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Adult neurogenesis augmentation attenuates anhedonia and HPA axis dysregulation in a mouse model of chronic stress and depression

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ABSTRACT

Major depressive disorder is a common debilitating mental health problem that represents one of the leading causes of disability. Up to date, the therapeutic targets and approaches are still limited. Adult hippocampal neurogenesis (AHN) has been proposed as a critical contributor to the pathophysiology and treatment of depression, altering the hippocampal control over stress response at network, neuroendocrine and behavioral levels. These findings together have suggested that manipulating AHN may be a promising therapeutic strategy for depression. To investigate this question, we assessed whether increasing adult neurogenesis would be sufficient to produce antidepressant-like effects at behavioral and neuroendocrine levels in a mouse model of depression; the unpredictable chronic mild stress (UCMS). For this purpose, we used a bi-transgenic mouse line (iBax) in which AHN increase was induced by deletion of the pro-apoptotic gene Bax from the neural progenitors following the tamoxifen-dependent action of CreERT2 recombinases. UCMS induced a syndrome that is reminiscent of depression-like states, including anhedonia (cookie test), physical changes (coat deterioration, reduced weight gain), anxiety-like behaviors (higher latency in the novelty-suppressed feeding –NSF– test), passive stress-coping behaviors (immobility in the forced swim test –FST–) and a blunted hypothalamo-pituitary-adrenal (HPA) axis reactivity to acute stress in addition to AHN decrease. Tamoxifen injection reversed the AHN decrease as well as partly counteracted UCMS effects on the cookie test and HPA axis but not for the coat state, weight gain, NSF test and FST. Taken together, our results suggest that a strategy directing at increasing AHN may be able to alleviate some depression-related behavioral and neuroendocrine dimensions of UCMS, such as anhedonia and HPA axis reactivity deficits, but may be hardly sufficient to produce a complete recovery.

Keywords : Stress, Adult Hippocampal Neurogenesis, Depression, Anhedonia, Glucocorticoids, HPA axis.

Introduction

Major depressive disorder (MDD) is a chronic psychiatric condition associated with a large range of symptoms including mood disturbance, anhedonia, sleep abnormalities, weight changes, cognitive abnormalities and multifaceted psychological distress. MDD represents a widespread, debilitating health problem and one of the leading causes of disability worldwide. Whereas pharmacological interventions can be beneficial for patients, many of them are resistant to the current treatments. Unfortunately, decades of research and drug development have not brought out more effective therapeutic strategies to treat MDD. Therefore, it is crucial to characterize better the neurobiological mechanisms underlying MDD and to identify novel targets that could improve recovery.

Several studies pointed toward the hippocampus as one of the corticolimbic structures that is critically involved in anxiety- and depression-related disorders (Seminowicz et al., 2004). For instance, hippocampal volume has been revealed to be reduced in correlation with the total duration of MDD episodes (Bremner et al., 2000). These effects are reminiscent to the morphological changes found in a subregion of the hippocampus, the dentate gyrus (DG), of mice exposed to chronic stress and to models of depression (Datson et al., 2012; David et al., 2009; Surget et al., 2008).

Stress-related neuroplastic alterations have been suggested to support the hippocampus' morphological changes found in MDD patients as well as in animal models of depression (Sapolsky, 2000). A remarkable form of neuroplasticity that is robustly affected by stress is the adult hippocampal neurogenesis (AHN). It consists in the addition of adult-generated neurons into the DG of the adult brain. Chronic stress substantially downregulates AHN in various mammal species (Bond et al., 2015; Eliwa et al., 2017), affecting both the early phase of neurogenesis (cell proliferation) and later stages like neuronal survival and integration into the DG circuitry. Such stress-induced AHN downregulations are counteracted by chronic antidepressants, which are able to promote neural progenitor proliferations as well as maturation, integration and survival of adult-generated neurons (Czeh et al., 2001; Santarelli, 2003; Wang et al., 2008). Evidence for a role of AHN in MDD and antidepressant effects may not only be correlative, but also causative (Anacker et al., 2018; Surget et al., 2011). Using experimental methods to inhibit adult neurogenesis in rodent models, several authors have indeed demonstrated that AHN may be necessary for behavioral effects of antidepressants (David et al., 2009; Surget et al., 2008). However, if the role of AHN in antidepressant effects has been shown to be crucial, it seems not to be the case in MDD etiology and pathophysiology as neurogenesis inhibition hardly provokes depression-like syndromes or makes rodents more vulnerable to chronic stress (Bessa et al., 2009; David et al., 2009; Surget et al., 2008).

Questioning the functional relevance of AHN in the therapeutic effects of antidepressants, we previously demonstrated that antidepressants promote and recruit adult-generated hippocampal neurons to restore, in chronic stress conditions, an operative control on stress response, and particularly at the neuroendocrine level on the hypothalamo-pituitary-adrenal (HPA) axis (Surget et al., 2011). Indeed, the hippocampus is a modulator of HPA axis activity (Herman et al., 1989), able to trigger negative feedback loops on glucocorticoid

releases (Mizoguchi et al., 2003). As MDD patients, rodents under chronic stress can display HPA axis abnormalities including disrupted negative feedback, while antidepressant-induced recovery is paralleled with the normalisation of HPA axis activity. Interestingly, this normalization has been shown to be dependent on AHN in animal models of depression, suggesting that adult-generated neurons strengthen hippocampal regulation of stress response (Snyder et al., 2011; Surget et al., 2011)

Accordingly, all these studies together established that AHN is necessary for the therapeutic effects of antidepressants and for the operative control of hippocampus over HPA axis and stress response, identifying AHN as a promising target for developing novel therapeutic strategies in MDD. However, is increasing hippocampal neurogenesis alone sufficient to mimic antidepressant effects and to reinforce the hippocampal control on stress response? A couple of studies in mouse tried to approach the question but did not provide persuasive responses: highlighting only some protective effects against chronic corticosterone treatment (Hill et al., 2015) and a marginal positive effects on coat state along with paradoxical effects after chronic stress (Culig et al., 2017). Accordingly, it is crucial to provide new, more precise answers to these questions in order to assess the appropriateness of drug developments in this direction.

The current study aimed therefore at investigating whether increasing AHN would be sufficient (1) to counteract the different symptomatic dimensions of a depression-like syndrome produced by a 8-week unpredictable chronic mild stress (UCMS), a validated mouse model of depression (Surget and Belzung, 2008), and (2) to improve behavioral and neuroendocrine (i.e. HPA axis) reactivity to stress conditions. For this purpose, we used a bitransgenic mouse line (i.e. iBax) allowing us to increase adult neurogenesis in an inducible manner following tamoxifen injections (Sahay et al. 2011). The iBax mice hold two floxed alleles of the *Bax* gene as well as one *CreERT2* allele (i.e. a Cre recombinase (Cre) fused to a mutant estrogen ligand-binding domain (ERT2) requiring tamoxifen for activity) under the control of the Nestin promoter, which is selectively active in neural stem cells and progenitors in adult mice. The protein Bax is a pro-apoptotic factor that can form oligomeric pores in the mitochondrial outer membrane leading to its permeabilisation and the release of cytochrome C (Dewson and Kluck, 2009; Westphal et al., 2014a, 2014b). Within the cytoplasm, the cytochrome C can activate caspase-3 leading to cell death (Westphal et al., 2014b; Zhang et al., 2004). Accordingly, this genetic construction enables to inducibly delete Bax gene in neural progenitors at the time of tamoxifen injection (i.e. UCMS week 3) and, from then onward, to promote the survival of the newly-generated neurons. Depression- and anxiety-related behaviors were evaluated in addition to the immunohistochemical estimation of AHN. At the neuroendocrine level, plasma corticosterone was assessed following an acute stress (or not). Here, we found that increasing AHN dampened depression-related effects of UCMS on behavioral and neuroendocrine levels but was hardly sufficient to induce a complete recovery.

Materials and Methods

A more detailed description is provided in Supplementary Methods.

Animals

Experiments were conducted on 140 male *iBax* mice (mice carrying two loxP-flanked *Bax* alleles; *Bax^{ff}* and one *Nes-CreERT2* allele) aged 2-4 months at the beginning of the experiment. These *iBax* mice were generated as previously mentioned by Sahay et al., 2011 and were maintained on a mixed C57BL/6 and 129/SvEv background. All animals were housed under standard conditions (22±2 °C, 12h-light/dark cycle with lights on at 20h30, food and water ad libitum). All the animals caring and experiments were conducted following the European Commission Council directive 2010/63/EU and approved by local ethical committee and French authorities.

Experimental design (fig.1a)

Animals were divided into four experimental groups: non-stressed vehicle (NS/Veh), non-stressed tamoxifen (NS/TAM), UCMS vehicle (UCMS/Veh), UCMS tamoxifen (UCMS/TAM) (34-35 per group in total). UCMS mice were daily exposed to various mild socio-environmental stressors in an unpredictable schedule for a complete period of 8 weeks. UCMS mice were isolated in individual cages (24x11x12cm) while non-stressed mice were housed together in a separate room, 6-8 mice per cage (42x27x16cm) containing 2 tubes and 2 shelters. At the third week, animals were administered intraperitoneally (ip) with tamoxifen (55 mg/kg) or vehicle (corn oil) for five consecutive days. At the fourth week, mice received intraperitoneal injections of BrdU (150 mg/kg) twice a day for 4 days. At the 7th week, mice were randomly divided into two cohorts, both containing mice from the four experimental groups. Mice from the 1st cohort went through a battery of behavioral tests: cookie test, Novelty-Suppressed Feeding (NSF) test, splash test, light/dark box test, and tail suspension test (TST) (fig. 1a). The 2nd cohort was used to investigate the effects of UCMS and AHN increase on neuroendocrine and behavioral reactivity to acute stress. During the 8th week, half of the mice were exposed to the Forced Swim Test (FST) as a source of acute stress while the other half was maintained in home cage. Either in the home cage or 45 or 90 minutes after the acute stress, blood samples (≈200 µl) were collected from submandibular vein and used to measure plasma corticosterone (CORT) levels. This procedure was repeated 4 days later, but inverting “home cage” and “swim stress” conditions for each mouse. Finally, mice underwent NSF test at the ninth week, with half of the mice pre-exposed to TST test as a source of acute stress immediately before performing the NSF test while the other half were kept in the home cage. All the mice were finally euthanised on the 9th week by transcardiac perfusion following deep anaesthesia (pentobarbital, 40 mg/kg ip).

UCMS

The UCMS procedure was performed as previously described (Surget and Belzung, 2008). UCMS mice were repeatedly exposed daily to various forms of socio-environmental stressors of mild intensity in an unpredictable schedule, including different alterations of

cage beddings (wet saw dust, saw dust change or removal, replacement of saw dust with 22 °C water), social stress (mouse placed in the cage of another mouse), cage tilting by 45°, restraint stress in small tubes (L: 6.5 cm, d: 3.7 cm), and altered durations of light/dark cycle. At the beginning of each week, coat state and weight were measured. The deterioration of coat state was measured as previously described (Nollet et al, 2013). As the coat state depends on the frequency and extent of grooming behavior, it is considered to be a measure of the animal's motivation toward self-centered activities (Nollet et al, 2013). The coat state might vary from smooth and clear in normal, control animals to bristling with spikes in those subjects that are most affected by the stressors. Hence, the fur of the mice was weekly assessed by two experimenters blind of the genotype, treatment and environmental conditions of each animal. Each experimenter examined independently seven different parts of the animal's body: head, neck, back, down back, tail, abdomen, and paws, and scored the coat state for each area as follows: 0 (good) for smooth and shiny fur, with no tousled or spiky patches; 0.5 (moderate) for slightly fluffy fur with some spiky patches; 1 (bad) for fluffy fur with slight staining. The final score obtained represents the sum of the scores of all the seven body parts.

Cookie test

Cookie test was performed as previously described (Surget et al., 2011) to evaluate the motivation for a palatable reward (butter cookie, Saint-Michel). The apparatus consisted of three aligned compartments of same dimensions (20x20x20 cm) and connected by two openings: compartment 1 – opening – compartment 2 – opening – compartment 3. Only the colours of the walls and the floor were different between the compartments; white for the first one, grey for the second one, and black for the third one. At the start, the mouse was always introduced in compartment 1 with its head facing the wall opposite to the opening that leads to compartment 2, while the piece of cookie ($1g \pm 1$) was always placed at the other end of the device: in the center of compartment 3.

Each session lasted for 5 min under red light. At each session, the 1st door was closed directly after the complete passage of the mouse into compartment 2 (maximum time for reaching the second compartment: 2 minutes – if not, the mouse was thereafter gently directed to compartment 2 and the door closed). Latencies to reach the second and third compartment as well as the latency to smell the cookie and the latency to bite the cookie were recorded.

The test consisted of 4 sessions, each separated by 3 days. Each session was always performed during the second half of the dark phase (starting from 14h00). On the day of testing, mice were food-deprived for 1 hour before the onset of the test. Ten days before the first session, all the mice were familiarized with the cookie by introducing a quarter of cookie in the home cage. The familiarisation was pursued every 2 days for 10 days before the start of the test.

Novelty Suppressed Feeding test (NSF)

As formerly described (Surget et al., 2009), mice were food-deprived in their home cage for 12h before the test. The apparatus consisted of a squared arena (30x30x30cm) with floor

covered with 2 cm sawdust illuminated by indirect red light. At the time of testing, a single regular pellet was placed on a square white paper (2x2cm) in the centre of the arena. The mouse was placed in the corner of the arena with the face directed to the corner and then allowed to freely move. The latency to start to eat the pellet was recorded (up to 5 min). Once the mouse started to bite the pellet, the mouse was removed from the arena, placed into home cage and allowed to eat the pellet. The quantity of the pellet eaten for 5 min (home food consumption) was recorded.

Splash test

This test was conducted as previously described (Surget et al, 2008). Non-stressed mice were individually isolated in cages 2h before testing. Mouse received two splashes of 10 % sucrose solution on his back fur in its home cage under red light. After the spraying, the grooming duration was recorded for 5 minutes.

Light-dark box

As previously described (Culig et al., 2017), the device was composed of a couple of boxes (20x20x15cm), connected by an opaque tunnel (5.5x6.5x10cm) which allowed the mouse to transfer freely between the two boxes. Mice were introduced first into the lit box, and the total duration the mouse spent in the lit box was recorded for 5 min.

Forced swim test (FST)

Mice were introduced inside a plexiglass cylinder (height: 40cm; diameter: 18cm) filled with 22 °C water with no escape for 5 minutes. The total immobility duration was recorded.

Tail suspension test (TST)

Mice were suspended by their tails to a metal rod 60 cm above the floor. The test was conducted for total 6 min recording the overall immobility duration.

Corticosterone levels

Blood was centrifugated 15 min later after collection (5000 rpm for 10 min) to extract plasma. Then plasma was stored in -20 °C until analysed for total corticosterone levels using an Enzyme-Linked ImmunoSorbent Assay (ELISA) Kit (Enzo Life Sciences (ELS) AG, Switzerland).

Immunohistochemistry

Animals were deeply anaesthetized using sodium pentobarbital, 40 mg/kg i.p., and transcardially perfused with 50 ml heparinized saline (0.9% sodium chloride, 1000 IU heparine; 10 mg/ml) for 4 minutes, followed by 100 ml 4% paraformaldehyde (PFA) in 0.1 M phosphate-buffer (PB, pH=7.4). Brains were collected and post-fixed for 24 h in 4% PFA/ 0.1 M PB and then cryoprotected in 10 % sucrose/0.1 M PB-saline (PBS, pH=7.4) at 4 °C for 24h, then in 20% sucrose/0.1 M PBS at 4 °C for 2 nights, and freezed in cold isopentane and stored at -80 °C.

For BrdU labelling, one out six series of brain sections (40 μ m) were mounted on slides. The detailed immunohistochemical procedure is provided in Supplementary Methods. Briefly, brain sections were subjected to: (1) heat antigen retrieval in citrate buffer (10mM pH=6) for 4-6 min at 90-95 °C, (2) 2N HCl for 1h at 37 °C followed by 0.1 M sodium borate buffer (pH=8.5, 25min), (3) 0.3% H₂O₂ in 1/1 PBS:Ethanol for 30min, (4) primary antibody (1/1000, rat monoclonal anti-BrdU, abcam ab6326) at 4 °C for 60h, (5) secondary antibody (1/200, biotinylated donkey anti-rat, Jackson ImmunoResearch, 712-066-150) for 2h, (6) avidin-biotin complex (ABC) kit (Vector Labs) for 3h at RT, and (7) staining using 3,3'-Diaminobenzidine Peroxidase Substrate (DAB) (SIGMAFAST™ DAB with metal enhancer, SLBP7387V).

BrdU+ cells in the SGZ and the GCL of DG (from bregma -0.94 mm to -3.88 mm) were counted bilaterally along the septotemporal axis (13-15 sections per animal) using a light microscope (Leica DM 2000) under x10 and x20 magnification.

Statistics

All data were analysed using ANOVA or general linear models (GLM). GLM analysis were undergone for physical measures (coat state, body weight change) and cookie test using: 'environment' (NS, UCMS) and 'treatment' (Veh, TAM) as categorical factors, as well as 'weeks of UCMS' (for physical measures) or 'sessions' (for cookie test) as repeated-measures. The behavioral tests and BrdU+ counting were analysed by factorial ANOVAs using two categorical factors: 'environment' (NS, UCMS) and 'treatment' (Veh, TAM). In case of significant effects ($p < 0.05$), Tukey posthoc tests were applied. All results are presented as mean \pm SEM unless otherwise indicated. Effect sizes are presented as partial η^2 et Hedge's g . Detailed statistical results are provided in supplementary Statistics.

Results

UCMS reduced adult hippocampal neurogenesis, an effect reversed by *Bax* gene deletion in neural progenitors

Our procedure allowed us to label adult-generated granule cells aged from 35 days to 40 days (fig. 1a). We observed a significant decrease in the number of BrdU-labeled cells in the dentate gyrus of mice exposed to UCMS (environment: $F_{1,64}=18.873$, $p<0.001$, $\eta^2=0.228$) (fig. 1b). Indeed, the UCMS/Veh mice exhibited a 33% reduction of BrdU-labeled cells (81.94 ± 4.26) compared to NS/Veh mice (122.59 ± 8.90 ; Hedge's $g=1.034$). By contrast, UCMS/TAM mice exhibited 21% reduction of BrdU+ cells (112.35 ± 9.9) compared to NS/TAM (142.06 ± 8.20 ; Hedge's $g=0.756$) (fig. 1c). We also found a significant effect of tamoxifen treatment ($F_{1,64}=9.487$, $p=0.003$, $\eta^2=0.129$), resulting in 1.16-fold (15.9%) and 1.37-fold (37.1%) of AHN increase in NS/TAM (Hedge's $g=0.496$) and UCMS/Tam (Hedge's $g=0.774$) mice respectively (fig. 1c). Therefore, our study recapitulated previous results showing a significant AHN increase in tamoxifen-treated *iBax* mice (Sahay et al., 2011), with a higher effect size in chronically stressed mice (Culig et al., 2017) resulting in the reversal of UCMS-induced AHN decrease.

UCMS induced a “depression-like” syndrome that is partially alleviated by *Bax* gene deletion in neural progenitors

The effects of UCMS and tamoxifen-induced AHN increase were probed through physical assessments (coat state and body weight) as well as a behavioral test battery assessing different depression-related dimensions: anhedonia (cookie test), anxiety-like (Light/Dark box, NSF test), altered self-directed (Splash test) and stress-coping (FST and TST) behaviors.

UCMS induced a significant, gradual deterioration of the coat state (week*environment: $F_{8,1064}=84.818$, $p<0.001$, $\eta^2=0.389$) all along the procedure starting to reach significance after two weeks of UCMS (fig. 2a). Body weight change, another physical readout, whose gradual increase was observed during the 8 weeks of UCMS (Fig. 2b). UCMS significantly reduced the weight gain ($F_{1,133} = 8.91$, $p=0.003$, $\eta^2=0.063$) obviously after the 3rd week, and gradually until the end of UCMS (week*environment: $F_{7,931}=26.698$, $p<0.001$, $\eta^2=0.167$). Tamoxifen treatment did not alter both variables and, specifically, was unable to reverse the UCMS effects (fig.2b), suggesting that reversing UCMS-induced AHN decrease alone was not sufficient to alleviate the physical consequences of UCMS exposure.

The cookie test was aimed to assess reward-related behaviors in cohort 1 (fig.1a), as a proxy for assessing anhedonia (a core symptom of depression) in UCMS. Our results revealed a significant reduction of the latency to eat the cookie over the sessions for all the groups (sessions: $F_{3,165}=20.342$, $p<0.001$, $\eta^2=0.270$). Moreover, a significant interaction between environment and treatment was found ($F_{1,55}=4.093$, $p=0.048$, $\eta^2=0.069$) in relation to a higher latency to eat the cookie in UCMS/veh mice all along the 4 sessions, emphasizing possible anhedonia-like effects of UCMS (fig. 2c). Indeed, this effect cannot be explained by changes in locomotion or exploratory behavior as no difference between groups was found for the latency to enter into the first and second chambers at each session (supplementary statistics) and to smell the cookie (fig.2d). The increased latency to eat the cookie was only

found in UCMS/veh mice, as tamoxifen-treated UCMS mice did not exhibit any difference with NS mice (fig.2c). These results indicate therefore that reversing UCMS-induced AHN decrease alone might help in alleviating the “anhedonia-like” symptoms induced by UCMS exposure.

Acute stress-coping behavioral strategy was evaluated in both FST (cohort 2) and TST (cohorts 1 and 2) (fig. 1a). These tests are widely used as bioassays for antidepressant-like effects that are commonly associated with high active behaviors and less immobility. Here we found a significant UCMS effect in the FST (environment: $F_{1,75}=19.790$, $p<0.001$, $\eta^2=0.209$), resulting in higher immobility in both groups of UCMS mice (vehicle and tamoxifen), an effect that did not reach significance in the TST. Tamoxifen had no effect on both FST and TST. These results indicate that reversing UCMS-induced AHN decrease alone was not sufficient to alter the changes in stress-coping behaviors induced by UCMS exposure in the FST.

Anxiety-related behaviors were first evaluated in the Light/Dark test, where an increased time passed in the lit compartment is commonly interpreted as lower anxiety-related behaviors. Our results revealed no significant effects of either UCMS or treatment in Light/Dark test (fig. 3a). In the same vein, no significant differences were found in the Splash test, a test assessing self-directed grooming behaviors (fig. 3b).

We also used the NSF test as (1) it assesses a different anxiety-related dimension, based on conflicting motivational drives (drive for eating a food pellet versus avoidance of the center area of a novel arena), (2) it is sensitive to chronic antidepressant treatments (i.e. resulting in a reduced latency to eat), and (3) it has been shown to be neurogenesis-sensitive (as AHN inhibition prevents antidepressant effects in this test) particularly in stress contexts (as AHN inhibition potentiates the influence of a preceding acute stress). We therefore carried out the NSF test in two conditions: a first condition where the NSF test was performed alone, without any particular interventions preceding the test, and a second condition where mice were exposed to the NSF test immediately following the TST in order to assess whether increased neurogenesis might modulate acute stress behavioral sensitivity.

Our results showed that UCMS increased the latency to eat in the NSF test ($F_{1,95}=19.333$, $p<0.001$, $\eta^2=0.169$), an effect that was not reversed by tamoxifen treatment (fig. 3c). As the test is based on conflicting motivation, the drive for food must be controlled in case a higher latency to eat would be explained by a lower hunger. For this purpose, home food consumption was examined for 5 minutes immediately after the NSF test. A significant environment effect was found ($F_{1,95}=8.881$, $p=0.004$, $\eta^2=0.085$). However, this effect was associated with a larger quantity of food consumed by UCMS mice, indicating that the larger latency to eat in the NSF test found in UCMS mice was not caused by a reduced motivation for food. Tamoxifen treatment also affected home food consumption ($F_{1,95}=5.394$, $p=0.022$, $\eta^2=0.054$), resulting in lower food consumption, a potential confounding factor that might have impeded tamoxifen reversing UCMS effects in the NSF test.

On the other hand, when the mice were pre-exposed to a source of acute stress (6'-TST) before performing the NSF test, we found no impact of neither UCMS, nor treatment, nor

their interaction on the latency to eat (fig. 3e). However, we found an effect of environment ($F_{1,32}=11.675$, $p=0.002$, $\eta^2=0.267$), but not treatment on home food consumption (fig. 3f). It resulted in a higher drive for food in home cage for UCMS mice. This may be of importance as it represents a potential confounding factor for interpreting the latency of the prestressed mice in the NSF test, concealing potential UCMS-induced increased latency in the pre-stress condition.

UCMS altered HPA axis reactivity to acute stress, an effect partially counteracted by *Bax* gene deletion in neural progenitors

To assess the neuroendocrine reactivity to acute stress, animals were either kept into their home cage or exposed to an acute swim stress (5-min FST). Blood was then collected in 3 different conditions: 45' or 90' following the swim stress or directly into the home cage (fig. 4a). This procedure allowed us to assess plasma corticosterone concentrations at baseline as well as for peak secretion (45' post-stress) and recovery capabilities (90' post-stress), representing different stages of HPA axis reactivity.

Our results revealed significant effects of environment, conditions and their interaction ($F_{1,140}=18.993$, $p<0.001$, $\eta^2=0.119$; $F_{2,140}=52.823$, $p<0.001$, $\eta^2=0.430$; $F_{2,140}=6.496$, $p=0.002$, $\eta^2=0.085$ respectively) (fig. 4b, 4c). Indeed, acute stress induced a strong upsurge of plasma corticosterone 45' following the FST (fig. 4c, 4d), but with a differential effect according to the conditions. Indeed, the corticosterone upsurge was mitigated in UCMS mice compared to NS mice, indicating that UCMS exposure blunted corticosterone release to acute stress. This effect was particularly pronounced for UCMS/Veh mice that exhibited significantly lower corticosterone levels at 45' post-stress (fig. 4b, 4c) and a lower upsurge when compared to other groups (fig. 4d), while UCMS/TAM mice did not display any difference with NS mice, indicating that reversing UCMS-induced neurogenesis decrease alone might alleviate UCMS-induced alterations of HPA reactivity to acute stress. No difference in corticosterone levels between baseline and 90' post-stress was observed for all groups (fig. 4e).

Finally, we also measured the corticosterone peak decrease between 45' and 90' after acute stress ($\Delta\text{CORT } 90'-45'$) as a proxy for HPA negative feedback effectiveness (fig. 4f). Our results showed that the peak decrease was of higher magnitude in NS/Veh, NS/TAM and UCMS/TAM compared to UCMS/Veh, suggesting a lower negative feedback effectiveness in the latter group, although this observation may have been distorted by the lower corticosterone upsurge at 45' post-stress.

Discussion

Our study aimed at investigating whether increasing AHN would be sufficient to (1) reverse the different symptomatic dimensions of a depression-like syndrome elicited by UCMS, and (2) improve the behavioral and neuroendocrine response to stress. Using a transgenic approach, our results showed that increasing AHN alleviated the UCMS-induced anhedonia-like behavior together with the blunted reactivity of HPA axis to acute stress, although it did not correct UCMS-associated physical indicators, anxiety-related behaviors and stress-coping strategies. Taken together, our study indicates that, if increasing AHN may be able to partially relieve some relevant depression-related effects of chronic stress (UCMS) at behavioral and neuroendocrine levels, it might be hardly sufficient to yield to a complete recovery of a MDD-like syndrome.

Increasing adult neurogenesis alleviates anhedonia-like behaviors in chronically stressed mice

To investigate the therapeutic benefits of increasing AHN in the UCMS model, we used the transgenic mice iBax where the survival of adult-generated neurons can be promoted following tamoxifen injections. Exposing vehicle-treated iBax mice to an 8-week UCMS led to a significant reduction of newly-generated neurons in the DG (BrdU+ cells aged between 5 and 6 weeks) while treating UCMS-exposed mice with tamoxifen normalized the number of newly-generated neurons to levels observed in control non-stressed mice. These findings recapitulate previous results that labelled doublecortin-positive immature neurons in iBax (Culig et al., 2017). Because the reliability of doublecortin as a marker of AHN has recently been questioned (Mendez-David et al., 2020), our study provides new, stronger evidence of the capability of Bax deletion in neural progenitors to counteract the UCMS-induced reduction of AHN. On the other hand, the AHN increase observed in the tamoxifen-treated non-stressed mice (1.16-fold increase) appeared non-significant and lower than the magnitudes found in two previous studies (3-4 fold increases) (Hill et al., 2015; Sahay et al., 2011) although others found no significant difference (Culig et al., 2017). This discrepancy might be attributed to the lower dose of tamoxifen used in our study (55mg/kg vs 75mg/kg), to the shorter duration between tamoxifen injection and brain collection (5-6 weeks vs 6-8 weeks), or to a higher enrichment of the control conditions in our study (more cage tubes, shelters and area per animal) that might have promoted a higher survival rate in our non-stressed mice (Praag et al., 2000). At least we were able in this way to reproduce the antidepressants' property to reverse the AHN reduction selectively in chronically stressed mice. Interestingly, the magnitude of tamoxifen-induced reversal was similar to the one found with antidepressant treatments in UCMS (Surget et al., 2011), allowing us to test whether the normalization of AHN was sufficient to induce therapeutic effects in the UCMS model, without altering the other pathways that usually go along with antidepressant action (e.g. monoaminergic neurotransmitter systems among others).

Our results have shown that exposing iBax mice to an 8-week UCMS regimen elicits a depression-like syndrome comprising different physical and behavioral dimensions such as deterioration of coat state, attenuation of weight gain, anhedonia-like behaviors (cookie test), passive stress-coping strategy (FST), and anxiety-like behaviors (NSF test). These

findings are consistent with previous UCMS studies (Ibarguen-Vargas et al., 2008; Nollet et al., 2019), which demonstrated that UCMS can yield to a wide pattern of alterations recapitulating several symptomatic features of the MDD syndrome, including anhedonia, changes in appetite and weight, sleep disturbances, increased anxiety behaviors, altered stress-coping responses at the behavioral and physiological levels (Nollet et al., 2019; Planchez et al., 2020; Surget and Belzung, 2008). In such studies, chronic antidepressant treatments reverse most of the physical and behavioral alterations induced by UCMS. Accordingly, it makes this model suitable to determine the therapeutic potential of neurogenesis augmentation strategy and particularly whether the spectrum of action of this strategy can encompass the full syndrome evoked by UCMS or is limited to specific endophenotypes. In our study, tamoxifen treatment was able to prevent the UCMS-induced increased latency to eat in the cookie test but let the other symptomatic manifestations unchanged (i.e. for coat state, body weight, FST and NSF test). Accordingly, reversing UCMS-induced AHN decrease might help alleviating the “anhedonia-like” symptom although not sufficient to reverse the full syndrome evoked by UCMS. This result is consistent with previous studies that used neurogenesis ablation and showed that AHN is required for some but not all antidepressant effects, suggesting that the recovery from some depression-related alterations goes through neurogenesis-dependent mechanisms while recovery from others goes through neurogenesis-independent pathways (David et al., 2009; Surget et al., 2008).

The antidepressant-like effects of AHN augmentation under stress conditions may, therefore, be restricted to selective endophenotypes including anhedonia as shown by our study. Indeed, the cookie test (also called reward maze test, Surget and Belzung, 2008) is meant to probe the mouse’s motivational drive for a palatable reward (i.e. cookie). Any deficit or reduction in this drive, emphasized by a higher latency to start eating the cookie or by a lower consumption, is commonly interpreted as anhedonia (Isingrini et al., 2010). However, changes in the motivational drive just as much as the exploratory drive and habituation to the experimental device might well explain differences for latency to eat or for cookie consumption too. Accordingly, we controlled exploration and habituation over sessions by measuring the latency to reach each chamber of the device and to smell the cookie in each session. As we reported no group differences on these variables (Supplementary statistics), the higher latency to eat observed in UCMS/Veh mice has been considered to reflect anhedonia-like behaviors. Interestingly, we previously found that AHN blockade prevents antidepressant effects in the cookie test (Surget et al., 2011), indicating that AHN may mediate anhedonia reversal by antidepressants. This is now supported by our current results, which even suggest that increasing AHN would be sufficient to reverse anhedonia elicited by chronic stress.

How to explain the capacity of AHN augmentation to reverse anhedonia?. Although hippocampal functions have long been associated with episodic memory and spatial navigation, there is growing evidence indicating a critical role of hippocampus in encoding goal value of locations and processing reward-related information (Poucet and Hok, 2017). Hippocampal principal cells exhibit spatially modulated activity, almost each active cell firing in particular location(s) of the environment, which delineates their place fields (O’Keefe and

Dostrovsky, 1971). These cells and their place fields are thought to carry collectively a representation of an allocentric spatial map of the environment and to provide individually animal's self-localization. Interestingly, it has been shown that place field distribution does not depend strictly on path-integration or sensory cues but can be biased and overrepresented at goal and reward locations (Hollup et al., 2001; Kobayashi et al., 2003). Goal locations and reward-related information seem therefore to be encoded by the hippocampus: dynamically at the population level, with fields accumulation and/or reorganization at goal and reward locations (Dupret et al., 2010; Hok et al., 2007); and at single-cell levels, with the presence of goal-direction cells and reward-associated cells (Gauthier and Tank, 2018; Sarel et al., 2017). Reward valuation itself may also be computed directly into the hippocampus or in conjunction with more specialized downstream brain areas like the Nucleus Accumbens (NAc), as it has been shown that hippocampal CA1 neurons projecting to NAc are more likely to be active near reward than CA1 neurons projecting to other areas (Ciocchi et al., 2015) and that the activity of their synapses to NAc neurons regulates reward behaviors (LeGates et al., 2018). The DG subregion within the hippocampus is also critically involved in goal and reward value encoding as its optogenetic activation enhances dopaminergic neurotransmission in the NAc (Tritschler et al., 2018), its lesion impairs goal-directed behaviors associated to reward value (Kirk et al., 2017) and it is engaged in the generation of novel reward representations (Woods et al., 2020). AHN continuously gives rise to adult-generated DG neurons that integrate hippocampal circuitry and therefore can potentially influence goal- and reward-value processing by actively modulating hippocampal network activity (Ikhar et al., 2013; Lacefield et al., 2012), by differentially gating related information coming from entorhinal cortex (Luna et al., 2019) and by actively promoting the encoding of novel representations (Danielson et al., 2016). As changes in AHN alter these functions, AHN augmentation may potentially contribute to shaping reward-related behaviors and correct anhedonia-like behaviors in chronic stress conditions as found in our study. This is in line with studies showing that AHN depletion is correlated with lower sucrose consumption in the sucrose preference test (Egeland et al., 2017; Snyder et al., 2011) and prevents antidepressant effects in the cookie test (Surget et al., 2011).

The other behavioral dimensions affected by UCMS included a higher immobility time in the FST and a higher latency to eat in the NSF test. These measures are commonly associated with increased passive stress coping and anxiety-like behaviors respectively, and are regarded as distinctive depression-related endophenotypes (Planchez et al., 2019). Both behaviors were not counteracted by tamoxifen in UCMS mice in our study suggesting that AHN augmentation alone is not sufficient to reverse these UCMS-related behavioral dimensions. Regarding the FST, this is consistent with previous findings showing that antidepressant effects in the FST are independent of AHN in mouse models of depression (David et al., 2009). By contrast, antidepressants have previously been shown to require AHN to induce their effects in the NSF test (Santarelli et al. 2003; Surget et al. 2008). Accordingly, AHN increase might be necessary but not sufficient to reverse stress effects in NSF test and that other mechanisms might be engaged concomitantly. It is worth mentioning that significant effects of UCMS were in the FST but not in the TST, while both

conceptually akin (i.e. the so-called “despair” tests) and represent tests for stress-coping behaviors (Porsolt et al., 2001; Steru et al., 1985). Several explanations may support FST-TST differences in our study. First, it is important to note that the direction of UCMS effects was similar in both tests with an increased immobility (fig. 2e, 2f); the effect size of the UCMS effects (Supplementary Statistics) was just more moderate in the TST (Hedge’s $g = 0.441$ NS-vehicle vs. UCMS-vehicle) than in the FST (Hedge’s $g = 0.989$ NS-vehicle vs. UCMS-vehicle), which causes not reaching statistical significance for TST, but indicates that the results were not fundamentally different between both tests. Second, mice were tested in the FST and TST at different time points of experiment schedule (fig. 1a): FST has been performed during the 8th week when socio-environmental stressors of the UCMS were still applied, while TST was carried out during the 9th week when stressors were not applied anymore, which also may have contributed to the lower effect size and lack of significance observed in TST. Third, despite their conceptual similarities, the FST and TST do not always display congruent results and sensitivities to experimental conditions, suggesting that neurobiological substrates mediating behavioral performances in both test are not identical (Bai et al., 2001; Bourin et al., 2005). In the same vein, we found UCMS effects in the NSF test but not in the L/D box, while both tests are considered to assess behavioral dimensions related to anxiety (Gould, 2009). However, it is well documented that animal performances in anxiety-related tests display high heterogeneities across studies as well as across paradigms and that these different paradigms assess diverse psychobiological dimensions of anxiety (Ramos, 2008; van Gaalen and Steckler, 2000). This is particularly right for the NSF test in the UCMS model *versus* other anxiety-related tests like the L/D box and the elevated plus-maze. Indeed, we previously found less consistencies in detecting UCMS effects on anxiety-like behaviors with L/D box, elevated plus maze or open-field than with the NSF test (Surget et Belzung 2008). This is corroborated by a recent study showing that the NSF test belongs to symptomatic-like dimensions that, together, strongly capture the most behavioral variance attributed to the UCMS model in contrast to other anxiety paradigms (Prevot et al., 2019).

Increasing adult neurogenesis improves HPA axis reactivity to acute stress in chronically stressed mice

Several lines of evidence have suggested that adult-generated DG neurons have a critical role in the hippocampal control of stress response at both behavioral and neuroendocrine levels. Indeed, AHN depletion has been shown to alter the responsiveness to acute stress resulting in an amplification of anxiety-like behaviors and of corticosterone releases (Snyder et al., 2011). Because chronic stress can also give rise to enhanced responsiveness to stress with elevated anxiety-like behaviors and corticosterone excess via a disrupted negative feedback of HPA axis (Surget et al., 2011), we aimed at testing whether AHN increase counteracts the effects of UCMS on stress response and improve reactivity to stress at behavioral and neuroendocrine levels.

The behavioral reactivity to stress was probed in the NSF test preceded by an acute pre-stress (i.e. 6’-TST). The significant differences existing between UCMS and control mice in the absence of pre-stress vanished when mice were exposed to a pre-stress, indicating that acute stress may differentially affect the subsequent behavioral response of control and

chronically stressed animals. However, our results revealed differences in the motivational drive to eat the cookie which may bias the latter interpretation. Considering our hypothesis, we did not find any effects of tamoxifen treatment in the latency to eat in the NSF test, suggesting that reversing UCMS-induced AHN decrease is not sufficient to affect the behavioral reactivity to acute stress in our conditions.

On the other hand, our results highlighted a tamoxifen-induced reversal of the UCMS-induced deficits in HPA axis reactivity to acute stress. Indeed, UCMS exposure significantly blunted the HPA axis reactivity to acute stress in iBax mice, highlighted by a reduced peak release at 45' post-stress as well as a minimal corticosterone decay between 45' and 90' suggestive of a dampened negative feedback, although the latter should be interpreted carefully as the lower corticosterone peak may mechanically reduce the decay. Interestingly, all these UCMS-evoked features were attenuated by tamoxifen treatment, indicating that reversing UCMS-induced neurogenesis decrease alone alleviates UCMS-induced alterations of HPA reactivity to acute stress. Our finding contrasts with results obtained by Hill and colleagues (2015), which used chronic corticosterone treatments to induce a depression-like state in iBax mice (i.e. CORT model). The iBax mice that were exposed to CORT model exhibited a blunted corticosterone release in response to a 30'-restraint stress, that was not reversed by tamoxifen-induced neurogenesis increase. However, this result may be misleading as this model is directly built on chronic supplementation of exogenous corticosterone, which yields to adrenal gland atrophy, corticotropin resistance and blunted CORT releases (David et al., 2009; Hernandez et al., 2013; Stanić et al., 2017). As a consequence HPA axis reactivity is mechanically depleted, with or without antidepressant treatments as the corticosterone supplementation is constantly maintained. These characteristics make this model unsuitable to probe endogenous HPA axis activity and responsiveness. By contrast, UCMS mimics in a naturalistic way the role of socio-environmental stressors to precipitate a depression-like neuropathology accompanied with HPA axis alterations that can be reversed by antidepressant treatments as observed in clinical conditions (Ising et al., 2007). These observations support the relevance of our results and the possibility that increasing AHN might help improve HPA axis reactivity under chronic stress and depression-like conditions.

Our time points for collecting blood were 45' and 90' after mice were exposed to the FST. The most likely is that 45' is still representative to the peak period of glucocorticoid response to acute stress, as glucocorticoid secretion generally peaks within 15'-60' following psychological stressors induced by threatening situations as the FST (Herman, 2013; Herman et al., 2016; Solomon et al., 2015). However, we cannot exclude the possibility of an earlier peak and that glucocorticoid concentration is starting its "falling" phase at 45'. Within this scenario, the reduced plasma corticosterone response to FST observed at 45' in UCMS-vehicle mice might be itself indicative of an increased negative feedback. The latter possibility would be in contrast with previous findings indicating that UCMS impairs HPA axis negative feedback, but would still indicate that UCMS alter HPA axis reactivity and negative feedback functionality and that these alterations may be attenuated by AHN increase.

Conclusion

In summary, our study indicates that a strategy directing at increasing AHN in chronically stressed mice is able to alleviate some depression-related behavioral and neuroendocrine dimensions of UCMS, such as anhedonia and HPA axis reactivity deficits, but may be hardly sufficient to produce a complete recovery. Our strategy did only reverse the AHN neurogenesis reduction induced by UCMS but did not result in a significant increase above baseline. Therefore it would be instructive to investigate whether the range of the beneficial effects of AHN augmentation is dependent on the magnitude of AHN augmentation and whether a strategy with higher effect size would be more beneficial. Finally, the augmentation strategy of the iBax model is based on promoting the survival of adult-generated neurons that are meant to be eliminated. Indeed, adult-generated neurons in the hippocampus engage into a selective survival or death in an input-specific, cell-dependent manner (Tashiro et al., 2006). If input activity is defective, neurons are stepped into programmed cell death. The iBax model by interfering with the pro-apoptotic pathway enforces the survival of adult-generated neurons intended to be eliminated, possibly less functional or integrated. Accordingly, it would be interesting to confirm our results using novel AHN augmentation strategy that does not share the same shortcoming.

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FIGURES

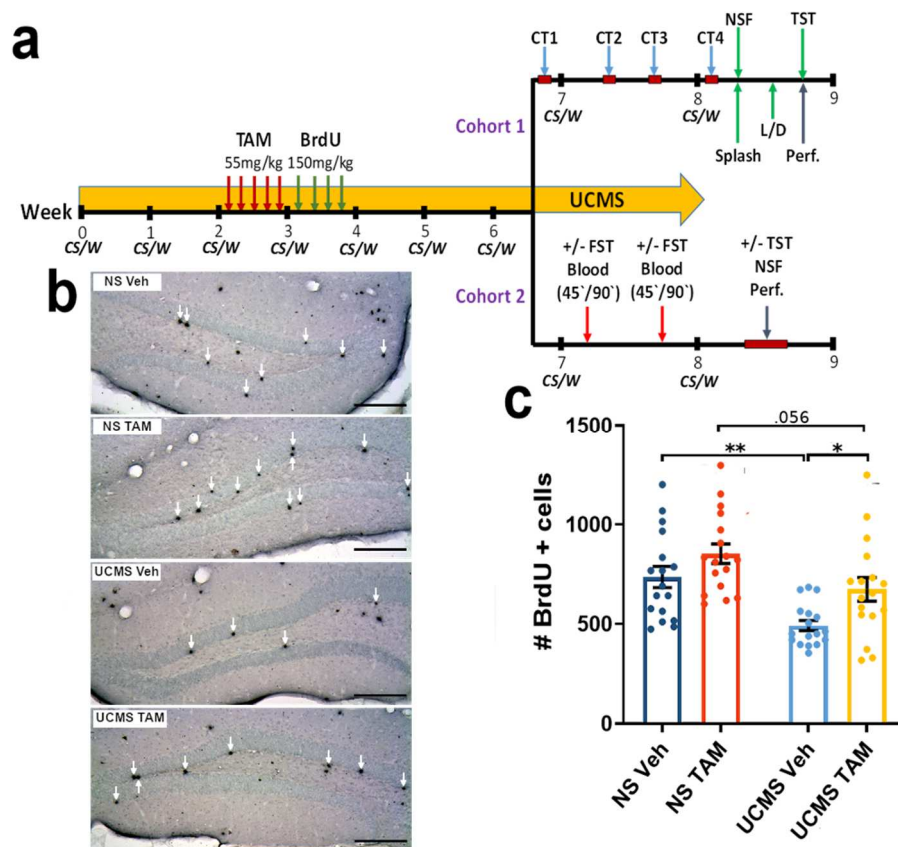


Figure 1. Experimental design and effects of UCMS and TAM on adult hippocampal neurogenesis of iBax mice. (a) Schematic representation of the experimental design of the study. Half of the mice were exposed to complete 8-weeks UCMS protocol or kept under control conditions. Coat state and weight were evaluated weekly. Either TAM (55 mg/kg, ip) or Veh was administered for five consecutive days at week 3. BrdU (150 mg/kg, ip) was administered twice per day for 4 days at week 4. From week 7, mice were randomly divided into two different cohorts allowing us to obtain different readouts: a battery of tests assessing different behavioural dimensions relevant to depression-like states for cohort 1, HPA axis and behavioural reactivity to acute stress for cohort 2. (b) A representative images of BrdU+ cells from the hippocampal dentate gyrus of the 4 different groups (scale bar, 100 μ m). (c) The result of total BrdU+ cell counting in the dentate gyrus. UCMS induced a significant decrease of the number of BrdU+ cells while TAM treatment increased this number in UCMS mice, Tukey's post-hoc: * $p < .05$, ** $p < .01$ ($n = 17$ mice/group). Abbreviations: CS, coat state; CT, cookie test; FST, forced swim test; NS, non-stressed; NSF, novelty-suppreseed feeding test; Perf., perfusion; TAM, tamoxifen; TST, tail suspension test; UCMS, unpredictable chronic mild stress; Veh, vehicle; W, weight assessment.

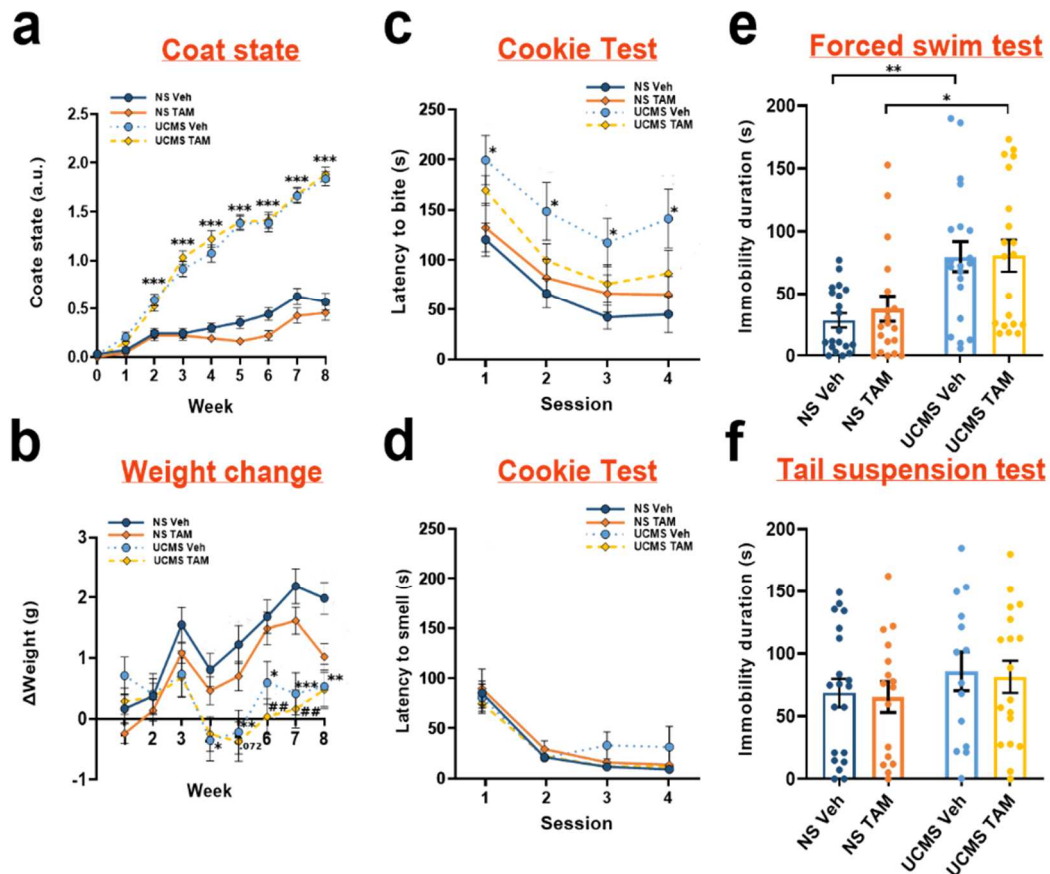


Figure 2: Effects of UCMS and TAM on physical measures, motivational behavior and stress-coping behavioral strategy. (a) UCMS induced a gradual deterioration of the coat state in both Veh- and TAM-treated iBax mice, highlighted by significant higher score from week 2, Tukey post-hoc: *** $p < .001$, UCMS versus NS ($n = 34-35$ mice/group). (b) UCMS induced a reduced body weight gains, reaching significance from week 4 to week 8 in Veh-treated mice (Tukey post-hoc * $p < .05$, ** $p < .01$, *** $P < .001$ versus NS Veh mice) and from week 5 to week 7 in TAM-treated mice (Tukey post-hoc $p = .072$, ## $p < .01$ versus NS TAM mice) ($n = 34-35$ mice/group). (c) Motivational behaviors for a palatable food were assessed through latency to smell and latency to bite a cookie in the cookie test in cohort 1. UCMS Veh mice exhibited a significantly higher latency in all the four sessions. Tukey post-hoc: * $p < .05$ ($n = 14-15$ mice/group). (d) Exploratory drive in the cookie test was controlled by measuring the latency to reach and smell the cookie in each session ($n = 14-15$ mice/group). Stress-coping behavioural strategies were assessed in (e) FST and (f) TST. UCMS promoted passive stress-coping behaviors in the FST, highlighted by significantly higher immobility duration in the FST in both Veh – and TAM treated mice: Tukey post-hoc * $p < .05$, ** $p < .01$ ($n = 19-20$). Significant differences in stress-coping behaviors were not found in the TST ($n = 16-20$ mice/group). Abbreviations: FST, forced swim test; NS, non-stressed; TAM, tamoxifen; TST, tail suspension test; UCMS, unpredictable chronic mild stress; Veh, vehicle.

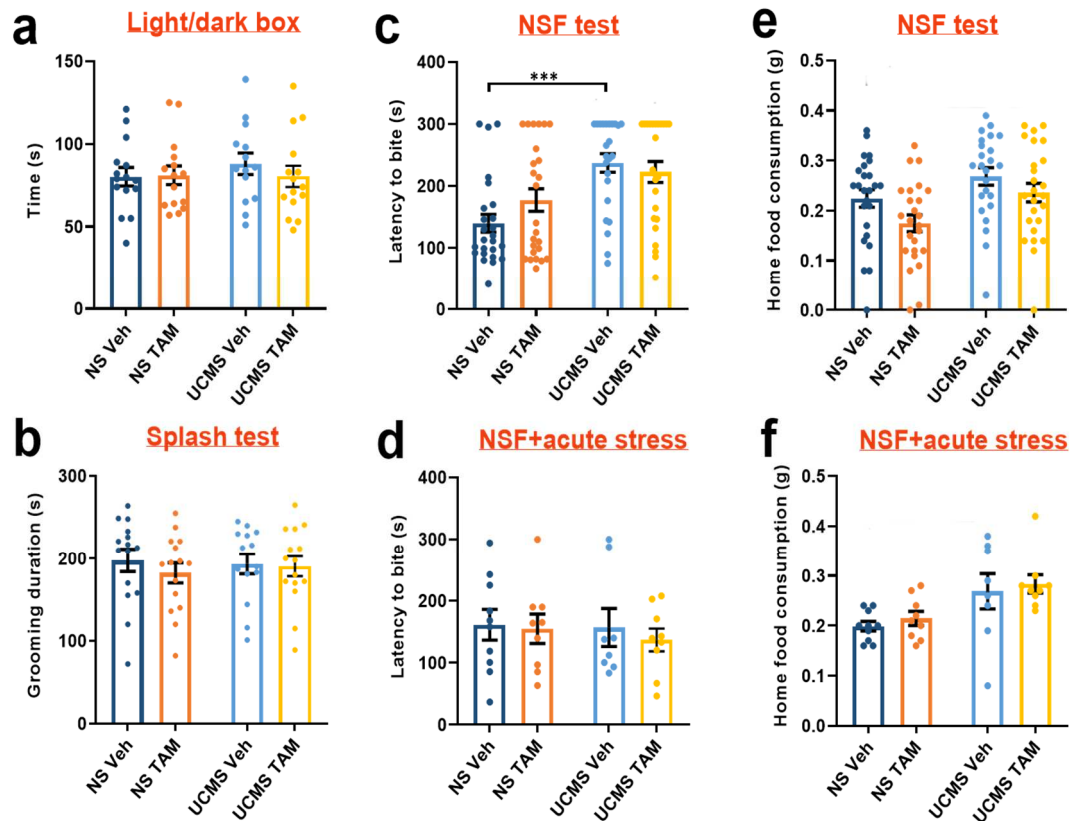


Figure 3: Effects of UCMS and TAM on anxiety-related behavioral tests. (a) Neither UCMS nor TAM had a significant effect on the time passed into the lit compartment of the light/dark box ($n=14-15$ mice/group). (b) Neither UCMS nor TAM had a significant effect on the total duration of grooming in the Splash test ($n=14-15$ mice/group). (c) UCMS induced a significant increase in the latency to bite the food pellet in the NSF test. No significant TAM effect was revealed (Tukey post-hoc: $***p < .001$, $n=24-25$ mice/group). (d) The UCMS effect in the NSF test was not apparent anymore when the test is preceded by an acute stress (TST) ($n= 8-10$ mice/group). (e) The UCMS effects on the latency to bite cannot be explained by changes in the motivational drive for regular food as no difference in home food consumption was detected in the 5 minute following the NSF test ($n=24-25$ mice/group). (f) No significant difference in home food consumption was found following the NSF test preceded by acute stress ($n= 8-10$, mice/group). Abbreviations: NS, non-stressed; NSF, novelty-suppressed feeding; TAM, tamoxifen; TST, tail suspension test; UCMS, unpredictable chronic mild stress; Veh, vehicle.

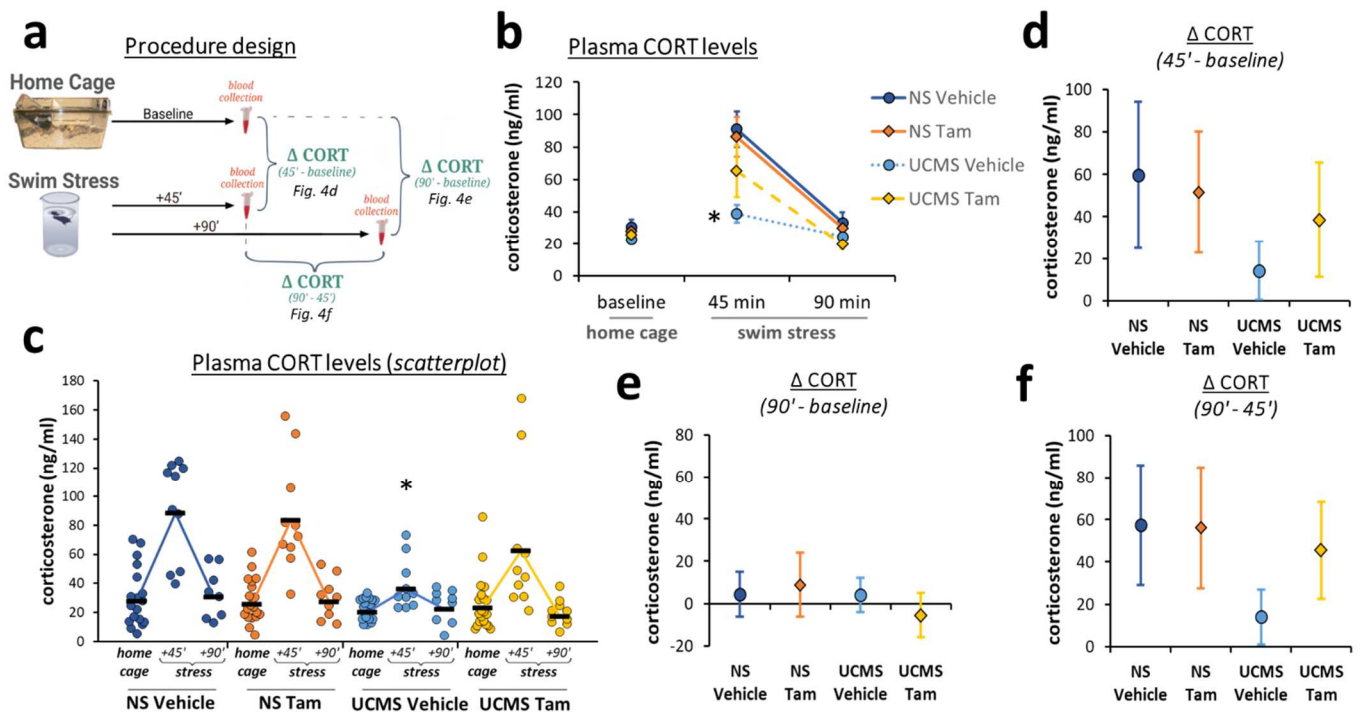


Figure 4: Effects of UCMS and TAM on HPA axis reactivity to acute swim stress. (a) iBax mice from cohort 2 were either exposed or not to 5-min swim stress (FST) as a source of acute stress, blood was collected 45' or 90' minute following FST or from home cage for baseline ($n = 18-20$ mice/group). (b) Effects of UCMS and TAM treatment on plasma corticosterone (CORT) levels (ng/ml) under baseline (home cage) as well as 45' and 90' following swim stress. UCMS induced a significant lower CORT peak at 45' compared to NS mice (Hedge's $g = 1.269$). Tukey post-hoc: $*p < .05$ UCMS vehicle vs. NS Vehicle. This effect was only found in Veh-treated mice and was attenuated by TAM treatment (Hedge's $g = 0.638$). (c) Scatterplot including the individual datapoints for the plasma CORT results. Confidence intervals (95%) of the difference between conditions and timepoints are provided in panels (d), (e) and (f). The results revealed low overlap for the CORT peak (Δ CORT 45' - baseline, d) and minimal overlap for the CORT post-stress decrease (Δ CORT 90' - 45', f) of the UCMS vehicle mice with the other groups. By contrast, TAM-treated UCMS mice displayed high overlaps with NS mice for Δ CORT 45' - baseline (d) and Δ CORT 90' - 45' (f). Abbreviations: CORT, corticosterone; Δ CORT, corticosterone level differences; NS, non-stressed; Tam, tamoxifen; UCMS, unpredictable chronic mild stress.