Resilience to Early Life Adversity Effects on Stress Reactivity by Postnatal Knockdown of 5-HT_{1A} Autoreceptors

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ABSTRACT

Early Life Adversity (ELA) predisposes to stress hypersensitivity in adulthood, but neurobiological mechanisms that can protect from long-lasting effects of ELA are poorly understood. Serotonin 1A ($5HT_{1A}$) autoreceptors in the raphé nuclei regulate adult stress vulnerability, but if $5HT_{1A}$ could be targeted to prevent ELA effects on susceptibility to future stressors is unknown. Here, we exposed mice with postnatal knockdown of $5HT_{1A}$ autoreceptors to the limited bedding and nesting model of ELA from postnatal day (P)3-10. We then tested behavioral, neuroendocrine, neurogenic, and neuroinflammatory responses to an acute swim stress in male and female mice in adolescence (P35) and in adulthood (P56). In ELA-exposed females, adult swim stress exposure increased passive coping and despair-like behavior, corticosterone levels at baseline and after stress, and neuronal activity and corticotropin releasing hormone levels in the paraventricular nucleus of the hypothalamus. ELA also reduced neurogenesis and increased microglia activation in the ventral dentate gyrus (DG) of the hippocampus – an important mediator of individual differences in stress susceptibility. These effects of ELA were specific to females, but not males, and manifested predominantly in adulthood, but not earlier on in adolescence. Postnatal $5HT_{1A}$ autoreceptor knockdown prevented ELA effects on stress reactivity and on neurogenesis and neuroinflammation in the DG, indicating that reducing $5HT_{1A}$ autoreceptors confers resilience to ELA. Our findings demonstrate that ELA induces long-lasting and sex-specific impairments in stress reactivity and ventral DG function across development, and identify $5HT_{1A}$ autoreceptors as potential targets to prevent these persistent effects of ELA.

Keywords: Hypothalamus-Pituitary-Adrenal Axis, Monoamines, Glucocorticoids, Neural Circuits, Childhood Trauma

INTRODUCTION

Early life adversity (ELA) is a major risk factor for cognitive and emotional disturbances, and ~30% of mental illnesses in adulthood can be attributed at least in part to ELA.[1] Despite this high prevalence and the potent and long-lasting effects of ELA on mental health, we still lack a comprehensive understanding of the biological mechanisms that determine individual differences in vulnerability and resilience to adverse early experiences.[2]

Enhanced vulnerability for psychopathology following ELA has been associated with altered physiological and emotional reactivity to stressors that are experienced later in life.[3-5] Accordingly, dysregulated hypothalamus-pituitary-adrenal (HPA) axis responses have consistently been found in individuals with a history of ELA,[5,6] as shown by increased adrenocorticotropic hormone (ACTH) and cortisol responses to an acute psychosocial laboratory stressor in adult subjects with a history of childhood trauma.[7,8]

Rodent models recapitulate ELA effects on HPA axis dysregulation and have shown that maternal separation or early life limited bedding and nesting (LBN) conditions lead to higher corticosterone (CORT) levels at baseline and following an acute stressor in adulthood.[9-16] These ELA effects on HPA axis hyperactivity in rodents have been associated with enhanced neural activity in the paraventricular nucleus (PVN) of the hypothalamus.[17,18] PVN hyperactivity in turn leads to increased synthesis and release of corticotropin releasing hormone (CRH), subsequently triggering the release of ACTH from the anterior pituitary gland, which stimulates the production and release of CORT from the adrenals.[19]

We and others have previously shown that high CORT levels reduce adult hippocampal neurogenesis in the dentate gyrus (DG)[20-22] - a region of the hippocampus that regulates behavioral and neuroendocrine responses to stress in adulthood.[23,24] ELA causes several alterations in the structure and cellular composition of the rodent DG, including decreases in dendritic arborization, neurogenesis, granule cell numbers, and overall DG volume.[25-31] Abnormalities in hippocampal microstructure and volume are also found in human subjects at high familial risk for depression[32,33] and in children with a history of ELA.[31,34-36] Hippocampal impairments resulting from ELA, including reduced neurogenesis, may thus predispose to heightened vulnerability for developing psychiatric disorders later in life.

Chronically elevated CORT levels have also been associated with increased inflammation, and hypercortisolemia and inflammation are both frequently observed in subsets of patients with major depression.[37-40] Accordingly, ELA enhances peripheral blood inflammatory responses following an acute psychosocial stressor,[41,42] and individuals with low early life socioeconomic status show elevated peripheral inflammation and higher baseline cortisol levels.[43] Such increased inflammation has been suggested to mediate an association between ELA and neurobiological correlates of mental illness.[44,45] Inflammatory effects of ELA on the brain are supported by studies showing higher numbers of activated microglia in the mouse and human hippocampus,[46,47] as well as disrupted microglia function in the developing PVN ,which promotes HPA axis hyperactivity following an acute stressor in adulthood.[17] ELA-dependent alterations in the function of microglia may thus contribute to heightened stress reactivity in adulthood.

The extent to which ELA determines vulnerability to future stressors, HPA axis reactivity, and psychopathology can be influenced profoundly by genetic predisposition and biological sex.[48-53] The serotonin 1A receptor ($5HT_{1A}$) has been implicated in the regulation of neuroendocrine responses to stress in juvenile and adult animals,[548-12] and the C(-1019)G polymorphism in the promoter region of the human *5HT1AR* gene is associated with depression and antidepressant treatment resistance.[13,14] $5HT_{1A}$ autoreceptors in the raphé nuclei of the brainstem inhibit the activity of 5HT neurons and thereby reduce 5HT release at terminal projection sites in limbic and cortical projection areas, including the hippocampus.[20] 5HT is a potent modulator of hippocampal activity, DG neurogenesis, and neuroinflammation.[55-57] Accordingly, brain-wide deficiencies in 5HT increase vulnerability to stress in adult rodents,[58] while increasing 5HT confers resilience to adult stressors.[59] Whether $5HT_{1A}$ autoreceptors also mediate differences in vulnerability to ELA on future stress reactivity at the behavioral, endocrine, and neurobiological level is not known.

Previous work has comprehensively characterized behavioral impairments following ELA in adolescence and adulthood.[51,52,60] However, neurobiological and molecular mechanisms underlying vulnerability and resilience to ELA effects at different developmental stages remain largely unexplored. In this study, we tested if $5HT_{1A}$ autoreceptors determine individual differences in ELA effects on stress reactivity, and how these effects develop throughout adolescence and early adulthood in male and female mice. We find that ELA-exposed females show increased despair-like behavior, elevated serum CORT levels, PVN activity and CRH levels, reduced adult hippocampal neurogenesis, and increased neuroinflammation in the ventral DG in response to acute stress later in life. These impairments are specific to female mice and develop primarily in

adulthood. Postnatal knockdown of $5HT_{1A}$ autoreceptors rescued these effects of ELA, indicating that $5HT_{1A}$ determines individual differences in stress reactivity resulting from ELA. Our findings shed new light on the biological mechanisms underlying vulnerability and resilience to ELA and provide novel leads into potential future targets for intervention.

MATERIALS AND METHODS

Experimental mice

Procedures were conducted in accordance with the US National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, the New York State Psychiatric Institute (NYSPI) Institutional Animal Care and Use Committee (IACUC) at Columbia University, and the Research Foundation for Mental Hygiene (RFMH). Mice were housed 3–5 per cage with *ad libitum* access to food and water on a 12:12h light/dark cycle. Behavioral testing was conducted during the light period between 10 am and 2 pm. To induce postnatal knockdown of $5HT_{1A}$ autoreceptors in raphé 5-HT neurons, we used Pet1-tTS;tetO-1A mice containing the tetracycline operator (tetO) in the promoter region of the *5htr1a* gene and the tetracycline-dependent transcriptional suppressor (tTS) under control of the 540Z Pet-1 promoter fragment (Pet1-tTS), which is specific to raphé serotonin neurons.[59] In the presence of doxycycline (DOX), raphé $5HT_{1A}$ levels are indistinguishable between mice with the *Pet1-tTS* transgene (Pet1-tTS$^+$) and mice without the *Pet1-tTS* transgene (Pet1-tTS$^-$). Postnatal removal of DOX causes suppression of *5htr1a* expression in transgenic Pet1-tTS$^+$ mice (from here on referred to as 5-HT1A K.D.) but not in Pet1-tTS$^-$ mice (from here on referred to as WT).[59] Pet1-tTS$^-$ females were bred with Pet1-tTS$^+$ males and maintained on DOX diet. DOX was removed three days before parturition. Female and male Pet1-tTS$^-$ (WT) and Pet1-tTS$^+$ pups (5-HT1A K.D.) were raised on regular chow.

Limited Bedding and Nesting (LBN)

Half of all litters were exposed to the previously described limited bedding and nesting (LBN) model, which causes fragmented and unpredictable maternal care as a form of ELA.[15] Briefly, dams and their newborn litters were placed on a wire mesh grid and provided with 1/3 of nesting material and 1/4 of bedding material from P3-10.[15,61] Mice were returned to standard-rearing conditions on P10. Standard-reared control cages were changed at P3 and P10 but otherwise left undisturbed. All litters were weaned at P21.

Behavioral Testing

Please see *Supplemental Methods* for a complete description of behavioral procedures.

Corticosterone (CORT) EIA assay

Peripheral blood was obtained by submandibular blood draw at baseline 3 days before the Forced Swim Test (FST), and again 30 min post-FST. Blood was centrifuged at 3000rpm for 15 min and serum extracted and stored at -80°C. CORT was quantified using EIA assays (ArborAssays, Ann Arbor, MI).[62]

Immunohistochemistry

For immunohistochemistry, brains were collected 1h following FST to assess cFos^{+} cells in the PVN, and Dcx, CD68, and Iba1 in the DG. Mice were anesthetized with ketamine/xylazine and transcardially perfused with saline and ice-cold 4% paraformaldehyde (PFA). Brains were post-fixed overnight in 4% PFA at 4 °C, cryoprotected in 30% sucrose for 48 hours, frozen in optimum cutting temperature (OCT) compound, and sectioned at 50 µm along the dorsal-ventral axis of the hippocampus.[62] See also *Supplemental Information*.

CRH quantification by Western Blot

PVN tissue biopsies were collected from fresh-frozen brain tissue and protein extracted using Neuronal Protein Extraction Reagent (N-PER™, Thermo Scientific) for determination of CRH protein levels by Western Blot. Please see *Supplemental Information* for complete description of protein extraction and Western Blot analyses.

Statistics

Data was confirmed to be normally distributed using the Shapiro–Wilk test. All statistical analyses were conducted using GraphPad Prism 9. Behavior and CORT data were analyzed using Two-Way repeated measure (RM) Analysis of Variance (ANOVA) comparing ELA effects across time, and Three-Way RM ANOVA to assess ELA x genotype x time interactions and ELA x sex x time interactions. Three-way ANOVA was used to compare body weights. Immunohistochemistry data was analyzed using Two-Way ANOVA to compare rearing ELA x genotype interactions, and Three-Way ANOVA to assess ELA x genotype x sex interactions. *Post hoc* tests (Fisher's LSD for Two-Way RM ANOVA; Tukey for all other tests) were used when significant interactions were detected. Detailed statistics for all experiments are reported in **Supp. Table 1**.

RESULTS

Behavioral responses to stress in adulthood are elevated by ELA and rescued by $5HT_{1A}$ autoreceptor knockdown

We first sought to determine how ELA affects behavioral responses to stress later in life and how these responses are modulated by $5HT_{1A}$ autoreceptors. We, therefore, used a transgenic mouse model in which we can suppress $5HT_{1A}$ autoreceptors on raphé serotonin neurons throughout postnatal development (Pet1-tTS;tetO-1A mice).[59,63] We exposed dams and their litters to the limited bedding and nesting (LBN) model of ELA from P3 to P10. Pups carrying the tTS transgene exhibit $5HT_{1A}$ knockdown (Pet1-tTS^{+}, from here on referred to as "$5HT_{1A}$ K.D." mice) of ~40% (as shown in[64]) while littermates not carrying the tTS transgene express wild-type $5HT_{1A}$ levels (Pet1-tTS^{-}, referred to as "wild-type, WT"; **Fig. 1a**). LBN exposed WT and $5HT_{1A}$ K.D. offspring showed a reduction in body weight until P28, which recovered by P35 (**Supp. Fig. 1**). We then tested behavioral responses of WT and $5HT_{1A}$ K.D. male and female littermates to an acute 6 min forced swim test (FST) in an adolescent cohort (at P35) and in an adult cohort (at P56) (**Fig. 1b**).

At the adolescent time point (P35), neither ELA nor $5HT_{1A}$ K.D. affected immobility in the FST in female or male mice compared to standard-reared controls (**Fig. 1c-f**). At the adult time point (P56), WT females that had been exposed to ELA from P3-10 showed significantly higher levels of immobility in the FST than standard-reared control females, which is usually interpreted as an expression of increased passive coping or despair-like behavior in mice (**Fig. 1g**). In mice with postnatal knockdown of $5HT_{1A}$ no difference in immobility was observed between standard-reared controls and ELA-exposed females (**Fig. 1h**). Moreover, ELA-exposed females with $5HT_{1A}$ K.D. showed less immobility compared to WT ELA-exposed females (**Fig. 1g,h, orange lines;** *see Supp Table 1 for detailed statistical comparisons*). No effect of ELA or $5HT_{1A}$ K.D. was observed in adult males (**Fig. 1i-j**).

Together, these data indicate that ELA in the form of LBN increases behavioral responses to an acute stressor in adult females, and that this heightened behavioral sensitivity to stress can be prevented by lowering $5HT_{1A}$ autoreceptor levels.

Baseline and stress-induced CORT responses are elevated by ELA and rescued by $5HT_{1A}$ autoreceptor knockdown

Because we saw that ELA exaggerates behavioral responses to acute stress in adulthood, we next asked whether this heightened stress vulnerability may be associated with increased neuroendocrine responses. To this end, we collected submandibular blood samples 3 days before exposure to acute swim stress ("baseline") and 30 min after swim stress exposure ("post-FST" sample; **Fig. 2a**). We then measured serum CORT levels in female and male mice in the adolescent cohort (at P35) and in the adult cohort (at P56).

At P35, ELA-exposed WT females show elevated serum CORT levels compared to standard-reared control females both at baseline and following acute swim stress (**Fig. 2b**). $5HT_{1A}$ K.D. prevented this elevation in baseline and post-stress CORT in ELA-exposed females (**Fig. 2b**). At P56, ELA-exposed WT females showed more pronounced elevations in baseline CORT compared to the adolescent time point. Moreover, CORT responses to the acute swim stress were exaggerated in ELA-exposed WT females compared to standard-reared controls and compared to ELA-exposed adolescent females (**Fig. 2c**). These effects of ELA on baseline and post-stress CORT in females were prevented by $5HT_{1A}$ knockdown (**Fig. 2c**). Similar to the absence of heightened behavioral sensitivity to acute stress in ELA-exposed male mice, neither ELA nor $5HT_{1A}$ K.D. altered baseline or post-FST CORT levels in males at P35 (**Fig. 2d**) or at P56 (**Fig. 2e**).

Collectively, these data show that ELA causes long-lasting elevations in female CORT levels that can already been seen in adolescence before heightened behavioral sensitivity to stress manifests. Baseline and stress-induced CORT responses further rise into adulthood, and this effect of ELA is prevented in mice with lower levels of $5HT_{1A}$ autoreceptors.

PVN activity and CRH release are elevated by ELA and rescued by 5HT1A autoreceptor knockdown

A key step in initiating the HPA axis and CORT release is the activation of neurons in the PVN of the hypothalamus. Because we have seen that ELA increases CORT levels that are rescued by $5HT_{1A}$ knockdown, we next wanted to determine if ELA causes long-lasting elevations in PVN neuron activity and CRH release that are ameliorated by $5HT_{1A}$ knockdown. We, therefore, first

used immunohistochemistry for the immediate early gene, cFos, as a proxy marker of neural activity in the PVN of ELA-exposed mice after adult stress exposure at P56 (**Fig. 3a-b**). ELA-exposed WT females show higher levels of cFos$^+$ immunoreactive cells in the PVN compared to standard-reared WT controls, indicating higher levels of neural activity. This effect of ELA on PVN hyperactivity was rescued in $5HT_{1A}$ K.D. females (**Fig. 3c**), consistent with reduced baseline and stress-induced CORT levels in these mice (as shown in **Fig. 2**). We did not observe effects ELA or $5HT_{1A}$ knockdown on PVN activity in males (**Fig. 3d**), who also do not show higher CORT levels or behavioral sensitivity to stress following ELA.

To test if the higher PVN activity in ELA-exposed females is associated with higher CRH levels, we quantified CRH using Western Blot. We find that ELA-exposed WT females have higher CRH levels in the PVN compared to standard-reared WT controls, and that these elevated CRH levels are rescued in mice with $5HT_{1A}$ knockdown (**Fig. 3e**). No differences in CRH were detected between ELA- and standard-reared male mice with or without $5HT_{1A}$ knockdown (**Fig. 3f**).

These data support the notion that ELA increases HPA axis activity at the level of the PVN in female mice, and that this effect can be prevented by $5HT_{1A}$ autoreceptor knockdown.

Adult hippocampal neurogenesis is reduced following ELA in adulthood and rescued by $5HT_{1A}$ autoreceptor knockdown

We and others have previously shown that high levels of CORT reduce adult hippocampal neurogenesis in rodents[22] and in human cells,[20,21,65] and that a reduction in neurogenesis in turn increases stress susceptibility[23] and HPA axis hyperactivity.[24] Because we found that ELA increases stress reactivity and HPA axis hyperactivity in females (**Figs. 1-3**), we asked if ELA may also reduce hippocampal neurogenesis as a potential mechanism predisposing to heightened stress vulnerability later in life, and if this effect could be prevented by $5HT_{1A}$ knockdown. We found no effect of ELA on the number of doublecortin (Dcx)-positive adult-born neurons in either dorsal or ventral DG of females or males at the adolescent testing time point at P35 (**Fig. 4b,c; Supp. Fig 2b,c**). However, at the adult timepoint (P56), we find that ELA reduces the number of Dcx$^+$ adult-born neurons predominantly in the ventral DG of WT females (**Fig. 4d; first two bars**), but not in the female dorsal DG (**Supp. Fig 2d**), or in the dorsal and ventral DG of male mice (**Fig 4e; Supp. Fig 2e**). Postnatal knockdown of $5HT_{1A}$ autoreceptors counteracted this reduction in Dcx$^+$ adult-born neurons in ELA-exposed females (**Fig 4d; last two bars**).

Together, these data indicate that ELA reduces adult hippocampal neurogenesis in female mice and that this reduction in neurogenesis can be prevented by postnatal knockdown of $5HT_{1A}$ autoreceptors.

Microglia activation is increased following ELA in adulthood and rescued by $5HT_{1A}$ autoreceptor knockdown

Previous studies have shown that ELA increases inflammatory processes in the rodent hippocampus.[46,47] Inflammation also decreases hippocampal neurogenesis and increases behavioral and neuroendocrine responses to stress – effects that we see specifically in female mice exposed to ELA. To test if ELA and $5HT_{1A}$ autoreceptor knockdown cause sex-specific effects on neuroinflammation following an acute stressor in adulthood, we used immunohistochemistry for the microglia-specific calcium binding protein, Iba1, and for the transmembrane glycoprotein, CD68, which increases in phagocytic microglia following their activation in response to neuroinflammatory processes (**Fig. 5a; Supp. Fig 3a**). We then quantified the number of $CD68^+/Iba1^+$ cells to determine microglia activation in the dorsal and ventral dentate gyrus 1h after exposure to acute swim stress.

In adolescent mice at P35, we find no significant effect of ELA on the number of activated $CD68^+/Iba1^+$ microglia in either the ventral DG (**Fig. 5b,c**) or dorsal DG (**Supp. Fig. 3b,c**) of WT males and females. However, $5HT_{1A}$ autoreceptor knockdown reduced overall microglia activation in adolescent females (**Fig. 5b**). In adulthood at P56, microglia activation was increased in the ventral DG of ELA-exposed WT females, and this effect was rescued by $5HT_{1A}$ autoreceptor knockdown (**Fig. 5d**). No effects of ELA or of $5HT_{1A}$ knockdown were observed in the ventral DG of males (**Fig. 5e**) or in the dorsal DG of either males or females at P56 (**Supp. Fig. 3d,e**).

These data show that ELA causes sex-dimorphic effects on neuroinflammation that are specific to the ventral DG, manifest predominantly in adult female mice, and can be prevented by $5HT_{1A}$ autoreceptor knockdown.

DISCUSSION

Identifying neurobiological mechanisms that can prevent ELA effects on future stress reactivity may help develop novel strategies to treat or prevent psychiatric disorders that have their origin early in life. Here, we show that ELA in the form of LBN in mice has profound and persistent sex effects on behavioral, endocrine, and neurobiological responses to stress. We find that these stress vulnerability-promoting effects of ELA are specific to females, develop primarily in adulthood, and can be prevented by postnatal knockdown of $5HT_{1A}$ autoreceptors. Our findings shed new light on the biological mechanisms that promote resilience to ELA effects on stress response regulation across development, and highlight the $5HT_{1A}$ receptor as a potential target for intervention.

To determine how ELA affects stress reactivity later in life, we exposed dams and their litters to LBN conditions from P3-10. We then exposed adolescent and adult offspring raised under LBN or standard rearing conditions to an acute swim stress and assessed behavioral and neuroendocrine responses in females and males. In female offspring, we find that LBN increases the time mice spent immobile during the FST, possibly indicating a more passive approach to coping with the inescapable swim stress or greater levels of despair-like behavior. These differences in the behavioral response to stress between LBN- and standard-reared females emerge in young adulthood (at P56), but not at the earlier testing time point in adolescence (at P35) (**Fig. 1**). Accordingly, we find heightened neuroendocrine responses to stress in ELA-exposed females, as evidenced by higher levels of baseline CORT, higher CORT responses to the acute stressor, higher activity of PVN neurons, and higher levels of CRH in the PVN of ELA-exposed females compared to standard-reared controls (**Figs. 2,3**). While these neuroendocrine effects of ELA are most pronounced in adulthood, we also find small but significant elevations in CORT levels in adolescent females, indicating that ELA-induced neuroendocrine impairments may precede the development of behavioral abnormalities. These behavioral and neuroendocrine effects of ELA were prevented in mice with postnatal knockdown of the $5HT_{1A}$ autoreceptor, suggesting that lowering $5HT_{1A}$ autoreceptor levels or activity can protect from ELA effects on future stress vulnerability. Our findings of enhanced stress reactivity following ELA are consistent with previous reports of elevated CORT responses to adult stress in the same LBN model that we used in our study.[6] It is important to note, however, that some studies have also reported blunted behavioral and neuroendocrine responses to adult stress in ELA-exposed rodents.[66] These blunted stress responses have been explained by the match/mismatch hypothesis, which

postulates that adverse early life experiences may “prepare” an individual for life in an overall highly adverse environment and initiate neurobiological changes that are adaptive for survival during stressful situations later in life.[4] These differences in ELA effects on *hyper*- vs *hypo*reactivity to future stressors may depend on the timing, intensity, and duration of early adversities.[3,6,61,67] Moreover, ELA effects on CORT responses have been reported to differ between different strains of mice,[50] indicating a potential role for genotypic differences in moderating ELA effects on stress reactivity.

We also investigated ELA effects on neurogenesis and neuroinflammation in the DG of the hippocampus - neurobiological processes that are sensitive to stress and glucocorticoid hormones, and that have been implicated in stress vulnerability.[5,20,21,23,65,68,69] We find that in adult females, ELA reduced hippocampal neurogenesis (**Fig. 4**) and increased microglia activation predominantly in the ventral DG as compared to the dorsal DG (**Fig. 5**). Similar to our behavioral and neuroendocrine findings, postnatal $5HT_{1A}$ knockdown prevented these effects of ELA on the DG, further supporting the notion that reducing $5HT_{1A}$ autoreceptor function confers resilience to ELA.

Interestingly, neither the exaggerated behavioral, neuroendocrine, and neuroinflammatory responses, nor the reduction in neurogenesis following ELA were observed in males. These pronounced sex differences are consistent with previous studies in the same ELA model, which have shown a predominant vulnerability of female offspring to LBN conditions in behavioral despair, anhedonia, hyponeophagia, and reversal learning.[51,52] It is worth noting, however, that LBN-reared male offspring in our study show similar, if not more pronounced reductions in body weight as females before P35, suggesting that LBN conditions do indeed have some effect also on males (**Supp. Fig. 1b**). Accordingly, previous studies have shown that LBN does cause some behavioral and biological impairments also in male mice, such as altered spatial recognition abilities,[60] feeding behavior,[70] and mitochondria function.[71] It is thus possible that males are not simply more resilient to the effects of ELA, but that ELA may affect different neurobiological mechanisms and their associated behavioral outcomes in male vs female mice. We also cannot exclude the possibility that male mice develop changes in stress reactivity at earlier or later time points than those examined in our study (i.e., before P35 or later than P56). Investigating ELA effects on aged mice could address this possibility in future studies.

We find that knockdown of raphé $5HT_{1A}$ autoreceptors has potent effects on preventing the development of heightened stress vulnerability and DG abnormalities following ELA. Under physiological conditions, $5HT_{1A}$ autoreceptors regulate 5HT release from raphé 5HT neurons at their terminal projection sites, including in the hippocampus. Knockdown of $5HT_{1A}$ releases this inhibition onto 5HT neurons and increases their spontaneous firing and hippocampal 5HT levels.[59] It is thus likely that disinhibition of 5HT neurons and the resulting elevation in hippocampal 5HT levels are responsible for the pro-resilience effects of the $5HT_{1A}$ knockdown in our study. Indirect evidence for an interaction between 5HT and susceptibility to ELA has been provided by reports of reduced 5HT metabolites in humans and monkeys,[72-74] and by studies showing that carriers of the short 5HT transporter allele, which is associated with low 5HT levels,[75] are more vulnerable to ELA effects on later psychopathology.[3,76] However, direct effects of ELA on the 5HT system have only been investigated by a few studies which have shown reduced 5HT content in raphé neurons of ELA exposed mice.[77,47] Whether ELA causes long-lasting impairments in the firing of 5HT neurons that are rescued by $5HT_{1A}$ autoreceptor knockdown will be a crucial question to address by future studies aimed at further investigating the biological mechanisms underlying the pervasive effects of ELA on brain function and stress vulnerability.

The $5HT_{1A}$ receptor has been implicated in the pathogenesis of anxiety disorders, major depression, suicidal ideation, and in the mechanism of action of antidepressants.[63,64,78-90] Indeed, new compounds targeting $5HT_{1A}$ have emerged over recent years that are currently being used or tested for their antidepressant- or anxiolytic effects.[91-94] Our data support the notion that reducing the function of presynaptic $5HT_{1A}$ autoreceptors in the raphé nuclei prevents ELA effects on future stress sensitivity. Our recent work has also shown that activating *postsynaptic* $5HT_{1A}$ *hetero*receptors in the DG promotes resilience to chronic stress,[54] in addition to their previously reported role in mediating antidepressant effects on behavior.[90] Recent advances in the development of biased $5HT_{1A}$ ant/agonists with functional selectivity at either $5HT_{1A}$ autoreceptors or $5HT_{1A}$ heteroreceptors may thus enable harnessing the differential potential of these receptors in specific cell types and brain regions in the future.[95] Identifying novel drugs with pharmacological profiles that can target specific subsets of $5HT_{1A}$ receptors or their downstream signaling pathways will be particularly important for psychiatric patients with a history of ELA who respond poorly to currently available antidepressants.[96]

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AUTHOR CONTRIBUTIONS

RSD, LM, RT, SW, NS, and CA, contributed to data acquisition, analysis, and interpretation. CA designed the work. CA and LM drafted the work. All authors contributed to revising the work.

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FIGURE LEGENDS

Figure 1: 5-HT1A autoreceptor knockdown rescues ELA effects on the behavioral response to adult stress in females. a. Schematic of the genetic knockdown of 5-HT1A autoreceptors in Pet1-tTS^{-} mice ("WT") vs Pet1-Tts^{+} ("5-HT1A K.D."). **b.** Experimental timeline of ELA (P3-10) and acute swim stress exposure (at P35 or P56). **c.-f.** Adolescent data (P35). No difference in immobility time between adolescent WT control (blue) vs. ELA-exposed females (orange), or **d.** adolescent 5-HT1A K.D. control vs. ELA-exposed females. **e.** No difference in immobility time between adolescent WT control vs. ELA-exposed males, or between **f.** adolescent 5-HT1A K.D. control vs. ELA-exposed males. **g.-j.** Adult data (P56). **g.** In WT mice, adult ELA-exposed females show increased immobility compared to standard-reared control females. **h.** In 5-HT1A K.D. mice, no difference in immobility was observed between adult control and ELA-exposed females. **i.** No difference in immobility time between adult WT control vs. ELA-exposed males, or between **j.** adult 5-HT1A K.D. control vs. ELA-exposed males. FST – Forced Swim Test. Mean±SEM; n=10-15 mice. *P<0.013 indicates main effect of Two-Way RM ANOVA.

Figure 2: 5-HT1A autoreceptor knockdown rescues elevated baseline and stress-induced CORT responses in ELA-exposed females. a. Experimental timeline of blood collection at baseline three-days prior to FST and 30 minutes post-FST. **b. Left panel:** ELA increases baseline and post-FST CORT in WT adolescent females (orange) vs. controls (blue). **Right panel:** ELA does not affect CORT levels in adolescent 5-HT1A K.D. females vs. controls. **c. Left panel:** ELA increases baseline CORT levels and exaggerates post-FST CORT responses in WT adult females vs. controls. **Right panel:** ELA does not affect CORT levels in adult 5-HT1A K.D. females vs. controls. **d.** ELA or 5-HT1A K.D. does not affect baseline or post-FST CORT responses in adolescent males, or in **e.** adult males. CORT – Corticosterone. FST – Forced Swim Test. Mean±SEM; n=6-11; *P<0.05; **P<0.01 indicate main effects of Two-Way ANOVA and *post hoc* tests for significant interactions.

Figure 3: 5-HT1A autoreceptor knockdown rescues PVN hyperactivity and CRH release in ELA-exposed females. a. Experimental timeline. **b.** Schematic and representative image of cFos+ cells in the PVN. **c.** ELA increases the number of cFos+ cells in the PVN of WT females compared to standard-reared control females. This effect was prevented in ELA-exposed 5-HT1A K.D. females. **d.** ELA or 5-HT1A K.D. did not affect cFos+ cells in the male PVN. **e.** ELA increased CRH in the PVN of WT females compared to controls. This effect was prevented in ELA-exposed 5-HT1A K.D. females. **f.** ELA or 5-HT1A K.D. did not affect CRH protein levels in the male PVN. Mean±SEM; n=4-7; *P<0.05 indicates *post hoc* tests for significant interactions.

Figure 4: 5-HT1A autoreceptor knockdown rescues ELA effects on adult hippocampal neurogenesis in the female vDG. a. Representative images of DCX^+ cells in the female vDG. **b.** In adolescence at P35, ELA or 5-HT1A K.D. did not affect the number of DCX^+ cells in the vDG of females, or **c.** males. **d.** In adulthood at P56, ELA decreased the number of DCX^+ cells in the vDG of WT females compared to controls. This ELA effect was rescued in females with 5-HT1A K.D. **e.** ELA or 5-HT1A K.D. did not affect the number of DCX^+ cells in the vDG of adult males. DCX – Doublecortin. Mean±SEM; n=4-6; *P<0.05; **P<0.001 indicate *post hoc* tests for significant interactions.

Figure 5: 5-HT1A autoreceptor knockdown rescues ELA effects on microglia activation in the female vDG. a. Representative images of $Iba1^+$ microglia (green) and $CD68^+$ active microglia (red) in the female vDG. Arrowheads indicate $CD68^+/Iba1^+$ active microglia. **b.** 5-HT1A K.D. decreased the % active microglia in adolescent females regardless of ELA exposure. **c.** ELA or 5-HT1A K.D. did not affect the % of active microglia in adolescent males. **d.** ELA increased the % active microglia in adult WT females compared to controls. This effect of ELA was prevented in 5-HT1A K.D. females. **e.** ELA or 5-HT1A K.D. did not affect the % active microglia in adult males. Mean±SEM; n=4-7; *P<0.05; **P<0.001 indicate main effects of Two-Way ANOVAs and *post hoc* tests for significant interactions.

REFERENCES

1. Kessler RC, McLaughlin KA, Green JG, et al. Childhood adversities and adult psychopathology in the WHO World Mental Health Surveys. *Br J Psychiatry*. Nov 2010;197(5):378-85. doi:10.1192/bjp.bp.110.080499
2. Nutt DJ. Neurobiological mechanisms in generalized anxiety disorder. *J Clin Psychiatry*. 2001;62 Suppl 11:22-7; discussion 28.
3. Malave L, van Dijk MT, Anacker C. Early life adversity shapes neural circuit function during sensitive postnatal developmental periods. *Transl Psychiatry*. Aug 01 2022;12(1):306. doi:10.1038/s41398-022-02092-9
4. Daskalakis NP, Bagot RC, Parker KJ, Vinkers CH, de Kloet ER. The three-hit concept of vulnerability and resilience: toward understanding adaptation to early-life adversity outcome. *Psychoneuroendocrinology*. Sep 2013;38(9):1858-73. doi:10.1016/j.psyneuen.2013.06.008
5. Anacker C, O'Donnell KJ, Meaney MJ. Early life adversity and the epigenetic programming of hypothalamic-pituitary-adrenal function. *Dialogues Clin Neurosci*. Sep 2014;16(3):321-33.
6. van Bodegom M, Homberg JR, Henckens MJAG. Modulation of the Hypothalamic-Pituitary-Adrenal Axis by Early Life Stress Exposure. *Front Cell Neurosci*. 2017;11:87. doi:10.3389/fncel.2017.00087
7. Heim C, Newport DJ, Wagner D, Wilcox MM, Miller AH, Nemeroff CB. The role of early adverse experience and adulthood stress in the prediction of neuroendocrine stress reactivity in women: a multiple regression analysis. *Depress Anxiety*. 2002;15(3):117-25. doi:10.1002/da.10015
8. Heim C, Newport DJ, Heit S, et al. Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. *JAMA*. Aug 02 2000;284(5):592-7. doi:10.1001/jama.284.5.592
9. Lajud N, Roque A, Cajero M, Gutiérrez-Ospina G, Torner L. Periodic maternal separation decreases hippocampal neurogenesis without affecting basal corticosterone during the stress hyporesponsive period, but alters HPA axis and coping behavior in adulthood. *Psychoneuroendocrinology*. Mar 2012;37(3):410-20. doi:10.1016/j.psyneuen.2011.07.011
10. Murgatroyd C, Patchev AV, Wu Y, et al. Dynamic DNA methylation programs persistent adverse effects of early-life stress. *Nat Neurosci*. Dec 2009;12(12):1559-66. doi:10.1038/nn.2436
11. Wu Y, Patchev AV, Daniel G, Almeida OF, Spengler D. Early-life stress reduces DNA methylation of the Pomc gene in male mice. *Endocrinology*. May 2014;155(5):1751-62. doi:10.1210/en.2013-1868
12. Chen J, Evans AN, Liu Y, Honda M, Saavedra JM, Aguilera G. Maternal deprivation in rats is associated with corticotrophin-releasing hormone (CRH) promoter hypomethylation and enhances CRH transcriptional responses to stress in adulthood. *J Neuroendocrinol*. Jul 2012;24(7):1055-64. doi:10.1111/j.1365-2826.2012.02306.x
13. Uchida S, Hara K, Kobayashi A, et al. Early life stress enhances behavioral vulnerability to stress through the activation of REST4-mediated gene transcription in the medial prefrontal cortex of rodents. *J Neurosci*. Nov 10 2010;30(45):15007-18. doi:10.1523/JNEUROSCI.1436-10.2010

14. Daskalakis NP, Diamantopoulou A, Claessens SEF, et al. Early experience of a novel-environment in isolation primes a fearful phenotype characterized by persistent amygdala activation. *Psychoneuroendocrinology*. Jan 2014;39:39-57. doi:10.1016/j.psyneuen.2013.09.021
15. Rice CJ, Sandman CA, Lenjavi MR, Baram TZ. A novel mouse model for acute and long-lasting consequences of early life stress. *Endocrinology*. Oct 2008;149(10):4892-900. doi:10.1210/en.2008-0633
16. Arp JM, Ter Horst JP, Loi M, et al. Blocking glucocorticoid receptors at adolescent age prevents enhanced freezing between repeated cue-exposures after conditioned fear in adult mice raised under chronic early life stress. *Neurobiol Learn Mem*. Sep 2016;133:30-38. doi:10.1016/j.nlm.2016.05.009
17. Bolton JL, Short AK, Othy S, et al. Early stress-induced impaired microglial pruning of excitatory synapses on immature CRH-expressing neurons provokes aberrant adult stress responses. *Cell Rep*. Mar 29 2022;38(13):110600. doi:10.1016/j.celrep.2022.110600
18. Gunn BG, Cunningham L, Cooper MA, et al. Dysfunctional astrocytic and synaptic regulation of hypothalamic glutamatergic transmission in a mouse model of early-life adversity: relevance to neurosteroids and programming of the stress response. *J Neurosci*. Dec 11 2013;33(50):19534-54. doi:10.1523/JNEUROSCI.1337-13.2013
19. Anacker C, Zunszain PA, Carvalho LA, Pariante CM. The glucocorticoid receptor: pivot of depression and of antidepressant treatment? *Psychoneuroendocrinology*. Apr 2011;36(3):415-25. doi:10.1016/j.psyneuen.2010.03.007
20. Anacker C, Cattaneo A, Luoni A, et al. Glucocorticoid-related molecular signaling pathways regulating hippocampal neurogenesis. *Neuropsychopharmacology*. Apr 2013;38(5):872-83. doi:10.1038/npp.2012.253
21. Anacker C, Cattaneo A, Musaelyan K, et al. Role for the kinase SGK1 in stress, depression, and glucocorticoid effects on hippocampal neurogenesis. *Proc Natl Acad Sci U S A*. May 2013;110(21):8708-13. doi:10.1073/pnas.1300886110
22. David DJ, Samuels BA, Rainer Q, et al. Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. *Neuron*. May 28 2009;62(4):479-93. doi:S0896-6273(09)00298-0 [pii]
10.1016/j.neuron.2009.04.017
23. Anacker C, Luna VM, Stevens GS, et al. Hippocampal neurogenesis confers stress resilience by inhibiting the ventral dentate gyrus. *Nature*. 07 2018;559(7712):98-102. doi:10.1038/s41586-018-0262-4
24. Snyder JS, Soumier A, Brewer M, Pickel J, Cameron HA. Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. *Nature*. Aug 3 2011;doi:nature10287 [pii]
10.1038/nature10287
25. Ivy AS, Rex CS, Chen Y, et al. Hippocampal dysfunction and cognitive impairments provoked by chronic early-life stress involve excessive activation of CRH receptors. *J Neurosci*. Sep 29 2010;30(39):13005-15. doi:10.1523/JNEUROSCI.1784-10.2010
26. Bath KG, Manzano-Nieves G, Goodwill H. Early life stress accelerates behavioral and neural maturation of the hippocampus in male mice. *Horm Behav*. 06 2016;82:64-71. doi:10.1016/j.yhbeh.2016.04.010

27. Youssef M, Atsak P, Cardenas J, Kosmidis S, Leonardo ED, Dranovsky A. Early life stress delays hippocampal development and diminishes the adult stem cell pool in mice. *Sci Rep*. Mar 2019;9(1):4120. doi:10.1038/s41598-019-40868-0
28. Lucassen PJ, Oomen CA, Naninck EF, et al. Regulation of Adult Neurogenesis and Plasticity by (Early) Stress, Glucocorticoids, and Inflammation. *Cold Spring Harb Perspect Biol*. Sep 2015;7(9):a021303. doi:10.1101/cshperspect.a021303
29. Naninck EF, Hoeijmakers L, Kakava-Georgiadou N, et al. Chronic early life stress alters developmental and adult neurogenesis and impairs cognitive function in mice. *Hippocampus*. Mar 2015;25(3):309-28. doi:10.1002/hipo.22374
30. Boldrini M, Galfalvy H, Dwork AJ, et al. Resilience Is Associated With Larger Dentate Gyrus, While Suicide Decedents With Major Depressive Disorder Have Fewer Granule Neurons. *Biol Psychiatry*. May 15 2019;85(10):850-862. doi:10.1016/j.biopsych.2018.12.022
31. van Dijk MT, Talati A, Kashyap P, et al. Dentate Gyrus Microstructure is associated with Resilience after Exposure to Maternal Stress Across Two Human Cohorts. *Biol Psychiatry*. Jun 29 2023;doi:10.1016/j.biopsych.2023.06.026
32. van Dijk MT, Cha J, Semanek D, et al. Altered Dentate Gyrus Microstructure in Individuals at High Familial Risk for Depression Predicts Future Symptoms. *Biol Psychiatry Cogn Neurosci Neuroimaging*. Jan 2021;6(1):50-58. doi:10.1016/j.bpsc.2020.06.006
33. Chen MC, Hamilton JP, Gotlib IH. Decreased hippocampal volume in healthy girls at risk of depression. *Arch Gen Psychiatry*. Mar 67(3):270-6. doi:67/3/270 [pii] 10.1001/archgenpsychiatry.2009.202
34. Teicher MH, Anderson CM, Polcari A. Childhood maltreatment is associated with reduced volume in the hippocampal subfields CA3, dentate gyrus, and subiculum. *Proc Natl Acad Sci U S A*. Feb 28 2012;109(9):E563-72. doi:10.1073/pnas.1115396109
35. Hanson JL, Nacewicz BM, Sutterer MJ, et al. Behavioral problems after early life stress: contributions of the hippocampus and amygdala. *Biol Psychiatry*. Feb 15 2015;77(4):314-23. doi:10.1016/j.biopsych.2014.04.020
36. Luby J, Belden A, Botteron K, et al. The effects of poverty on childhood brain development: the mediating effect of caregiving and stressful life events. *JAMA Pediatr*. Dec 2013;167(12):1135-42. doi:10.1001/jamapediatrics.2013.3139
37. Anacker C, Zunszain PA, Carvalho LA, Pariante CM. The glucocorticoid receptor: pivot of depression and of antidepressant treatment? *Psychoneuroendocrinology*. Apr 2011a;36(3):415-25. doi:S0306-4530(10)00085-5 [pii] 10.1016/j.psyneuen.2010.03.007
38. Osimo EF, Pillinger T, Rodriguez IM, Khandaker GM, Pariante CM, Howes OD. Inflammatory markers in depression: A meta-analysis of mean differences and variability in 5,166 patients and 5,083 controls. *Brain Behav Immun*. Jul 2020;87:901-909. doi:10.1016/j.bbi.2020.02.010
39. Juruena MF, Pariante CM, Papadopoulos AS, Poon L, Lightman S, Cleare AJ. Prednisolone suppression test in depression: prospective study of the role of HPA axis dysfunction in treatment resistance. *Br J Psychiatry*. Apr 2009;194(4):342-9. doi:194/4/342 [pii] 10.1192/bjp.bp.108.050278

40. Pariante CM. Why are depressed patients inflamed? A reflection on 20 years of research on depression, glucocorticoid resistance and inflammation. *Eur Neuropsychopharmacol*. Jun 2017;27(6):554-559. doi:10.1016/j.euroneuro.2017.04.001
41. Pace TW, Mletzko TC, Alagbe O, et al. Increased stress-induced inflammatory responses in male patients with major depression and increased early life stress. *Am J Psychiatry*. Sep 2006;163(9):1630-3. doi:10.1176/ajp.2006.163.9.1630
42. Danese A, Moffitt TE, Pariante CM, Ambler A, Poulton R, Caspi A. Elevated inflammation levels in depressed adults with a history of childhood maltreatment. *Arch Gen Psychiatry*. Apr 2008;65(4):409-15. doi:10.1001/archpsyc.65.4.409
43. Miller GE, Chen E, Fok AK, et al. Low early-life social class leaves a biological residue manifested by decreased glucocorticoid and increased proinflammatory signaling. *Proc Natl Acad Sci U S A*. Aug 25 2009;106(34):14716-21. doi:10.1073/pnas.0902971106
44. Yuan JP, Coury SM, Ho TC, Gotlib IH. Early life stress moderates the relation between systemic inflammation and neural activation to reward in adolescents both cross-sectionally and longitudinally. *Neuropsychopharmacology*. Sep 06 2023;doi:10.1038/s41386-023-01708-y
45. Yuan JP, Ho TC, Coury SM, Chahal R, Colich NL, Gotlib IH. Early life stress, systemic inflammation, and neural correlates of implicit emotion regulation in adolescents. *Brain Behav Immun*. Oct 2022;105:169-179. doi:10.1016/j.bbi.2022.07.007
46. Reemst K, Kracht L, Kotah JM, et al. Early-life stress lastingly impacts microglial transcriptome and function under basal and immune-challenged conditions. *Transl Psychiatry*. Dec 08 2022;12(1):507. doi:10.1038/s41398-022-02265-6
47. Yam KY, Schipper L, Reemst K, et al. Increasing availability of ω-3 fatty acid in the early-life diet prevents the early-life stress-induced cognitive impairments without affecting metabolic alterations. *FASEB J*. Apr 2019;33(4):5729-5740. doi:10.1096/fj.201802297R
48. McIlwrick S, Rechenberg A, Matthes M, et al. Genetic predisposition for high stress reactivity amplifies effects of early-life adversity. *Psychoneuroendocrinology*. Aug 2016;70:85-97. doi:10.1016/j.psyneuen.2016.04.023
49. Klengel T, Mehta D, Anacker C, et al. Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. *Nat Neurosci*. Jan 2013;16(1):33-41. doi:10.1038/nn.3275
50. Ayriyants KA, Ryabushkina YA, Sapronova AA, et al. A comparison of stress reactivity between BTBR and C57BL/6J mice: an impact of early-life stress. *Exp Brain Res*. Mar 2023;241(3):687-698. doi:10.1007/s00221-022-06541-1
51. Goodwill HL, Manzano-Nieves G, Gallo M, et al. Early life stress leads to sex differences in development of depressive-like outcomes in a mouse model. *Neuropsychopharmacology*. 03 2019;44(4):711-720. doi:10.1038/s41386-018-0195-5
52. Goodwill HL, Manzano-Nieves G, LaChance P, et al. Early Life Stress Drives Sex-Selective Impairment in Reversal Learning by Affecting Parvalbumin Interneurons in Orbitofrontal Cortex of Mice. *Cell Rep*. 11 2018;25(9):2299-2307.e4. doi:10.1016/j.celrep.2018.11.010
53. Zoladz P, Reneau K, Weiser J, et al. Childhood Maltreatment in Females Is Associated with Enhanced Fear Acquisition and an Overgeneralization of Fear. *Brain Sci*. Nov 12 2022;12(11)doi:10.3390/brainsci12111536
54. Bickle JG, Li Y, Millette A, et al. 5HT1A receptors on dentate gyrus granule cells confer stress resilience. *Biol Psychiatry*. Oct 18 2023;doi:10.1016/j.biopsych.2023.10.007

55. Richter-Levin G, Segal M. Effects of serotonin releasers on dentate granule cell excitability in the rat. *Exp Brain Res*. 1990;82(1):199-207. doi:10.1007/BF00230852
56. Nozaki K, Kubo R, Furukawa Y. Serotonin modulates the excitatory synaptic transmission in the dentate granule cells. *J Neurophysiol*. 06 01 2016;115(6):2997-3007. doi:10.1152/jn.00064.2016
57. Ahern GP. 5-HT and the immune system. *Curr Opin Pharmacol*. Feb 2011;11(1):29-33. doi:10.1016/j.coph.2011.02.004
58. Sachs BD, Ni JR, Caron MG. Brain 5-HT deficiency increases stress vulnerability and impairs antidepressant responses following psychosocial stress. *Proc Natl Acad Sci U S A*. Feb 2015;112(8):2557-62. doi:10.1073/pnas.1416866112
59. Richardson-Jones JW, Craige CP, Guiard BP, et al. 5-HT1A autoreceptor levels determine vulnerability to stress and response to antidepressants. *Neuron*. Jan 2010;65(1):40-52. doi:10.1016/j.neuron.2009.12.003
60. Bath KG, Nitenson AS, Lichtman E, et al. Early life stress leads to developmental and sex selective effects on performance in a novel object placement task. *Neurobiol Stress*. Dec 2017;7:57-67. doi:10.1016/j.ynstr.2017.04.001
61. Walker CD, Bath KG, Joels M, et al. Chronic early life stress induced by limited bedding and nesting (LBN) material in rodents: critical considerations of methodology, outcomes and translational potential. *Stress*. 09 2017;20(5):421-448. doi:10.1080/10253890.2017.1343296
62. Anacker C, Sydnor E, Chen BK, et al. Behavioral and neurobiological effects of GnRH agonist treatment in mice-potential implications for puberty suppression in transgender individuals. *Neuropsychopharmacology*. Sep 2020;doi:10.1038/s41386-020-00826-1
63. Richardson-Jones JW, Craige CP, Nguyen TH, et al. Serotonin-1A autoreceptors are necessary and sufficient for the normal formation of circuits underlying innate anxiety. *J Neurosci*. Apr 2011;31(16):6008-18. doi:10.1523/JNEUROSCI.5836-10.2011
64. Donaldson ZR, Piel DA, Santos TL, et al. Developmental effects of serotonin 1A autoreceptors on anxiety and social behavior. *Neuropsychopharmacology*. Jan 2014;39(2):291-302. doi:10.1038/npp.2013.185
65. Anacker C, Zunszain PA, Cattaneo A, et al. Antidepressants increase human hippocampal neurogenesis by activating the glucocorticoid receptor. *Mol Psychiatry*. Jul 2011;16(7):738-50. doi:10.1038/mp.2011.26
66. Santarelli S, Zimmermann C, Kalideris G, et al. An adverse early life environment can enhance stress resilience in adulthood. *Psychoneuroendocrinology*. Apr 2017;78:213-221. doi:10.1016/j.psyneuen.2017.01.021
67. Daskalakis NP, De Kloet ER, Yehuda R, Malaspina D, Kranz TM. Early Life Stress Effects on Glucocorticoid-BDNF Interplay in the Hippocampus. *Front Mol Neurosci*. 2015;8:68. doi:10.3389/fnmol.2015.00068
68. Iwata M, Ota KT, Duman RS. The inflammasome: pathways linking psychological stress, depression, and systemic illnesses. *Brain Behav Immun*. Jul 2013;31:105-14. doi:10.1016/j.bbi.2012.12.008
69. Koo JW, Duman RS. IL-1beta is an essential mediator of the antineurogenic and anhedonic effects of stress. *Proc Natl Acad Sci U S A*. Jan 2008;105(2):751-6. doi:10.1073/pnas.0708092105

70. Ruigrok SR, Kotah JM, Kuindersma JE, et al. Adult food choices depend on sex and exposure to early-life stress: Underlying brain circuitry, adipose tissue adaptations and metabolic responses. *Neurobiol Stress*. Nov 2021;15:100360. doi:10.1016/j.ynstr.2021.100360
71. Ruigrok SR, Yim K, Emmerzaal TL, et al. Effects of early-life stress on peripheral and central mitochondria in male mice across ages. *Psychoneuroendocrinology*. Oct 2021;132:105346. doi:10.1016/j.psyneuen.2021.105346
72. Roy A. Self-rated childhood emotional neglect and CSF monoamine indices in abstinent cocaine-abusing adults: possible implications for suicidal behavior. *Psychiatry Res*. Sep 2002;112(1):69-75. doi:10.1016/s0165-1781(02)00176-2
73. Maestripieri D, Higley JD, Lindell SG, Newman TK, McCormack KM, Sanchez MM. Early maternal rejection affects the development of monoaminergic systems and adult abusive parenting in rhesus macaques (Macaca mulatta). *Behav Neurosci*. Oct 2006;120(5):1017-24. doi:10.1037/0735-7044.120.5.1017
74. Higley JD, Suomi SJ, Linnoila M. A longitudinal assessment of CSF monoamine metabolite and plasma cortisol concentrations in young rhesus monkeys. *Biol Psychiatry*. Jul 1992;32(2):127-45. doi:10.1016/0006-3223(92)90016-s
75. Bennett AJ, Lesch KP, Heils A, et al. Early experience and serotonin transporter gene variation interact to influence primate CNS function. *Mol Psychiatry*. 2002;7(1):118-22. doi:10.1038/sj.mp.4000949
76. Caspi A, Sugden K, Moffitt TE, et al. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science*. Jul 18 2003;301(5631):386-9. doi:10.1126/science.1083968
301/5631/386 [pii]
77. Konno K, Matsumoto M, Togashi H, et al. Early postnatal stress affects the serotonergic function in the median raphe nuclei of adult rats. *Brain Res*. Oct 2007;1172:60-6. doi:10.1016/j.brainres.2007.05.022
78. Gordon JA, Hen R. The serotonergic system and anxiety. *Neuromolecular Med*. 2004;5(1):27-40. doi:10.1385/NMM:5:1:027
79. Arango V, Underwood MD, Boldrini M, et al. Serotonin 1A receptors, serotonin transporter binding and serotonin transporter mRNA expression in the brainstem of depressed suicide victims. *Neuropsychopharmacology*. Dec 2001;25(6):892-903. doi:10.1016/S0893-133X(01)00310-4
80. Arango V, Underwood MD, Mann JJ. Serotonin brain circuits involved in major depression and suicide. *Prog Brain Res*. 2002;136:443-53.
81. Arango V, Huang YY, Underwood MD, Mann JJ. Genetics of the serotonergic system in suicidal behavior. *J Psychiatr Res*. 2003 Sep-Oct 2003;37(5):375-86.
82. Boldrini M, Underwood MD, Mann JJ, Arango V. Serotonin-1A autoreceptor binding in the dorsal raphe nucleus of depressed suicides. *J Psychiatr Res*. May 2008;42(6):433-42. doi:10.1016/j.jpsychires.2007.05.004
83. Drevets WC, Thase ME, Moses-Kolko EL, et al. Serotonin-1A receptor imaging in recurrent depression: replication and literature review. *Nucl Med Biol*. Oct 2007;34(7):865-77. doi:10.1016/j.nucmedbio.2007.06.008

84. Kaufman J, Sullivan GM, Yang J, et al. Quantification of the Serotonin 1A Receptor Using PET: Identification of a Potential Biomarker of Major Depression in Males. *Neuropsychopharmacology*. Jun 2015;40(7):1692-9. doi:10.1038/npp.2015.15
85. Parsey RV, Olvet DM, Oquendo MA, Huang YY, Ogden RT, Mann JJ. Higher 5-HT1A receptor binding potential during a major depressive episode predicts poor treatment response: preliminary data from a naturalistic study. *Neuropsychopharmacology*. Aug 2006;31(8):1745-9. doi:10.1038/sj.npp.1300992
86. Parsey RV, Ogden RT, Miller JM, et al. Higher serotonin 1A binding in a second major depression cohort: modeling and reference region considerations. *Biol Psychiatry*. Jul 15 2010;68(2):170-8. doi:10.1016/j.biopsych.2010.03.023
87. Ramboz S, Oosting R, Amara DA, et al. Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. *Proc Natl Acad Sci U S A*. Nov 24 1998;95(24):14476-81. doi:10.1073/pnas.95.24.14476
88. Donaldson ZR, le Francois B, Santos TL, et al. The functional serotonin 1a receptor promoter polymorphism, rs6295, is associated with psychiatric illness and differences in transcription. *Transl Psychiatry*. Mar 01 2016;6:e746. doi:10.1038/tp.2015.226
89. Gross C, Santarelli L, Brunner D, Zhuang X, Hen R. Altered fear circuits in 5-HT(1A) receptor KO mice. *Biol Psychiatry*. Dec 15 2000;48(12):1157-63. doi:S0006322300010416 [pii]
90. Samuels BA, Anacker C, Hu A, et al. 5-HT1A receptors on mature dentate gyrus granule cells are critical for the antidepressant response. *Nat Neurosci*. Sep 2015;doi:10.1038/nn.4116
91. Zhang YM, Ye LY, Li TY, Guo F, Li Y, Li YF. New monoamine antidepressant, hypidone hydrochloride (YL-0919), enhances the excitability of medial prefrontal cortex in mice via a neural disinhibition mechanism. *Acta Pharmacol Sin*. Jul 2022;43(7):1699-1709. doi:10.1038/s41401-021-00807-0
92. Dedic N, Jones PG, Hopkins SC, et al. SEP-363856, a Novel Psychotropic Agent with a Unique, Non-D. *J Pharmacol Exp Ther*. Oct 2019;371(1):1-14. doi:10.1124/jpet.119.260281
93. Dawson LA, Watson JM. Vilazodone: a 5-HT1A receptor agonist/serotonin transporter inhibitor for the treatment of affective disorders. *CNS Neurosci Ther*. 2009;15(2):107-17. doi:10.1111/j.1755-5949.2008.00067.x
94. Celada P, Bortolozzi A, Artigas F. Serotonin 5-HT1A receptors as targets for agents to treat psychiatric disorders: rationale and current status of research. *CNS Drugs*. Sep 2013;27(9):703-16. doi:10.1007/s40263-013-0071-0
95. Newman-Tancredi A, Depoortère RY, Kleven MS, Kołaczkowski M, Zimmer L. Translating biased agonists from molecules to medications: Serotonin 5-HT 1A receptor functional selectivity for CNS disorders. *Pharmacol Ther*. Jan 2022;229:107937. doi:10.1016/j.pharmthera.2021.107937
96. Williams LM, Debattista C, Duchemin AM, Schatzberg AF, Nemeroff CB. Childhood trauma predicts antidepressant response in adults with major depression: data from the randomized international study to predict optimized treatment for depression. *Transl Psychiatry*. May 03 2016;6(5):e799. doi:10.1038/tp.2016.61

Figure 1

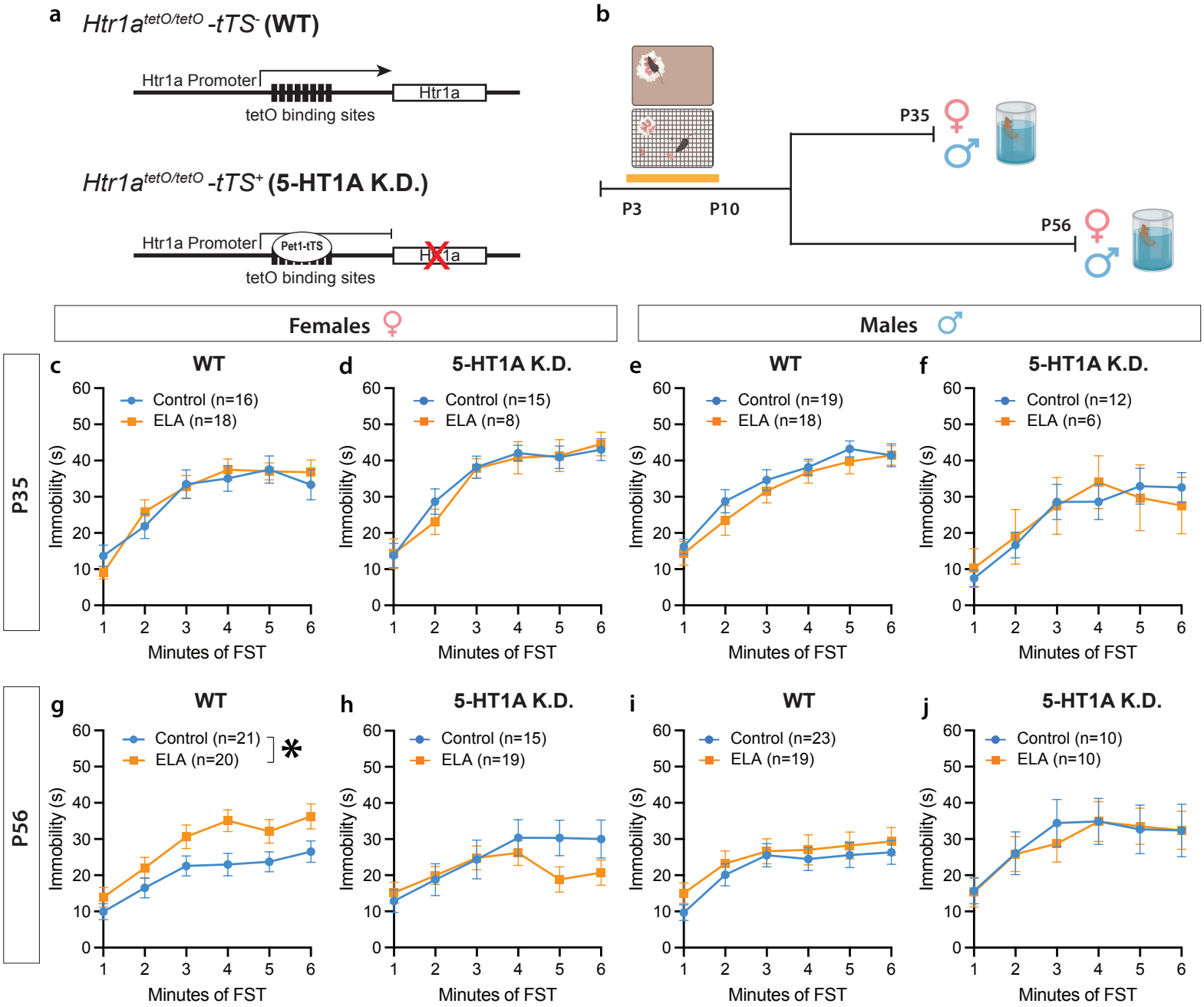

a
Htr1a tetO/tetO -tTS- (WT)
Htr1a Promoter
Htr1a
tetO binding sites
Htr1a tetO/tetO -tTS+ (5-HT1A K.D.)
Htr1a Promoter
Pet1-tTS
Htr1a
tetO binding sites
b
P3
P10
P35
P56
Females
Males
P35
P56
c WT
Control (n=16)
ELA (n=18)
d 5-HT1A K.D.
Control (n=15)
ELA (n=8)
e WT
Control (n=19)
ELA (n=18)
f 5-HT1A K.D.
Control (n=12)
ELA (n=6)
g WT
Control (n=21)
ELA (n=20)
*
h 5-HT1A K.D.
Control (n=15)
ELA (n=19)
i WT
Control (n=23)
ELA (n=19)
j 5-HT1A K.D.
Control (n=10)
ELA (n=10)
Immobility (s)
Minutes of FST

Figure 2

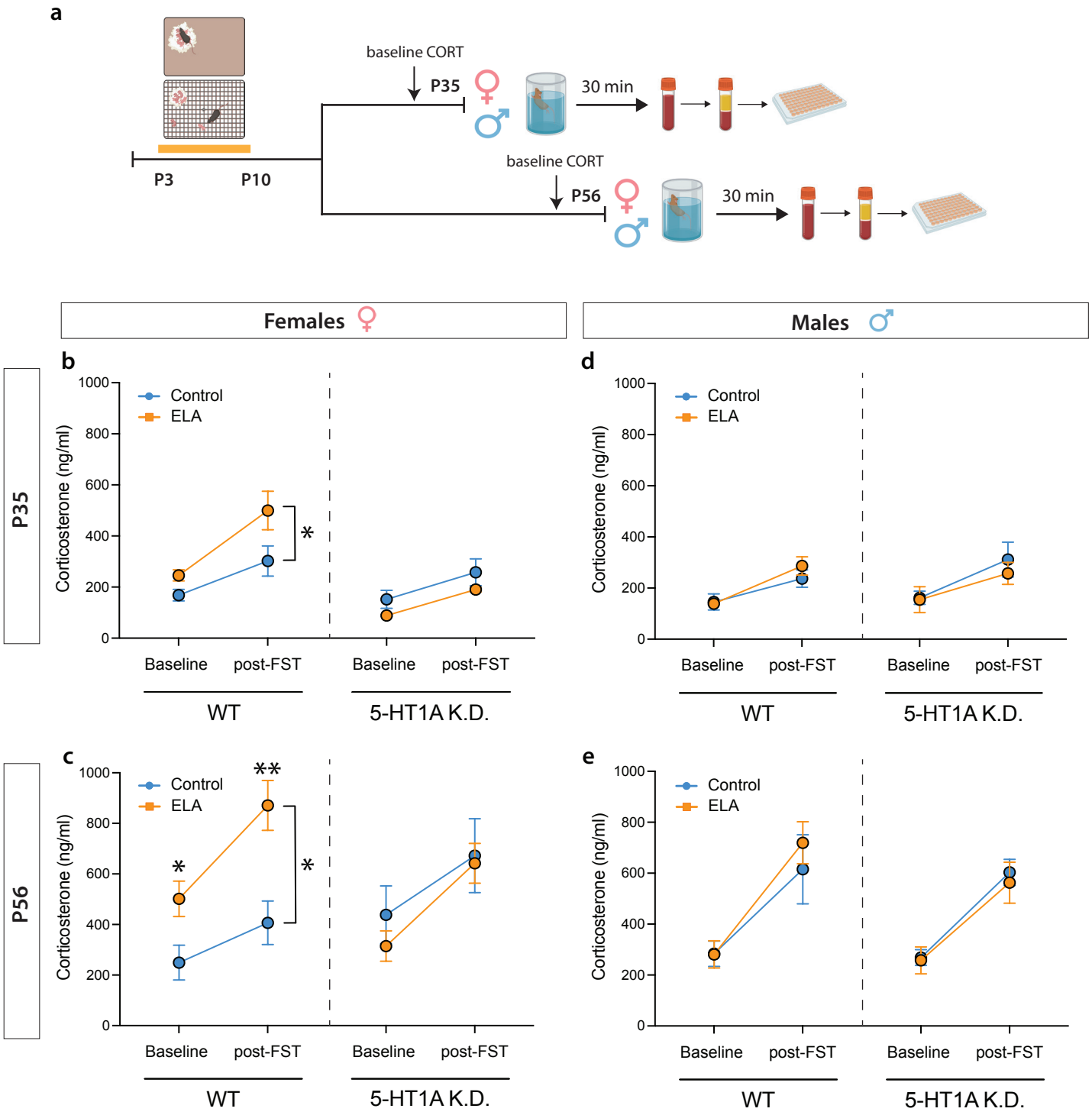

a
P3
P10
baseline CORT
P35
30 min
baseline CORT
P56
30 min
Females
Males
b
c
d
e
P35
P56
Control
ELA
Corticosterone (ng/ml)
1000
800
600
400
200
0
Baseline
post-FST
WT
5-HT1A K.D.
*
**

Figure 3

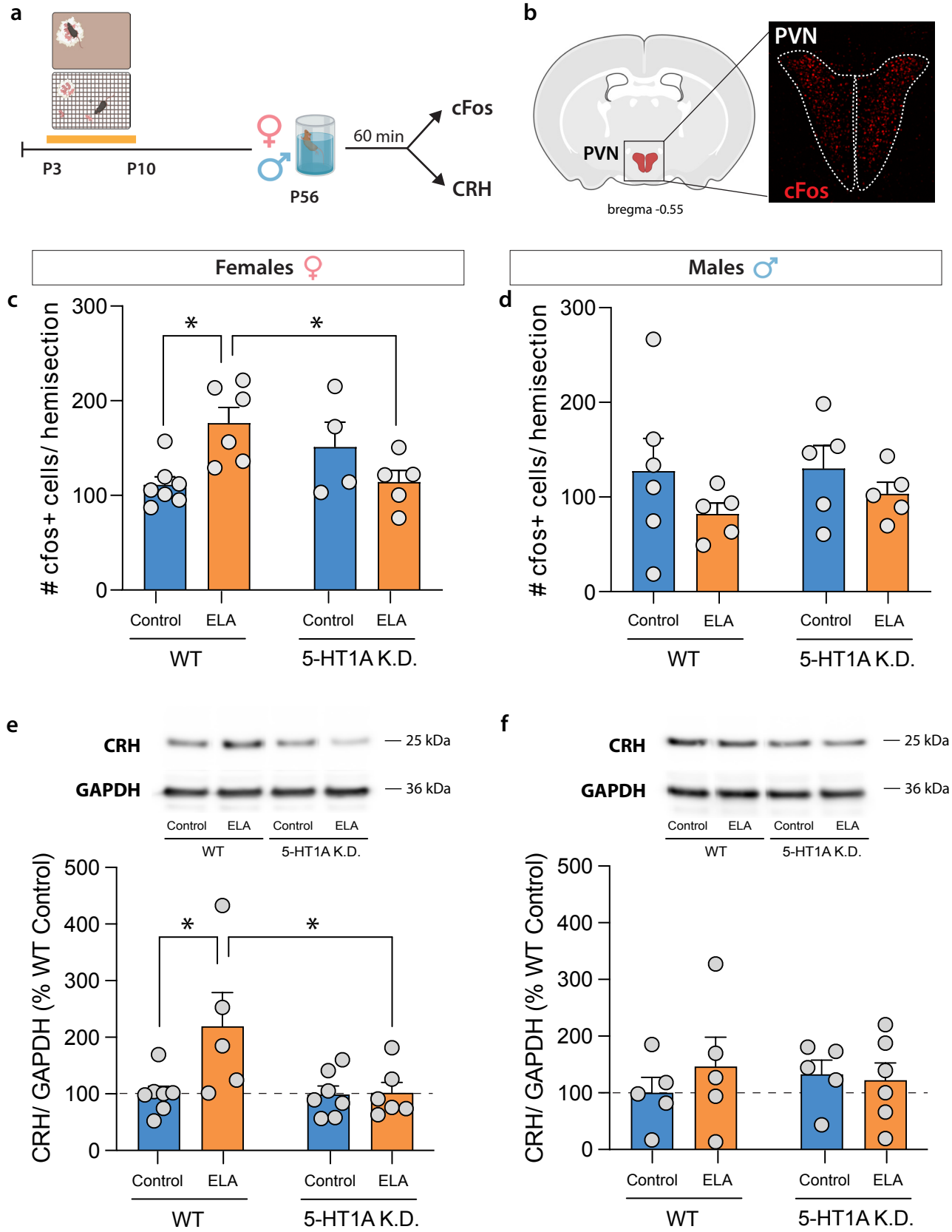

a
P3
P10
P56
60 min
cFos
CRH
b
PVN
PVN
cFos
bregma -0.55
Females ♀
Males ♂
c
cfos+ cells/ hemisection
300
200
100
0
*
*
Control
ELA
Control
ELA
WT
5-HT1A K.D.
d
cfos+ cells/ hemisection
300
200
100
0
Control
ELA
Control
ELA
WT
5-HT1A K.D.
e
CRH
GAPDH
25 kDa
36 kDa
Control
ELA
Control
ELA
WT
5-HT1A K.D.
CRH/ GAPDH (% WT Control)
500
400
300
200
100
0
*
*
Control
ELA
Control
ELA
WT
5-HT1A K.D.
f
CRH
GAPDH
25 kDa
36 kDa
Control
ELA
Control
ELA
WT
5-HT1A K.D.
CRH/ GAPDH (% WT Control)
500
400
300
200
100
0
Control
ELA
Control
ELA
WT
5-HT1A K.D.

Figure 4

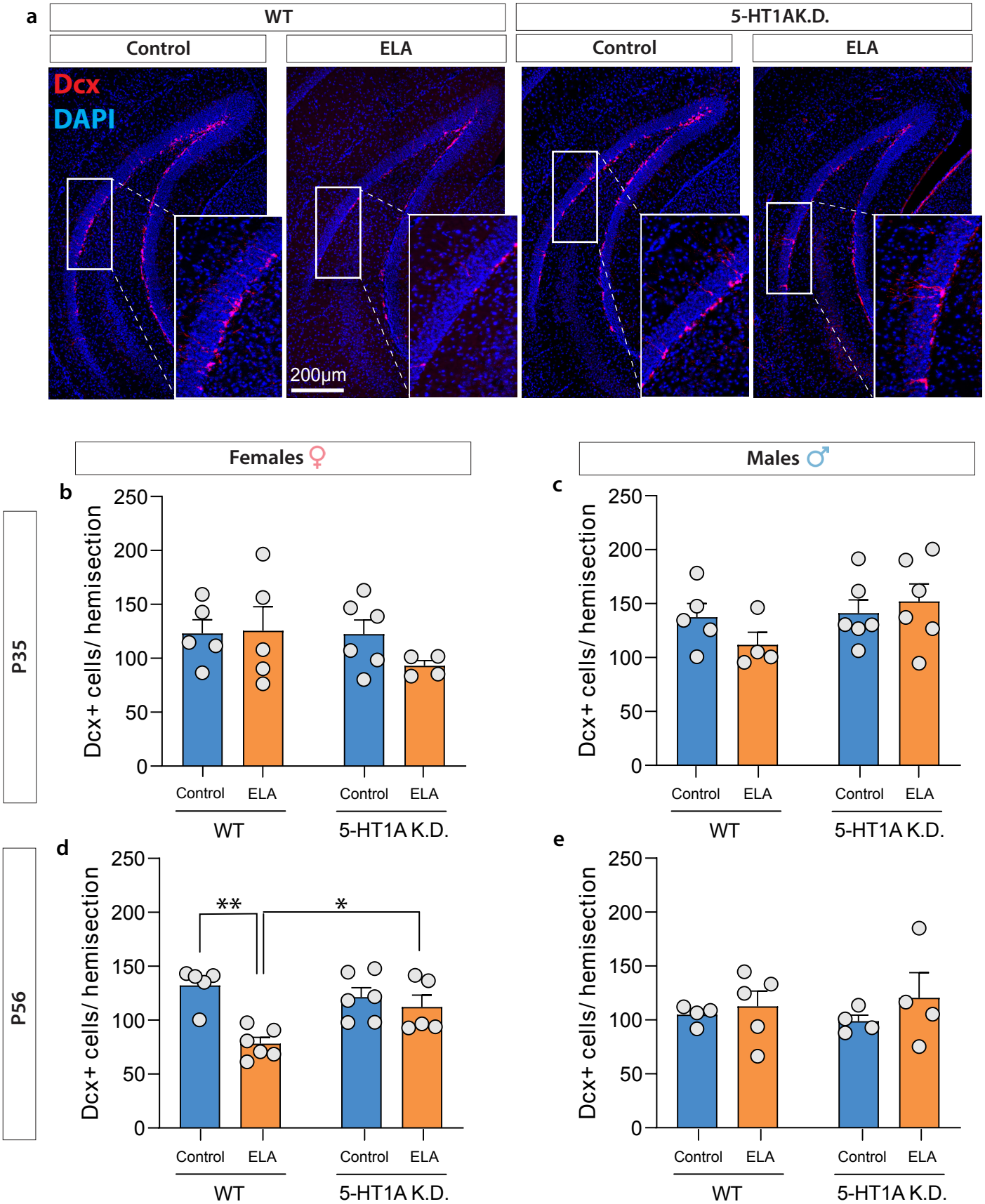
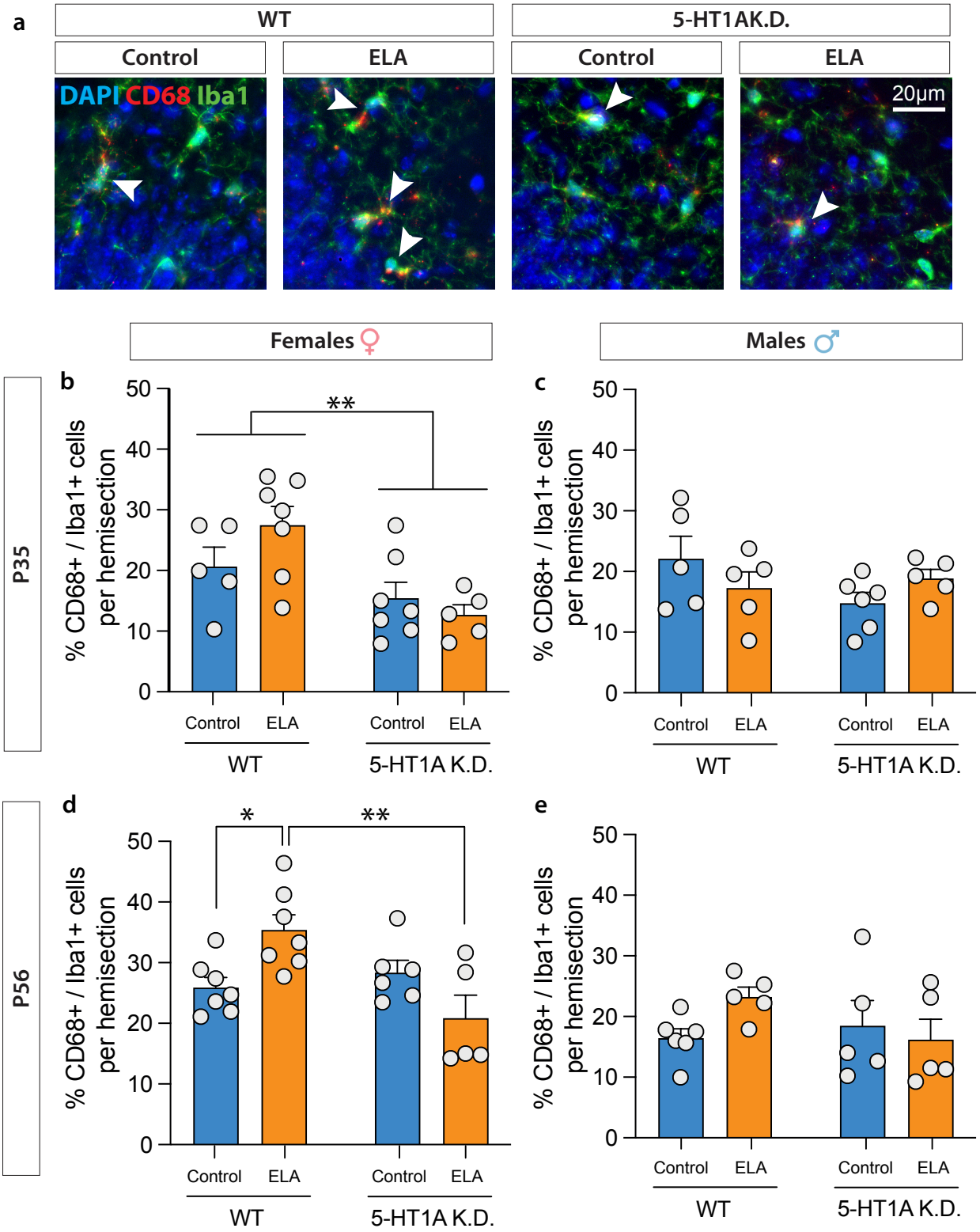

a
WT
5-HT1AK.D.
Control
ELA
Control
ELA
Dcx
DAPI
200µm
Females ♀
Males ♂
P35
P56
b
c
d
e
Dcx+ cells/ hemisection
250
200
150
100
50
0
Control
ELA
Control
ELA
WT
5-HT1A K.D.
**
*

Figure 5


a
WT
5-HT1AK.D.
Control
ELA
Control
ELA
DAPI CD68 Iba1
20µm
Females ♀
Males ♂
P35
b
c
% CD68+ / Iba1+ cells per hemisection
50
40
30
20
10
0
**
Control
ELA
Control
ELA
WT
5-HT1A K.D.
P56
d
e
*
**

Supplementary Table 1. Detailed statistical analyses for all experiments.

Name	Abbrev	Measurement	Statistical	Comparison	F	° of	p	*	Fig.	Post hoc Test
Forced Swim test immobility	FST	P35 Female WT	2-Way RM ANOVA	Time x ELA	1.14	5, 160	0.34	ns	1c	n.a.
				Time	43.84	3.802, 121.7	<0.0001	***		
				ELA	0.03	1, 32	0.86	ns		
		P35 Female 5-HT1A K.D.	2-Way RM ANOVA	Time x ELA	0.67	5, 105	0.65	ns	1d	n.a.
				Time	56.49	3.325, 69.83	<0.0001	***		
				ELA	0.36	1, 21	0.85	ns		
		P35 Male WT	2-Way RM ANOVA	Time x ELA	0.54	5, 175	0.74	ns	1e	n.a.
				Time	66.95	3.202, 112.1	<0.0001	***		
				ELA	0.49	1, 35	0.49	ns		
		P35 Male 5-HT1A K.D.	2-Way RM ANOVA	Time x ELA	0.87	5, 80	0.50	ns	1f	n.a.
				Time	18.98	2.468, 39.49	<0.0001	***		
				ELA	0.001	1, 16	0.98	ns		
		P56 Female WT	2-Way RM ANOVA	Time x ELA	0.88	5, 195	0.50	ns	1g	n.a.
				Time	22.47	2.92, 113.9	<0.0001	***		
				ELA	6.86	1, 39	0.013	*		
		P56 Female 5-HT1A K.D.	2-Way RM ANOVA	Time x ELA	2.96	5, 160	0.0140	*	1h	n.a.
				Time	9.21	3.79, 121.4	<0.0001	***		
				ELA	0.58	1, 32	0.45	ns		
		P56 Male WT	2-Way RM ANOVA	Time x ELA	0.24	5, 200	0.94	ns	1i	n.a.
				Time	17.81	2.924, 117	<0.0001	***		
				ELA	0.54	1, 40	0.47	ns		
		P56 Male 5-HT1A K.D.	2-Way RM ANOVA	Time x ELA	0.4	5, 90	0.85	ns	1j	n.a.
				Time	14.56	2.406, 43.31	<0.0001	***		
				ELA	0.02	1, 18	0.90	ns		
		P35 Female	3-Way RM ANOVA	Time	90.02	3.831, 203.1	<0.0001	***	1c, d	n.a.
				GT	2.56	1, 53	0.12	ns		
				ELA	0.0004	1, 53	0.98	ns		
				Time x GT	1.09	5, 265	0.37	ns		
				Time x ELA	0.45	5, 265	0.81	ns		
				GT x ELA	0.07	1, 53	0.80	ns		
				Time x GT x ELA	1.19	5, 265	0.32	ns		
		P35 Male	3-Way RM ANOVA	Time	67.09	3.170, 161.7	<0.0001	***	1e, f	n.a.
				GT	4.9	1, 51	0.03	*		
				ELA	0.1	1, 51	0.75	ns		
				Time x GT	1.1	5, 255	0.37	ns		
				Time x ELA	0.73	5, 255	0.60	ns		
				GT x ELA	0.15	1, 51	0.70	ns		
				Time x GT x ELA	0.98	5, 255	0.43	ns		
		P56 Female	3-Way RM ANOVA	Time	28.93	3.45, 244.8	<0.0001	***	1g, h	Tukey
				GT	0.38	1, 71	0.54	ns		
				ELA	0.69	1, 71	0.41	ns		
				Time x GT	1.53	5, 355	0.18	ns		
				Time x ELA	1.04	5, 355	0.39	ns		
				GT x ELA	4.58	1, 71	0.036	*		
				Time x GT x ELA	2.96	5, 355	0.012	*		
		P56 Male	3-Way RM ANOVA	Time	29.47	2.819, 163.5	<0.0001	***	1i, j	n.a.
				GT	2.07	1, 58	0.16	ns		
				ELA	0.07	1, 58	0.79	ns		
				Time x GT	0.75	5, 290	0.59	ns		
				Time x ELA	0.49	5, 290	0.78	ns		
				GT x ELA	0.56	1, 58	0.61	ns		
				Time x GT x ELA	0.17	5, 290	0.97	ns		
		P35 WT	3-Way RM ANOVA	Time	106.3	3.651, 244.6	<0.0001	***	1c,e	n.a.
				Sex	1.37	1, 67	0.25	ns		
				ELA	0.13	1, 67	0.72	ns		
				Time x Sex	1.39	5, 335	0.23	ns		
				Time x ELA	0.86	5, 335	0.51	ns		
				Sex x ELA	0.39	1, 67	0.54	ns		
				Time x Sex x ELA	0.97	5, 335	0.43	ns		
		P35 5-HT1A K.D.	3-Way RM ANOVA	Time	66.12	3.425, 126.7	<0.0001	***	1d, f	n.a.
				Sex	5.97	1, 37	0.02	*		
				ELA	0.01	1, 37	0.94	ns		
				Time x Sex	1.21	5, 185	0.31	ns		
				Time x ELA	0.44	5, 185	0.82	ns		
				Sex x ELA	0.02	1, 37	0.90	ns		
				Time x Sex x ELA	1.24	5, 185	0.29	ns		
		P56 WT	3-Way RM ANOVA	Time	39.72	2.986, 235.9	<0.0001	***	1g,i	n.a.
				Sex	0.13	1, 79	0.72	ns		
				ELA	4.67	1, 79	0.03	*		
				Time x Sex	1.19	5, 395	0.31	ns		
				Time x ELA	0.33	5, 395	0.90	ns		

				Sex x ELA	0.98	1, 79	0.33	ns		
				Time x Sex x ELA	0.86	5, 395	0.50	ns		
		P56 5-HT1A K.D.	3-Way RM ANOVA	Time	21.73	3.51, 175.5	<0.0001	***	1h, j	Tukey
				Sex	2.33	1, 50	0.13	ns		
				ELA	0.29	1, 50	0.59	ns		
				Time x Sex	0.84	5, 250	0.52	ns		
				Time x ELA	0.95	5, 250	0.45	ns		
				Sex x ELA	0.11	1, 50	0.75	ns		
				Time x Sex x ELA	1.89	5, 250	0.10	ns		
Body weight	n.a.	Female	3-Way ANOVA	Age	365.5	3, 195	<0.0001	***	Supp. 1a	n.a.
				Gentoype	0.36	1, 195	0.55	ns		
				ELA	8.88	1, 195	0.003	**		
				Age x Gentoype	0.1	3, 195	0.96	ns		
				Age x ELA	9.63	3, 195	<0.0001	***		
				Gentoype x ELA	0.00002284	1, 195	1.00	ns		
				Age x Gentoype x ELA	0.13	3, 195	0.94	ns		
		Male	3-Way ANOVA	Age	503.7	3, 195	<0.0001	***	Supp. 1b	n.a.
				Gentoype	1.27	1, 195	0.26	ns		
				ELA	16.94	1, 195	<0.0001	***		
				Age x Gentoype	0.12	3, 195	0.95	ns		
				Age x ELA	3.21	3, 195	0.02	*		
				Gentoype x ELA	2.42	1, 195	0.12	ns		
				Age x Gentoype x ELA	0.87	3, 195	0.46	ns		
Corticosterone Levels	Cort	P35 Female WT	2-Way RM ANOVA	Time x ELA	1.74	1, 24	0.20	ns	2b	n.a.
				Time	18.1	1, 24	0.0003	***		
				ELA	6.3	1, 24	0.02	*		
		P35 Female 5-HT1A K.D.	2-Way RM ANOVA	Time x ELA	0.003	1, 12	0.96	ns		n.a.
				Time	8.12	1, 12	0.01	*		
				ELA	1.72	1, 12	0.21	ns		
		P56 Female WT	2-Way RM ANOVA	Time x ELA	4.5	1, 18	0.05	ns	2c	Fisher's LSD
				Time	27.79	1, 18	<0.0001	***		
				ELA	10.17	1, 18	0.005	**		
		P56 Female 5-HT1A K.D.	2-Way RM ANOVA	Time x ELA	1.4	1, 18	0.25	ns		n.a.
				Time	50.44	1, 18	<0.0001	***		
				ELA	0.32	1, 18	0.58	ns		
		P35 Male WT	2-Way RM ANOVA	Time x ELA	1.04	1, 18	0.32	ns	2d	n.a.
				Time	18.89	1, 18	0.0004	***		
				ELA	0.36	1, 18	0.55	ns		
		P35 Male 5-HT1A K.D.	2-Way RM ANOVA	Time x ELA	0.31	1, 17	0.59	ns		n.a.
				Time	8.89	1, 17	0.008	**		
				ELA	0.23	1, 17	0.64	ns		
		P56 Male WT	2-Way RM ANOVA	Time x ELA	0.5	1, 23	0.49	ns	2e	n.a.
				Time	25.94	1, 23	<0.0001	***		
				ELA	0.22	1, 23	0.64	ns		
		P56 Male 5-HT1A K.D.	2-Way RM ANOVA	Time x ELA	0.09	1, 16	0.77	ns		n.a.
				Time	41.02	1, 16	<0.0001	***		
				ELA	0.11	1, 16	0.75	ns		
		P35 Female	3-Way RM ANOVA	Time	18.29	1, 36	0.0001	***	2b	n.a.
				GT	9.55	1, 36	0.004	**		
				ELA	0.71	1, 36	0.40	ns		
				Time x GT	1.67	1, 36	0.20	ns		
				Time x ELA	0.7	1, 36	0.41	ns		
				GT x ELA	5.65	1, 36	0.02	*		
				Time x GT x ELA	0.79	1, 36	0.38	ns		
		P56 Female	3-Way RM ANOVA	Time	73.18	1, 36	<0.0001	***	2c	n.a.
				GT	0.01	1, 36	0.91	ns		
				ELA	2.54	1, 36	0.12	ns		
				Time x GT	0.07	1, 36	0.79	ns		
				Time x ELA	5.77	1, 36	0.02	*		
				GT x ELA	6.08	1, 36	0.02	*		
				Time x GT x ELA	0.87	1, 36	0.36	ns		
		P35 Male	3-Way RM ANOVA	Time	24.44	1, 35	<0.0001	***	2d	n.a.
				GT	0.29	1, 35	0.59	ns		
				ELA	0.02	1, 35	0.90	ns		
				Time x GT	0.02	1, 35	0.90	ns		
				Time x ELA	0.01	1, 35	0.93	ns		
				GT x ELA	0.53	1, 35	0.47	ns		
				Time x GT x ELA	1.07	1, 35	0.31	ns		
		P56 Male	3-Way RM ANOVA	Time	49.89	1, 39	<0.0001	***	2e	n.a.
				GT	0.52	1, 39	0.48	ns		
				ELA	0.03	1, 39	0.86	ns		
				Time x GT	0.43	1, 39	0.52	ns		

			ANOVA	Time x ELA	0.15	1, 39	0.70	ns		
				GT x ELA	0.28	1, 39	0.60	ns		
				Time x GT x ELA	0.47	1, 39	0.50	ns		
		P35 WT	3-Way RM ANOVA	Time	29.62	1, 42	<0.0001	***	2b, 2d	n.a.
				Sex	8.48	1, 42	0.006	**		
				ELA	5.13	1, 42	0.03	*		
				Time x Sex	1.66	1, 42	0.20	ns		
				Time x ELA	2.34	1, 42	0.13	ns		
				Sex x ELA	2.74	1, 42	0.11	ns		
				Time x Sex x ELA	0.31	1, 42	0.58	ns		
		P35 5-HT1A K.D.	3-Way RM ANOVA	Time	15.37	1, 29	0.0005	***	2b, 2d	n.a.
				Sex	1.29	1, 29	0.26	ns		
				ELA	1.24	1, 29	0.27	ns		
				Time x Sex	0.14	1, 29	0.71	ns		
				Time x ELA	0.19	1, 29	0.67	ns		
				Sex x ELA	0.16	1, 29	0.69	ns		
				Time x Sex x ELA	0.13	1, 29	0.72	ns		
		P56 WT	3-Way RM ANOVA	Time	45.36	1, 41	<0.0001	***	2c, 2e	n.a.
				Sex	0.17	1, 41	0.68	ns		
				ELA	6.81	1, 41	0.013	*		
				Time x Sex	1.59	1, 41	0.21	ns		
				Time x ELA	2.74	1, 41	0.11	ns		
				Sex x ELA	3.85	1, 41	0.06	ns		
				Time x Sex x ELA	0.3	1, 41	0.59	ns		
		P56 5-HT1A K.D.	3-Way RM ANOVA	Time	90.81	1, 34	<0.0001	***	2c, 2e	n.a.
				Sex	1.34	1, 34	0.26	ns		
				ELA	0.4	1, 34	0.53	ns		
				Time x Sex	0.38	1, 34	0.54	ns		
				Time x ELA	0.26	1, 34	0.62	ns		
				Sex x ELA	0.1	1, 34	0.75	ns		
				Time x Sex x ELA	0.95	1, 34	0.34	ns		
cFos in Paraventricular Nucleus	PVN	P56 Female	2-Way ANOVA	GT x ELA	10.89	1, 18	0.004	**	3c	Tukey
				GT	0.51	1, 18	0.48	ns		
				ELA	0.83	1, 18	0.37	ns		
		P56 Male	2-Way ANOVA	GT x ELA	0.14	1, 17	0.71	ns	3d	n.a.
				GT	0.24	1, 17	0.63	ns		
				ELA	2.19	1, 17	0.16	ns		
		P56	3-Way ANOVA	GT	0.001	1, 35	0.97	ns	3c,d	Tukey
				Sex	3.64	1, 35	0.06	ns		
				ELA	0.59	1, 35	0.45	ns		
				GT x Sex	0.65	1, 35	0.43	ns		
				GT x ELA	2.13	1, 35	0.15	ns		
				Sex x ELA	3.06	1, 35	0.09	ns		
				GT x Sex x ELA	4.42	1, 35	0.04	*		
Corticotropin Releasing Hormone	CRH/GAPDH	P56 Female	2-Way ANOVA	GT x ELA	4.46	1, 21	0.05	ns	3e	Tukey
				GT	4.6	1, 21	0.04	*		
				ELA	4.9	1, 21	0.04	*		
		P56 Male	2-Way ANOVA	GT x ELA	0.66	1, 17	0.43	ns	3f	n.a.
				GT	0.02	1, 17	0.90	ns		
				ELA	0.26	1, 17	0.62	ns		
		P56	3-Way ANOVA	GT	1.55	1, 38	0.22	ns	3e,f	n.a.
				Sex	0.05	1, 38	0.83	ns		
				ELA	3.21	1, 38	0.08	ns		
				GT x Sex	2.08	1, 38	0.16	ns		
				GT x ELA	3.89	1, 38	0.06	ns		
				Sex x ELA	0.97	1, 38	0.33	ns		
				GT x Sex x ELA	0.46	1, 38	0.50	ns		
		P35 Female vDG	2-Way ANOVA	GT x ELA	1.08	1, 16	0.31	ns	4b	n.a.
				GT	1.15	1, 16	0.30	ns		
				ELA	0.77	1, 16	0.39	ns		
		P35 Male vDG	2-Way ANOVA	GT x ELA	1.63	1,17	0.22	ns	4c	n.a.
				GT	2.38	1,17	0.14	ns		
				ELA	0.27	1,17	0.61	ns		
		P56 Female vDG	2-Way ANOVA	GT x ELA	7.01	1, 18	0.02	*	4d	Tukey
				GT	1.88	1, 18	0.19	ns		
				ELA	13.83	1, 18	0.002	**		
		P56 Male vDG	2-Way ANOVA	GT x ELA	0.25	1, 13	0.63	ns	4e	n.a.
				GT	1.06	1, 13	0.32	ns		
				ELA	0.005	1, 13	0.94	ns		
		P35 Female dDG	2-Way ANOVA	GT x ELA	0.13	1, 16	0.73	ns		n.a.
				GT	0.9	1, 16	0.36	ns		

			ANOVA	ELA	3.03	1, 16	0.10	ns	Supp. 2b	
		P35 Male dDG	2-Way ANOVA	GT x ELA	1.35	1, 17	0.26	ns		n.a.
				GT	1.5	1, 17	0.24	ns		
				ELA	3.5	1, 17	0.08	ns	Supp. 2c	
		P56 Female dDG	2-Way ANOVA	GT x ELA	4.26	1, 18	0.05	ns		n.a.
				GT	0.2	1, 18	0.66	ns		
				ELA	3.84	1, 18	0.07	ns	Supp. 2d	
		P56 Male dDG	2-Way ANOVA	GT x ELA	2.01	1, 13	0.18	ns		n.a.
				GT	0.09	1, 13	0.77	ns		
				ELA	0.08	1, 13	0.78	ns	Supp. 2e	
Double Cortin Staining	DCX	P35 vDG	3-Way ANOVA	GT	0.07	1, 33	0.80	ns		n.a.
				Sex	3.51	1, 33	0.07	ns		
				ELA	0.99	1, 33	0.33	ns		
				GT x Sex	3.38	1, 33	0.08	ns	4b,c	
				GT x ELA	0.01	1, 33	0.92	ns		
				Sex x ELA	0.09	1, 33	0.77	ns		
				GT x Sex x ELA	2.67	1, 33	0.11	ns		
		P56 vDG	3-Way ANOVA	GT	2.8	1, 31	0.10	ns		n.a.
				Sex	0.05	1, 31	0.82	ns		
				ELA	3.76	1, 31	0.06	ns		
				GT x Sex	0.04	1, 31	0.84	ns	4d,e	
				GT x ELA	3.52	1, 31	0.07	ns		
				Sex x ELA	4.27	1, 31	0.05	ns		
				GT x Sex x ELA	0.95	1, 31	0.34	ns		
		P35 dDG	3-Way ANOVA	GT	0.02	1, 33	0.90	ns		n.a.
				Sex	25.17	1, 33	<0.0001	***		
				ELA	6.53	1, 33	0.02	*	Supp. 2b,c	
				GT x Sex	2.33	1, 33	0.14	ns		
				GT x ELA	0.27	1, 33	0.61	ns		
				Sex x ELA	0.0003	1, 33	0.99	ns		
				GT x Sex x ELA	1.1	1, 33	0.30	ns		
		P56 dDG	3-Way ANOVA	GT	0.26	1, 31	0.61	ns		n.a.
				Sex	0.05	1, 31	0.82	ns		
				ELA	2.51	1, 31	0.12	ns	Supp. 2d,e	
				GT x Sex	0.01	1, 31	0.90	ns		
				GT x ELA	5.8	1, 31	0.02	*		
				Sex x ELA	1.44	1, 31	0.24	ns		
				GT x Sex x ELA	0.28	1, 31	0.60	ns		
		P35 Female vDG	2-Way ANOVA	GT x ELA	2.79	1, 20	0.11	ns		n.a.
				GT	12.21	1, 20	0.002	**		
				ELA	0.5	1, 20	0.49	ns	5b	
		P35 Male vDG	2-Way ANOVA	GT x ELA	3.15	1, 17	0.09	ns		n.a.
				GT	1.32	1, 17	0.27	ns		
				ELA	0.02	1, 17	0.88	ns	5c	
		P56 Female vDG	2-Way ANOVA	GT x ELA	11.74	1, 21	0.003	**		Tukey
				GT	5.91	1, 21	0.024	*		
				ELA	0.15	1, 21	0.70	ns	5d	
		P56 Male vDG	2-Way ANOVA	GT x ELA	2.6	1, 17	0.13	ns		n.a.
				GT	0.79	1, 17	0.39	ns		
				ELA	0.65	1, 17	0.43	ns	5e	
		P35 Female dDG	2-Way ANOVA	GT x ELA	0.24	1, 20	0.63	ns		n.a.
				GT	7.7	1, 20	0.01	*		
				ELA	4.39	1, 20	0.049	*	Supp. 3b	
		P35 Male dDG	2-Way ANOVA	GT x ELA	0.18	1, 17	0.67	ns		n.a.
				GT	3.2	1, 17	0.09	ns		
				ELA	1.47	1, 17	0.24	ns	Supp. 3c	
		P56 Female dDG	2-Way ANOVA	GT x ELA	2.49	1, 20	0.13	ns		n.a.
				GT	0.09	1, 20	0.77	ns		
				ELA	0.51	1, 20	0.48	ns	Supp. 3d	
		P56 Male dDG	2-Way ANOVA	GT x ELA	1.03	1, 18	0.32	ns		n.a.
				GT	0.03	1, 18	0.87	ns		
				ELA	0.022	1, 18	0.89	ns	Supp. 3e	
Active Microglia	n.a.	P35 vDG	3-Way ANOVA	GT	11.19	1, 37	0.002	**		Tukey
				Sex	0.17	1, 37	0.68	ns		
				ELA	0.18	1, 37	0.67	ns		
				GT x Sex	3.41	1, 37	0.07	ns	5b,c	
				GT x ELA	0.01	1, 37	0.93	ns		
				Sex x ELA	0.39	1, 37	0.53	ns		
				GT x Sex x ELA	5.74	1, 37	0.02	*		
		P56 vDG	3-Way ANOVA	GT	5.22	1, 38	0.03	*		n.a.
				Sex	23.38	1, 38	<0.0001	***		
				ELA	0.75	1, 38	0.39	ns		
				GT x Sex	0.89	1, 38	0.35	ns	5d,e	
				GT x ELA	12.17	1, 38	0.001	**		
				Sex x ELA	0.12	1, 38	0.73	ns		

				GT x Sex x ELA	1.12	1, 38	0.30	ns		
		P35 dDG	3-Way ANOVA	GT	0.5	1, 37	0.49	ns	Supp. 3b,c	n.a.
				Sex	12.46	1, 37	0.001	**		
				ELA	5.43	1, 37	0.03	*		
				GT x Sex	10.34	1, 37	0.003	**		
				GT x ELA	0.002	1, 37	0.97	ns		
				Sex x ELA	0.4	1, 37	0.53	ns		
				GT x Sex x ELA	0.41	1, 37	0.52	ns		
		P56 dDG	3-Way ANOVA	GT	0.1	1, 38	0.92	ns	Supp. 3d,e	n.a.
				Sex	1	1, 38	0.32	ns		
				ELA	0.38	1, 38	0.54	ns		
				GT x Sex	0.11	1, 38	0.74	ns		
				GT x ELA	3.39	1, 38	0.07	ns		
				Sex x ELA	0.18	1, 38	0.67	ns		
				GT x Sex x ELA	0.22	1, 38	0.64	ns		
Total microglia	n.a.	P35 Female vDG	2-Way ANOVA	GT x ELA	0.003	1, 20	0.96	ns	Supp. 4a	n.a.
				GT	2.77	1, 20	0.11	ns		
				ELA	0.0002	1, 20	0.99	ns		
		P35 Male vDG	2-Way ANOVA	GT x ELA	3.95	1, 17	0.06	ns	Supp. 4b	n.a.
				GT	0.00004	1, 17	1.00	ns		
				ELA	5.96	1, 17	0.03	*		
		P56 Female vDG	2-Way ANOVA	GT x ELA	0.05	1, 19	0.82	ns	Supp. 4c	n.a.
				GT	0.03	1, 19	0.86	ns		
				ELA	3.31	1, 19	0.08	ns		
		P56 Male vDG	2-Way ANOVA	GT x ELA	0.0007	1, 21	0.98	ns	Supp. 4d	n.a.
				GT	3.13	1, 21	0.09	ns		
				ELA	0.33	1, 21	0.57	ns		
		P35 Female dDG	2-Way ANOVA	GT x ELA	0.01	1, 20	0.91	ns	Supp. 4e	n.a.
				GT	0.54	1, 20	0.47	ns		
				ELA	0.05	1, 20	0.82	ns		
		P35 Male dDG	2-Way ANOVA	GT x ELA	6.03	1, 16	0.03	*	Supp. 4f	Tukey
				GT	6.24	1, 16	0.02	*		
				ELA	6.71	1, 16	0.02	*		
		P56 Female dDG	2-Way ANOVA	GT x ELA	0.07	1, 20	0.79	ns	Supp. 4g	n.a.
				GT	1.2	1, 20	0.29	ns		
				ELA	0.0004	1, 20	0.98	ns		
		P56 Male dDG	2-Way ANOVA	GT x ELA	0.91	1, 18	0.35	ns	Supp. 4h	n.a.
				GT	0.17	1, 18	0.69	ns		
				ELA	0.91	1, 18	0.35	ns		
		P35 vDG	3-Way ANOVA	GT	1.51	1, 37	0.23	ns	Supp. 4a,b	n.a.
				Sex	0.06	1, 37	0.80	ns		
				ELA	2.62	1, 37	0.11	ns		
				GT x Sex	1.5	1, 37	0.23	ns		
				GT x ELA	1.82	1, 37	0.19	ns		
				Sex x ELA	2.55	1, 37	0.12	ns		
				GT x Sex x ELA	1.61	1, 37	0.21	ns		
		P56 vDG	3-Way ANOVA	GT	1.47	1, 40	0.23	ns	Supp. 4c,d	n.a.
				Sex	5.15	1, 40	0.03	*		
				ELA	2.6	1, 40	0.11	ns		
				GT x Sex	2.07	1, 40	0.16	ns		
				GT x ELA	0.016	1, 40	0.90	ns		
				Sex x ELA	0.56	1, 40	0.46	ns		
				GT x Sex x ELA	0.03	1, 40	0.87	ns		
		P35 dDG	3-Way ANOVA	GT	2.82	1, 36	0.10	ns	Supp. 4e,f	n.a.
				Sex	0.03	1, 36	0.85	ns		
				ELA	1.68	1, 36	0.20	ns		
				GT x Sex	0.19	1, 36	0.66	ns		
				GT x ELA	0.89	1, 36	0.35	ns		
				Sex x ELA	0.81	1, 36	0.37	ns		
				GT x Sex x ELA	1.3	1, 36	0.26	ns		
		P56 dDG	3-Way ANOVA	GT	0.31	1, 38	0.58	ns	Supp. 4 g,h	n.a.
				Sex	1.85	1, 38	0.18	ns		
				ELA	0.41	1, 38	0.53	ns		
				GT x Sex	1.17	1, 38	0.29	ns		
				GT x ELA	0.18	1, 38	0.67	ns		
				Sex x ELA	0.37	1, 38	0.55	ns		
				GT x Sex x ELA	0.67	1, 38	0.42	ns		