Sex and stressor modality influence acute stress-induced dynamic changes in corticolimbic endocannabinoid levels in adult Sprague Dawley rats

Haley A. Vecchiarelli a,c,d,1,2, Maria Morena b,c,d,1,3, Tiffany T.Y. Lee b,c,d,1,e,1, Andrei S. Nastase a,c,d, Robert J. Aukema a,c,d, Kira D. Leitl b,c,d, J. Megan Gray b,c,d, Gavin N. Petrie a,c,d, Kristin J. Tellez-Monnery b,c,d, Matthew N. Hill b,c,d,*

a Neuroscience Graduate Program, University of Calgary, Calgary, AB, T2N 4N1, Canada
b Departments of Cell Biology & Anatomy and Psychiatry, Cumming School of Medicine, University of Calgary, Calgary, AB, T2N 4N1, Canada
c Hotchkiss Brain Institute, Cumming School of Medicine, University of Calgary, Calgary, AB, T2N 4N1, Canada
d Mathison Centre for Mental Health Research and Education, Cumming School of Medicine, University of Calgary, Calgary, AB, T2N 4N1, Canada
e Department of Psychology, University of British Columbia, Vancouver, BC, V6T 1Z4, Canada

ARTICLE INFO

Keywords:
Endocannabinoid
Stress
Stressor modality
Sex differences
Rats
Corticolimbic

ABSTRACT

Research over the past few decades has established a role for the endocannabinoid system in contributing to the neural and endocrine responses to stress exposure. The two endocannabinoid ligands, anandamide (AEA) and 2-arachidonoyl glycerol (2-AG), both play roles in regulating the stress response and both exhibit dynamic changes in response to stress exposure. Most of this previous research, however, was conducted in male rodents. Given that, especially in rodents, the stress response is influenced by sex, an understanding of how these dynamic responses of endocannabinoids in response to stress is influenced by sex could provide insight into sex differences of the acute stress response. We exposed adult, Sprague Dawley rats to different commonly utilized acute stress modalities, specifically restraint, swim and foot shock stress. Thirty minutes following stress onset, we excised the amygdala, hippocampus and medial prefrontal cortex, corticolimbic brain regions involved in the stress response, to measure endocannabinoid levels. When AEA levels were altered in response to restraint and swim stress, they were reduced, whereas exposure to foot shock stress led to an increase in the amygdala. 2-AG levels, when they were altered by stress exposure were only increased, specifically in males in the amygdala following foot shock stress. This increase in 2-AG levels following stress only in males was the only sex difference found in stress-induced changes in endocannabinoid levels. There were no consistent sex differences observed. Collectively, these data contribute to our further understanding of the interactions between stress and endocannabinoid function.

1. Introduction

The endocannabinoid system was first identified as the molecular target through which delta-9-tetrahydrocannabinol (THC), the primary psychoactive constituent of cannabis, exerts its effects on the brain and body (Hillard, 2015). While the cannabinoid type 1 receptor (CB1) is established as the primary mediator of the effects of THC, this receptor is endogenously activated by a class of lipid signaling molecules known as endocannabinoids, with the arachidonate-derived anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) being the two primary endocannabinoid signaling molecules (Hillard, 2015). As reduction of stress and anxiety is often listed as a primary motivation for using cannabis

Abbreviations: THC, delta-9-tetrahydrocannabinol; CB1, cannabinoid type 1 receptor; AEA, anandamide; 2-AG, 2-arachidonoyl glycerol; CRH, corticotropin releasing hormone; HPA, hypothalamic-pituitary-adrenal; SEM, standard error of the mean; FAAH, fatty acid amide hydrolase; CRH-R1, CRH receptor type 1.

* Corresponding author. University of Calgary, Canada.
E-mail address: mnhill@ucalgary.ca (M.N. Hill).
1 Equal contribution.
2 Dr. Haley A. Vecchiarelli, Division of Medical Sciences, University of Victoria, Victoria, BC, V8W 2Y2.
3 Dr. Maria Morena, Department of Physiology and Pharmacology, Sapienza University of Rome, European Center for Brain Research, Santa Lucia Foundation, Rome, Italy.

https://doi.org/10.1016/j.ynstr.2022.100470
Received 16 April 2022; Received in revised form 27 June 2022; Accepted 15 July 2022
Available online 30 July 2022
2352-2895/© 2022 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
upregulate CB1 receptor expression (Reich et al., 2009). These sex differences in receptors in males, however, chronic stress in females was found to downregulate CB1 endocannabinoid signaling in response to various challenges or external stimuli. For example, while elevating AEA signaling in males has typically been found to promote the extinction of fear, we recently reported that augmentation of AEA signaling appears to do the opposite and promote fear behaviors in female rodents (Morena et al., 2021). In a similar vein, chronic stress has been reliably found to downregulate CB1 signaling, primarily 2-AG, which then acts to reduce neural activity throughout stress-responsive neural circuits and contributes to the normative termination of the stress response (Di et al., 2003; Evanson et al., 2010; Hill et al., 2011; Ragozzino et al., 2020). These dynamic changes in endocannabinoid signaling in response to stress have been well characterized, and several translational studies have also demonstrated similar dynamic changes in endocannabinoid signaling in humans (Crombie et al., 2019; Hill et al., 2009b; Mayo et al., 2020a; Spohrs et al., 2022), and verified that the endocannabinoid system is also an important regulator of stress responses and affective states in humans (Gunduz-Cinar et al., 2013; Mayo et al., 2020a, 2020b).

To date, the overwhelming majority of research on endocannabinoids and stress has focused explicitly on male subjects. Particularly in rodents, stress reactivity is well documented to be influenced by the sex of the subject, predominately with respect to the activation of the hypothalamic-pituitary-adrenal (HPA) axis and the subsequent release of glucocorticoid hormones, such as corticosterone (Rincón-Cortés et al., 2019). As more research has begun to focus on sex differences, it has also become apparent that the endocannabinoid system is influenced by sex, both in terms of the impact that fluctuations in endocannabinoid signaling have on behavior and the dynamic changes that occur in endocannabinoid signaling in response to various challenges or external stimuli. For example, while elevating AEA signaling in males has typically been found to promote the extinction of fear, we recently reported that augmentation of AEA signaling appears to do the opposite and promote fear behaviors in female rodents (Morena et al., 2021). In a similar vein, chronic stress has been reliably found to downregulate CB1 receptors in males, however chronic stress in females was found to upregulate CB1 receptor expression (Reich et al., 2009). These sex differences are not surprising, however, given that cannabis itself can produce opposing or differential effects and there are sex differences in both the expression of CB1 receptors and endocannabinoid molecules in humans and rodents (Cooper and Craft, 2018).

As there are well established sex differences in stress reactivity and endocannabinoid function, we sought to examine if the effects of acute stress on dynamic changes in endocannabinoid function were influenced by sex. Moreover, given that the modality and nature of the stressor applied can influence many of the effects of stress, we performed a detailed analysis of changes in endocannabinoid content throughout stress-responsive corticolimbic structures following exposure to three different common used experimental models to induce stress: restraint, swim and foot shock. Our results indicate that both sex and stressor modality have a significant influence on dynamic changes in endocannabinoid function.

2. Methods

2.1. Animals

Adult (~post-natal 70 days old) male and female Sprague Dawley rats were utilized for this study. All rats were obtained from Charles River (Saint Constant, QC, Canada, RGD Cat# 734476, RRID: RGD_734476). Rats were acclimated for 1 week prior to experiments. Rats were pair housed; from each pair-housed duo, one rat was collected immediately for inclusion in the basal group while the other was used for the stress group. Rats were kept on a 12:12 lightdark cycle and had ad libitum access to food and water. All experiments were performed during the start of the light phase. All protocols were approved by the University of Calgary Animal Care Committee and Canadian Council for Animal Care. All experiments took place in the light portion of the light cycle.

2.2. Acute stress protocols

From each pair-housed duo of rats, one was placed in the stress apparatus and the other was immediately euthanized for use in the corresponding basal group.

2.2.1. Restraint stress

Rats were exposed to 30 min of acute restraint stress in a clear, Plexiglass restrainer. Immediately following restraint termination, blood and tissue samples were collected. Graphical representation of methodology is found in Fig. 1A (Created in Biorender).

2.2.2. Swim stress

Rats were exposed to 15 min of acute swim stress in an opaque 5 gal container, containing 4 gal water, followed by 15 min in the home cage. Water temperature was 25 °C ± 1 °C. Graphical representation of methodology is found in Fig. 2A (Created in Biorender).

2.2.3. Foot shock stress

Foot shock stress consisted of ten foot-shocks (0.65 mA, 2 s) delivered in 15 min using MED Associates fear-conditioning chambers (St. Albans, VT, USA). Rats were returned to their home cage for 15 min after stress termination, before collection of blood and brain samples. Graphical representation of methodology is found in Fig. 3A (Created in Biorender).

2.3. Endocannabinoid ligand level quantification

Corticolimbic brain regions (amygdala, hippocampus and medial prefrontal cortex) were excised as previously described (Del et al., 2010a), immediately snap frozen and stored at −80 °C. Specifically, excised brain regions were defined as: amygdala (central, basolateral, medial and cortical nuclei); hippocampus (both dorsal and ventral, including all sub-regions); and, medial prefrontal cortex and anterior cingulate cortex (dorsal to the anterior olfactory nucleus and medial to the corpus callosum and claustrum) (Hill et al., 2010a). Samples were homogenized for analysis of the bulk tissue level of AEA and 2-AG using liquid chromatography/tandem mass spectrometry as previously described (Morena et al., 2019; Qi et al., 2015; Vecchiarelli et al., 2021). Samples were homogenized in 2 mL of acetonitrile with 5 pmol d8-2-AG (Cayman Chemical Company, Ann Arbor, Michigan, USA, #390050) and 5 nmol d8-2-AG (Cayman Chemical Company, #362160) in a borosilicate glass tube with a glass rod. Samples were sonicated, incubated overnight at −20 °C, centrifuged at 1500×g, supernatants containing lipids were isolated, evaporated with nitrogen gas, washed with acetonitrile and evaporated with nitrogen gas again. Final reconstitution for liquid chromatography/tandem mass spectrometry was in 200 μL of acetonitrile before storage at −80 °C.

2.4. Plasma corticosterone level quantification

Plasma corticosterone levels were determined using an ELISA as previously described, according to the manufacturer’s protocol (Cayman Chemical Company, Ann Arbor, Michigan, USA, #500655, RRID: AB_2868564—for restraint and swim stress cohorts (Vecchiarelli et al.,
Fig. 1. Restraint Stress-Induced Alterations in Endocannabinoid Levels.
(A) Representative methods schematic (created in Biorender). (B) Corticosterone (CORT) levels increased with restraint stress exposure and were greater in females compared to males both basally and in response to stress. Anandamide (AEA) levels were higher in females compared to males in the (C) amygdala, (D) hippocampus and (E) medial prefrontal cortex, but were only altered by stress in the (E) medial prefrontal cortex, where they were decreased. 2-arachidonoyl glycerol (2-AG) levels were not altered by sex or restraint stress exposure in the (F) amygdala, (G) hippocampus and (H) medial prefrontal cortex. (I) Correlation matrix. Estradiol, progesterone and uterine weights only compared in females. Significant bars inside the axes represent specific comparisons between groups, while those outside of the axes represent main effects (sex and stress). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Basal = left pair, Stress = right pair. In each pair, the left bars are the males (grape, horizontal lines, diamonds) and the right bars are the females (teal, vertical lines, triangle).
Fig. 2. Swim Stress-Induced Alterations in Endocannabinoid Levels.

(A) Representative methods schematic (created in Biorender). (B) Corticosterone (CORT) levels increased with swim stress exposure and were greater in females compared to males in response to stress. Anandamide (AEA) levels were reduced in the (C) amygdala, (D) hippocampus and (E) medial prefrontal cortex following swim stress and this was primarily driven by males in the (E) medial prefrontal cortex. 2-arachidonoyl glycerol (2-AG) levels were lower in females compared to males in the (F) amygdala, (G) hippocampus and (H) medial prefrontal cortex. 2-AG levels were increased with swim stress in the (F) amygdala and this was primarily driven by males, with no stress-induced changes in the (G) hippocampus or (H) medial prefrontal cortex. (I) Correlation matrix. Estradiol, progesterone and uterine weights only compared in females. Significant bars inside the axes represent specific comparisons between groups, while those outside of the axes represent main effects (sex and stress). **p < 0.01, ***p < 0.001, ****p < 0.0001. Basal = left pair, Stress = right pair. In each pair, the left bars are the males (grape, horizontal lines, diamonds) and the right bars are the females (teal, vertical lines, triangle).
Fig. 3. Foot Shock Stress-Induced Alterations in Endocannabinoid Levels.

(A) Representative methods schematic (created in Biorender). (B) Corticosterone (CORT) levels increased with foot shock stress exposure. Anandamide (AEA) levels were increased in the (C) amygdala following foot shock stress with no effect of sex. Sex or stress exposure did not alter AEA levels in the (D) hippocampus or (E) medial prefrontal cortex. 2-arachidonoyl glycerol (2-AG) levels were increased in the (G) hippocampus and (H) medial prefrontal cortex with exposure to foot shock stress with no effect of sex. Sex or stress did not alter 2-AG levels in the (F) amygdala. (I) Correlation matrix. Estradiol, progesterone and uterine weights only compared in females. Significant bars inside the axes represent specific comparisons between groups, while those outside of the axes represent main effects (sex and stress). *p < 0.05, **p < 0.01, ***p < 0.001. Basal = left pair, Stress = right pair. In each pair, the left bars are the males (grape, horizontal lines, diamonds) and the right bars are the females (teal, vertical lines, triangle).
2.5. Estrus cycle determination

The phase of the estrus cycle that females were in was determined by using a composite of plasma estradiol, plasma progesterone, uterine weight and vaginal cytology all taken immediately post-mortem concomitantly with brain tissue collection. Plasma estradiol (Alpco Diagnostics, Salem, New Hampshire, USA, #55-ESTRT-E01) and progesterone (Alpco Diagnostics, #55-PROMS-E01) levels were ascertained using ELISAs per manufacturer’s protocols. For analysis, rats that were in the proestrus phase were compared to rats in remaining phases. However, there were no overall significant effects of estrus cycle phase on endocannabinoid levels, so the female data were collapsed for analysis.

2.6. Statistical analysis

Statistical analysis was performed using Prism v9 (GraphPad, San Diego, California, USA). For all experiments, statistical analysis was performed using a two-way ANOVA, analyzing for an interaction between stress and sex, as well as, main effects for stress and sex, followed by post-hoc comparisons with Bonferroni corrections between relevant groups. For all non-correlative data, F-values and p-values are reported in Supplemental Table 1; all data are represented as mean ± standard error of the mean (SEM). Correlations were assessed between all parameters within each stressor using Pearson r; r-, F- and p-values are reported in Supplemental Table 1.

3. Results

3.1. Restraint stress

Plasma corticosterone levels in the restraint cohort were increased with exposure to restraint stress (Fig. 1B); furthermore, plasma corticosterone levels were higher in females than males, both basally and in response to restraint stress exposure (Fig. 1B). In the restraint cohort, females had higher basal levels of AEA than males in the amygdala, hippocampus and medial prefrontal cortex (Fig. 1C–E). Overall, in the medial prefrontal cortex, there was a reduction in AEA levels with acute restraint stress (Fig. 1E), but there was no main effect of acute restraint stress on AEA levels in the amygdala or hippocampus (Fig. 1C and D). With restraint, there was no an effect of sex, acute restraint stress or the interaction of the two, on 2-AG levels in the amygdala, hippocampus or medial prefrontal cortex (Fig. 1F–H).

3.2. Swim stress

Plasma corticosterone levels in the swim stress cohort were increased with exposure to swim stress (Fig. 2B); furthermore, plasma corticosterone levels were higher in females than males in response to swim stress exposure (Fig. 2B). With exposure to swim, there was an overall reduction in AEA levels across the amygdala, hippocampus and medial prefrontal cortex (Fig. 2C–E); this was perhaps driven by males in the medial prefrontal cortex, as in this region males had significantly lower levels of AEA with swim stress (Fig. 2E). Amygdala 2-AG levels were increased with stress exposure in males, but not females (Fig. 2F). There was also an overall effect of sex on 2-AG levels in this cohort, with males across groups having greater 2-AG levels than females in the amygdala, hippocampus and medial prefrontal cortex (Fig. 2F–H); in the hippocampus, this may have been driven by significant sex differences specifically in the basal group, with the basal male group having higher levels than basal female group (Fig. 2G).

3.3. Foot shock stress

Plasma corticosterone levels in the foot shock stress cohort were increased with exposure to stress (Fig. 3B); there were no observed sex differences in plasma corticosterone levels, basally or as a response to stress exposure. Following acute foot shock in the amygdala, there was an increase in AEA levels (Fig. 3C), and no changes in the hippocampus or medial prefrontal cortex (Fig. 3D and E). With foot shock stress, there was an increase in 2-AG levels in the hippocampus and medial prefrontal cortex (Fig. 3G and H), but there were no changes in 2-AG levels in the amygdala (Fig. 3F).

3.4. Correlations

While there were no significant effects of estrous cycle on endocannabinoid ligand levels, basally or following stress exposure, it was possible that individual aspects of our composition score, i.e. estradiol, progesterone, uterine weight, had effects on endocannabinoids. We correlated, across all groups, between endocannabinoid levels, corticosterone, estradiol, progesterone and uterine weights, in the restraint stress cohort (Fig. 11), swim stress cohort (Fig. 2I) and foot shock stress (Fig. 3I). There were no significant correlations between female endocannabinoid ligand levels and estradiol, progesterone or uterine weight (Supplemental Table 1). When looking at any overall patterns, there were generally negative correlations between plasma estradiol and progesterone and AEA levels; progesterone was also generally negatively correlated with 2-AG levels. While each cohort had some significant correlations, there were no significant correlations across all three stressors.

4. Discussion

This study builds on previous work investigating the impact of acute stress on endocannabinoid dynamics in the brain by comparing a host of stressors of differing modalities, as well as exploring sex differences in the responses by utilizing both male and female subjects. Generally consistent with previous reports, we found stressor- and sex-specific changes in endocannabinoid levels across corticolimbic brain regions. Surprisingly, there was no consistent effect of all three stressors employed on any endocannabinoid change in any brain region, suggesting that these changes produced by stress are heavily influenced by the nature of the stressor being employed. More so, we did not detect any reliable sex difference, either in basal or stress-induced changes in endocannabinoid contents in any brain region examined.

Surprisingly, exposure to restraint stress had very little impact on endocannabinoid levels, with a stress-induced reduction in AEA content in the medial prefrontal cortex in both sexes (although largely carried by males) being the only significant outcome. While stress-induced reductions in amygdalar AEA levels are also typically reported after acute restraint, this effect in rats is typically quite subtle (~10–15% reduction in rats) (Hill et al., 2009a) and is typically found to be more robust in mice (Bedse et al., 2017; Mayo et al., 2020a; Patel et al., 2005), suggesting that there may be species differences in terms of the magnitude of this response. More so, previous work has indicated that some of the restraint-induced changes in endocannabinoid levels, particularly with respect to 2-AG levels, do not become apparent until later time points following stress cessation (Hill et al., 2011); however, analysis of the temporal kinetics of stressors was beyond the scope of this study and we focused on the time point associated with peak neuroendocrine responses to stress. We focused on 30 min post-stress onset for all of our analysis, as this is when corticosterone levels generally peak in the brain. Given that the other stress groups were given a period of 15 min following stress cessation in their home cage, this could have contributed to differences seen across cohorts. Additionally, longer exposure to other stress protocols could have introduced confounding variables to the interpretation, potentially. Repeated exposure to restraint in both pathways...
suggests the possibility of a stress intensity threshold to produce
stressors act to dampen AEA and augment 2-AG signaling, but suggest
the intracellular mechanisms linking CRH-R1 and FAAH have yet to
be elucidated, this could indicate that specific intracellular cascades
activated by CRH-R1, which trigger FAAH activity, may differ in males
and females or stress-induced changes in AEA levels occur at a different
time course in males than females.

Foot shock has previously been reported to be the one stressor that
results in an increase in AEA signaling (Hohmann et al., 2005; Morena
et al., 2014), as opposed to a decrease, and consistent with those reports
we similarly found that repeated shock exposure increased AEA levels
within the amygdala. In line with previous reports, and consistent with
other stressors (Morena et al., 2016), foot shock was also found to in-
crease endocannabinoid levels in the hippocampus and prefrontal cortex. Previous
work using a shock paradigm has indicated that this mobilization of
endocannabinoid signaling could be involved in the generation of
stress-induced analgesia (Hohmann et al., 2005), which suggests that
the inclusion of a noxious component to the stressor itself could alter
endocannabinoid dynamics, as the presence of a painful stimuli could
recruit endocannabinoid signaling as an endogenous analgesic mecha-

mechanisms that drive these responses.

Interestingly, there was no consistent effect of sex on either basal or
stress-induced changes in endocannabinoid signaling across these
studies. Previous work has suggested that endocannabinoid levels,
particularly that of AEA, may be sensitive to reproductive hormones
such as estrogen (Hill et al., 2007; Maccarrone et al., 2002; Maia et al.,
2017; Tabatadze et al., 2015). In the cohort of rats used for the restraint
study, we found that females had globally higher AEA then males,
regardless of stress condition; however, this was not seen in either of
the other two cohorts. Additionally, in the cohort of rats used for the swim
stress study, females exhibited globally lower 2-AG levels than males,
which again was not observed in the other cohorts. We did not perform
serial lavages to establish cycle stage in the days preceding the stress
exposure (due to the potential confounds this introduces to the study
given that the lavage itself is mildly stressful, and is only performed in
females and not males, and thus represents a confounding variable that
could independently influence female stress responses). We used a
combination of estrogen/progesterone levels at time of tissue collection,
uterine weight and a single post-mortem lavage to identify estrus stage
and we did not see any association with differences in estrus stage
(proestrus versus other stages) or estradiol levels that could explain the
differences in endocannabinoid levels seen in one cohort but not the
other. We also saw no significant correlations between estradiol or
progesterone levels or uterine weights and endocannabinoid levels.
We did see differences in basal corticosterone across the cohorts, however,
which could be reflective of either life stress history (in breeding facility
or during shipping) or current differences in ambient stress in the
housing facility in which they reside, suggesting that perhaps additional
variables beyond our control could be interacting with sex to produce
spurious differences in endocannabinoid levels that are being attributed
explicitly to sex. Regardless of the reason as to why we saw inconsistent
sex effects across the cohorts, our data generally do not suggest that
there are robust sex differences across modalities in stress-induced
endocannabinoid dynamics within corticolimbic brain structures.
Collectively, these data add to the growing body of literature
regarding the interactions between stress and endocannabinoid func-
tion. Our data generally support previous findings suggesting that
stressors act to dampen AEA and augment 2-AG signaling, but suggest
that the impacts of these effects may be modified by stressor modality,
with more mixed or physical modalities producing more robust and
consistent changes in endocannabinoid levels. More so, the inclusion of
a noxious component to the stressor could influence the directionality of
endocannabinoid changes due to its co-involvement in central pain
networks. Surprisingly, biological sex of the subject seemed to have
minimal or inconsistent impact on basal or stress-induced endocanna-
binoid changes. Several previous studies have investigated sex differ-
ences in the context of the impacts of pharmacological modulation of
endocannabinoid signaling on the neuroendocrine response to stress
(Atkinson et al., 2010; Roberts et al., 2014), and similar to what we saw
here the overall conclusion from those studies is minimal or inconsis-
tent. Interestingly, previous work has found that there are, however, sex
differences in the impact of endocannabinoid signaling on stress-related
affective behaviors such as fear and anxiety (Albrechet-Souza et al.,
2021; Bowers and Ressler, 2016; Morena et al., 2021; Simone et al.,
2018). Given that pharmacological tools which target endocannabinoid
signaling are well into clinical development for the treatment of
stress-related and affective disorders (Mayo et al., 2020b; Panlus et al.,
2021; Schmidt et al., 2021), understanding the parameters by which
stress regulates endocannabinoid signaling is important to establish.
Future work will need to focus on the influence of sex on endocanna-
binoid dynamics to chronic or repeated stress exposure, as this may be
particularly relevant for disease states, and endocannabinoid signaling
has already been identified to play an important role in stress adaptation
and plasticity of the HPA axis.
Funding

This work was supported by a foundation grant from the Canadian Institutes of Health Research (CIHR) FDN333950 to MNH; HAV received stipend funding from CIHR (Vanier CGS), the University of Calgary (UofC; Killam Pre-doctoral Laureate), Alberta Innovates-Health Solutions (AIHS) and Branch Out Neurological Foundation (BONF); MM received fellowship support from AIHS and CIHR; ASN received stipend funding from CIHR (CGS-M), and the UofC (Mathison Centre Graduate Recruitment Scholarship in Mental Health and Cumming School of Medicine Graduate Scholarship); RJA received stipend funding from the University of Calgary (Mathison Centre Graduate Recruitment Scholarship in Mental Health); JMG received stipend funding from CIHR (CGS-D) and fellowship support from AIHS; MNH was the recipient of a Tier II Canada Research Chair in the Neurobiology of Stress.

Funding agencies had no influence on the design, execution or publishing of this work.

CRediT authorship contribution statement

Haley A. Vecchiarelli: Conceptualization, Formal analysis, Investigation, Writing – original draft, Visualization. Maria Morena: Conceptualization, Formal analysis, Investigation, Writing – original draft. Tiffany T.Y. Lee: Conceptualization, Investigation, Writing – review & editing. Andrei S. Nastase: Investigation, Writing – review & editing. Robert J. Aukema: Investigation, Writing – review & editing. Kirà D. Leíl: Investigation, Writing – review & editing. J. Megan Gray: Investigation, Writing – review & editing. Gavin N. Petrie: Investigation, Writing – review & editing. Kristín J. Tellez-Monney: Investigation, Writing – review & editing. Matthew N. Hill: Conceptualization, Methodology, Investigation, Resources, Writing – original draft, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare no conflict of interest. Our funding acknowledgement statement can be found above, and funding agencies had no influence on the design, execution or publishing of this work.

Acknowledgements

This manuscript is dedicated to the memory of Dr. Bruce McEwen. We acknowledge that this work was conducted on the traditional territories of the people of the Treaty 7 region in Southern Alberta, which includes the Blackfoot Confederacy (comprising the Siksika, Piikani, and Kainai First Nations), as well as the Tsuut'ina First Nation, and the Stoney Nakoda (including the Chiniki, Bearspaw, and Wesley First Nations). The City of Calgary is also home to Metis Nation of Alberta, Region III.

We acknowledge the work of the University of Calgary Health Sciences Animal Research Centre for care of the rats, particularly Krista Jensen and Brittany Munro.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ynst.2022.100470.

References

Atkinson, H.C., Leggett, J.D., Wood, S.A., Castricue, E.S., Kershaw, Y.M., Lightman, S.L., 2010. Regulation of the hypothalamic-pituitary-adrenal axis circular rhythm by endocannabinoids is sexually dimorphic. Endocrinology 151, 3720–3727. https://doi.org/10.1210/en.2010-0101.

Bangasser, D.A., 2013. Sex differences in stress-related receptors: ”micro” differences with “macro” implications for mood and anxiety disorders. Biol. Sex Differ. 4, 2. https://doi.org/10.1186/2042-6410-4-2.

Bangasser, D.A., Curtis, A., Reyes, B.A.S., Beatha, T.T., Parastatidis, I., Ischiropoulos, H., Van Bockstaele, E.J., Valentino, R.J., 2010. Sex differences in corticotropin-releasing factor receptor signaling and trafficking: potential role in female vulnerability to stress-related psychopathology. Mol. Psychiatr. 15 (077), 896–904. https://doi.org/10.1038/mp.2010.66.

Bangasser, D.A., Wiersielis, K.R., 2018. Sex differences in stress responses: a critical role for corticotropin-releasing factor. Hormones (Basel) 17, 5–13. https://doi.org/10.3390/hormones17010005.

Bedse, G., Hartley, N.D., Neale, E., Gaulden, A.D., Patrick, T.A., Kingsley, P.J., Uddin, M.J., Plath, N., Marnett, L.J., Patel, S., 2017. Functional redundancy between canonical endocannabinoid systems in the modulation of pain. Biol. Psychiatr. 82, 488–499. https://doi.org/10.1016/j.biopsych.2017.03.002.

Bluett, R.J., Gamble-George, J.C., Herrington, D.J., Marnett, L.J., Patel, S., 2014. Central anandamide deficiency predicts stress-induced anxiety: behavioral reversal through endocannabinoid signaling. Transl. Psychiatr. 4, e608. https://doi.org/10.1038/mp.2014.55.

Bowers, M.E., Resledger, K.J., 2016. Sex dependence of anxiety-like behavior in cannabinoid receptor 1 (Cnr1) knockout mice. Behav. Brain Res. 300, 65–69. https://doi.org/10.1016/j.bbr.2015.12.005.

Commons, K.G., Cholanians, A.B., Babb, J.A., Ehlinger, D.G., 2017. The rodent forced swim test measures stress coping strategy, not depression like behavior. ACS Chem. Neurosci. 8, 955–960. https://doi.org/10.1021/acschemneuro.7b00042.

Cooper, Z.D., Craft, R.M., 2018. Sex-Dependent effects of cannabis and cannabinoids: a translational perspective. Neuropsychopharmacology 43, 34–51. https://doi.org/10.1038/npp.2017.140.

Cordova, A., Gimenez, M., Escanero, J.F., 1990. Effect of swimming to exhaustion, at low temperatures, on serum, brain, spinal cord and CA in rats. Physiol. Behav. 48, 595–598. https://doi.org/10.1016/S0031-8135(05)80719-7.

Crombie, K.M., Leitzelar, B.N., Brellelhin, A.G., Hillard, C.J., Koltyn, K.F., 2019. Loss of exercise- and stress-induced increases in circulating 2-arachidonoylgllycerol concentrations in adults with chronic PTSD. Biol. Psyched. 145, 1–7. https://doi.org/10.1016/j.biopsycho.2019.04.002.

Daiwile, A.P., Jayanthi, S., Cadet, J.L., 2021. Sex- and brain region-specific changes in gene expression in male and female rats as consequences of methamphetamine self-administration and abstinence. Neuroscience 492, 265–279. https://doi.org/10.1016/j.neuroscience.2020.11.025.

DeVuono, M.V., Hrelja, K.M., Petitie, G.N., Limebeer, C.L., Rock, E.M., Hill, M.N., Parker, L.A., 2020. Nauma-induced conditioned gaping reactions in rats produced by high-dose synthetic cannabinoid, JWH-018. Cannabis Cannabinoid Res. 5, 298–304. https://doi.org/10.1089/can.2019.0103.

Di, S., Malcer-Lopez, R., Halmoc, K.C., Tasker, J.G., 2003. Nongenomic glucocorticoid inhibition via endocannabinoid release in the hypothalamus: a fast feedback mechanism. J. Neurosci. 23, 4850–4857.

Dubreucq, S., Matias, I., Cardinal, P., Haring, M., Lutz, B., Mariscano, G., Chaouloff, F., 2012. Genetic dissection of the role of cannabinoid type-1 receptors in the emotional consequences of repeated social stress in mice. Neuropsychopharmacology 37, 1885–1900. https://doi.org/10.1038/npp.2012.36.

Evanos, N.K., Tasker, J.G., Hill, M.N., Hillard, C.J., Herman, J.P., 2010. Fast feedback inhibition of the HPA axis by glucocorticoids is mediated by endocannabinoid binding. Endocrinology 151, 4811–4819. https://doi.org/10.1210/en.2010-0285.

Gray, J.M., Vecchiarelli, H.A., Morena, L., Lee, T.T.Y., Hermanson, D.J., Kim, A.B., Cooper, Z.D., Craft, R.M., 2018. Sex-Dependent effects of cannabis and cannabinoids: a translational perspective. Neuropsychopharmacology 43, 34–51. https://doi.org/10.1038/npp.2017.140.

Hill, M.N., Carrier, E.J., McLaughlin, R.J., Morrish, A.C., Meier, S.E., Hillard, C.J., Gorzalka, B.B., 2008. Regional alterations in the endocannabinoid system in an animal model of depression: effects of concurrent antidepressant treatment. J. Neurochem. 106, 2322–2336. https://doi.org/10.1111/j.1471-4159.2008.05567.x.

Hill, M.N., Karacabeyli, E.S., Gorzalka, B.B., 2007. Environ recruits the endocannabinoid system to modulate emotionality. Psychoneuroendocrinology 32, 350–357. https://doi.org/10.1016/j.psyneuen.2007.02.003.

Hill, M.N., Karatosanos, I.N., Hillard, C.J., McEwen, B.S., 2010a. Rapid elevations in limbic endocannabinoid content by glucocorticoids hormones in vivo. Psychoneuroendocrinology 35, 1333–1338. https://doi.org/10.1016/j.psyneuen.2010.03.005.

Hill, M.N., Kumar, S.A., Filipski, S.B., Iverson, M., Stuhr, K.L., Keith, J.M., Cravatt, B.F., Hillard, C.J., Chatterji, S., McEwen, B.S., 2013. Disruption of fatty acid amide hydrolase activity prevents the amelioration of anxiety and amygudalar...
H.A. Vecchiarelli et al.

Neurobiology of Stress 20 (2022) 100470

Morena, M., Roozendaal, B., Trezza, V., Ratano, P., Peloso, A., Hauer, D., Atsak, P., Trebah, L., Guzmán-Buhr, J.H., Schelling, G., Campolongo, P., 2014. Endogenous cannabinoid release within prefrontal-limbic pathways affects memory consolidation of emotional training. Proc. Natl. Acad. Sci. U. S. A. 111, 1057–1062. https://doi.org/10.1073/pnas.1313232111.

Natividad, L.A., Buczynski, M.W., Herman, M.A., Kirson, D., Oleva, C.S., Irimia, C., Polis, I., Ciccioppo, R., Roberto, M., Parsons, L.H., 2017. Constitutive increases in amygdalar corticotropin-releasing factor and fatty acid amide hydrolase drive an anxiogenic phenotype. Biol. Psychiatr. 82, 500–510. https://doi.org/10.1016/j.biopsych.2017.01.005.

Patel, S., Roelke, C.T., Rademacher, D.J., Hillard, C.J., 2005. Inhibition of restraint-stress-induced neural and behavioural activation by endogenous cannabinoid signalling. Eur. J. Neurosci. 21, 1057–1069. https://doi.org/10.1111/j.1460-9568.2005.04916.x.

Paulus, M.P., Stein, M.B., Simmons, A.N., Rischvery, V.B., Halter, R., Chaplan, S.R., 2021. The effects of FAAH inhibition on the neural basis of anxiety-related processing in healthy male subjects: a randomized clinical trial. Neuropsychopharmacology 46, 1011–1019. https://doi.org/10.1038/s41386-020-0936-w.

Qi, M., Morena, M., Vecchiarelli, H.A., Hill, M.N., Schriemer, D.C., 2015. A robust capillary liquid chromatography/tandem mass spectrometry method for quantitation of neurmodulatory endocannabinoids. Rapid Commun. Mass Spectrom.: RCM (Rapid Commun. Mass Spectrom.) 29, 1889–97. https://doi.org/10.1002/rcm.7277.

Rademacher, D.J., Meier, S.E., Shi, L., Ho, W.-S.V., Jarrahian, A., Hillard, C.J., 2008. Effects of acute and repeated restraint stress on endocannabinoid content in the amygdala, ventral striatum, and medial prefrontal cortex in mice. Neuropharmacology 54, 108–116. https://doi.org/10.1016/j.neuropharm.2007.06.012.

Ragazzino, F.J., Arnold, R.A., Kowalski, C.W., Savenkov, M.I., Karatoson, I.N., Peters, J.H., 2020. Corticosterone inhibits vagal afferent glumatate release in the nucleus of the solitary tract via retrograde endocannabinoid signaling. Am. J. Physiol. Cell Physiol. 319, C1097. https://doi.org/10.1152/ajpcell.00190.2020. –C1106.

Reich, C.G., Taylor, M.E., McCarthy, M.M., 2009. Differential effects of chronic unpredictable stress on hippocampal CB1 receptors in male and female rats. Brain Res. 1125, 263–269. https://doi.org/10.1016/j.brainres.2009.05.013.

Rincón-Cortes, M., Herman, J.P., Lupien, S., Maguire, J., Shanks, R.M., 2019. Stress: influence of sex, reproductive status and gender. Neurobiol. Stress 10, 100155. https://doi.org/10.1016/j.nustr.2020.100155.

Roberts, C.J., Stuh, K.L., Hutz, M.J., Raff, H., Hillard, C.J., 2014. Endocannabinoid signaling in hypothalamic-pituitary-adrenocortical axis recovery following stress: effects of indirect agonists and comparison of male and female mice. Pharmacol. Biochem. Behav. 117, 17–24. https://doi.org/10.1016/j.pbb.2013.11.026.

Schmitz, M.E., Liebowitz, M.B., Stein, M.B., Grunfeld, J., Van Hove, I., Simmons, W.K., Van Der Ark, P., Palmer, J.A., Saad, Z.S., Pemberton, D.J., Van Nueten, L., Drevets, W.C., 2021. The effects of inhibition of fatty acid amide hydrolase (FAAH) by JNJ-42165279 in social anxiety disorder: a double-blind, randomized, placebo-controlled proof-of-concept study. Neuropsychopharmacology 46, 1004–1010. https://doi.org/10.1038/s41386-020-00888-1.

Simone, J.J., Baumbach, J.L., McCormick, C.M., 2018. Sex-specific effects of CB1 receptor antagonism and stress in adolescence on anxiety, corticosterone concentrations, and contextual fear in adulthood in rats. Int. J. Dev. Neurosci. 69, 119–131. https://doi.org/10.1016/j.ijdevneuro.2017.08.011.

Spohrs, J., Prost, M., Ulrich, M., Plener, P.L., Bindlind, L., Ahler, B., 2022. Endocannabinoid system reactivity during stress processing in healthy humans. Biol. Psychol. 169, 75–92. https://doi.org/10.1016/j.biopsycho.2022.03.052.

Tabatadze, N., Huang, G., May, R.M., Jain, A., Woolley, C.S., 2015. Sex differences in molecular signaling at inhibitory synapses in the Hippocampus. J. Neurosci. 35, 11252–11265. https://doi.org/10.1523/JNEUROSCI.2331-18.2018.

Morena, M., Nastase, A.S., Santori, A., Cravatt, B.F., Shansky, R.M., Hill, M.N., 2020b. Elevated anandamide, enhanced recall of fear extinction, and attenuated stress responses following inhibition of fatty acid amide hydrolase: a randomized, controlled experimental medicine trial. Biol. Psychiatry. 87, 548–547. https://doi.org/10.1016/j.biopsycho.2019.07.034.

Vecchiarelli, H.A., Akaishi, Y., Colangeli, R., Morena, M., Villalobos, M., Watanabe, K., Yasmin, F., Colangeli, R., Morena, M., Filipski, S., van der Stelt, M., Pittman, Q.J., 2014. Sex- and region-specific pubertal maturation of the corticotropin-releasing factor system in the rat. J. Comp. Neurol. 522, 1284–1298. https://doi.org/10.1002/jcn.23475.

Vainnin, F., Colangeli, R., Morena, M., Filipski, S., van der Steh, M., Pittman, Q.J., Hillard, C.J., Teskey, G.C., McEwen, B.S., Hill, M.N., Chattarji, S., 2020. Stress-induced modulation of endocannabinoid signaling leads to delayed strengthening of synaptic connectivity in the amygdala. Proc. Natl. Acad. Sci. U. S. A. 117, 655–665. https://doi.org/10.1073/pnas.1901221117.