$, p < 0.001
			Age/ELS	$F_{(1, 119)} = 10.26$, p = 0.018
			Gen/ELS	$F_{(2, 117)} = 0.44$, p = 0.64
			Gen/ELS/Age	$F_{(2, 111)} = 1.25$, p = 0.289
			Gen/ELS/Sex	$F_{(2, 111)} = 0.29$, p = 0.74
			Gen/ELS/Age/Sex	

Table 1 (*continued*)

Figure	Measured condition	Groups analyzed	Factor	W or F statistic and p-value
				$F_{(2, 99)} = 0.57$, p = 0.565
4A-D	Fear expression	Young Males	Genotype	$F_{(2, 28)} = 8.029$, p = 0.002
			ELS	$F_{(1, 29)} = 0.013$, p = 0.91
			Gen/ELS	$F_{(2, 25)} = 0.181$, p = 0.83
		Aged Males	Genotype	$F_{(2, 27)} = 1.413$, p = 0.26
			ELS	$F_{(1, 28)} = 0.511$, p = 0.48
			Gen/ELS	$F_{(2, 24)} = 2.665$, p = 0.09
		Young females	Genotype	$F_{(2, 28)} = 3.94$, p = 0.03
			ELS	$F_{(1, 29)} = 0.209$, p = 0.65
			Gen/ELS	$F_{(2, 25)} = 0.008$, p = 0.99
		Aged females	Genotype	$F_{(2, 31)} = 4.503$, p = 0.02
			ELS	$F_{(1, 32)} = 0.678$, p = 0.41
			Gen/ELS	$F_{(2, 28)} = 0.140$, p = 0.87
6B	IHC: HPC NeuN/CV	All groups	Genotype	$F_{(2, 55)} = 2.72$, p = 0.076
			ELS	$F_{(1, 56)} = 1.01$, p = 0.320
			Age	$F_{(1, 56)} = 4.78$, p = 0.034
			Age/ELS	$F_{(1, 54)} = 8.155$, p = 0.006
			Gen/ELS	$F_{(2, 52)} = 0.09$, p = 0.910
			Gen/ELS/Age	$F_{(2, 46)} = 1.61$, p = 0.211
6C	IHC: HPC Volume	All groups	Genotype	$F_{(2, 45)} = 0.34$, p = 0.713
			ELS	$F_{(1, 56)} = 0.002$, p = 0.968
			Age	$F_{(1, 56)} = 0.34$, p = 0.563
			Age/ELS	$F_{(1, 54)} = 0.002$, p = 0.968
			Gen/ELS	$F_{(2, 52)} = 0.71$, p = 0.497
			Gen/ELS/Age	$F_{(2, 46)} = 0.04$, p = 0.959

CORT: corticosterone, ELS: Early life stress, GEN: Genotype, TST: tail suspension test, OF: open field, IHC: immunohistochemistry, HPC: hippocampus, NeuN/CV: neuronal nuclear antigen/cresyl violet, W: Wald statistic, F: F statistic. The CORT data followed a normal distribution with unequal variances. This was analyzed using linear regression with Huber-White correction followed by Bonferroni post hoc test as part of the generalized linear model in SPSS. All other group data was analyzed by factorial analysis of variance (ANOVA) using the general linear model (GLM) procedure in the SPSS Statistics software.

Massachusetts, USA,CS-4604; 1:300), anti-GFAP (Millipore, Burlington, MA, USA, MAB360, 1:10,000), anti-Iba1 (WAKO, Richmond, VA, USA, 016-26461, 1:10,000). Images were taken at 20× using a Zeiss Axi-oScan.Z1 slide scanner (Zeiss, Oberkochen, Germany) and tissue segmentation was performed using the NearCYTE software (www.nearcyte.org).

2.7. CORT ELISA assay

We collected blood from the submandibular area ten days before behavioral testing. Blood samples were taken during the morning between 10:00 h and 12:00 h within a 3-min period to prevent disturbances (Vahl et al., 2005). Samples from five animals were excluded in the analysis because of noticeable stress during blood extraction. Samples were centrifuged for serum separation (SAI Infusion Technologies, Lake Villa, IL, #PMTS-SG-1.1) and stored at −80 °C until analyzed. CORT levels were quantified with an enzyme-linked immunosorbent assay (ELISA) kit (Enzo Life Sciences, Farmingdale NY, USA, # ADI-901-097) according to the manufacturer's instructions using 10 μL of serum.

2.8. Statistical analysis

Total distance travelled in the OF test was measured using ANY-Maze. Data distributions were tested using Shapiro-Wilk's test and equality of variances using Levene's test. Behavioral data were analyzed by multifactorial analysis of variance (ANOVA) using the general linear model (GLM) procedure in the SPSS Statistics software (v25). GLM repeated measures was used for associative fear conditioning stimuli. When there was a group difference, GLM was followed by Bonferroni multiple comparison test. Three- and two-way ANOVA was used to analyze interactions between variables. Significant difference was considered if $p < 0.05$. Data are presented as mean ± SEM. Main and interactive effects by genotype, ELS and age on dependent variables (immobility, distance travelled, fear expression, and neuronal number) are described in Table 1. Generalized linear models with a Huber-White correction (robust error estimator) were used to analyze CORT and tissue expression (Ki-67, DCX, GFAP and Iba1) as equal variances were not achieved even after log transformation of data. When statistically significant, analysis was followed by Bonferroni post hoc test. Interactions and main effects are described in Table 1 (CORT) and Table 2 (tissue).

3. Results

3.1. High FKBP5 lowers CORT levels

Because CORT secretion is tightly regulated by the HPA axis and dysfunction of this neuroendocrine system has been implicated in the pathophysiology of depression, we collected blood from mice prior to behavioral testing (see experimental timeline in Fig. 1A and animal groups in Fig. 1B). We evaluated whether basal serum CORT levels were affected by genotype, age and/or ELS. We found significant effects of genotype ($p < 0.001$), ELS ($p = 0.003$), sex ($p < 0.001$) and age ($p = 0.011$), as well as interactive effects of ELS x age ($p < 0.001$), genotype x ELS ($p = 0.005$), and genotype x ELS x sex ($p = 0.01$).

Post-hoc tests demonstrated that the rTgFKBP5 genotype was associated with significantly reduced CORT levels compared to both other genotypes overall, regardless of sex or age (Fig. 2A). ELS also reduced

Table 2

Summary of statistical analyses of neurogenesis and neuroinflammatory markers.

Figure	Measured condition	Groups analyzed	Factor	Wald Chi-Square	N	df	p-value
5A-B	Ki-67	All groups	**Genotype**	8.687	61	2	.013
			ELS	2.279	61	1	.131
			Age	54.058	61	1	.000
			Age/ELS	0.253	61	1	.615
			Age/Genotype	4.929	61	2	.085
			Gen/ELS	10.367	61	2	.006
			Gen/ELS/Age	4.527	61	2	.104
5A-B	Ki-67	Young	**Genotype**	1.028	31	2	.598
			ELS	1.563	31	1	.211
			Gen/ELS	1.582	31	2	.453
		Aged	**Genotype**	8.261	30	2	.016
			ELS	1.208	30	1	.272
			Gen/ELS	8.857	30	2	.012
5C-D	DCX	All groups	**Genotype**	0.336	63	2	.845
			ELS	0.462	63	1	.497
			Age	58.069	63	1	.000
			Age/ELS	0.000	63	1	.991
			Age/Genotype	0.126	63	2	.939
			Gen/ELS	1.841	63	2	.398
			Gen/ELS/Age	0.580	63	2	.748
5C-D	DCX	Young	**Genotype**	10.981	32	2	.004
			ELS	5.184	32	1	.023
			Gen/ELS	4.426	32	2	.109
		Aged	**Genotype**	0.030	31	2	.985
			ELS	0.114	31	1	.736
			Gen/ELS	1.145	31	2	.564
7A-B	GFAP	All groups	**Genotype**	23.902	59	2	.000
			ELS	1.027	59	1	.311
			Age	106.628	59	1	.000
			Age/ELS	10.713	59	1	.001
			Age/Genotype	4.354	59	2	.113
			Gen/ELS	10.502	59	2	.005
			Gen/ELS/Age	2.487	59	2	.288
7A-B	GFAP	Young	**Genotype**	25.559	28	2	.000
			ELS	3.472	28	1	.062
			Gen/ELS	4.079	28	2	.130
		Aged	**Genotype**	7.341	31	2	.025
			ELS	7.265	31	1	.007
			Gen/ELS	8.849	31	2	.012
7C-D	Iba1	All groups	**Genotype**	0.363	60	2	.834
			ELS	1.104	60	1	.293
			Age	14.402	60	1	.000
			Age/ELS	1.166	60	1	.280
			Age/Genotype	0.472	60	2	.790
			Gen/ELS	11.781	60	2	.003
			Gen/ELS/Age	11.883	60	2	.003
7C-D	Iba1	Young	**Genotype**	4.552	30	2	.103
			ELS	0.724	30	1	.395
			Gen/ELS	0.276	30	2	.871
		Aged	**Genotype**	0.416	30	2	.812
			ELS	1.135	30	1	.287
			Gen/ELS	11.834	30	2	.003

DCX: doublecortin, GFAP: Glial fibrillary acidic protein, Iba1: Ionized calcium binding adaptor molecule 1, ELS: early life stress, Gen: genotype, df: degrees of freedom, N: number of samples. The relative intensity expression of each protein (Ki-67, DCX, GFAP, Iba1) was analyzed by Generalized Linear Models with Huber-White correction (robust error estimator) using the SPSS Statistics software. When significant, this analysis was followed with a Bonferroni correction post hoc test.
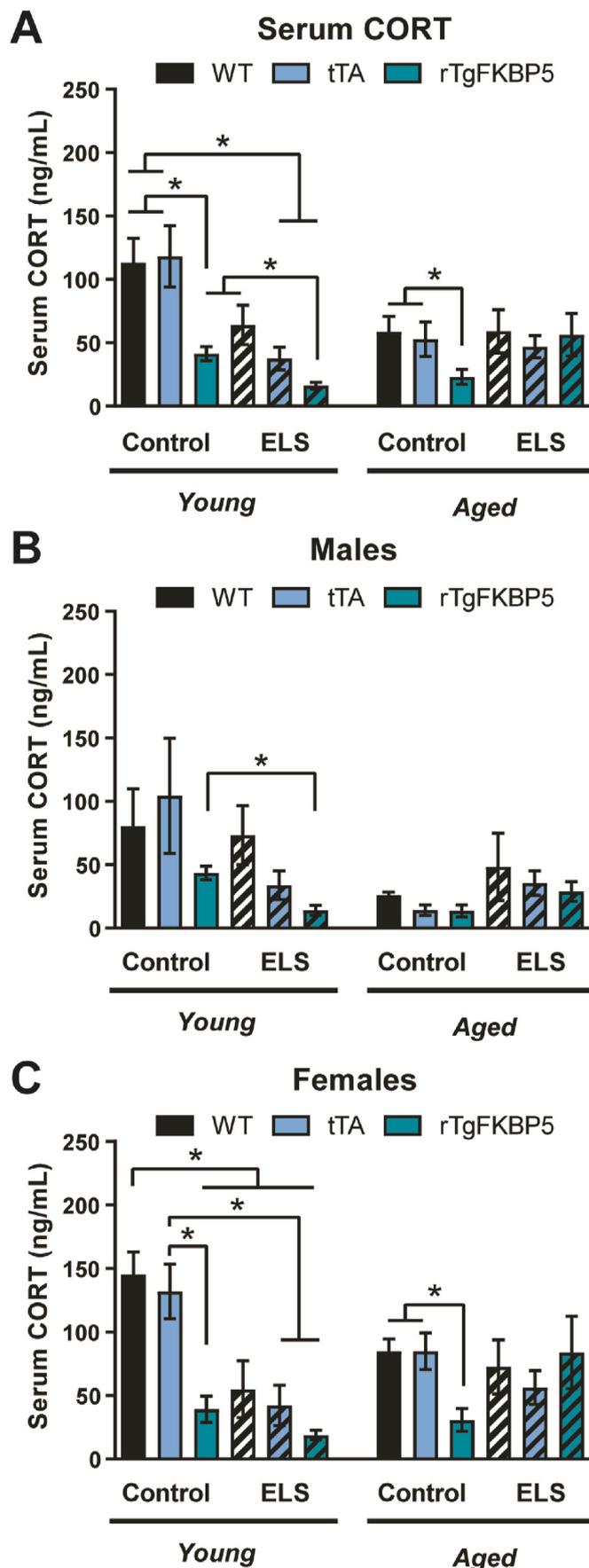
Fig. 2. High FKBP5 reduces basal CORT levels. (**A**) Blood was collected from mice at 50 (young) or 200 (aged) postnatal days. After centrifugation, serum was collected, and total CORT levels were analyzed by ELISA. (**B**) Basal CORT levels in males and (**C**) females. WT = wild-type, tTA = CamKIIα-tTA, rTgFKBP5 = FKBP5 overexpressing mice, CORT, corticosterone, ng = nanogram, mL = milliliter, ELS = early life stress. Data are represented as standard error of the mean (SEM) and analyzed by linear regression followed by Bonferroni post hoc test (see Table 1). Significant results were considered when *p < 0.05.

CORT levels, and FKBP5 and ELS produced a synergistic decrease in CORT levels, but only in young mice (p < 0.05). On the other hand, CORT levels were reduced in aged mice independent of any other factors (p < 0.05). These findings were all greater in female mice, due primarily to the greater levels of CORT at both ages in female vs. male control genotypes (Fig. 2B and C). This suggests that malfunction of FKBP5 alone at young ages can impact HPA function, and that this impact may be greater in females.

3.2. ELS counteracts FKBP5-induced susceptibility to depressive-like symptoms in aged mice

We previously showed that the interaction of high FKBP5 and ELS increases anxiety levels in young mice (Criado-Marrero et al., 2019). Here, we investigated whether this interaction also drives depressive behavior, since depression symptoms are highly comorbid with anxiety. Using the tail suspension test (TST), we found that aged mice spent less time immobile than young mice (age effect, $F_{1,98}$ = 129.7, p < 0.0005; Fig. 3A). While overexpression of FKBP5 in aged mice led to more time immobile, ELS prevented this effect (control x ELS, p < 0.05). This suggests that FKBP5 counteracts the effect of aging on depressive-like behavior in this model. In both age groups, WT and tTA were not affected by ELS. Using the open field test, we assessed whether animals had mobility differences that may have confounded the TST observations. Overall locomotion, as measured by total distance travelled, was not altered between genotypes, although both ELS and aging were associated with a significant decrease in locomotor activity. A follow-up analysis on these effects revealed that there were no differences in either sex between age groups (see supporting data for Fig. 3B in Table 1), confirming that sex differences in TST were not due locomotor variations (Fig. 3B). Although sex alone did not affect active escape-oriented behavior during TST monitoring, we found a significant interactive effect of sex with age and genotype on total time immobile (p < 0.006). Hence, we analyzed immobility differences in males (Fig. 3C) and females (Fig. 3D) separately. For the males, aging was associated with a significant decrease in time immobile independent of ELS or genotype, whereas ELS increased time immobile in FKBP5 mice. Time immobile was also reduced by age in WT and tTA, but not rTgFKBP5 female mice. In aged rTgFKBP5 females, ELS counteracted the FKBP5-induced susceptibility to depressive-like symptoms by reducing time immobile.

3.3. Age, but not FKBP5, influences fear learning

In addition to anxiety and depression, gene and protein changes in FKBP5 have been associated with enhanced fear responses, which is a common symptom in PTSD (Fani et al., 2016, 2012). Using a fear conditioning paradigm, we examined whether fear learning was affected by overexpression of FKBP5, age or exposure to ELS. Fear expression was higher in aged control mice when compared to young animals (p < 0.05) (Fig. 4A and B). This age-related increase in fear expression was independent of ELS (p < 0.05, young x aged) (Fig. 4C and D). Although there were main effects of genotype and sex on fear learning, the interplay of each factor with ELS exposure (two-way interaction) was not significant. Both aged female and male mice showed slightly higher fear expression compared to young mice (Supplementary Fig. 1 and Table 1), but no significant differences between the groups were found after following up with a Bonferroni multiple comparison analysis.

(caption on next column)
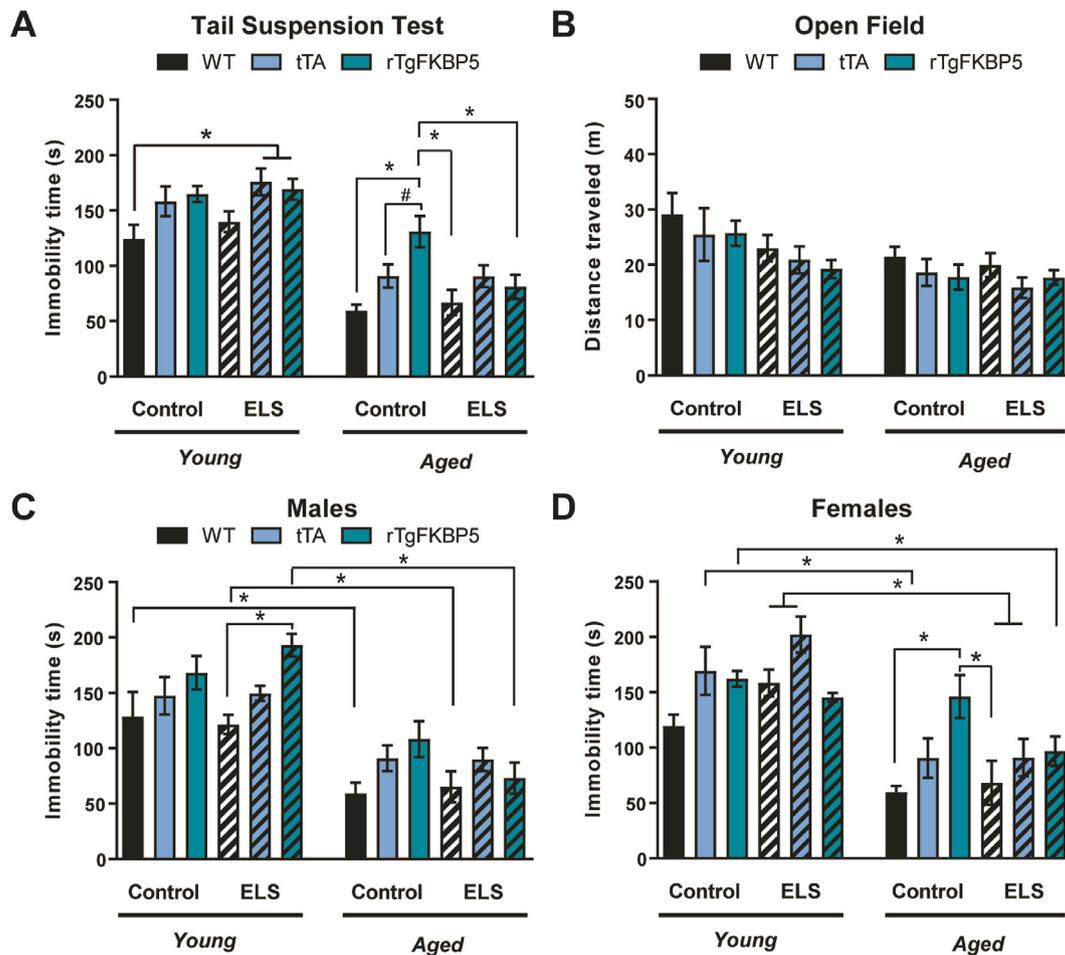
Fig. 3. ELS prevents FKBP5-induced susceptibility to depressive-like behavior in aged mice. (**A**) Total immobile time in the tail suspension test and (**B**) distance travelled during the open field test as measure of locomotor activity. Immobile time spent in the tail suspension test by (**C**) males and (**D**) females. WT = wild-type, tTA = CamKIIα-tTA, rTgFKBP5 = FKBP5 overexpressing mice, s = seconds, m = meters, ELS = early life stress. Mice were 60-days-old (young) and 210-days-old (aged) when subjected to behavioral testing; N = 9-11 per group. Data are represented as standard error of the mean (SEM) and analyzed by three- and two-way Analysis of variance (ANOVA)s (see Table 1). Significant results were considered when *p < 0.05 and were followed by Bonferroni post hoc tests. #p = 0.08.

3.4. Neuronal loss in rTgFKBP5 mice is age-dependent

Prolonged exposure to stress or oral administration of CORT are known to increase FKBP5 expression and suppress hippocampal neurogenesis (Lee et al., 2010; Scharf et al., 2011; Zhang et al., 2016). Multiple studies have also shown that aging reduces hippocampal neurogenesis (Kuhn et al., 1996; Ben Abdallah et al., 2010; Lugert et al., 2010). Thus, we tested whether FKBP5 genotype and ELS interact to impact hippocampal neurogenesis and if this change is affected by aging. First, we measured the levels of Ki-67 (a proliferation cell marker). There was a statistically significant difference in Ki-67 among the groups (p < 0.0001; Fig. 5A and B), which was mainly driven by age, genotype and the interaction of ELS x genotype (Table 2). Specifically, aged rTgFKBP5 mice showed higher Ki-67 expression than aged tTA (Fig. 5A and B). Young WT and tTA animals did not show differences from FKBP5 mice in Ki-67 expression. Then, we measured levels of doublecortin (DCX, immature progenitor and migration cell marker). We found an overall group difference (p < 0.001) and a main effect of age (p < 0.0001). DCX was decreased in aged mice, suggesting an age-related decline in cell differentiation or migration. ELS interacted with FKBP5 significantly in young mice, but not in aged mice. Specifically, ELS reduced DCX expression in FKBP5 mice compared to tTA (p < 0.05) mice (Fig. 5C and D). In both age groups, ELS-rTgFKBP5 animals showed the greatest suppression in DCX expression, highlighting the importance of gene x environment in cellular processes. Since levels of these cell markers,

Ki-67 and DCX, may vary based on the cell phase transition (Miller et al., 2018), we counted the total number of neurons to determine whether there were significant changes in neuronal counts. Different from what we expected, ELS did not cause significant loss of NeuN-positive neurons within young (Fig. 6A) or aged (Fig. 6B) groups. However, a Bonferroni multiple comparison analysis revealed a significant reduction of neurons in aged rTgFKBP5 mice when compared to young WT and rTgFKBP5 mice (Fig. 6C). The hippocampal volume was not affected by age, ELS or genotype (Fig. 6D).

3.5. Microglial expression is increased in aged rTgFKBP5 mice

Astrocyte and microglia alterations are linked to early life adversities and depression (Abbink et al., 2019; Rajkowska and Stockmeier, 2013; Réus et al., 2019). Therefore, we investigated changes in astrocyte and microglial counts, since their numbers can be affected by stress and aging (Réus et al., 2019; Tynan et al., 2010). Chronic activation of these cells has been implicated in various brain diseases including major depressive disorder (Rajkowska and Stockmeier, 2013). We stained for the glial fibrillary acidic protein (GFAP) and ionized calcium binding adaptor molecule 1 (Iba1), which are highly expressed in astrocytes and microglia, respectively. In the young cohort, tTA-ELS animals had the highest levels of GFAP (Fig. 7A). GFAP levels were increased in tTA animals as well as in the rTFKBP5 group, but there was no difference between tTA and FKBP5 genotypes. Different from what we expected, GFAP was
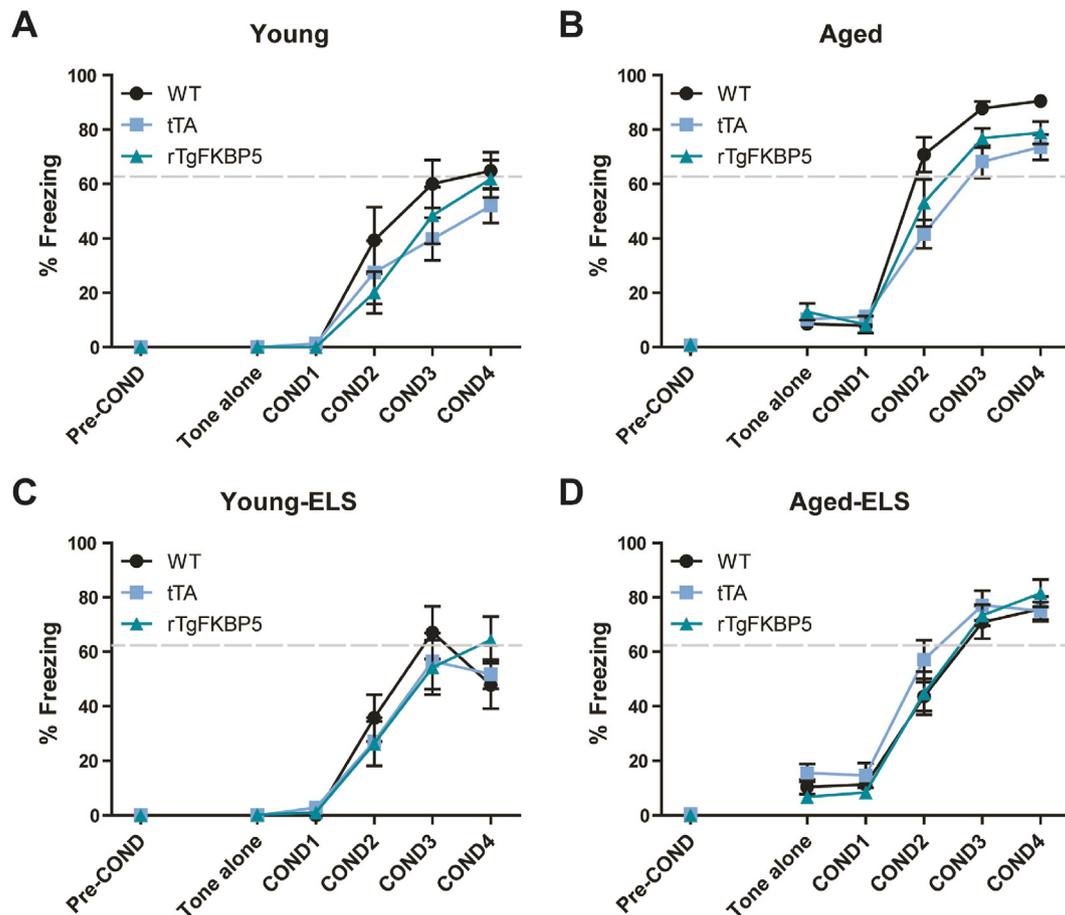
Fig. 4. High FKBP5 does not impact auditory fear learning. Percentage of time freezing during the fear conditioning test by non-stressed (**A**) young and (**B**) aged animals. Freezing time for (**C**) young and (**D**) aged mice exposed to ELS. WT = wild-type, tTA = CamKIIα-tTA, rTgFKBP5 = FKBP5 overexpressing mice, ELS = early life stress; N = 9-11 per group. Data are represented as standard error of the mean (SEM) and analyzed by repeated measures three- and two-way Analysis of variance (ANOVA)s (see Table 1 for analysis).

reduced by ELS in aged rTgFKBP5 and WT mice when compared to animals of the same genotype without stress exposure. Also, in the aged group, there was a significant genotype x ELS interaction due to significantly higher GFAP expression in rTgFKBP5 mice compared to WT-ELS (Fig. 7B). Like GFAP, we found significantly higher levels of Iba1 in aged mice and an interaction in genotype x ELS (Fig. 7C and D). Surprisingly, this microglial marker was highly induced in aged rTgFKBP5 mice. ELS induced lba1 in aged WT and tTA mice but blocked the age-induced increase in lba1 in FKBP5 mice (Fig. 7D). These results demonstrate an age-related increase in microglial markers in rTgFKBP5 mice and that hippocampal levels of these markers can be differentially affected by ELS.

4. Discussion

4.1. Summary

Over the last decade, there have been numerous studies on how the environment fosters epigenetic, genetic and molecular changes in the brain, increasing predisposition to mental disorders (Daskalakis et al., 2013; Eid et al., 2019; Hui et al., 2011). This study adds to our previous finding demonstrating that an FKBP5 x ELS interaction increases anxiety levels (Criado-Marrero et al., 2019). Here, we tested whether this interaction, FKBP5 x ELS, may affect depressive-like symptoms. We tested this possibility using a mouse model overexpressing FKBP5 in the brain (rTgFKBP5) and two control groups, WT and tTA (similar to rTgFKBP5, tTA express the tetracycline-off transactivator driven by the CamKIIα

promoter). Since ELS can have long-lasting effects in the brain, we also evaluated depressive-like symptoms and brain changes at two ages. First, we found that high FKBP5 affected neuroendocrine regulation by reducing basal CORT levels in rTgFKBP5 mice, especially in females. Second, we observed an age-dependent effect wherein young mice displayed higher depressive-like symptoms than older mice during the tail suspension test. Aged, but not young, rTgFKBP5 mice with prior exposure to ELS were resistant to these depressive-like symptoms. Third, ELS decreased Ki-67-positive cells in aged rTgFKBP5 mice, while affecting DCX expression in young animals. Lastly, FKBP5 overexpression increased microglial markers and promoted neuronal loss in the hippocampus of aged mice. This suggests that high expression of FKBP5 may accelerate an age-related activated microglia response to neuronal damage or neuroinflammation. Overall, our findings indicate that the interplay of FKBP5 with ELS impacts brain function and physiology throughout the lifecycle, affecting emotional responding. Importantly, our data demonstrate that key factors in the vulnerability for mental disorders, like age and sex, can influence behavioral and neurobiological outcomes.

4.2. FKBP5 affects the HPA axis in a sex-dependent manner

Clinical reports have documented an association between CORT levels and depression (see review (Sanjay Nandam et al., 2020)); however, we did not find a correlation between basal CORT levels and depressive-like symptoms in this study. Instead, we found that animals overexpressing FKBP5 showed reduced basal CORT levels. This reduction
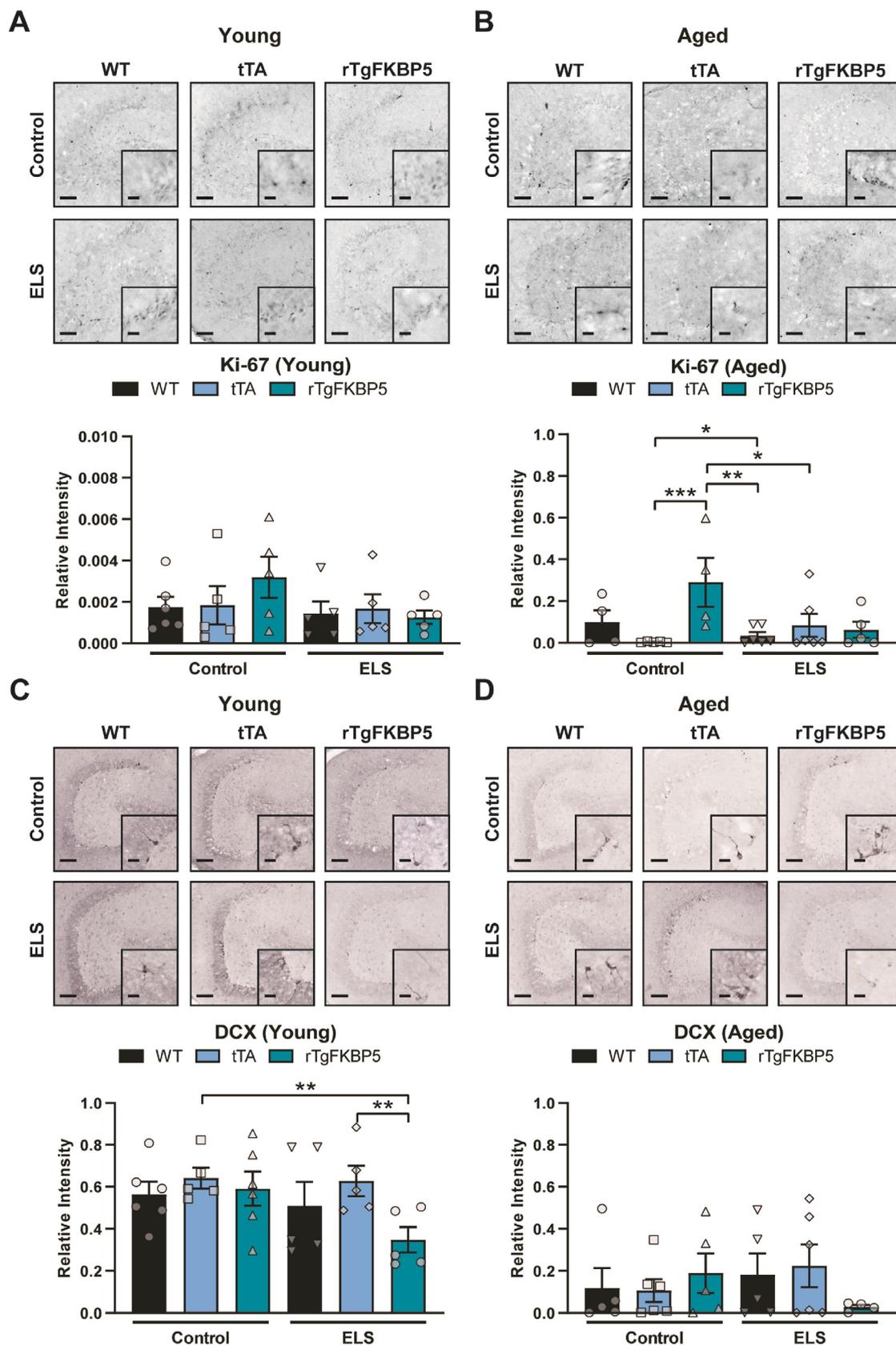
Fig. 5. FKBP5 x ELS reduces DCX-positive cells in the dentate gyrus of young mice. Representative images and relative expression of Ki-67 (cell proliferation marker) in the hippocampal dentate gyrus of (**A**) young and (**B**) aged animals. (**C-D**) Representative images and DCX expression in the same mice. Hippocampal sections were collected from mice at 150 (young) and 300 (aged) days of age; N = 5-6 per group. Interaction of stress x genotype was analyzed by linear regression followed by Bonferroni post hoc test (see Table 2). Data represented as standard error of the mean (SEM). Statistical significance was considered when *p < 0.05, **p < 0.01, ***p < 0.001. Scale bar represents 200 μm; inset scale represents 20 μm. DCX = doublecortin; ELS, early life stress.
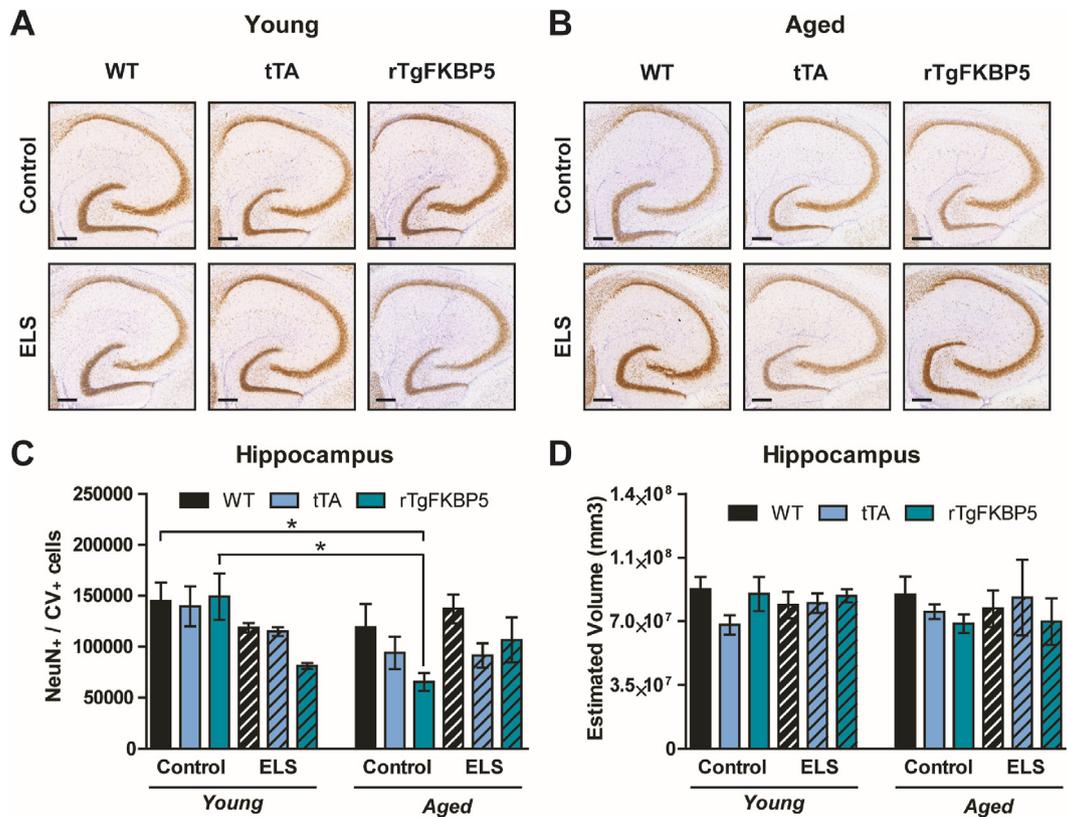
Fig. 6. FKBP5 overexpression x age interaction reduces neuronal number in the hippocampus. Representative images of hippocampal sections and analysis from (**A**) young and (**B**) animals. Analysis of total neurons in the hippocampus from (**C**) young and (**D**) aged mice. Hippocampal sections collected from mice at 150 (young) and 300 (aged) days of age were stained with NeuN (brown) and cresyl violet (violet). N = 4-6 animals per group. ELS = early life stress. Scale bar represents 200 μm. Values are represented as standard error of the mean (SEM). Significant results were considered when *p < 0.05 and were followed by Bonferroni post hoc tests (see Table 1). WT = wild-type, tTA = CamKIIα-tTA, rTgFKBP5 = FKBP5 overexpressing mice, ELS = early life stress.

in basal CORT levels was most evident in females. This is interesting because similar observations have been reported in *FKBP5* knockout mice where females, but not males, showed reduced basal CORT levels (Hoeijmakers et al., 2014; Touma et al., 2011). This indicates the negative feedback sensitivity of the HPA axis can be affected by levels of FKBP5 in a sex-dependent manner. Specifically, the FKBP5 SNP rs1360780 has been linked to increased risk for depression where TT carriers have higher basal FKBP5 and present with GR resistance. Notably, the susceptibility for depressive symptoms in individuals with these FKBP5 variants are sex-dependent and moderated by adverse experiences at early ages (Appel et al., 2011; VanZomeren-Dohm et al., 2015).

In contrast to our hypothesis, FKBP5 lowered CORT levels in young mice, implying a hypocortisolism phenotype. A different FKBP5 SNP, rs9296158, has been linked to childhood abuse and low levels of CORT in depressed patients (Kohrt et al., 2015). Moreover, hypocortisolism has been described in patients with atypical depression and in females with depression, suggesting that FKBP5 may affect specific endophenotypes in depression (Bremmer et al., 2007; Juruena et al., 2018; Maripuu et al., 2014; Penninx et al., 2007). Moreover, women with other mental illnesses, like PTSD, have shown lower CORT levels than healthy women (Freidenberg et al., 2009; Meewisse et al., 2007), which suggests that GR dysregulation in females is not exclusive to depression. In the present study, we found that young rTgFKBP5 females had lower CORT levels than young males as well as older animals. The exact mechanism underlying low basal CORT levels in females is still unknown. Based on previous studies, activity of steroid receptors and hormone levels (e.g. estrogen) may contribute to sex differences (see review (Bourke et al., 2012). Particularly, in an aged population, a greater CORT variability should be anticipated in women because of the impact exerted by

menopausal status. Another possibility is that the half-life of cortisol is shorter in women as compared to men (Roelfsema et al., 2017). Investigators reported that these differences were found in individuals younger than 50 years old, and no differences were found in older adults. This is in line with our findings showing that sex differences were evident in young but not aged mice. Follow-up studies with additional timepoints throughout the day are needed to fully evaluate the CORT dynamics in these animals to determine if they have decreased CORT overall, if the circadian dynamics are shifted so that the peak and trough of CORT varies between groups, or if the amplitude of the CORT rhythm varies between groups.

4.3. FKBP5 reduces hippocampal neurons in aged mice

Multiple studies have reported the adverse impact of chronic and early life stress on brain maturation and development (Chocyk et al., 2013, 2011; Delpech et al., 2016; Dioli et al., 2019). Specifically, stress induces adverse effects in the hippocampus by impairing both neuron maturation and formation of synaptic circuits; these developmental deficits are associated with changes in mood and affect (Delpech et al., 2016; Wei et al., 2015, 2011). Here, we observed that high FKBP5 increases hippocampal Ki-67 proliferation in aged animals, while ELS prevents this effect. However, ELS reduced DCX in young animals without affecting aged mice. This suggests that stress exerts a stronger effect on hippocampal cell growth and differentiation. We previously showed that long-term potentiation is modestly increased in rTgFKBP5 mice (Blair et al., 2019). Thus, it is possible that the enhancement of long-term potentiation in these mice is secondary to an increase in cell proliferation and in brain-derived neurotrophic factor (BDNF) expression, as previously reported (Chun et al., 2006; Gassen et al., 2015). Also,

A Young

B Aged

C Young

D Aged
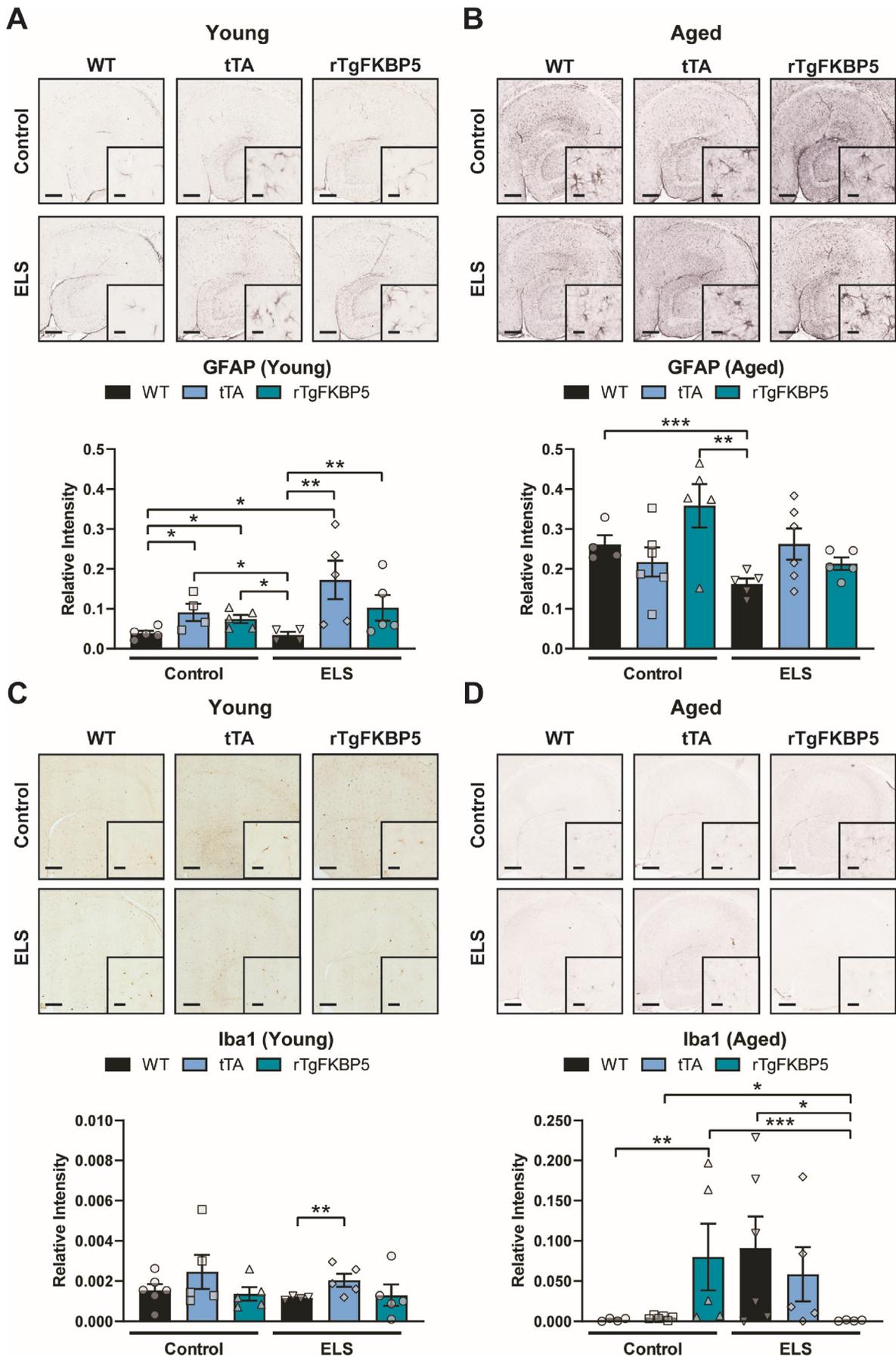
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rTgFKBP5 mice exhibited reversal spatial learning deficits in the Morris water maze (Blair et al., 2019). Reduced neurogenesis has been linked to cognitive impairments (Anacker and Hen, 2017), thus the spatial learning deficit in rTgFKBP5 mice may be due to loss of hippocampal neurons as reported in this study. Age was a determining factor in high FKBP5-expressing animals, as only aged control rTgFKBP5 mice demonstrated this reduction in neurons. Our observations are in line with prior reports showing that proliferation and neurogenesis do not necessarily correlate and that ELS induces its effects in an age- and sex-dependent manner (Hulshof et al., 2011; Loi et al., 2014). However, we cannot exclude possible ELS-induced changes in the structure of hippocampal neurons. Based on a previous study, ELS can promote expression of immature spines while reducing the number of mature spines in CA1 neurons (Wei et al., 2015). The authors also showed that ELS can impair synaptic maturation in the hippocampus, implying a direct effect by ELS on hippocampal-dependent processes like emotional responses.

4.4. Markers of microglia are increased by FKBP5 in aged mice

Lastly, we found that Iba1 levels were significantly increased by FKBP5 in control animals and by ELS in aged WT and tTA mice. This is interesting because Iba1 is upregulated in microglia in response to peripheral inflammation and brain damage as well as after CORT exposure (see review (Delpech et al., 2015)). As shown in previous studies, it is possible that high FKBP5 recruits more microglia by increasing proinflammatory cytokines like TNFa, promoting NF-κB–driven peripheral inflammation (Klengel et al., 2013; Lively and Schlichter, 2018; Zannas et al., 2019). However, ELS prevented upregulation of microglia in aged rTgFKBP5 mice, possibly via inhibiting GR. Depending on length of stress or CORT exposure, CORT is known to exert both suppressive and stimulatory effects on microglia (Rohan Walker et al., 2013; Tanaka et al., 1997). Since aging and stress can each induce the expression of FKBP5, it is possible that the enhanced overexpression of FKBP5 is enough to abolish the GR-induced inflammatory response to stress. As previously reported, this will inhibit microglial activation and proinflammatory cytokine release (Frank et al., 2012). Importantly, we must note that independent of FKBP5, aging also determined the levels of microglia, which may correlate with GR activation in the hippocampus and cytokine activation. For instance, Barrientos et al. showed that there is greater microglial activation in the hippocampus of middle-aged rats compared to young rats (Barrientos et al., 2015). They also showed that GR inhibition reduced the expression of lipopolysaccharide (LPS)-induced proinflammatory cytokines (IL-1β and TNF-α) in microglia cultures. In a separate study, peripheral LPS injection produced a greater induction of cytokines from isolated microglia in aged animals when compared to adults (Henry et al., 2009). Interestingly, FKBP5-deficient mice have reduced levels of IL-1β at 6 months, but no differences were detected at older timepoints (>10 month of age) (Sabbagh et al., 2014). These findings suggest that the influence of FKBP5 on inflammatory markers, and possibly on microglia, may depend on age and stress factors.

4.5. Limitations and future studies

We limited our study to measure basal CORT levels at a single time point. Although no association was found between CORT and depressive-like symptoms, more research is needed to examine whether FKBP5 alters CORT feedback regulation and dynamics after chronic stress. To test

this, the DEX/CRH test should be used, since it is more suitable to detect abnormalities in the HPA axis in response to stress than measuring basal levels. Also, repeating stress later in life may reflect whether resiliency is altered in rTgFKBP5-ELS animals by dysregulating CORT levels and identifying behavioral disturbances. This is from the perspective of the previously described three-hit hypothesis where brain and HPA axis responses adapt to ongoing gene-environment interactions involving (1-hit) genetic predisposition [high FKBP5], (2-hit) early-life environment [maternal separation], and (3-hit) later-life stressful environment [e.g. chronic variable stress] (Daskalakis et al., 2013). Moreover, we did not observe an effect of FKBP5 x ELS on associative fear conditioning. Nevertheless, follow up studies should evaluate other types of memory that depend on extra-hippocampal brain areas, like prefrontal cortex-dependent working memory. Specifically, healthy aged individuals (50+ years old) carrying the T allele of the FKBP5 variant rs1360780 had poor working memory in two independent memory tests (Fujii et al., 2014). These individuals also presented with lower CORT reactivity in the DEX/CRH test. Thus, it is probable that FKBP5 and stress trigger other symptoms, like the cognitive deficits commonly reported in depression (Mcintyre et al., 2013).

Additionally, future research should seek to uncover epigenetic changes induced by FKBP5 x ELS in young and aged groups. In the ELS mice, we would expect a decrease in DNA methylation since corticosterone exposure is known to reduce FKBP5 methylation in the CpG4 of two intronic regions: intron 1 and 5 (Lee et al., 2010). In humans, lower methylation in two FKBP5 CpGs, cg20813374 and cg00130530, correlates with high FKBP5 mRNA levels and is associated with aging and stress-related phenotypes (Zannas et al., 2016). In a separate study, it was reported that the strongest DNA demethylation is in the CpG Intron 7 of FKBP5 after DEX-induction of GR in human hippocampal progenitor cells (Klengel et al., 2013). They also reported that early trauma and its interaction with this high-risk allele of FKBP5 were significant predictors for lifetime PTSD risk, supporting the link between epigenetics and environmental factors with mood disorders.

Another limitation of our study is the reduced power to establish sex differences in tissue analyses due the small sample size per sex/group. ELS is known to induce sex-specific differences in neurogenesis, thus we may expect variations between males and females. For example, one study showed that males exposed to ELS had higher DCX-positive neurons where females had significantly fewer (Oomen et al., 2009). In another study, adult male mice had fewer neurons after ELS, but no change was found in females (Naninck et al., 2015). Sex can also affect microglia in the hippocampus. Female rats possess more activated microglia during juvenile and early adulthood; this may help to explain FKBP5 and stress effects in microglia at different ages. Thus, due to the influence of sex and age in stress-related disorders, it will be important to further investigate whether FKBP5 differentially affects neurogenesis, microglia and cytokine population in both sexes.

Finally, we have focused this and our previous investigation on understanding the impact of stress on non-cognitive (affective) symptoms related to mood disorders. We have examined changes in the hippocampus, but other areas expressing CamKII in the limbic system like the amygdala and cortex may also contribute to our results. Additionally, considering that ELS can promote amyloid-beta plaque deposition (Lesuis et al., 2018) and FKBP5 has been shown to promote protein oligomerization (Blair et al., 2013), our findings are relevant to understand whether high FKBP5 and early exposure to stress may influence the age of onset and severity of age-related diseases like Alzheimer's disease (AD).

Fig. 7. FKBP5 induces microglial markers in aged mice. Representative images and relative fold change of GFAP (astrocyte marker) in the hippocampal dentate gyrus of (**A**) young and (**B**) aged animals. (**C-D**) Representative images and fold-change of Iba1 expression in the dentate gyrus of the hippocampus in the same mice. Hippocampal sections were collected from mice at 150 (young) and 300 (aged) days of age; N = 5-6 per group. Interaction of stress and genotype was analyzed by linear regression followed by Bonferroni post hoc test (see Table 2). Data represented as standard error of the mean (SEM). Statistical significance was considered when *p < 0.05, **p < 0.01, ***p < 0.001. Scale bar represents 200 μm; inset scale represents 20 μm. DCX = doublecortin; ELS, early life stress; GFAP, glial fibrillary acidic protein; Iba1, Ionized calcium binding adaptor molecule 1.

Table 3
Summary of ELS and FKBP5 interaction results.

Measurements	Factors					
	Genotype	ELS	Sex	Age	Interactions	Key finding(s)
CORT levels	Yes	Yes	Yes	Yes	Gen x ELS	Young mice are susceptible to high levels of FKBP5
Depressive-like symptoms	Yes	No	No	Yes	None	ELS protects aged effect on rTgFKBP5 mice
Fear expression	Yes	No	Yes	Yes	None	Age modestly increases fear expression
Neural proliferation and differentiation	Yes (Ki-67)	No	–	Yes (Ki-67)	Gen x ELS (Ki-67)	Aged rTgFKBP5 mice showed increased proliferation.
HPC neurons	No	No	–	Yes	None	Age negatively impacts number of neurons in rTgFKBP5 mice
Neuroinflammation	Yes (GFAP)	No	–	Yes	Gen x ELS (GFAP, Iba1)	FKBP5 induces microglia expression in aged mice and ELS prevents this effect.

DCX: doublecortin, GFAP: Glial fibrillary acidic protein, Iba1: Ionized calcium binding adaptor molecule 1, ELS: early life stress, Gen: genotype, CORT: corticosterone, HPC: hippocampus.

It would be highly valuable to investigate whether the interplay of FKBP5-environment plays a role in the progression and development of AD neuropathology.

5. Conclusions

Our findings, as an extension of previous studies, demonstrate that FKBP5 contributes to the susceptibility for stress-related disorders, which is highly influenced by age and sex. Like in patients with depression, we found endocrine and inflammatory alterations in mice overexpressing FKBP5. Specifically, we found that high levels of FKBP5 reduce basal CORT levels, reflecting its role in HPA axis function. Additionally, FKBP5 had a robust effect on the levels of microglial markers in the hippocampus, suggesting that it may promote neuronal loss by altering glial cells in the brain. Interestingly, we observed that ELS inhibits the effects induced by FKBP5 in microglia. Recent evidence indicates that childhood adversity increases levels of FKBP5 and leukocyte activity and reduces cortisol levels in patients with anxious depression (Menke et al., 2018). Therefore, together with our previous study showing that ELS promotes higher anxiety in mice overexpressing FKBP5, these findings are highly relevant to understanding this subtype of depression due to similarity of observations. It is important to highlight that age was a common factor affecting depressive-like behavior and cellular changes in the hippocampus (summarized in Table 3). Certainly, further investigation is needed to better understand age-related effects on the complex etiology and heterogeneity of depression. Overall, our findings provide important evidence of changes at the neuroendocrine, behavioral as well cellular and molecular levels in the mouse hippocampus that may underlie mechanisms of how risk genes and stress promote susceptibility to mental health disorders during older age.

Author contributions

Conceptualization, original draft preparation, and writing, M.C.-M. and L.J.B.; Behavioral testing and analysis, M.C.-M. and T.M.S.; CORT testing and analysis, M.C.-M, D.G. and Z.S. Histopathological and immunohistochemistry analysis, M.C.-M., L.A.G., D.G. and S.K.; Stereological analyses, H.J.P.; Resources and funding acquisition, C.A.D. and L.J.B.; Supervision and project administration, L.J.B. All authors participated in reviewing the manuscript, except for Dr. Chad Dickey who is deceased.

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Declaration of competing interest

The authors declare the following competing financial interest(s): #C.A.D. and L.J.B. are co-inventors of the following patent application: "Transgenic mouse model for conditional fkbp51 expression and related methods." US Patent Application# US20150327523 A1. The other authors have no competing financial interest to disclose. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2020.100143.

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