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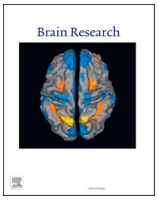

Neurobehavioral and molecular changes in a rodent model of ACTH-induced HPA axis dysfunction

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ABSTRACT

Hypothalamic-pituitary-adrenal (HPA) axis dysregulation is linked to the pathophysiology of depression. Although exogenous adrenocorticotropic hormone (ACTH) is associated with a depressive-like phenotype in rodents, comprehensive neurobehavioral and mechanistic evidence to support these findings are limited. Sprague-Dawley rats (male, n = 30; female, n = 10) were randomly assigned to the control (male, n = 10) or ACTH (male, n = 20; female n = 10) groups that received saline (0.1 ml, sc.) or ACTH (100 μg/day, sc.), respectively, for two weeks. Thereafter, rats in the ACTH group were subdivided to receive ACTH plus saline (ACTH_S; male, n = 10; female, n = 5; 0.2 ml, ip.) or ACTH plus imipramine (ACTH_I; male, n = 10; female, n = 5;10 mg/kg, ip.) for a further four weeks. Neurobehavioral changes were assessed using the forced swim test (FST), the sucrose preference test (SPT), and the open field test (OFT). Following termination, the brain regional mRNA expression of *BDNF* and *CREB* was determined using RT-PCR. After two-weeks, ACTH administration significantly increased immobility in the FST ($p = 0.03$), decreased interaction with the center of the OFT ($p < 0.01$), and increased sucrose consumption ($p = 0.03$) in male, but not female rats. ACTH administration significantly increased the expression of *BDNF* in the hippocampus and *CREB* in all brain regions in males ($p < 0.05$), but not in female rats. Imipramine treatment did not ameliorate these ACTH-induced neurobehavioral or molecular changes. In conclusion, ACTH administration resulted in a sex-specific onset of depressive-like symptoms and changes in brain regional expression of neurotrophic factors. These results suggest sex-specific mechanisms underlying the development of depressive-like behavior in a model of ACTH-induced HPA axis dysregulation.

1. Introduction

Major depressive disorder (MDD) is a mental health disorder that is characterized by symptoms that include feelings of anxiety, hopelessness and despair, anhedonia, insomnia, chronic fatigue, a sense of worthlessness, and suicidal ideation (Association, 2013). In severe cases, MDD may lead to cognitive dysfunction, social isolation, hostility, and physical debilitation which affects quality of life (Knight et al., 2020). MDD is one of the leading causes of disability, suicide, and morbidity (World Health Organization, 2014). Currently, MDD affects approximately 300 million people globally, with this number expected to increase. Indeed, the WHO predicts depression to be one of the major contributors to the global disease burden, by 2030 (World Health Organization, 2008).

Abbreviations: ADX, Adrenalectomized; BDNF, Brain-derived neurotrophic factor; CREB, Cyclic AMP response-element binding protein; DSM, Diagnostic and Statistical Manual of Mental Disorders; FST, Forced swim test; IMI, Imipramine; MDD, Major depressive disorder; OFT, Open field test; SPR, Sucrose preference ratio; SPT, Sucrose preference test; WRAF, Wits Research Animal Facility.

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The pathophysiology of depression is complex and multifactorial. MDD has been associated with changes in hippocampal volume, executive function, and memory, circulating cortisol concentrations, neuroinflammation, neurotransmitter signaling, and expression of neurotrophic and transcription factors such as brain-derived neurotrophic factor (BDNF) and cAMP response element-binding protein (CREB) (Young et al., 2001; O'Brien et al., 2004). Dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis is associated with high circulating cortisol levels and has been proposed as a major contributor in the development of MDD (Mlyniec, 2015). Indeed, patients with chronically increased cortisol concentrations are at an increased risk for the development of MDD (Lupien et al., 1999). Clinical evidence shows increased adrenocorticotrophic hormone (ACTH) and cortisol concentrations in individuals diagnosed with MDD (Choi et al., 2018; Posener et al., 2000). Moreover, HPA axis dysregulation is associated with the altered expression of neurotrophic factors, such as BDNF and CREB, which are strongly related to MDD (Kunugi et al., 2010). In this regard, studies have shown that HPA-axis dysregulation and increased glucocorticoid (GC)/corticosterone levels decrease BDNF expression, that directly impacts the expression and function of the BDNF receptor, Trkb (Kumamaru et al., 2008; Revest et al., 2014). In turn, decreased BDNF expression results in decreased phosphorylation of CREB, which is associated with synaptic plasticity and neuronal survival (Wang et al., 2019). In addition, increased glucocorticoids have also been linked to decreased CREB phosphorylation and expression (Blendy, 2006; Li et al., 2017). These findings suggest a strong link between HPA axis dysregulation and the clinical presentation of depression (Nandam et al., 2020). However, the exact mechanisms whereby HPA dysregulation contributes to depression are uncertain.

To understand the relationship between dysregulation of the HPA axis and MDD, several animal studies have demonstrated that chronic administration of ACTH resulted in the development of depressive-like symptoms when assessed using neurobehavioral tools (Sterner and Kalynchuk, 2010; Liu et al., 2018; Porsolt et al., 1977; Kraeuter et al., 2019; Song et al., 2019). In this regard, symptoms of anhedonia (Liu et al., 2018), helplessness (Porsolt et al., 1977), and anxiety (Kraeuter et al., 2019) have been reported following chronic ACTH administration as assessed by the sucrose preference test (SPT), forced swim test (FST), and open field test (OFT), respectively (Zhao et al., 2018; Kitamura et al., 2002). In contrast, others have not shown any depressive-like behaviors post- ACTH administration (Kitamura et al., 2008; Pereira et al., 2019). A possible explanation for these discrepancies are methodological variations and differences in interpretation of neurobehavioral assessments in animal models (Sousa et al., 2006; Hasanpour et al., 2021).

Although neurobehavioral tests are widely used to identify and quantify depressive-like symptoms in animal models of depression, it remains uncertain whether the reporting, interpretation, and translation of these results are reliable and reproducible, especially when using a single test (Bennabi et al., 2013). In addtion, it is important to substantiate neurobehavioral changes with mechanistic molecular data. The relationship between ACTH-induced neurobehavioral changes and the expression of neurotrophic molecular markers, frequently associated with depressive-like symptoms, are limited. Additionally, whether the use of a robust battery of neurobehavioral tests will improve the interpretation and translational ability of depressive-like symptoms in ACTH-induced HPA dysregulation requires investigation. Therefore, this study aimed to assess the effect of ACTH-induced HPA axis dysregulation on depressive-like symptoms using a battery of neurobehavioral tests in rodents, and its effect on the expression of BDNF and CREB.

2. Materials and methods

2.1. ***Ethical approval

All experimental protocols were approved by the Animal Research Ethics Committee of the University of the Witwatersrand (Reference number: 2022/03/06/C). All procedures were conducted in accordance with the regulations of the South African National Legislation for animal husbandry, welfare, and experimentation using laboratory animals. All *in vivo* experiments are reported in accordance with ARRIVE guidelines.

2.2. Study design

2.2.1. Animals

Forty Sprague-Dawley rats (male, n = 30; female, n = 10) with an initial weight of 250 ± 50 g were obtained from the Wits Research Animal Facility (WRAF) at the University of the Witwatersrand. Rats were allowed to acclimatize for seven days prior to model induction or neurobehavioral analysis. Male and female rats were housed in separate rooms, and neurobehavioral tests were conducted in a dedicated neurobehavioral testing room. Rats were housed two per cage with *ad libitum* access to water and standard rat chow (Epol, Johannesburg, South Africa), except during the SPT. The housing and testing rooms were maintained at an ambient temperature of 25 °C ± 1 °C with relative humidity of 24–26 %. A 12 h:12 h light/dark cycle was followed (lights on at 07 *h*00; testing was conducted at 09 *h*00).

2.2.2. Treatment regimen

Rats were randomly divided into the control group (male, n = 10) that received saline (0.1 ml/daily, sc) or the ACTH-treated groups (male, n = 20; female, n = 10) that received ACTH 100 μg/day, (0.1 ml, sc) for two weeks (Kitamura et al., 2002). Thereafter, the control group was subdivided to continue receiving, in addition to daily saline injections (0.1 ml, sc.), either saline (CON; 0.2 ml/day, ip) or imipramine (SAL_I; 10 mg/kg/day, ip). Rats in the ACTH-treated group were subdivided to receive, in addition to daily ACTH injections, either saline (ACTH_S; 0.2 ml/day, ip) or imipramine (ACTH_I; 10 mg/kg/day, ip) for four weeks (Kitamura et al., 2002).

2.2.3. Tissue harvest

Two hours following the completion of the neurobehavioral tests, rats were briefly exposed to isoflurane anesthesia, prior to being terminated by decapitation using a rodent guillotine. Rodent brain tissue was surgically dissected into the prefrontal cortex, striatum, midbrain, and hippocampus. Brain tissues were snap-frozen using liquid nitrogen and stored at −80 °C until molecular analysis.

2.3. Neurobehavioral assessments

EthoVision™ XT 17.5 software (Noldus Technologies, Wageningen, Netherlands) was used to automatically score all parameters of the FST and OFT and to manually score climbing behavior in the FST, as previously reported (Rastoldo et al., 2020; NI, 2024).

2.3.1. Forced swim test (FST)

The FST was used to evaluate the presence of depression-associated helplessness and despair behaviors in rodents, as previously reported (Kulkarni and Dhir, 2007; Slattery and Cryan, 2012). At the end of the acclimatization period, each rat underwent an FST adaptation, which consisted of a one-minute, three-minute, and five-minute habituation swim over three consecutive days, with a rest day, before being subjected to a five-minute FST to assess baseline behavior (baseline time point, week 0) (Supplementary Fig. 1) (Slattery and Cryan, 2012). The FST was repeated after the 2-week ACTH administration period (week 2) and again following the four-week ACTH plus saline or imipramine treatment period (week 6) (Supplementary Fig. 4). The baseline assessment (week 0) for each treatment group served as an individual control for the subsequent FSTs conducted at week 2 and week 6. All animals were allowed to acclimatize for five minutes, prior to the FST being conducted in a separate testing room. Analysis was performed using the standardized FST module in EthoVision software.

2.3.2. Open field test (OFT)

The OFT was used to assess locomotor activity and anxiety-like behavior associated with depressive-like symptoms in a subset of animals (males, n = 10; females, n = 10) (Seibenhener and Wooten, 2015). Rats were subjected to the OFT after the acclimatization period to determine baseline behavior (baseline time point, week 0), after the 2-week ACTH induction (week 2), and after the four-week ACTH plus saline or imipramine treatment period (week 6). All animals were allowed to acclimatize for five minutes, prior to the OFT being conducted in a separate testing room, as previously described (NI, 2024). Briefly, rats were placed in the center of a square open field arena (90 cm x 90 cm x 30 cm) under dimmed lighting, subsequent behaviors were recorded for five minutes (Rastoldo et al., 2020; NI, 2024). This was followed by analysis using the standardized OFT module in EthoVision software.

2.3.3. Sucrose preference test (SPT)

The SPT was performed to assess the hedonic tendency of the rats in all groups at the end of the drug treatment period (week 6), adapted from Liu et al. (2018) (Supplementary Fig. 4) (Liu et al., 2018). Each SPT included an adaptation period (72 h) and a preference test period (12 h) (Supplementary Fig. 2). During the SPT, the volume of normal drinking water and sucrose water consumed were recorded. The preference ratio (SPR) was calculated as sucrose intake / (water + sucrose intake) x 100.

2.4. Molecular analysis

2.4.1. Real Time-Polymerase chain reaction (RT-PCR)

For PCR analyses, brain samples were homogenized using a probe sonicator (Fisher Scientific, Waltham, Massachusetts, USA). RNA was extracted using the Illustra Minispin RNA extraction kit (GE Healthcare, USA), according to the manufacturer's instructions. cDNA was synthesized using the SuperScript VILO cDNA synthesis kit (ThermoFisher, California, USA). RNA and cDNA were quantified using a spectrophotometer (NanoDrop One^{C}, Thermo Scientific, Waltham, MA, USA) and were stored at −80 °C until needed.

BDNF and *CREB* mRNA expressions were determined using RT-PCR TaqMan assays (ThermoScientific, Waltham, MA, USA), with *Tbp* as the endogenous control. The TaqMan assay IDs of the probes used were: *BDNF* (Rn02531967_s1), *CREB* (Rn01451883_m1), and *Tbp* (Rn01455646_m1). The genes of interest were amplified using a StepOne Real-Time PCR System (Applied Biosystems, Foster City, USA). Changes in mRNA expression were determined using the $2^{-\Delta\Delta CT}$ method and are represented as a fold change relative to the control (Livak and Schmittgen, 2001).

2.5. Data analysis

Data analysis was performed using SAS software, version 9.4 (SAS Institute Inc., Cary, North Carolina, USA). A Shapiro-Wilk test was used to assess the normality of the data. Molecular data were not normally distributed and were logarithmically transformed for statistical analysis. Data are presented as mean and standard deviation (±SD). A mixed model analysis of variance (ANOVA) (GLM procedure in SAS) was used to assess differences between the groups for the SPT and RT-PCR results with treatment and sex as the main effects. To determine changes in the FST and OFT in response to the ACTH intervention and determine the effects of imipramine treatment in male and female rats, data were analyzed using a repeated measures two-way ANOVA, followed by a Tukey's *post hoc* test. In a repeated measures ANOVA including both within and between factors for four groups, to achieve statistical power at 80 % with a two-sided α value of < 0.05 a sample size of 20 was required. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Forced swim test

ACTH administration resulted in a significant change in the time spent highly mobile (model $F = 9.68$, $p < 0.0001$) in male rats, but not in female rats (sex effect, $p < 0.0001$) (Table 1). Treatment with ACTH for two weeks resulted in significantly less time spent being highly mobile compared to baseline in male rats ($p = 0.0001$). Moreover, after two weeks and six weeks of ACTH administration, male rats spent less time highly mobile compared to female rats ($p = 0.003$ and $p = 0.01$, respectively). Similarly, the number of episodes spent highly mobile were significantly decreased after ACTH administration for two weeks in males ($p = 0.0008$), but not female rats ($p > 0.05$) (model $F = 4.14$, $p = 0.0006$). Male rats treated with ACTH for two weeks had fewer episodes highly mobile when compared to females ($p = 0.04$). After treatment with imipramine, time spent highly mobile and highly mobile episodes were not different from baseline in male rats or female rats (all $p > 0.05$).

ACTH administration resulted in a significant increase in freezing time ($F = 10.58$, $p < 0.0001$) and freezing episodes ($F = 10.8$, $p < 0.0001$) in male, but not female rats (sex effect, $p < 0.0001$ for both) (Table 1). At baselines, males had a higher freezing duration ($p = 0.03$) and freezing episodes ($p = 0.002$) compared to females. After two weeks of ACTH administration, male rats had a higher freezing duration compared to baseline ($p = 0.003$). After two weeks of ACTH administration, males had higher freezing duration and episodes compared to females (both $p < 0.0001$). After six weeks of ACTH administration, freezing duration and episodes increased compared to week 2 ($p = 0.04$ and $p = 0.03$) in male rats. After imipramine treatment, there were no differences in freezing duration or episodes compared to baseline or ACTH administration in either male or female rats (all $p > 0.05$).

There was an overall significant model effect for time spent climbing ($F = 4.4$, $p = 0.0004$), but not for climbing episodes ($F = 1.97$, $p = 0.07$). There was a significant treatment effect ($p = 0.03$) and sex effects ($p = 0.0001$) for time spent climbing. However, *post hoc* tests did not show significant interaction effects (Table 1).

3.2. Open field test

Although there were significant differences in the model for the mean speed ($F = 7.14$, $p < 0.0001$; Fig. 1C) and the distance traveled ($F = 7.12$, $p < 0.0001$; Fig. 1D), there were no significant interaction effects (all $p > 0.05$). ACTH administration significantly increased the duration of time spent immobile in the OFT ($F = 10.39$, $p < 0.0001$; Fig. 1E). After two and six weeks of ACTH administration, the immobility duration increased in male rats compared to baseline ($p < 0.0001$ and $p = 0.0002$, respectively), which were not impacted by imipramine treatment. Similarly in female rats, six weeks of ACTH treatment increased the immobility duration when compared to baseline ($p = 0.01$). ACTH administration for two weeks significantly decreased the time spent in the center of the arena ($F = 3.72$, $p = 0.003$) and percentage time spent in the center of the open field ($F = 3.69$, $p = 0.02$) in male (Fig. 1A), but not female rats (Fig. 1B). Interestingly, after six weeks of either ACTH plus saline or imipramine treatment, there were no differences in the interaction with the center area of the OFT arena compared to baseline (all $p > 0.05$).

3.3. Sucrose preference test

Regular water consumption was significantly different between treatment groups ($F = 7.36$, $p = 0.0001$; treatment effect, $p = 0.004$; sex effect, $p < 0.0001$) (Fig. 2A). In male rats, ACTH plus imipramine (ACTH_I) treatment significantly increased regular water consumption compared to the CON ($p = 0.03$) and the ACTH_S rats ($p = 0.03$). In female rats, ACTH plus imipramine treatment resulted in an increased

Table 1
FST results after ACTH administration and imipramine treatment.

FST Parameter		Week 0 Baseline		Week 2 ACTH		Week 6			
						ACTH_S		ACTH_I	
		Male (n = 19)	Female (n = 10)	Male (n = 19)	Female (n = 10)	Male (n = 10)	Female (n = 5)	Males (n = 9)	Female (n = 5)
Highly mobile	Time (s)	161.9 ± 47.5	202.0 ± 42.9	84.9 ± 48.0***	162.3 ± 50.5††	114.9 ± 60.1	217.3 ± 45.5$^{\#\#}$	176.6 ± 45.8	196.0 ± 54.1
	Episodes (n)	204.3 ± 31.1	190.9 ± 30.3	144.5 ± 51.7***	196.7 ± 18.0†	166.4 ± 68.4	145.8 ± 22.2	181.3 ± 43.5	209.4 ± 35.9
Freezing	Time (s)	42.9 ± 8.5	26.3 ± 2.7*	52.5 ± 11.9**	25.7 ± 6.9†††	38.2 ± 12.6†	23.6 ± 7.2	37.9 ± 12.7	31.0 ± 8.1
	Episodes (n)	400.3 ± 62.7	312.2 ± 25.3**	432.0 ± 57.4	296.9 ± 65.8†††	369.2 ± 72.5†	284.0 ± 63.8	361.7 ± 71.5	344.2 ± 65.9
Climbing	Time (s)	65.4 ± 58.2	13.8 ± 11.3	60.5 ± 45.6	30.7 ± 22.5	85.3 ± 58.2	13.8 ± 11.3	114.1 ± 77.2	59.9 ± 10.5
	Episodes (n)	8.9 ± 3.5	7.8 ± 1.1	7.2 ± 3.3	5.6 ± 2.5	6.3 ± 3.3	4.6 ± 4.6	7.0 ± 2.4	6.4 ± 2.9

FST data was collected at week 0: baseline measurement; week 2: after 2 weeks of ACTH (100 μg/day, sc) treatment; and week 6: after four weeks of ACTH plus saline (0.2 ml/day, ip) or imipramine (10 mg/kg/day, ip) treatment. Data are presented as mean ± SD. Data were analyzed using a repeated measures two-way ANOVA followed by a Tukey's *post hoc* test. ACTH_S, ACTH + saline; ACTH_I, ACTH + imipramine. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus males at week 0; † $p < 0.05$, †† $p < 0.01$, ††† $p < 0.001$ versus males at week 2; ## $p < 0.01$ versus ACTH_S males at week 6.

consumption of regular water compared to the CON male rats and male rats treated with ACTH_I ($p = 0.001$ and $p = 0.02$, respectively). Interestingly in males, imipramine treatment in the imipramine CON group significantly increased water consumption when compared to the untreated controls ($p = 0.04$) (Supplementary Fig. 5A). Sucrose solution consumption was significantly different between the male ACTH-treated rats and the controls ($F = 3.32$, $p = 0.02$; Fig. 2B). Six weeks of ACTH_S treatment in male rats resulted in significantly higher sucrose solution consumption when compared to the controls ($p = 0.03$). Total water and sucrose consumption was significantly different between the male control and ACTH-treated groups ($F = 3.22$, $p = 0.02$; Fig. 2C). ACTH_S males had greater total water and sucrose consumption compared to control rats ($p = 0.01$). Despite a significant model effect ($F = 4.01$, $p = 0.01$), treatment, and sex effects (both $p = 0.01$), in the sucrose preference ratio, there were no interaction effects ($p > 0.05$) (Fig. 2D).

3.4. mRNA expression of BDNF and CREB

ACTH administration did not result in changes in *BDNF* mRNA expression in the prefrontal cortex, striatum, or midbrain (Fig. 3A, 3B, and 3D). Hippocampal *BDNF* mRNA expression was significantly increased following ACTH administration in males ($F = 4.68$, $p = 0.004$), and was significantly different between males and females ($p = 0008$; Fig. 3C). Six weeks of ACTH_S treatment in males resulted in significantly higher hippocampal *BDNF* mRNA levels when compared to controls and ACTH_I males (both $p = 0.04$). There were no differences in *BDNF* mRNA expression in female groups (all $p > 0.05$).

CREB mRNA expression was significantly increased following ACTH treatment in all brain regions in male rats, but not in female rats (all $p < 0.05$). There were no differences in *CREB* mRNA expression between male ACTH_I and male control rats (all $p > 0.05$). *CREB* mRNA expression did not differ between female ACTH_S and ACTH_I groups (all $p > 0.05$; Fig. 4A–C).

4. Discussion

HPA axis dysregulation is strongly associated with the clinical symptoms and pathophysiology of depression (Keller et al., 2017). Animal models of HPA axis dysregulation have shown contrasting neurobehavioral outcomes, largely due to the use of a single neurobehavioral test in the face validation of these models (Kitamura et al., 2002; Kitamura et al., 2008). Moreover, molecular evidence to substantiate these neurobehavioral changes are limited. Hence, this study aimed to determine the effects of ACTH administration on the development of depressive-like symptoms in a rodent model, using a battery of neurobehavioral tests as well as by assessing associated neurotrophic molecular markers. We showed that in male rats, ACTH administration significantly increased immobility in the FST, which suggests despair-like behaviour. In the OFT, although ACTH administration significantly reduced locomotor behaviour in both males and females, only male rats showed a reduced interaction with the center of the open field, suggesting an increase in anxiety-like behaviour. In addition, ACTH administration resulted in a significant increase in the volume of sucrose solution consumed by males, however, there was no difference in the sucrose preference ratio. There were no differences in any of the neurobehavioral outcomes between rats receiving ACTH plus imipramine and those receiving ACTH plus saline, suggesting imipramine did not impact ACTH-induced changes in neurobehavior. Furthermore, in male, but not female rats, ACTH plus saline administration increased *BDNF* mRNA expression in the hippocampus, while *CREB* mRNA expressions were increased in all brain regions. The expression of these neurotrophic factors was not altered in rats receiving ACTH and imipramine. Taken together, these results show that ACTH administration resulted in depressive-like behaviour in male rats only when assessed using several neurobehavioral tests. These changes were accompanied by increased region-specific expression of neurotrophic factors.

4.1. Effects of HPA axis dysregulation on a neurobehavioral measure of despair

Immobility or freezing assessed in the FST is commonly used as a measure of behavioral despair in rodent models (Yankelevitch-Yahav et al., 2015). In the present study, we showed increases in immobility following two weeks of ACTH administration, as shown by a significant increase in freezing duration and episodes. Similarly, we showed decreased time and episodes of mobility during the swim. Interestingly, this phenomenon was observed only in male rats, demonstrating the sex-specific effects of ACTH. Previous studies have shown that chronic administration of ACTH significantly increases immobility in the FST, suggesting behavioral despair (Kitamura et al., 2002; Bennabi et al., 2013; Song et al., 2019; Kim et al., 2016). Other models of HPA axis dysregulation have also demonstrated changes in mobility in the FST. In this regard, in glucocorticoid receptor (GR) knockout rodents there was a reduced immobility (Tronche et al., 1999). Moreover, adrenalectomized (ADX) rats that received a GC antagonist showed a reversal in immobility behavior compared to the controls (Veldhuis et al., 1985).

Interestingly, after six weeks of ACTH administration, there were no differences in freezing or mobility compared to baseline. It has been suggested that the two-week administration of ACTH may align more closely with the acute phase of the stress response; where elevated ACTH levels lead to an increase in corticosterone levels, which, in turn, alters neural circuits involved in mood regulation (Song et al., 2019). These results suggest that the HPA axis may recover with longer treatment durations via an adaptive homeostatic response.

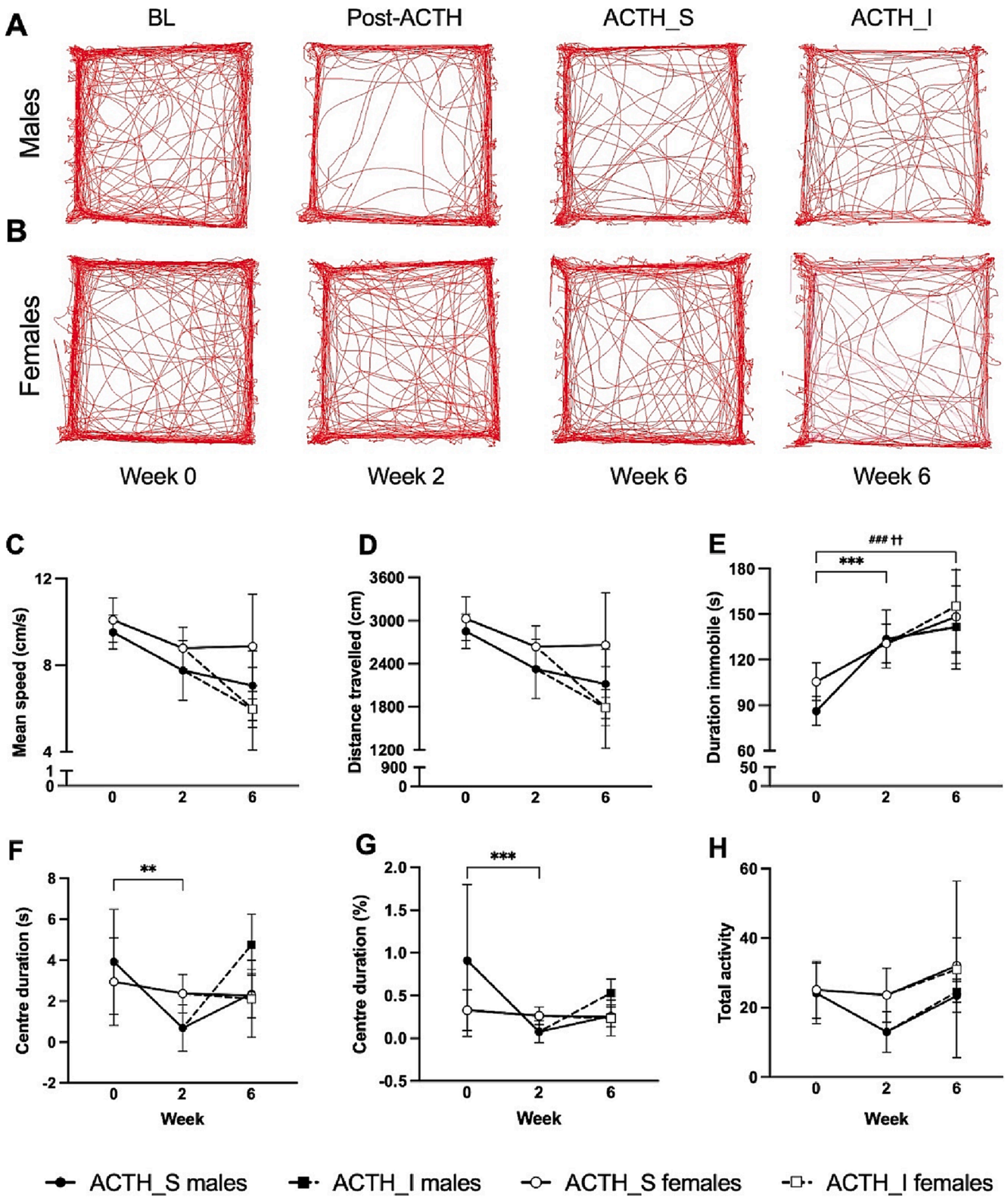

Fig. 1. Average track plots for all male rats (A) and female rats (B) across each experimental time point. Effect of ACTH and imipramine treatment on mean speed (C), distance travelled (D), time immobile (E), center duration (F), center duration (G), and total activity (H), as assessed by the OFT. OFT measurement time points include week 0: baseline measurement (males, n = 19; females, n = 10); week 2: after 2 weeks of ACTH (100 μg/day, sc) treatment (males, n = 19; females, n = 10); and week 6: after four weeks of ACTH plus saline (0.2 ml/day, ip) (males, n = 10; females, n = 5) or imipramine (10 mg/kg/day, ip) treatment (males, n = 9; females, n = 5). Data are presented as mean ± SD. Data were analyzed using a repeated measures two-way ANOVA followed by a Tukey's *post hoc* test. BL, baseline; ACTH_S, ACTH + saline; ACTH_I, ACTH + imipramine. ** $p < 0.01$, *** $p < 0.001$ week 0 vs week 2 between ACTH_S males, ### $p < 0.001$ week 0 vs week 6 between ACTH_S males, †† $p < 0.01$ week 0 vs week 6 between ACTH_S females.

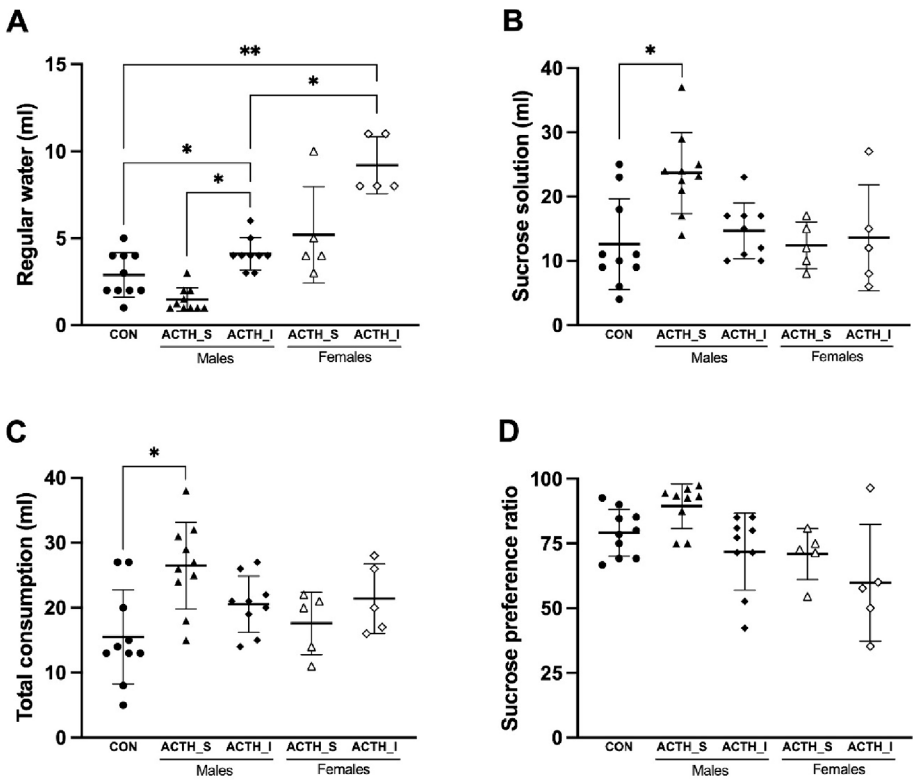

Fig. 2. Sucrose preference test showing the effect of ACTH and imipramine on regular water consumption (A), sucrose solution consumption (B), total volume of water and sucrose consumed (C), and the sucrose preference ratio (D). ACTH (100 μg/day, sc) and saline (0.1 ml, sc) were administered once daily for 6 weeks. Imipramine (10 mg/kg/day, ip) and saline (0.2 ml/day, ip) were administered once daily for 4 weeks (weeks 2–6). Data are presented as means ± SD. Data were analysed using a two-way ANOVA followed by a Tukey's *post hoc* test. CON, control (males, n = 10); ACTH_S, ACTH + saline (males, n = 10; females, n = 5); ACTH_I, ACTH + imipramine (males, n = 9; females, n = 5). * $p < 0.05$, ** $p < 0.01$.

In the present study, despite the ACTH effects on freezing behavior in male rats, there were no effects on behavioural despair in females. The lack of effects in females may be attributed to the suppressive effects of estrogen and progesterone on the HPA axis activity (Patchev and Almeida, 1996; Goel et al., 2011). Progesterone and estrogen have been shown to decrease glucocorticoid-induced expression of hypothalamic CRH and corticosteroid receptors in the hippocampus, leading to suppression of the HPA axis (Patchev and Almeida, 1996). A similar sex-specific effect was seen in a chronic restraint stress model, where an increase in corticosterone was associated with a decreased GR mRNA expression in the hippocampus of male but not female rats (Karandrea et al., 2000). Taken together, the interplay between the HPA axis and sex hormones in response to ACTH treatment and FST-induced stress plays a crucial role in modulating these sex-specific behavioral responses in the FST.

4.2. *Effects of HPA axis dysregulation on locomotion and exploration*

In the present study, we showed that ACTH administration resulted in a significant decrease in locomotor activity of male rats in the OFT, as measured by mean speed, distance travelled and duration immobile. Similarly, in females, the duration immobile in the OFT increased after ACTH administration. None of the changes in locomotor variables were impacted by imipramine treatment. These changes in locomotor activity may be explained by elevated ACTH levels which strongly correlate with altered dopaminergic activity in the brain via an increase in circulating glucocorticoids (Bloomfield et al., 2019; Abercrombie et al., 1989; Baik, 2020; Willner et al., 1991).

Interestingly, in male rats only, ACTH administration resulted in a decreased interaction with the center zone of the OFT. Similar to the results from the FST, after six weeks of ACTH administration center interaction was not different from baseline. Similarly, previous studies have shown that ACTH administration decreases locomotor activity and time in the central zone of the open field arena (Zhao et al., 2018).

It has been proposed that avoidance of the center of the OFT and more time seeking shelter along the periphery is associated with fear or anxiety-like behavior (Carola et al., 2002). Previous studies have shown that ACTH administration decreases locomotor activity and time in the central zone of the open field arena (Carola et al., 2002). Our results therefore suggest that ACTH administration may induce anxiety-like behaviour in male but not female rats. This is important considering that chronic anxiety is frequently comorbid with depression and has been associated with the exacerbation of depressive disorders (Kessler et al., 1996; Chen et al., 2015; Vreeburg et al., 2013). These changes in locomotor activity may be explained by elevated ACTH levels which strongly correlate with altered dopaminergic activity in the brain via an increase in circulating glucocorticoids (Bloomfield et al., 2019; Abercrombie et al., 1989; Baik, 2020; Willner et al., 1991). This suggests that specific dopaminergic pathways are responsible for neurobehavioral changes observed in ACTH-induced HPA axis dysregulation. In this regard, studies have shown that locomotion and avoidance behaviours related to anxiety are associated with the nigrostriatal pathway, while cognitive and emotional processes are associated with the mesolimbic and mesocorticolimbic pathways (Bertolucci-D'Angio et al., 1990; LeBlanc et al., 2020). Taken together, these results suggest that ACTH administration may alter dopaminergic activity which may lead to changes in locomotor and anxiety-like behaviour in the OFT (Ouakki et al., 2019).

4.3. *Effects of HPA axis dysregulation on sucrose preference*

In rodent studies, anhedonia is associated with a reduced preference

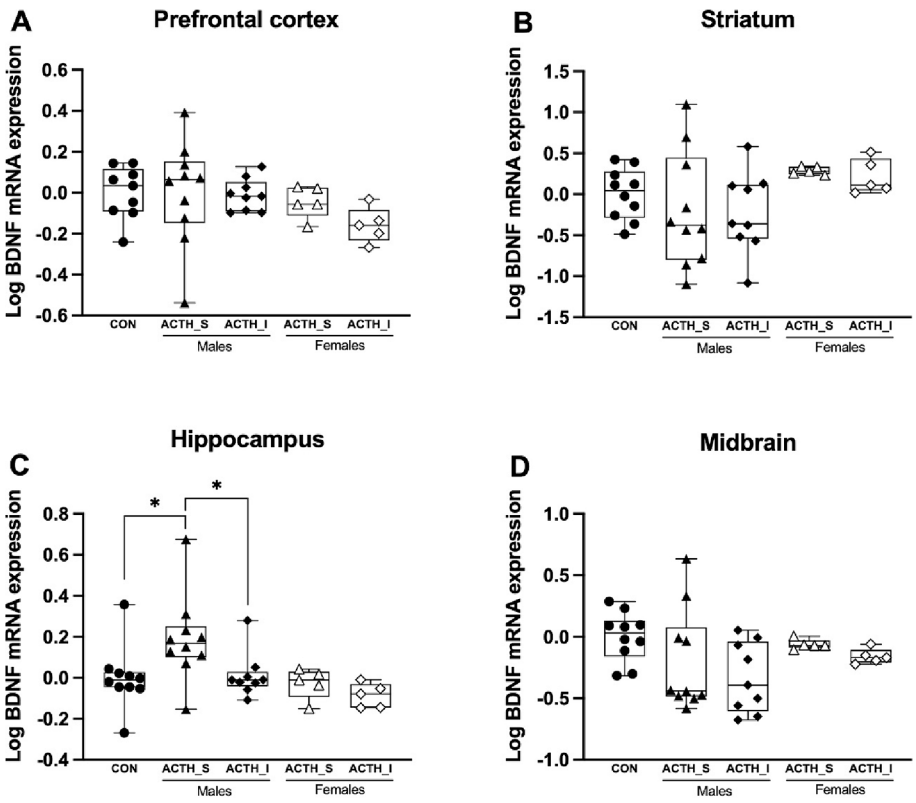

Fig. 3. RT-PCR results showing the effect of ACTH and imipramine treatment on the *BDNF* mRNA expression in the prefrontal cortex (A), striatum (B), hippocampus (C), and midbrain (D). ACTH (100 μg/day, sc) and saline (0.1 ml, sc) were administered once daily for 6 weeks. Imipramine (10 mg/kg/day, ip) and saline (0.2 ml/day, ip) were administered once daily for 4 weeks (weeks 2–6). Data are presented as mean ± SD relative to the housekeeping gene *Tbp*. Data was analysed using a two-way ANOVA followed by a Tukey's *post hoc* test. CON, control (males, n = 10); ACTH_S, ACTH + saline (males, n = 10; females, n = 5); ACTH_I, ACTH + imipramine (males, n = 9; females, n = 5). * $p < 0.05$.

for sucrose-sweetened water compared to normal drinking water. (Liu et al., 2018; Pizzagalli, 2014). Despite these expected outcomes, the results of the SPT in ACTH-treated rodent models are contradicting (Zhao et al., 2018; Kale et al., 2021). One study has reported an increased sucrose preference ratio, which was the only parameter reported after 3 weeks of ACTH administration (Kale et al., 2021). Another study reported that male ADX rats consumed more sucrose when treated with either ACTH or corticosterone replacement therapy (Liang and Nagy & Dallman., 2000). We found that ACTH administration increased the volume of sucrose solution consumed, with no effect on regular water consumption, in males. However, in females, ACTH treatment did not alter any of the parameters measured in the SPT when compared to the male rats.

The contrasting sucrose consumption findings may be explained by inter-individual differences in coping strategies. In this regard, MDD patients often present with varying hunger responses, with some having an increased appetite, while others have decreased appetite (Simmons et al., 2016). Variations in MDD-related appetite may explain why some studies report decreased sucrose preference in ACTH-treated rats while others showed the opposite (Zhao et al., 2018). Moreover, in MDD patients there is also a large variation in the preference for caloric-rich foods, again largely dependent on inter-individual differences in coping mechanisms (Koning et al., 2022). In rodents, these coping mechanisms may manifest as changes in preference for sucrose and may also be explained by altered dopaminergic activity in the mesocorticolimbic pathway (Meye and Adan, 2014). In MDD patients, increased appetite has been linked to hyperactivation of mesocorticolimbic reward pathways while a loss of appetite is associated with hypoactivation of insular regions responsible for homeostatic monitoring (Simmons et al., 2016). Activation of reward circuits increases the hedonic drive to consume substances high in sugar and fat in response to food cues (Adam and Epel, 2007). Indeed, patients with elevated cortisol concentrations have an increased preference for sweet and savoury foods over other foods (Geer, 2016). In our study, chronic ACTH administration in males may have caused a glucocorticoid-related increase in mesocorticolimbic reward signals, resulting in increased sucrose consumption. Due to the extensive adaptation protocol required for the SPT, the present study did not assess sucrose preference at baseline, and hence we could not assess the change in behaviour over time. Further studies need to assess sucrose preference changes over time to better understand the effects of the dysregulated HPA axis on inter-individual symptoms of anhedonia.

In contrast, imipramine treatment increased the volume of regular water consumed in both male and female rats. Despite an overall significant model effect, there were no differences in the sucrose preference ratio between treatment groups. The increase in water intake subsequent to imipramine treatment has been shown in previous rodent studies (Aulakh et al., 1987; Zabik et al., 1977). This imipramine-induced increase in water consumption may be attributed to the drug's side effects, which include dry mouth and increased thirst (Fayez and Gupta, 2020). Indeed, we have also shown that imipramine treatment alone causes an increase in regular water consumption (Supplementary Fig. 5). Taken together, similar to the OFT, imipramine treatment did not reverse the effects of ACTH in the SPT, further illustrating the drug's inefficacy in a model of HPA axis dysfunction.

4.4. Effects of HPA axis dysregulation on central BDNF and CREB mRNA expression

This study showed that six weeks of ACTH administration resulted in increased hippocampal *BDNF* and increased *CREB* mRNA expression across all brain regions in male, but not in female rats. These findings

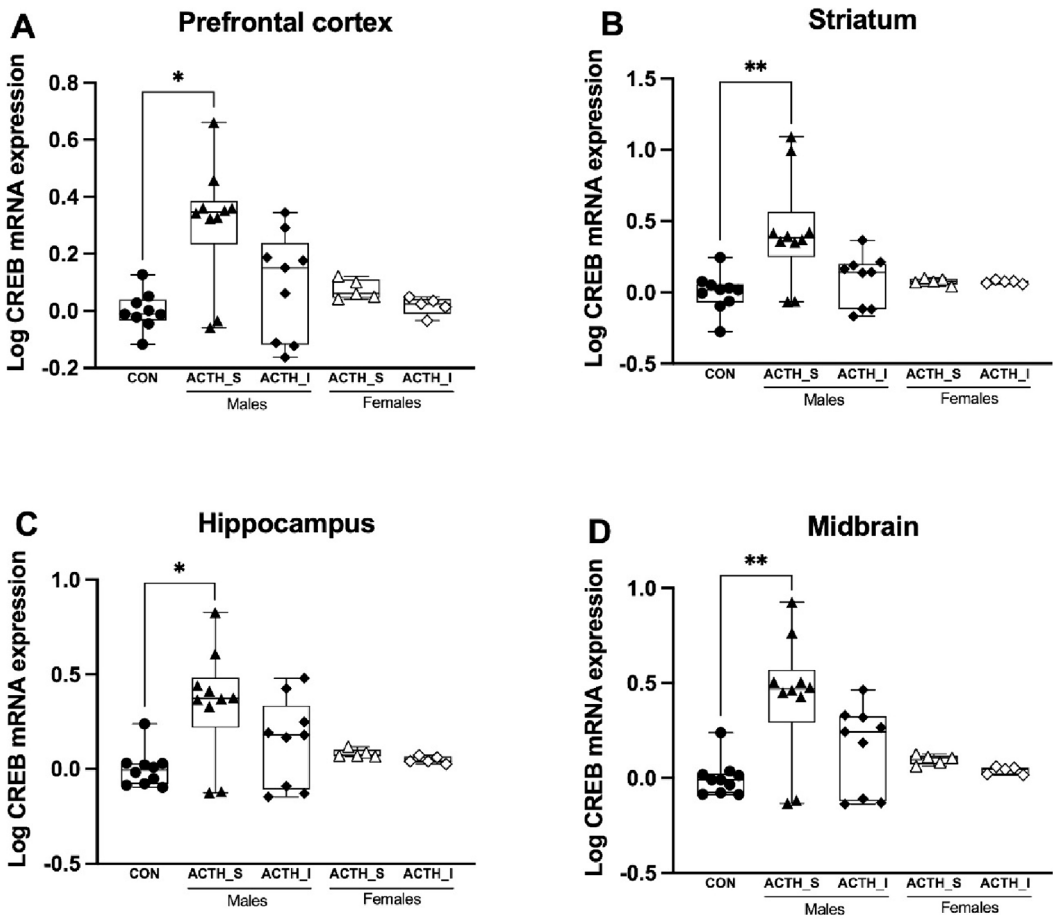

Fig. 4. RT-PCR results showing the effect of ACTH and imipramine on the *CREB* mRNA expression in the prefrontal cortex (A), striatum (B), hippocampus (C), and midbrain (D). ACTH (100 μg/day, sc) and saline (0.1 ml, sc) were administered once daily for 6 weeks. Imipramine (10 mg/kg/day, ip) and saline (0.2 ml/day, ip) were administered once daily for 4 weeks (weeks 2–6). Data are presented as mean ± SD relative to the housekeeping gene *Tbp*. Data were analyzed using a two-way ANOVA followed by a Tukey's *post hoc* test. CON, control (males, n = 10); ACTH_S, ACTH + saline (males, n = 10; females, n = 5); ACTH_I, ACTH + imipramine (males, n = 9; females, n = 5). * $p < 0.05$, ** $p < 0.01$.

differ from previous studies that administered ACTH to rodents for two weeks and found either a decrease (Antunes et al., 2015; Zabik et al., 1977) or no change in brain *BDNF* and *CREB* expression (Kuwatsuka et al., 2013; Li et al., 2006). The differences in the expression of neurotrophins in a model of ACTH-induced HPA axis dysfunction between studies may be explained by differences in the duration and dose of ACTH administration. The majority of studies investigating the depressive effects of this model administered ACTH for a maximum of three weeks (Antunes et al., 2015; Kuwatsuka et al., 2013; Li et al., 2006). Although a single dose of an ACTH analogue has been shown to significantly increase BDNF and its receptor, Trkb, expression in the hippocampus of male rats (Dolotov et al., 2006), others showed that two weeks of ACTH administration decreases both pro and mature BDNF and CREB expression (Kuwatsuka et al., 2013). During ACTH-induced HPA axis dysregulation, there is impaired negative feedback which results in the hypersecretion of cortisol, adrenal androgens, and mineralocorticoids (Torres et al., 2001). These adrenal androgens, dehydroepiandrosterone, pregnenolone, and their derivatives have been shown to increase BDNF expression in the hippocampus (Naert et al., 2007). Moreover, these neuroactive steroids also increase ACTH and CRH levels in rodents, which supports the suggestion of hypersecretion of adrenal hormones (Naert et al., 2007). GRs, mineralocorticoid receptors (MRs), and Trkb are co-expressed on hippocampal neurons highlighting the interplay between the GC-BDNF pathways (Jeanneteau et al., 2012). In this regard, further studies are required to explore the possible adaptive mechanism through which chronically altered HPA axis function leads to an upregulation of neurotrophins following ACTH administration.

The increased *CREB* mRNA expression in all brain regions in the present study may be explained by a GC-related increase in dopaminergic activity. As previously discussed, increased ACTH and circulating GC levels correlate with increased dopaminergic activity (Abercrombie et al., 1989; Baik, 2020; Willner et al., 1991). CREB regulates genes crucial for the function of dopaminergic neurons (Wang et al., 2018). Dopamine alters the phosphorylation of CREB via G-protein coupled receptors, upregulating its expression (Wang et al., 2018). Consistent with neurobehavioral results, chronic ACTH administration may have resulted in a GC-mediated increase in dopaminergic activity, upregulating CREB expression.

Our findings indicate a strong sex effect in HPA axis activity and the expression of neurotrophins in response to repeated exogenous ACTH administration. ACTH did not alter the mRNA expression of *BDNF* and *CREB* in the female rat brain. This may also be attributed to the suppressive effect of female sex hormones on the HPA axis (Patchev and Almeida, 1996; Goel et al., 2011). Meanwhile, in the rats treated with ACTH plus imipramine *BDNF* and *CREB* mRNA expression did not differ from the controls in both males and females. The mechanims whereby imipramine affects neurotrophic factors in HPA axis dysregulation requires further investigation.

5. Limitations and future perspectives

Additional limitations should be considered. Differential group

numbers may impact the statistical power of the study. The smaller number of female rats when compared to male rats used in this study should be considered when interpreting the effect of sex on neurobehavior and molecular expression. Nevertheless, a *post-hoc* power calculation showed adequate statistical power. Changes in the FST and OFT were not reported for control animals. However, since repeated measures were used in the neurobehavioral analysis, animals within each specific treatment group, served as their own control at baseline for which more robust comparisons could be made. In the SPT, due to the extended nature of the protocol (Liu et al., 2018), an assessment could not be performed prior to ACTH administration, hence changes in anhedonia over time could not be assessed and were only assessed prior to termination for group comparisons. Furthermore, although changes were observed in the sucrose preference test between groups, food intake was not recorded in this study. Future studies should include changes in feeding behavior to better interpret food motivation and anhedonia. Lastly, ACTH and corticosterone levels were not measured in this study and should be measured in future studies to assess HPA axis function.

6. Conclusion

This study demonstrates that ACTH administration for two weeks resulted in sex-specific responses, with decreases in overall mobility and locomotion and increases in sucrose consumption, in males. In females, ACTH had no effect on despair- or anxiety-like behaviours or on sucrose consumption. Additionally, after six weeks, there were no differences in depressive-like behaviour in the FST or OFT between the rats treated with ACTH plus imipramine and those treated with ACTH plus saline. However, imipramine treatment did increase water consumption. ACTH administration increased *BDNF* mRNA in the hippocampus and *CREB* mRNA in the prefrontal cortex, striatum, midbrain, and hippocampus in males, an effect not observed in female rats. Co-administration of ACTH and imipramine did not show any changes in *BDNF* and *CREB* mRNA expression. Taken together, chronic ACTH administration may result in despair and anxiety-like symptoms as well as depression-like increases in appetite in male rats, which is not altered by imipramine treatment. These results suggest that drugs targeting the monoaminergic system may not be effective on depressive-like symptoms in a model of HPA axis dysregulation. The contrasting results observed in the neurobehavioral and molecular outcomes between male and female rats underscore the differential sex-associated physiological mechanisms underlying depression.

CRediT authorship contribution statement

Farhanah N Sallie: Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Leandrie Pienaar:** Methodology, Investigation. **Andrea Lubbe:** Methodology, Investigation. **Sanelisiwe Xhakaza:** Methodology, Investigation. **Srinivasa R Manne:** Methodology, Investigation. **Beatriz G. de la Torre:** Resources, Methodology, Investigation. **Fernando Albericio:** Resources, Methodology, Investigation. **William MU Daniels:** Supervision, Methodology, Investigation. **Aletta ME Millen:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Sooraj Baijnath:** Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brainres.2024.148913.

References

Abercrombie, E.D., Keefe, K.A., DiFrischia, D.S., Zigmond, M.J., 1989. Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial frontal cortex. J. Neurochem. 52, 1655–1658.

Adam, T.C., Epel, E.S., 2007. Stress, eating and the reward system. Physiol. Behav. 91, 449–458.

Antunes, M.S., Ruff, J.R., de Oliveira, E.D., Piegas, M.B., de Brito, M.L.O., Rocha, K.A., de Gomes, M.G., Goes, A.T.R., Souza, L.C., Donato, F., 2015. Neuropeptide Y administration reverses tricyclic antidepressant treatment-resistant depression induced by ACTH in mice. Horm. Behav. 73, 56–63.

Association, A.P., 2013. Diagnostic and Statistical Manual of Mental Disorders, vol. 5. American psychiatric association, Washington, DC.

Aulakh, C.S., Cohen, R.M., Hill, J.L., Murphy, D.L., Zohar, J., 1987. Long-term imipramine treatment enhances locomotor and food intake suppressant effects of m-chlorophenylpiperazine in rats. Br. J. Pharmacol. 91, 747–752.

Baik, J.-H., 2020. Stress and the dopaminergic reward system. Exp. Mol. Med. 52, 1879–1890.

Bennabi, D., Vandel, P., Papaxanthis, C., Pozzo, T., Haffen, E., 2013. Psychomotor retardation in depression: a systematic review of diagnostic, pathophysiologic, and therapeutic implications. Biomed Res. Int. 2013 (158746), 2314–6133. https://doi.org/10.1155/2013/158746.

Bertolucci-D'Angio, M., Serrano, A., Scatton, B., 1990. Mesocorticolimbic dopaminergic systems and emotional states. J. Neurosci. Methods 34, 135–142.

Blendy, J.A., 2006. The role of CREB in depression and antidepressant treatment. Biol. Psychiatry 59, 1144–1150.

Bloomfield, M.A., McCutcheon, R.A., Kempton, M., Freeman, T.P., Howes, O., 2019. The effects of psychosocial stress on dopaminergic function and the acute stress response. Elife 8, e46797.

Carola, V., D'Olimpio, F., Brunamonti, E., Mangia, F., Renzi, P., 2002. Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. Behav. Brain Res. 134, 49–57.

Chen, F., Zhou, L., Bai, Y., Zhou, R., Chen, L., 2015. Hypothalamic-pituitary-adrenal axis hyperactivity accounts for anxiety-and depression-like behaviors in rats perinatally exposed to bisphenol a. J. Biomed. Res. 29, 250.

Choi, K.W., Na, E.J., Fava, M., Mischoulon, D., Cho, H., Jeon, H.J., 2018. Increased adrenocorticotropic hormone (ACTH) levels predict severity of depression after six months of follow-up in outpatients with major depressive disorder. Psychiatry Res. 270, 246–252.

Dolotov, O.V., Karpenko, E.A., Inozemtseva, L.S., Seredenina, T.S., Levitskaya, N.G., Rozyczka, J., Dubynina, E.V., Novosadova, E.V., Andreeva, L.A., Alfeeva, L.Y., 2006. Semax, an analog of ACTH (4–10) with cognitive effects, regulates BDNF and trkB expression in the rat hippocampus. Brain Res. 1117, 54–60.

Fayez, R., Gupta, V., 2020. Imipramine. StatPearls Publishing, United States, Florida, Treasure Island (FL).

Geer E. (2016). The Role of Cortisol in Food Intake and Food Choice Behavior: Two Studies in Patients with Cushing's. Available at https://csrf.net/doctors-articles/role-cortisol-food-intake-food-choice-behavior-two-studies-patients-cushings/. [Accessed July 12, 2023].

Goel, N., Workman, J.L., Lee, T.T., Innala, L., Viau, V., 2011. Sex differences in the HPA axis. Compr. Physiol. 4, 1121–1155.

Hasanpour, M., Mitricheva, E., Logothetis, N., Noori, H.R., 2021. Intensive longitudinal characterization of multidimensional biobehavioral dynamics in laboratory rats. Cell Rep. 35, 2211.

Jeanneteau FD, Lambert WM, Ismaili N, Bath KG, Lee FS, Garabedian MJ & Chao MV. (2012). BDNF and glucocorticoids regulate corticotrophin-releasing hormone (CRH) homeostasis in the hypothalamus. *Proceedings of the National Academy of Sciences* **109,** 1305-1310.

Kale, R.P., Nguyen, T.T.L., Price, J.B., Yates, N.J., Walder, K., Berk, M., Sillitoe, R.V., Kouzani, A.Z., Tye, S.J., 2021. Mood regulatory actions of active and sham nucleus accumbens deep brain stimulation in antidepressant resistant rats. Front. Hum. Neurosci. 15, 644921.

Karandrea, D., Kittas, C., Kitraki, E., 2000. Contribution of sex and cellular context in the regulation of brain corticosteroid receptors following restraint stress. Neuroendocrinology 71, 343–353.

Keller, J., Gomez, R., Williams, G., Lembke, A., Lazzeroni, L., Murphy, G.M., Schatzberg, A.F., 2017. HPA axis in major depression: cortisol, clinical symptomatology and genetic variation predict cognition. Mol. Psychiatry 22, 527–536.

Kessler, R.C., Nelson, C.B., McGonagle, K.A., Liu, J., Swartz, M., Blazer, D.G., 1996. Comorbidity of DSM–III–R major depressive disorder in the general population: results from the US National Comorbidity Survey. Br. J. Psychiatry 168, 17–30.

Kim, Y., Mcgee, S., Czeczor, J., Walker, A., Kale, R., Kouzani, A., Walder, K., Berk, M., Tye, S., 2016. Nucleus accumbens deep-brain stimulation efficacy in ACTH-pretreated rats: alterations in mitochondrial function relate to antidepressant-like effects. Transl. Psychiatry 6, e842–e.

Kitamura, Y., Araki, H., Gomita, Y., 2002. Influence of ACTH on the effects of imipramine, desipramine and lithium on duration of immobility of rats in the forced swim test. Pharmacol. Biochem. Behav 71, 63–69.

Kitamura, Y., Fujitani, Y., Kitagawa, K., Miyazaki, T., Sagara, H., Kawasaki, H., Shibata, K., Sendo, T., Gomita, Y., 2008. Effects of imipramine and bupropion on the duration of immobility of ACTH-treated rats in the forced swim test: involvement of the expression of 5-HT2A receptor mRNA. Biol. Pharm. Bull. 31, 246–249.

Knight, M.J., Lyrtzis, E., Baune, B.T., 2020. The association of cognitive deficits with mental and physical quality of life in major depressive Disorder. Compr. Psychiatry 97 (152147), 0010–0440. https://doi.org/10.1016/j.comppsych.2019.152147.

Koning, E., Vorstman, J., McIntyre, R.S., Brietzke, E., 2022. Characterizing eating behavioral phenotypes in mood disorders: a narrative review. Psychol. Med. 1–14.

Kraeuter, A.-K., Guest, P.C., Sarnyai, Z., 2019. The open field test for measuring locomotor activity and anxiety-like behavior. Pre-Clinical Models: Techniques and Protocols 1916, 99–103.

Kulkarni, S., Dhir, A., 2007. Effect of various classes of antidepressants in behavioral paradigms of despair. Prog. Neuropsychopharmacol. Biol. Psychiatry 31, 1248–1254.

Kumamaru, E., Numakawa, T., Adachi, N., Yagasaki, Y., Izumi, A., Niyaz, M., Kudo, M., Kunugi, H., 2008. Glucocorticoid prevents brain-derived neurotrophic factor-mediated maturation of synaptic function in developing hippocampal neurons through reduction in the activity of mitogen-activated protein kinase. Mol. Endocrinol. 22, 546–558.

Kunugi, H., Hori, H., Adachi, N., Numakawa, T., 2010. Interface between hypothalamic-pituitary-adrenal axis and brain-derived neurotrophic factor in depression. Psychiatry Clin. Neurosci. 64, 447–459.

Kuwatsuka, K., Hayashi, H., Onoue, Y., Miyazaki, I., Koyama, T., Asanuma, M., Kitamura, Y., Sendo, T., 2013. The mechanisms of electroconvulsive stimuli in BrdU-positive cells of the dentate gyrus in ACTH-treated rats. J. Pharmacol. Sci. 122, 34–41.

LeBlanc, K.H., London, T.D., Szczot, I., Bocarsly, M.E., Friend, D.M., Nguyen, K.P., Mengesha, M.M., Rubinstein, M., Alvarez, V.A., Kravitz, A.V., 2020. Striatopallidal neurons control avoidance behavior in exploratory tasks. Mol. Psychiatry 25, 491–505.

Li, Q., Qu, F.-L., Gao, Y., Jiang, Y.-P., Rahman, K., Lee, K.-H., Han, T., Qin, L.-P., 2017. Piper sarmentosum roxb. produces antidepressant-like effects in rodents, associated with activation of the CREB-BDNF-ERK signaling pathway and reversal of HPA axis hyperactivity. J. Ethnopharmacol. 199, 9–19.

Li, B., Suemaru, K., Cui, R., Kitamura, Y., Gomita, Y., Araki, H., 2006. Repeated electroconvulsive stimuli increase brain-derived neurotrophic factor in ACTH-treated rats. Eur. J. Pharmacol. 529, 114–121.

Liang, S., Nagy, Dallman, 2000. Voluntary sucrose ingestion, like corticosterone replacement, prevents the metabolic deficits of adrenalectomy. J. Neuroendocrinol. 12, 461–470.

Liu, M.-Y., Yin, C.-Y., Zhu, L.-J., Zhu, X.-H., Xu, C., Luo, C.-X., Chen, H., Zhu, D.-Y., Zhou, Q.-G., 2018. Sucrose preference test for measurement of stress-induced anhedonia in mice. Nat. Protoc. 13, 1686–1698.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods 25, 402–408.

Lupien, S., Nair, N., Briere, S., Maheu, F., Tu, M., Lemay, M., McEwen, B., Meaney, M., 1999. Increased cortisol levels and impaired cognition in human aging: implication for depression and dementia in later life. Rev. Neurosci. 10, 117–140.

Meye, F.J., Adan, R.A., 2014. Feelings about food: the ventral tegmental area in food reward and emotional eating. Trends Pharmacol. Sci. 35, 31–40.

Mlyniec, K., 2015. Zinc in the glutamatergic theory of depression. Curr. Neuropharmacol. 13, 505–513.

Naert, G., Maurice, T., Tapia-Arancibia, L., Givalois, L., 2007. Neuroactive steroids modulate HPA axis activity and cerebral brain-derived neurotrophic factor (BDNF) protein levels in adult male rats. Psychoneuroendocrinology 32, 1062–1078.

Nandam, L.S., Brazel, M., Zhou, M., Jhaveri, D.J., 2020. Cortisol and major depressive disorder—translating findings from humans to animal models and back. Front. Psych. 10 (974), 1664. https://doi.org/10.3389/fpsyt.2019.00974.

Technology NI. Basic behavioral neuroscience in rodents. In *Exploration & locomotion*, pp. 12-14. Noldus Information Technology, Noldus.

O'Brien, J.T., Lloyd, A., McKeith, I., Gholkar, A., Ferrier, N., 2004. A longitudinal study of hippocampal volume, cortisol levels, and cognition in older depressed subjects. Am. J. Psychiatry 161, 2081–2090.

Organization WH. (2008). The global burden of disease: 2004 update. Available at https://www.who.int/publications/i/item/9789241563710. [Accessed April 05, 2022].

Organization WH. (2014). Preventing suicide: A global imperative. Available at https://www.who.int/publications/i/item/9789241564779. [Accessed April 05, 2022].

Ouakki, S., Zghari, O., El Hessni, A., Mesfioui, A., Ouichou, A., 2019. Modulation of anxiolytic-like and antidepressant-like effects of melatonin by imipramine in Wistar rats: possible Interaction with central monoaminergic systems. Neuroscience and Medicine 10, 118–133.

Patchev, V.K., Almeida, O.F., 1996. Gonadal steroids exert facilitating and "buffering" effects on glucocorticoid-mediated transcriptional regulation of corticotropin-releasing hormone and corticosteroid receptor genes in rat brain. J. Neurosci. 16, 7077–7084.

Pereira, V.S., Joca, S.R., Harvey, B.H., Elfving, B., Wegener, G., 2019. Esketamine and rapastinel, but not imipramine, have antidepressant-like effect in a treatment-resistant animal model of depression. Acta Neuropsychiatrica 31, 258–265.

Pizzagalli, D.A., 2014. Depression, stress, and anhedonia: toward a synthesis and integrated model. Annu. Rev. Clin. Psychol. 10, 393–423.

Porsolt, R.D., Le Pichon, M., Jalfre, M., 1977. Depression: a new animal model sensitive to antidepressant treatments. Nature 266, 730–732.

Posener, J.A., DeBattista, C., Williams, G.H., Kraemer, H.C., Kalehzan, B.M., Schatzberg, A.F., 2000. 24-hour monitoring of cortisol and corticotropin secretion in psychotic and nonpsychotic major depression. Arch. Gen. Psychiatry 57, 755–760.

Rastoldo, G., Marouane, E., El Mahmoudi, N., Péricat, D., Bourdet, A., Timon-David, E., Dumas, O., Chabbert, C., Tighilet, B., 2020. Quantitative evaluation of a new posturo-locomotor phenotype in a rodent model of acute unilateral vestibulopathy. Front. Neurol. 11, 505.

Revest, J., Le Roux, A., Roullot-Lacarrière, V., Kaouane, N., Vallée, M., Kasanetz, F., Rougé-Pont, F., Tronche, F., Desmedt, A., Piazza, P., 2014. BDNF-TrkB signaling through Erk1/2MAPK phosphorylation mediates the enhancement of fear memory induced by glucocorticoids. Mol. Psychiatry 19, 1001–1009.

Seibenhener, M.L., Wooten, M.C., 2015. Use of the open field maze to measure locomotor and anxiety-like behavior in mice. JoVE (journal of Visualized Experiments) e52434, 1940. https://doi.org/10.3791/52434.

Simmons, W.K., Burrows, K., Avery, J.A., Kerr, K.L., Bodurka, J., Savage, C.R., Drevets, W.C., 2016. Depression-related increases and decreases in appetite: dissociable patterns of aberrant activity in reward and interoceptive neurocircuitry. Am. J. Psychiatry 173, 418–428.

Slattery, D.A., Cryan, J.F., 2012. Using the rat forced swim test to assess antidepressant-like activity in rodents. Nat. Protoc. 7, 1009–1014.

Song, J., Zhou, N., Ma, W., Gu, X., Chen, B., Zeng, Y., Yang, L., Zhou, M., 2019. Modulation of gut microbiota by chlorogenic acid pretreatment on rats with adrenocorticotropic hormone induced depression-like behavior. Food Funct. 10, 2947–2957.

Song, J., Ma, W., Gu, X., Zhao, L., Jiang, J., Xu, Y., Zhang, L., Zhou, M., Yang, L., 2019. Metabolomic signatures and microbial community profiling of depressive rat model induced by adrenocorticotrophic hormone. J. Transl. Med. 17, 1–12.

Sousa, N., Almeida, O., Wotjak, C., 2006. A hitchhiker's guide to behavioral analysis in laboratory rodents. Genes Brain Behav. 5, 5–24.

Sterner, E.Y., Kalynchuk, L.E., 2010. Behavioral and neurobiological consequences of prolonged glucocorticoid exposure in rats: relevance to depression. Prog. Neuropsychopharmacol. Biol. Psychiatry 34, 777–790.

Torres, J., Ruiz, E., Ortega, E., 2001. Effects of CRH and ACTH administration on plasma and brain neurosteroid levels. Neurochem. Res. 26, 555–558.

Tronche, F., Kellendonk, C., Kretz, O., Gass, P., Anlag, K., Orban, P.C., Bock, R., Klein, R., Schütz, G., 1999. Disruption of the glucocorticoid receptor gene in the nervous system results in reduced anxiety. Nat. Genet. 23, 99–103.

Veldhuis, H.D., De Korte, C.C., De Kloet, E.R., 1985. Glucocorticoids facilitate the retention of acquired immobility during forced swimming. Eur. J. Pharmacol. 115, 211–217.

Vreeburg, S.A., Hoogendijk, W.J., DeRijk, R.H., van Dyck, R., Smit, J.H., Zitman, F.G., Penninx, B.W., 2013. Salivary cortisol levels and the 2-year course of depressive and anxiety disorders. Psychoneuroendocrinology 38, 1494–1502.

Wang, G., Lei, C., Tian, Y., Wang, Y., Zhang, L., Zhang, R., 2019. Rb1, the primary active ingredient in Panax ginseng CA Meyer, exerts antidepressant-like effects via the BDNF–TrkB–CREB pathway. Front. Pharmacol. 10, 1034.

Wang, H., Xu, J., Lazarovici, P., Quirion, R., Zheng, W., 2018. cAMP response element-binding protein (CREB): a possible signaling molecule link in the pathophysiology of schizophrenia. Front. Mol. Neurosci. 11, 255.

Willner, P., Golembiowska, K., Klimek, V., Muscat, R., 1991. Changes in mesolimbic dopamine may explain stress-induced anhedonia. Psychobiology 19, 79–84.

Yankelevitch-Yahav, R., Franko, M., Huly, A., Doron, R., 2015. The forced swim test as a model of depressive-like behavior. JoVE (journal of Visualized Experiments) e52587.

Young, E.A., Carlson, N.E., Brown, M.B., 2001. Twenty-four-hour ACTH and cortisol pulsatility in depressed women. Neuropsychopharmacology 25, 267–276.

Zabik, J., Levine, R., Spaulding, J., Maickel, R., 1977. Interactions of tricyclic antidepressant drugs with deprivation-induced fluid consumption by rats. Neuropharmacology 16, 267–271.

Zhao, L., Zhang, Z., Zhou, M., Gou, X., Zeng, Y., Song, J., Ma, W., Xu, Y., 2018. A urinary metabolomics (GC-MS) strategy to evaluate the antidepressant-like effect of chlorogenic acid in adrenocorticotropic hormone-treated rats. RSC Adv. 8, 9141–9151.