Received: 19 December 2019 | Revised: 5 October 2020 | Accepted: 7 December 2020

DOI: 10.1111/bph.15341

RESEARCH PAPER

Sex-dependent effects of endocannabinoid modulation of conditioned fear extinction in rats

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Funding information
Fondation Brain Canada; Mathison Centre for Mental Health Research & Education; Branch Out Neurological Foundation (BONF); CIHR postdoctoral fellowships; Alberta Innovates Health Solutions (AIHS); National Institute of Mental Health, Grant/Award Number: R56 MH11493; Canadian Institutes of Health Research, Grant/Award Number: CIHR FDN-143329

Background and Purpose: Women are twice as likely as men to develop post-traumatic stress disorder (PTSD) making the search for biological mechanisms underlying these gender disparities especially crucial. One of the hallmark symptoms of PTSD is an alteration in the ability to extinguish fear responses to trauma-associated cues. In male rodents, the endocannabinoid system can modulate fear extinction and has been suggested as a therapeutic target for PTSD. However, whether and how the endocannabinoid system may modulate fear expression and extinction in females remains unknown.

Experimental Approach: To answer this question, we pharmacologically manipulated endocannabinoid signalling in male and female rats prior to extinction of auditory conditioned fear and measured both passive (freezing) and active (darting) conditioned responses.

Key Results: Surprisingly, we found that acute systemic inhibition of the endocannabinoid anandamide (AEA) or 2-arachidonoyl glycerol (2-AG) hydrolysis did not significantly alter fear expression or extinction in males. However, the same manipulations in females produced diverging effects. Increased AEA signalling at vanilloid TRPV1 receptors impaired fear memory extinction. In contrast, inhibition of 2-AG hydrolysis promoted active over passive fear responses acutely via activation of cannabinoid$_1$ (CB_1) receptors. Measurement of AEA and 2-AG levels after extinction training revealed sex- and brain region-specific changes.

Conclusion and Implications: We provide the first evidence that AEA and 2-AG signalling affect fear expression and extinction in females in opposite directions. These findings are relevant to future research on sex differences in mechanisms of fear extinction and may help develop sex-specific therapeutics to treat trauma-related disorders.

KEYWORDS
CB_1 receptors, endocannabinoid system, fear extinction, sex differences, TRPV1 receptors

Abbreviations: 2-AG, 2-arachidonoyl glycerol; AEA, anandamide; CPZ, capsazepine; CS, conditioned stimulus; dPAG, dorsal periaqueductal grey; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; PFC, prefrontal cortex; PTSD, post-traumatic stress disorder; US, unconditioned stimulus; vPAG, ventral periaqueductal grey.

1 | INTRODUCTION

Impaired fear extinction contributes to the development and persistence of post-traumatic stress disorder (PTSD) (Jovanovic & Norrholm, 2011; Milad et al., 2009). While only a small proportion of trauma-exposed individuals develop PTSD, women have a twofold greater risk, prevalence and duration of PTSD than men (Breslau, 2009). The biological mechanisms underlying these gender disparities remain unclear and controversial. Yet most preclinical studies on fear memory processes are exclusively performed in males and studies comparing the sexes are few and inconsistent (Shansky, 2015). In rodents, learned fear responses are traditionally assessed by quantifying freezing behaviour, a passive fear response defined as the absence of movements except for respiration (Fanselow, 1980), predominately expressed by males. In contrast, females generally exhibit lower freezing and express darting behaviour, a rapid, forward movement that resembles an active and escape-like fear response (Colom-Lapetina, Li, Pelegrina-Perez, & Shansky, 2019; Gruene, Flick, Stefano, Shea, & Shansky, 2015). A better understanding of the mechanisms that mediate these sex differences in fear responding may inform sex-specific pharmacological approaches to the management of PTSD (Velasco, Florido, Milad, & Andero, 2019).

Compelling evidence from studies in males demonstrates the importance of the endocannabinoid system in modulating fear responses and memory for aversive experiences (Lutz, Marsicano, Maldonado, & Hillard, 2015; Morena, Patel, Bains, & Hill, 2016). The endocannabinoid system consists of the two receptor types (**CB_1** and **CB_2**), two main endogenous ligands **anandamide (AEA)** and **2-arachidonoylglycerol (2-AG)**, and their respective degrading enzymes **fatty acid amide hydrolase (FAAH)** and **monoacylglycerol lipase (MAGL)** (Blankman & Cravatt, 2013). In addition to binding to cannabinoid receptors, AEA is also an endogenous ligand for the non-selective cation channel, **transient receptor potential vanilloid type 1 (TRPV1)** (Zygmunt et al., 1999). Both CB_1 receptor and TRPV1 are widely expressed in brain areas involved in anxiety and fear (Cristino et al., 2008; Mezey et al., 2000; Tsou, Mackie, Sañudo-Peña, & Walker, 1999), and like the CB_1 receptor, AEA activation of TRPV1 receptors can regulate synaptic plasticity (Chávez, Chiu, & Castillo, 2010; Grueter, Brasnjo, & Malenka, 2010). While CB_1 receptor activation has overall inhibitory effects through reduction of neurotransmitter release (Katona & Freund, 2012; Yasmin et al., 2020), activation of TRPV1 promotes membrane depolarization, increases neuronal firing rate and facilitates neurotransmitter release (Bialecki et al., 2020; Marinelli et al., 2003; Musella et al., 2009; Xing & Li, 2007). Behaviourally, activation of CB_1 receptor or TRPV1 in male rodents has also been shown to induce opposing responses. Specifically, CB_1 receptor stimulation reduces anxiety and facilitates fear extinction, while TRPV1 activation promotes anxiety-like behaviour and increases fear expression (Moreira, Aguiar, Terzian, Guimarães, & Wotjak, 2012).

Interestingly, compelling preclinical evidence has shown sex differences in endocannabinoid content and CB_1 receptor binding and affinity in different stress- and fear-related brain areas (Bradshaw, Rimmerman, Krey, & Walker, 2006; Castelli et al., 2014; Cooper & Craft, 2018; de Fonseca, Cebeira, Ramos, Martín, & Fernández-Ruiz, 1994; Riebe, Hill, Lee, Hillard, & Gorzalka, 2010). In humans, sex differences have been reported as well, showing higher CB_1 receptor binding in the limbic system and cortico-striato-thalamic-cortical circuit in males compared to females (Van Laere et al., 2008) but higher AEA levels in females compared to males (Neumeister et al., 2013). In parallel, cannabinoid compounds have been reported to have sex-divergent effects both in animal and human studies, due to direct gonadal hormone influence on the endocannabinoid system and pharmacokinetic and pharmacodynamic differences in drug metabolism (Wiley & Burston, 2014) and potency (Craft, Wakley, Tsutsui, & Laggart, 2012), reviewed in Cooper and Craft (2018).

We recently reported that repeatedly enhancing AEA signalling accelerated extinction learning in male rats (Morena et al., 2018). Specifically, the amygdala represents an important brain region for AEA regulation of fear extinction (Gunduz-Cinar et al., 2013). Recent evidence has shown that the endocannabinoid signalling regulates plasticity within the amygdala-prefrontal cortex (PFC) circuit under stressful experiences (Marcus et al., 2020) and fundamental sex differences have been identified within this amygdala-PFC fear circuit, underlying differences in fear expression (Gruene, Roberts, Thomas, Ronzio, & Shansky, 2015). Together with the amygdala and PFC, the periaqueductal grey (PAG) represents an important fear-related brain

What is already known

- In male rodents, endocannabinoids modulate fear extinction and have been suggested to treat PTSD.
- Increased anandamide signalling at CB_1 receptors promotes fear extinction in male rodents.

What this study adds

- Increased anandamide signalling impairs fear extinction in female rats via activation of vanilloid (TRPV1) receptors.
- Increased 2-arachidonoylglycerol signalling at CB_1 receptors promotes darting over freezing in female rats.

What is the clinical significance

- Our results provide new insights into the sex dimorphism documented in PTSD.
- This study facilitates the development of endocannabinoid-based sex-specific approaches to treat PTSD.

area (Maren, 2001), wherein the dorsal subregion (dPAG) primarily regulates innate and active fear responses (Bandler, Keay, Floyd, & Price, 2000; Watson, Cerminara, Lumb, & Apps, 2016), more prominent in females (Gruene, Flick, Stefano, Shea, & Shansky, 2015), while the ventral PAG (vPAG) seems to be more involved in the regulation of freezing behaviour (Watson, Cerminara, Lumb, & Apps, 2016), more prominent in males (Gruene, Flick, Stefano, Shea, & Shansky, 2015). To date, little is known about whether endocannabinoids regulate conditioned fear extinction in females. To answer this question, we employed systemic pharmacological inhibition of either AEA or 2-AG in male and female rats and determined the role of CB_1 receptor or TRPV1 in mediating any potential behavioural changes observed. Finally, we measured post-extinction AEA and 2-AG levels in the amygdala, PFC, dPAG and vPAG, to identify potential sex differences in these extinction-related brain regions (Maren, 2001). Results from this study may inform future research aiming at investigating sex differences in endocannabinoid regulation of fear memory dynamics within specific brain regions and neuronal circuits.

2 | METHODS

2.1 | Animals

Male and female Sprague Dawley rats (10–11 weeks old at the time of testing; Charles River, Montreal, QB, Canada; RRID: RGD_10395233) were pair housed in clear plastic cages (47 × 25 × 20 cm) in separate temperature-controlled (20 ± 1°C) rooms and maintained under a 12 h/12 h light/dark cycle (8:00 a.m. to 8:00 p.m. lights on) with ad libitum access to food and water and environmental enrichment (i.e., polycarbonate play tunnels and sizzle nest). This animal model was chosen because processing of emotional information, including memory of aversive experiences and expression of fear, rely on the activation of an evolutionary primitive subcortical and cortical circuit, highly conserved across species, including humans and pattern of fear response expressed by rats parallels that observed in humans (Lang, Davis, & Öhman, 2000). All tests were performed during the light phase of the cycle between 10:00 a.m. and 5:00 p.m. Animals were randomly assigned to the experimental groups. Male and female rats were tested separately, in different cohorts and different days. All experimental procedures were in compliance with protocols approved by the University of Calgary Animal Care Committee, guidelines from the Canadian Council on Animal Care. Animal studies are reported in compliance with the ARRIVE guidelines (Percie du Sert et al., 2020) and with the recommendations made by the *British Journal of Pharmacology* (Lilley et al., 2020). All efforts were made to minimize animal suffering and to reduce the number of animals used. Except for the animals used for brain endocannabinoid measurements, all rats were killed at the end of the behavioural experiments with CO_2 and rigor mortis confirmed. Recommendations set out in the *BJP* editorials, where relevant, were followed by the authors.

2.2 | Experimental procedures

2.2.1 | Auditory fear conditioning and extinction paradigm

Rats underwent auditory fear conditioning, extinction and extinction retrieval with a slightly different procedure as the ones previously described (Gruene, Flick, Stefano, Shea, & Shansky, 2015; Morena et al., 2019) (Figure 1a). Behavioural testing occurred in two different contexts (A and B). Context A consisted of a chamber with a grid floor, back and side metal walls, clear Plexiglas front door and ceiling, and white light. Context A was cleaned with 70% ethanol between rats. Context B consisted of a white opaque plastic floor and curved walls and was cleaned with Virkon solution between rats. To habituate the animals to the behavioural testing room, rats were transferred to the behavioural room and their home cages were placed in sound attenuating, ventilated and lighted cabinets for at least 30 min before and after the handling on day 1, 2 and 3, and for at least 90 min before and after testing, the following days. Fear conditioning chambers and cabinets were cleaned thoroughly with soapy water and ethanol at the end of each experimental run, in between male and female experimental cohorts. Rats were handled for 1 min each. On day 2 and 3, immediately after the handling procedure, animals were habituated to context A and B for 10 min. Auditory fear conditioning (day 4) was performed in context A. After a 5-min acclimation period, all rats were exposed to seven conditioning trials. Each conditioning trial involved a presentation of the conditioned stimulus (CS; 80 dB, 4 Hz tone) for 30 s, co-terminating with a 1 s unconditioned stimulus (US; 0.65 mA shock). Inter-trial interval (ITI) between two consecutive CS-US pairings was 3 min. After conditioning, each rat was returned to its home cage. On day 5, rats underwent the extinction training consisting of 20 CS presentations with an ITI between conditioned stimuli of 2 min, in context B. On day 6, rats received an extinction retrieval session in context B. After a 2-min acclimation period, rats were presented with five conditioned stimuli (2-min ITI). Behaviour was video-recorded, scored and analysed for freezing (i.e. absence of any movement except for those necessary for respiration) using Video Freeze software (Med Associates Inc., St. Albans, VT, USA; RRID: SCR_014574). Darting behaviour (i.e. rapid, forward movement across the chamber that resembles an escape-like response) was scored manually as number of discrete darting events and expressed as darting rate (dart·min^{-1}), by two trained observers blinded to the experimental conditions.

To test the effects of the AEA or 2-AG hydrolysis inhibitors on fear extinction, rats were injected intraperitoneally with **URB597** or **MJN110** (respectively), or their vehicle, 60 min prior to the extinction training. The CB_1 antagonist **AM251**, or its vehicle, was injected intraperitoneally 30 min before URB597 or MJN110 administration (i.e. 90 min before extinction training). A separate group of rats was injected with AM251 in combination with the TRPV1 antagonist **capsazepine** (CPZ), 30 min before URB597 injection.

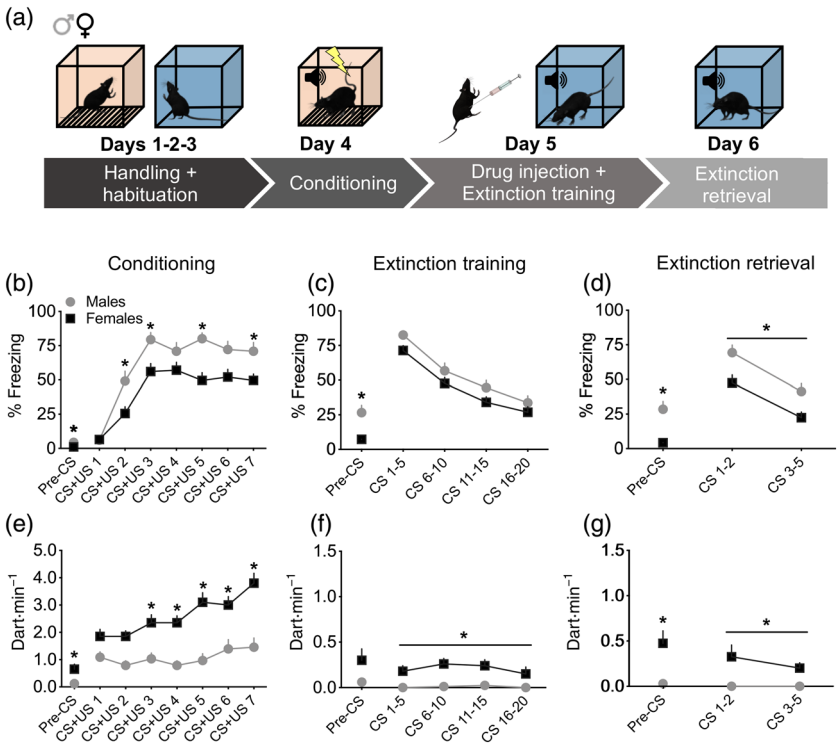

FIGURE 1 Sexually divergent expression of fear responses during the auditory fear conditioning paradigm. (a) Schematic representation of the experimental design. (b–d) Except during conditioned stimulus (CS) presentations at extinction training (c), male rats consistently showed higher freezing behaviour than females during the auditory fear conditioning paradigm. Percentage of freezing during auditory fear conditioning (b), extinction training (c) and extinction retrieval (d). (e–g) Female rats consistently showed higher darting behaviour than males throughout the auditory fear conditioning paradigm. Number of darting events·min^{-1} (dart·min^{-1}) during auditory fear conditioning (e), extinction training (f) and extinction retrieval (g). Data are expressed as mean ± SEM. $^{*}P < 0.05$, males versus females, the horizontal line below the star indicates main effect of sex (males, $n = 33$; females, $n = 40$)

2.3 | Endocannabinoid extraction and analysis

To assess whether learned fear expression and extinction learning elicits sex-specific patterns of endocannabinoid release, male and female rats were randomly assigned to either extinction [Ext] or no-extinction [No-Ext] groups. Ext groups underwent fear conditioning and extinction training as described above. No-Ext groups underwent fear conditioning but were exposed to the extinction context for an equivalent amount of time without CS presentations. Immediately after the extinction training, rats underwent rapid decapitation and the brain regions of interest (amygdala, PFC, dPAG and vPAG) were dissected, frozen on dry ice and stored at −80°C until endocannabinoid level determination. Lipid extraction to determine AEA and 2-AG levels was performed as described previously (Morena et al., 2015; Qi, Morena, Vecchiarelli, Hill, & Schriemer, 2015).

2.4 | Data and statistical analysis

The data and statistical analysis comply with the recommendations of the *British Journal of Pharmacology* on experimental design and analysis in pharmacology (Curtis et al., 2018). All data were analysed using GraphPad Prism 6 (RRID:SCR_002798) and are expressed in all figures as mean ± SEM. Statistical analysis was run using independent values and outliers were included in data analysis and presentation. To better evaluate any difference in drug effects in the early or late phases of the behavioural sessions, percentage of freezing or darting rate during extinction training sessions was averaged in four blocks of five consecutive conditioned stimuli each (CS1-5, CS6-10, CS11-15 and CS16-20); behavioural measures for extinction retrieval were averaged in two blocks (CS1-2 and CS3-5). CS-US- or CS-evoked freezing and darting were analysed with repeated measures (RM) ANOVA. Freezing and darting during the pre-CS period were analysed with Student's *t*-test or one-way ANOVA, when appropriate. Student's *t*-test was used to analyse brain endocannabinoid levels. Adjusted Bonferroni's multiple comparison post hoc tests were run when F achieved $P < 0.05$ and there was no significant variance in homogeneity. The correlation analyses were performed with the Pearson correlation test. A probability level of <0.05 was accepted as statistically significant. Group size, shown in the figure legends, is the number of independent values (i.e. number of rats). To achieve a power of 0.80–0.95, a sample size of at least 10 (for behavioural experiments) or eight (for biochemical experiments) animals per group was calculated. Studies were designed to generate groups of equal size, using randomisation and blinded analysis. However, sizes for the Vehicle, URB597 and MJN110 groups are higher than those of the remaining groups, as they were combined from separate sets of experiments which were originally run separately to generate pilot data and then replicated when the remaining groups were added to the study. Statistical analysis was undertaken only for studies where each group size was at least $n = 5$. All experiments and data analyses were carried out by operators blinded to the experimental conditions.

2.5 | Materials

The AEA hydrolysis inhibitor URB597 (0.3 mg·kg^{-1}; Cayman Chemical, Cedarlane®, Burlington, ON, Canada), the 2-AG hydrolysis inhibitor MJN110 (10 mg·kg^{-1}; provided by B.F. Cravatt), the CB_1 antagonist/inverse agonist AM251 (1 mg·kg^{-1}; Tocris, Cedarlane®,

Burlington, ON, Canada), the TRPV1 antagonist capsazepine (CPZ; 5 mg·kg^{-1}; Cayman Chemical, Cedarlane®, Burlington, ON, Canada), or their vehicle (5% polyethylene glycol, 5% Tween-80, 90% saline) were injected intraperitoneally at a volume of 1 ml·kg^{-1}. URB597, MJN110, or their vehicle were injected 60 min before the extinction training session; AM251, capsazepine or their vehicle were injected 90 min before the extinction training session.

Doses and timing were chosen based on previously published papers (Colangeli et al., 2017; Kathuria et al., 2003; Morena et al., 2018; Ratano, Palmery, Trezza, & Campolongo, 2017; Sticht et al., 2019) and pilot experiments performed in our laboratory. All drug solutions were freshly prepared before each experiment.

2.6 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY http://www.guidetopharmacology.org and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander et al., 2019a, 2019b, 2019c).

3 | RESULTS

3.1 | Sexually divergent expression of fear responses during auditory fear conditioning and extinction

We first examined whether sex-specific conditioned fear strategies emerged across the different sessions of the auditory fear conditioning paradigm by assessing CS-US- and CS-evoked freezing and darting behaviour of all rats that received an intraperitoneal injection of vehicle used in the subsequent experiments, pooled together (Figure 1). As shown in Figure 1b–d and Table S1, we found significant main effects of CS trial across all three test days and significant main effect of sex at conditioning and extinction retrieval indicating higher freezing in males. We also observed significant Sex × Trial interaction for fear conditioning training. Post hoc comparisons indicated that male rats showed significant higher freezing levels as compared to females during presentations of CS-US 2, 3, 5 and 7. Student's *t*-tests for freezing before CS presentations (pre-CS period) at conditioning, extinction training and retrieval indicated that male rats showed significant higher freezing levels as compared to females, potentially suggesting higher innate fear and context generalization in males. Figure 1e–g shows CS-US-evoked darting during conditioning, CS-evoked darting during extinction training and extinction retrieval. Analysis of darting behaviour during fear conditioning revealed significant main effects of trial and sex, and a significant Trial × Sex interaction. Post hoc comparisons revealed that females darted more than males at CS-US 3, 4, 5, 6 and 7. During both extinction training and retrieval, we found a main effect of sex. Student's *t*-tests for darting during the pre-CS period indicated higher darting in females as compared to males at conditioning and extinction retrieval. These results indicate that, as we have shown previously (Gruene, Flick, Stefano, Shea, & Shansky, 2015), males and females engage different fear responses, males consistently show greater freezing than females while females consistently show higher darting than males.

3.2 | AEA hydrolysis inhibition does not significantly affect auditory fear memory expression and extinction in males

Figure 2 shows behavioural data for pre-extinction administration of AEA hydrolysis inhibitor URB597 alone, CB_1 antagonist AM251 alone and URB597 + AM251 (a–f) in males; statistics are in Table S2. In freezing measures (Figure 2a–c), we observed significant main effects of trial for all 3 days of testing, suggesting successful fear conditioning and extinction learning. We found a main effect of drug at extinction training and a significant Trial × Drug interaction for extinction retrieval (Figure 2b,c). However, although URB597 treatment trended to decrease freezing, post hoc analyses did not reveal a significant difference compared to the vehicle group but did show a significant difference compared to the URB597 + AM251 group at CS3-5 at extinction retrieval (Figure 2c). In darting measures (Figure 2d–f), we found a significant main effect of trial during fear conditioning only and observed very little or no darting at all during extinction or extinction retrieval. Importantly, there was no main effect of drug during fear conditioning, suggesting that there were no pre-existing differences in these cohorts (Figure 2a,d). One-way ANOVAs for freezing or darting during the pre-CS period did not show significant effects in any of the testing sessions.

3.3 | 2-AG hydrolysis inhibition does not affect auditory fear memory expression and extinction in males

Figure 3 shows behavioural data for pre-extinction administration of 2-AG hydrolysis inhibitor MJN110 alone, AM251 alone and MJN110 + AM251 (a–f) in males; statistics are in Table S3. We only found significant main effects of trial for freezing at conditioning, extinction training and retrieval, indicating successful fear conditioning and extinction learning. We did not observe any main effects of drug or Drug × Trial interactions for freezing at conditioning, extinction training or retrieval (Figure 3a–c). No statistically significant effects were observed in darting measures (Figure 3d–f) or during the pre-CS period for freezing or darting in all the three testing sessions.

3.4 | Increased AEA signalling at TRPV1 augments freezing behaviour at extinction training and retrieval in females

Figure 4 shows the effects of systemic pre-extinction administration of URB597 alone, AM251 alone, URB597 + AM251, and

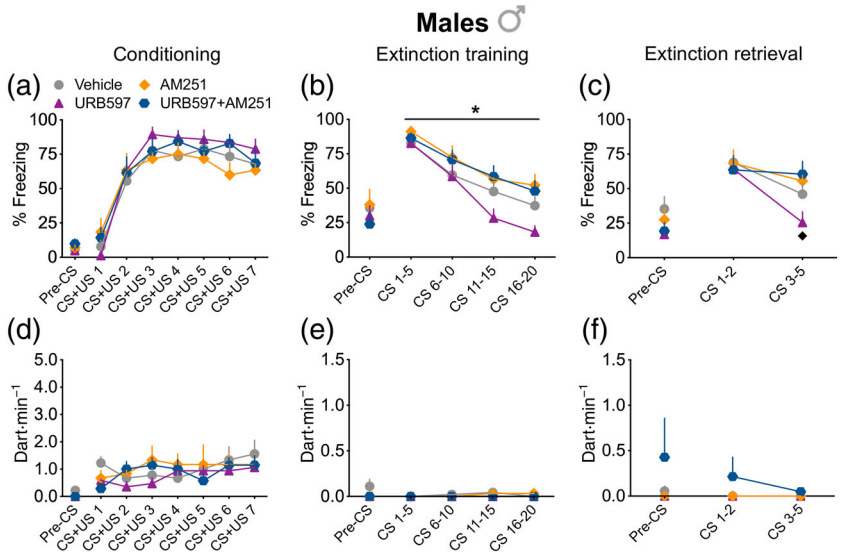

FIGURE 2 Increased anandamide (AEA) signalling did not significantly alter auditory fear memory expression and extinction in males. (a–f) Treatment with the AEA hydrolysis inhibitor URB597, the CB_1 antagonist AM251 or their combination (URB597 + AM251) did not significantly alter freezing or darting behaviour in male rats, although URB597 showed a trend towards reducing conditioned freezing as compared to the vehicle group during the late phases of extinction training and retrieval and significantly reduced freezing compared to URB597 + AM251 (at extinction retrieval). Percentage of freezing during auditory fear conditioning (a), extinction training (b) and extinction retrieval (c). Number of darting events·min^{-1} (dart·min^{-1}) during auditory fear conditioning (d), extinction training (e), and extinction retrieval (f). Vehicle, n = 18; URB597, n = 17; AM251, n = 12; URB597 + AM251, n = 14. Data are expressed as mean ± SEM. $^{*}P < 0.05$ main effect of drug; ◆$P < 0.05$ versus URB597 + AM251

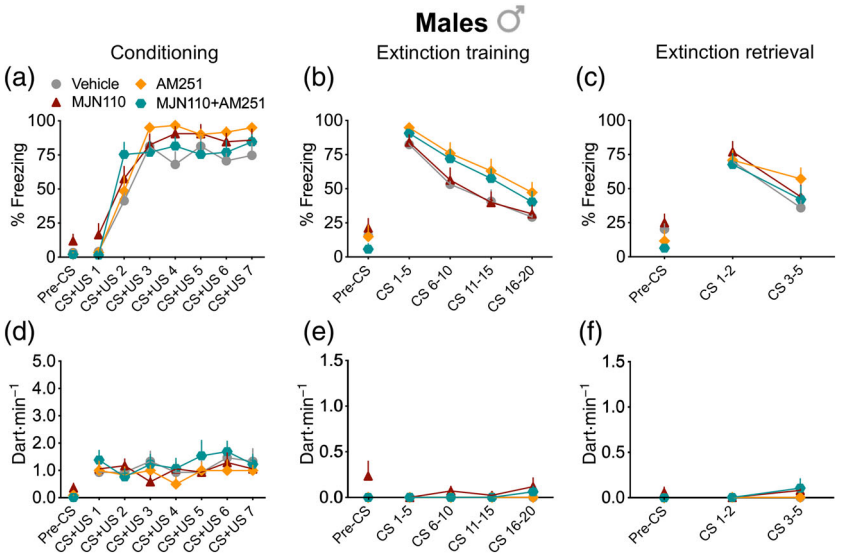

FIGURE 3 Increased 2-arachidonoyl glycerol (2-AG) signalling did not alter auditory fear memory expression and extinction in males. (a–f) Treatment with the 2-AG hydrolysis inhibitor MJN110, the CB_1 antagonist AM251 or their combination (MJN110 + AM251) did not alter freezing or darting behaviour in male rats. Percentage of freezing during auditory fear conditioning (a), extinction training (b) and extinction retrieval (c). Number of darting events·min^{-1} (dart·min^{-1}) during auditory fear conditioning (d), extinction training (e), and extinction retrieval (f). Vehicle, n = 15; MJN110, n = 17; AM251, n = 12; MJN110 + AM251, n = 13. Data are expressed as mean ± SEM

URB597 + AM251 together with the TRPV1 antagonist capsazepine on freezing (Figure 4a–c) and darting (Figure 4d–f) behaviour during the auditory fear conditioning paradigm. Detailed statistics is reported in Table S4. Analysis of freezing during fear conditioning revealed a significant main effect of trial but no significant drug treatment or Drug × Trial interaction (Figure 4a) or differences during the pre-CS period. This confirms no pre-existing differences between groups before drug treatment and shows that all groups exhibited fear learning. Analysis of freezing behaviour during extinction (Figure 4b) revealed significant main effects of trial, drug and a Trial × Drug interaction. Post hoc comparisons showed that rats treated with URB597 + AM251 exhibited higher freezing at later time blocks compared to vehicle (CS6-10, CS11-15 and CS16-20), URB597 alone (CS11-15 and CS16-20), AM251 alone (CS16-20) and URB597 + AM251 + capsazepine groups (CS11-15 and CS16-20; Figure 4b). The same group also showed higher freezing levels during the pre-CS period than vehicle-, URB597- and AM251-treated rats, suggesting higher context generalization. During extinction retrieval

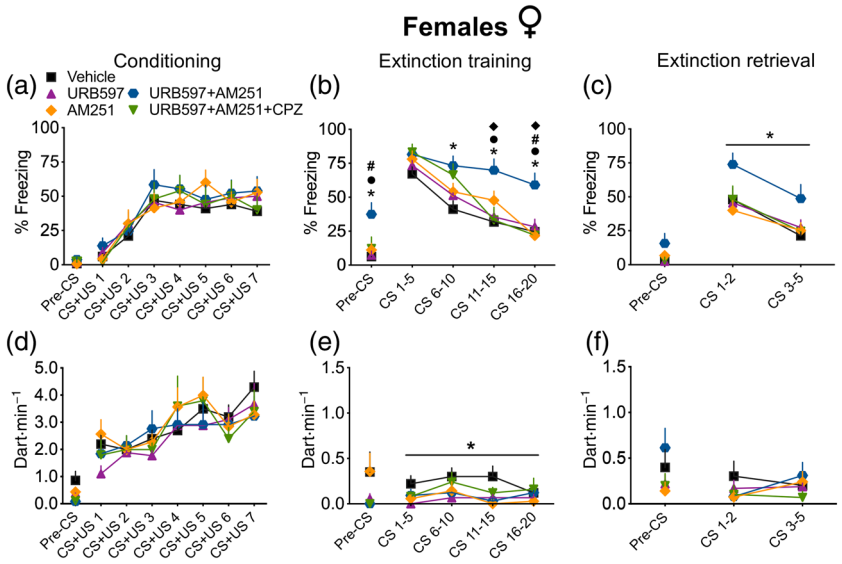

FIGURE 4 Increased anandamide (AEA) signalling at TRPV1s augmented freezing behaviour at extinction training and retrieval in females. (a–f) Treatment with the AEA hydrolysis inhibitor URB597 with concurrent blockade of CB_1 receptors with the antagonist AM251 (URB597 + AM251) induced fear generalization, impaired within-session extinction and extinction retrieval. These effects were mediated by AEA signalling at TRPV1s, as they were completely blocked by concomitant injection with the TRPV1 antagonist capsazepine (CPZ) (URB597 + AM251 + CPZ). Furthermore, treatment with URB597 alone induced an overall reduction of darting behaviour during conditioned stimulus (CS) presentation at extinction training compared to the vehicle group. Percentage of freezing during auditory fear conditioning (a), extinction training (b) and extinction retrieval (c). Number of darting events·min^{-1} (dart·min^{-1}) during auditory fear conditioning (d), extinction training (e) and extinction retrieval (f). Vehicle, n = 20; URB597, n = 18; AM251, n = 14; URB597 + AM251, n = 13; URB597 + AM251 + CPZ, n = 10. Data are expressed as mean ± SEM. $^{*}P < 0.05$ versus vehicle; $^{\bullet}P < 0.05$ versus URB597; $^{\#}P < 0.05$ versus AM251; $^{\blacklozenge}P < 0.05$ versus URB597 + AM251 + CPZ. Horizontal line below the star indicates a main effect of drug: $P < 0.05$, URB597 + AM251 group versus vehicle group (c) and URB597 group versus vehicle group (e)

(Figure 4c), we found significant main effects of trial and drug but no interaction. Post hoc comparisons showed that URB597 + AM251 rats exhibited overall higher freezing levels than vehicle-treated rats (Figure 4c). No differences were observed for freezing during the pre-CS period at extinction retrieval. Analysis of darting behaviour during conditioning, extinction training and retrieval (Figure 4d–f) revealed a significant main effect of trial for conditioning and a significant main effect of drug at extinction training but no other significant effects. Post hoc comparisons revealed that URB597-treated rats exhibited overall lower darting than the vehicle group did, across all the CS trials presented during the extinction training (Figure 4e). One-way ANOVAs for darting during the pre-CS period did not reveal any significant effects for conditioning, extinction training and retrieval.

3.5 | Increased 2-AG signalling at CB_1 receptor reduces freezing and enhances darting behaviour at extinction training in females

Figure 5 shows the effects of systemic pre-extinction administration of MJN110 alone, AM251 alone and MJN110 + AM251 on freezing (Figure 5a–c) and darting (Figure 5d–f) behaviour during the auditory fear conditioning paradigm. Detailed statistics is reported in Table S5. Analysis of freezing during conditioning (Figure 5a) revealed a significant main effect of trial but no significant drug treatment effect or significant Trial × Drug interaction or differences during the pre-CS period, thus confirming no pre-existing differences between groups before drug treatment. Analysis of freezing during extinction training (Figure 5b) revealed significant main effects of both trial and drug but no interaction. Post hoc comparisons showed that the MJN110 alone group exhibited overall less freezing than vehicle-treated rats did across all the CS presentations and there were no significant differences between the vehicle group and the MJN110 + AM251 group, suggesting that MJN110 reduces freezing behaviour through a CB_1 receptor mediated mechanism. Analysis of freezing during extinction retrieval (Figure 5c) showed only a significant main effect of trial. One-way ANOVAs for freezing during the pre-CS period at extinction training and retrieval did not reveal any significant effects. Analysis of darting (Figure 5d–f) revealed a significant main effect of trial during conditioning (Figure 5d), significant effects of trial and Trial × Drug interaction at extinction training (Figure 5e), but no significant effects at extinction retrieval (Figure 5f). Post hoc analyses for extinction training showed that treatment with MJN110 alone significantly increased darting during CS11-15 as compared with both the vehicle and the AM251 groups (Figure 5e). One-way ANOVAs for darting during the pre-CS periods for all the three testing days revealed a

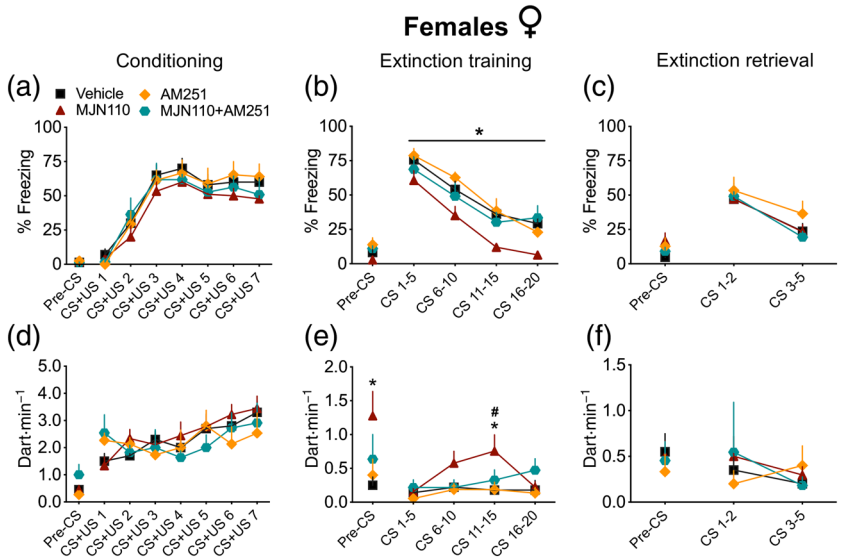

FIGURE 5 Increased 2-arachidonoyl glycerol (2-AG) signalling at CB_1 receptors reduced freezing and augmented darting behaviour at extinction training in females. (a–f) The 2-AG hydrolysis inhibitor MJN110 decreased freezing and increased darting behaviour at extinction training. These effects were mediated by activation of CB_1 receptors, as they were blocked by concurrent injection with the CB_1 antagonist AM251 (MJN110 + AM251). Percentage of freezing during auditory fear conditioning (a), extinction training (b) and extinction retrieval (c). Number of darting events·min^{-1} (dart·min^{-1}) during auditory fear conditioning (d), extinction training (e) and extinction retrieval (f). Vehicle, n = 20; MJN110, n = 18; AM251, n = 15; MJN110 + AM251, n = 11. Data are expressed as mean ± SEM. $^{*}P < 0.05$ versus vehicle; $^{\#}P < 0.05$ versus AM251. Horizontal line below the star indicates a main effect of drug: $P < 0.05$, MJN110 group versus vehicle group (b)

significant effect only for extinction training. Post hoc analysis showed that the MJN110 group presented higher darting than the vehicle group (Figure 5e).

3.6 | Sex-dependent effects of auditory fear extinction training on endocannabinoid brain levels

To assess how extinction training may differentially alter endocannabinoid levels in males and females, we fear conditioned new cohorts of animals and measured AEA and 2-AG in the amygdala, PFC, dPAG and vPAG immediately after extinction training (Ext) or a no-CS control session (No-Ext). All data are shown in Figure 6 and statistics are in Table S6. Surprisingly, we observed effects of extinction on endocannabinoid levels in males only. In the amygdala, we found that male Ext rats had significantly higher AEA levels than male No-Ext rats, while no differences in AEA levels between the No-Ext and Ext groups in females were detected (Figure 6a). No significant differences were found for amygdala 2-AG levels in either sex (Figure 6b). In the PFC, we found no significant differences in AEA or 2-AG levels between No-Ext and Ext groups in males or females (Figure 6c,d). In the dPAG, we found a reduction in AEA levels in Ext males compared to No-Ext males, but no significant differences for AEA levels in females (Figure 6e). No significant effects were observed for 2-AG levels in the dPAG (Figure 6f) or for AEA or 2-AG in the vPAG (Figure 6g,h). We also found significant positive correlations between amygdala 2-AG levels and darting rate and between vPAG AEA levels and freezing behaviour and a negative correlation between PFC AEA levels and darting rate shown during CS presentations at the extinction session in females (Table S7). Freezing and darting behaviour for rats in the No-Ext and Ext groups is shown in Figure S1.

4 | DISCUSSION

Although the effects of endocannabinoid system manipulation on fear memory in males have been well investigated, our study provides the first systematic pharmacological examination of endocannabinoid regulation of fear extinction in both sexes and reveals for the first time a strong sex-dependent effect of endocannabinoids in the acute modulation of fear extinction. Quite surprisingly, we found that acutely elevating AEA or 2-AG signalling at extinction training did not significantly alter fear expression or extinction in males, although increased AEA tended to facilitate fear extinction. Experiments in females revealed an opposite picture to what has been previously reported for males. We observed divergent effects of AEA versus 2-AG signalling manipulations, each mediated by distinct mechanisms.

Consistent with previous findings (Gruene, Flick, Stefano, Shea, & Shansky, 2015), we show a robust sexual dimorphism in behavioural expression of fear. While males predominately expressed freezing behaviour, females exhibited both freezing and darting. Although darting increased over time with CS-US presentations at conditioning, it remained mostly unvaried with progression of CS presentations during both extinction training and retrieval. Thus, under our experimental conditions, darting did not seem to strictly reflect a learned fear response. Moreover, accordingly with previous studies comparing conditioned freezing behaviour between sexes (Gupta, Sen, Diepenhorst, Rudick, & Maren, 2001; Maren, De Oca, & Fanselow, 1994;

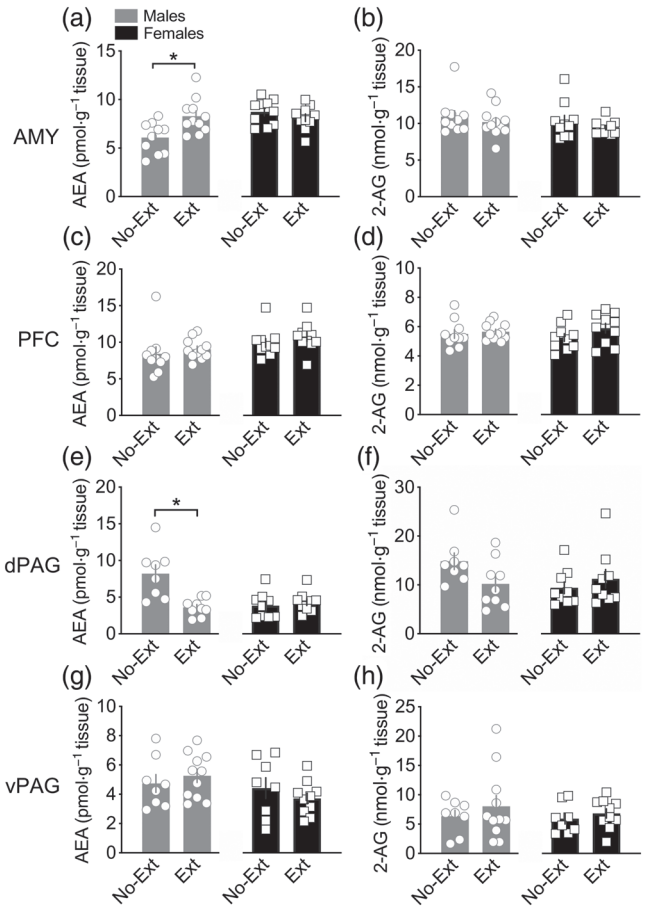

FIGURE 6 Sex-dependent effects of auditory fear extinction training on endocannabinoid brain levels. anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) brain levels (pmol·g^{-1} tissue and nmol·g^{-1} tissue, respectively) in the amygdala (AMY; a, b; for AEA and 2-AG: males No-Ext, *n* = 10, males Ext, *n* = 11, females No-Ext, *n* = 10, females Ext, *n* = 10), prefrontal cortex (PFC; c, d; for AEA and 2-AG: males No-Ext, *n* = 10, males Ext, *n* = 11, females No-Ext, *n* = 10, females Ext, *n* = 10), dorsal periaqueductal grey (dPAG; e, f; for AEA and 2-AG: males No-Ext, *n* = 8, males Ext, *n* = 9, females No-Ext, *n* = 9, females Ext, *n* = 9) and ventral periaqueductal grey (vPAG; g, h; for AEA and 2-AG: males No-Ext, *n* = 8, males Ext, *n* = 11, females No-Ext, *n* = 8, females Ext, *n* = 10) in male and female rats immediately after the extinction training session (Ext group) or in control groups only exposed to the extinction context without the conditioned stimulus (CS) presentations (No-Ext group). Data are expressed as mean ± SEM. $^{*}P < 0.05$

Pryce, Lehmann, & Feldon, 1999), we found that males showed significantly higher freezing than females at conditioning and extinction retrieval.

The endocannabinoid system has been consistently reported to modulate fear memory extinction and stress/fear coping strategies, in male rodents (Colangeli, Morena, Pittman, Hill, & Teskey, 2020; Gunduz-Cinar et al., 2013; Heinz, Genewsky, & Wotjak, 2017; Llorente-Berzal et al., 2015; Marsicano et al., 2002; Metna-Laurent et al., 2012; Morena et al., 2016, 2019, 2018). Increased AEA has been shown to promote fear extinction by reducing expression of freezing (Bitencourt, Pamplona, & Takahashi, 2008; Chhatwal, Davis, Maguschak, & Ressler, 2005; Gunduz-Cinar et al., 2013; Marsicano et al., 2002; Pamplona, Bitencourt, & Takahashi, 2008) via activation of CB_1 receptors on forebrain glutamatergic neurons (Llorente-Berzal et al., 2015). Elevated 2-AG signalling, however, has been reported to impair within-session extinction (Hartley et al., 2016) and increase freezing via activation of CB_1 receptors on forebrain GABAergic neurons (Llorente-Berzal et al., 2015) in male rodents. Surprisingly, our pharmacological manipulations did not significantly alter fear responses in males. However, consistent with previously published findings using somewhat different approaches (Bitencourt, Pamplona, & Takahashi, 2008; Chhatwal, Davis, Maguschak, & Ressler, 2005; Gunduz-Cinar et al., 2013; Pamplona, Bitencourt, & Takahashi, 2008), elevating AEA signalling did tend to facilitate fear extinction. Differences in species, experimental protocol, type, doses and administration regimen of drugs used, likely contributed to these discrepancies. Indeed, in the work by Gunduz-Cinar et al. (2013) it was used an inbred strain of mice with impaired fear extinction learning and retrieval. Furthermore, in all the above mentioned studies in rats by Chhatwal, Davis, Maguschak, and Ressler (2005), Bitencourt, Pamplona, and Takahashi (2008) and Pamplona, Bitencourt, and Takahashi (2008), to explore the effects of increased AEA levels, AM404 was used, which, in addition to inhibiting AEA uptake, has been shown to increase 2-AG signalling and act on many other different sites including TRPV1 and sodium channels (Hájos, Kathuria, Dinh, Piomelli, & Freund, 2004; Nicholson et al., 2003; Wiskerke et al., 2012; Zygmunt, Chuang, Movahed, Julius, & Högestätt, 2000). Consistent with our results, however, pre-extinction injection of URB597, at the same dose we used in the present study, has been reported to not affect fear extinction in male rats under basal conditions, but to only prevent the impairment in fear extinction induced by stress (Zer-Aviv & Akirav, 2016). It is also possible that doses different from the ones used in the present study or repeated dosing are necessary to produce consistent effects as repeated FAAH inhibition enhanced fear extinction in both male rats (Morena et al., 2018) and a mixed sample of males and females in humans (Mayo et al., 2020).

Interestingly, in females, elevated AEA signalling at TRPV1 increased freezing behaviour both acutely during the extinction training and the following day during extinction retrieval, unveiling an impairment of within-session extinction and recall of extinction memory. Furthermore, the same manipulation induced a strong fear generalization as indicated by elevated freezing shown before CS presentations in the extinction context, which was never associated to the aversive experience. Specifically, although it reduced darting across all CS trials at extinction training, we did not find that inhibition of AEA hydrolysis per se increased freezing behaviour. Surprisingly, concurrent blockade at CB_1 receptor, while elevating AEA signalling, robustly increased freezing response. Additional treatment with capsazepine revealed that this effect was mediated by activation of TRPV1. Since inhibition of AEA hydrolysis together with CB_1 blockade did not influence fear responses and memory in males, the TRPV1 antagonism experiment was carried out exclusively in females. These data indicate, for the first time, that AEA signalling at TRPV1 might be

biased towards facilitating freezing in female rats, thus unveiling sex differences in the affinity, expression and/or functionality of TRPV1s and endocannabinoid system components. An alternate possibility is that females could exhibit an up-regulation of TRPV1s in response to the noxious footshocks delivered during fear conditioning itself, which then favours AEA signalling at these receptors. Future work is required to understand this relationship in more depth, exploring the effects of direct TRPV1 agonism in discrete brain regions, as it would be challenging to examine this mechanism with a systemic manipulation and avoid confounding pain-related effects due to peripheral TRPV1 activation.

Elevated 2-AG signalling at CB_1 receptors in females modulated learned fear expression in the opposite direction. Pre-extinction treatment with MJN110 acutely reduced freezing at the last CS presentations, thus accelerating within-session extinction. This effect was CB_1 receptor-mediated as it was blocked by CB_1 antagonism. Interestingly, MJN110 affected darting behaviour in females in the opposite direction. Therefore, increased 2-AG signalling promoted active over passive fear responses acutely, without affecting the consolidation of extinction, as treatment with MJN110 did not affect rats' behaviour at extinction retrieval. This shift from passive to active forms of acute fear coping is consistent with an established role of CB_1 receptors on glutamatergic neurons (Metna-Laurent et al., 2012), suggesting that in females elevated 2-AG signalling may preferentially engage this receptor population to promote this behavioural transition. These collective findings in females are very reminiscent of a study in a line of male mice, bred to exhibit a high degree of anxiety, where elevated AEA signalling increased passive fear responses whereas inhibition of 2-AG hydrolysis increased active responses (Heinz, Genewsky, & Wotjak, 2017). While it is not immediately apparent as to why pharmacological manipulations of endocannabinoids in these anxious male mice parallel our results with female rats, it does indicate that bidirectional effects of manipulating endocannabinoid signalling on fear behaviours can occur across species and sexes.

Sex differences were also observed in AEA levels in several brain regions involved in the regulation of fear memory and fear responses. Corroborating previous findings in mice (Gunduz-Cinar et al., 2013; Marsicano et al., 2002), male rats undergone fear extinction exhibited higher amygdala AEA levels than males never exposed to CS extinction. Furthermore, among males, we found decreased dPAG AEA levels in rats that underwent fear extinction. Interestingly, a previous study showed increased dPAG AEA levels following a 3 min re-exposure to a context previously associated with a footshock (Olango, Roche, Ford, Harhen, & Finn, 2012), thus potentially indicating an opposing role in the regulation of early fear expression/extinction versus late extinction phases of fear memory. Fear extinction did not affect AEA levels in females nor 2-AG levels in either sex in the brain regions examined. However, correlational analyses in females revealed that rats presenting higher amygdala 2-AG levels showed increased darting during extinction training, paralleling our behavioural findings with MJN110 treatment. Interestingly, within the PFC, AEA levels negatively correlated with darting, which paralleled our finding that treatment with URB597 decreased darting across all conditioned stimulus (CS) presentations at extinction training in females. Furthermore, accordingly to our behavioural results in females showing increased freezing following AEA-mediated activation of TRPV1s, a positive correlation was also detected between freezing during extinction training and AEA levels in the vPAG, a brain region strongly involved in freezing and learned fear responses (Watson, Cerminara, Lumb, & Apps, 2016). Future work will employ site-specific pharmacological manipulations to establish the sites of action of AEA and 2-AG and to further identify subregion-specific endocannabinoid changes in the amygdala and PFC, also known to play important roles in different phases of fear memory.

Previous studies have shown that, although darting is not affected by oestrous cycle (Gruene, Flick, Stefano, Shea, & Shansky, 2015), freezing behaviour at extinction varies with oestrous phases (Gruene, Roberts, Thomas, Ronzio, & Shansky, 2015; Zeidan et al., 2011). Moreover, the oestrous cycle has been reported to modulate CB_1 receptor density and affinity (de Fonseca, Cebeira, Ramos, Martín, & Fernández-Ruiz, 1994), and AEA and 2-AG levels across different brain regions (Bradshaw, Rimmerman, Krey, & Walker, 2006; González et al., 2000). However, in the present study, oestrous cycle was not monitored; thus, future investigations are warranted to examine the influence of oestrous phases on endocannabinoid modulation of fear memory expression and extinction.

The opposing effects of enhanced AEA versus 2-AG signalling in the modulation of fear responses and the biphasic effects of cannabinoid drugs have been largely documented (Moreira, Aguiar, Terzian, Guimarães, & Wotjak, 2012; Morena & Campolongo, 2014). Beside the involvement of CB_1 receptors at different neuronal subpopulations (Heinz, Genewsky, & Wotjak, 2017; Llorente-Berzal et al., 2015; Lutz, Marsicano, Maldonado, & Hillard, 2015; Metna-Laurent et al., 2012; Rey, Purrio, Viveros, & Lutz, 2012), these opposing effects have also been ascribed to the recruitment of receptors other than CB_1 receptors (Casarotto et al., 2012; Colangeli et al., 2019; Di Maio, Colangeli, & Di Giovanni, 2019; Moreira, Aguiar, Terzian, Guimarães, & Wotjak, 2012; Patel, Hill, Cheer, Wotjak, & Holmes, 2017), such as TRPV1s, which can be activated by high AEA levels (Bialecki et al., 2020; Di Marzo, 2008; Zygmunt et al., 1999). Both CB_1 receptors and TRPV1s are widely expressed in brain areas involved in anxiety and fear, including the PFC, hippocampus, amygdala and PAG (Bialecki et al., 2020; Cristino et al., 2008; Mezey et al., 2000; Tsou, Mackie, Sañudo-Peña, & Walker, 1999). Consistent with our results, compelling evidence has reported opposing roles for both CB_1 receptors and TRPV1s in the modulation of fear and anxiety-related responses, where activation of TRPV1 has been shown to increase fear and anxiety-like behaviour, whereas CB_1 receptor activation attenuates these behavioural responses in male rodents (Campos & Guimarães, 2009; Moreira, Aguiar, Terzian, Guimarães, & Wotjak, 2012; Rubino et al., 2008). In agreement with our results, Laricchiuta, Centonze, and Petrosini (2013) found that a systemic-induced augmentation of AEA signalling at TRPV1 increased freezing and impaired extinction in a contextual fear conditioning paradigm in mice (Laricchiuta, Centonze, & Petrosini, 2013). Moreover,

TRPV1 knockout (male) mice show low anxiety-like behaviour and conditioned fear responses compared to their wild-type controls (Marsch et al., 2007). Further corroborating our findings, it has been recently reported in males that antagonism of CB_1 receptors or activation of TRPV1s in the dorsolateral PAG increased fear response, through a mechanism that seemed to involve increased glutamatergic transmission induced by either manipulations (Uliana, Hott, Lisboa, & Resstel, 2016).

Further supporting our sex-divergent results, a number of preclinical studies have reported sex-differences in the expression and functionality of endocannabinoid system components in fear-related brain regions in both baseline conditions and in models for stress/trauma-related disorders (Cooper & Craft, 2018; Fattore & Fratta, 2010; Reich, Taylor, & McCarthy, 2009; Xing et al., 2014; Zer-Aviv & Akirav, 2016).

In conclusion, our data provide the first evidence supporting fundamental sex differences of the endocannabinoid system in the modulation of fear expression and extinction. Augmenting AEA or 2-AG signalling did not significantly alter fear expression in male rats, whereas it did affect fear expression and extinction in females in opposite directions. While increased 2-AG signalling acutely reduced conditioned freezing, facilitated within-session extinction and enhanced darting via activation of CB_1 receptors, elevated AEA signalling at TRPV1s increased conditioned freezing, fear generalization and impaired fear extinction. Processes of fear extinction are profoundly altered in PTSD and clinical literature provides evidence that the prevalence of PTSD is twice as high in women compared to men (Breslau, 2009), with documented sex differences found in both disease severity and treatment efficacy. Moreover, human studies have reported sex-related changes of endocannabinoid system components in patients suffering from PTSD, showing a more pronounced up-regulation of CB_1 receptor in the amygdala-hippocampal-cortico-striatal neural circuit in women than men and a decrease in peripheral AEA levels in both sexes (Neumeister et al., 2013). Therefore, understanding how endocannabinoids modulate fear responses and processes of extinction in both sexes will provide new insights into the sex dimorphism documented in the pathophysiology of PTSD and possibly help facilitate the development of sex-specific therapeutic interventions.

ACKNOWLEDGEMENTS

We thank Min Qiao for technical assistance. This study was supported by operating funds from the Canadian Institutes of Health Research (CIHR FDN-143329; M.N.H.) and Fondation Brain Canada (M.N.H.) and National Institute of Mental Health R56 MH11493 grant to R.M.S. and M.N.H. M.M. received salary support from Alberta Innovates Health Solutions (AIHS) and CIHR postdoctoral fellowships, A.S.N. was the recipient of a studentship from the Branch Out Neurological Foundation (BONF), A.S. received salary support from the Mathison Centre for Mental Health Research & Education. All funding agencies had no influence on the design, execution or publishing of this work.

AUTHOR CONTRIBUTIONS

M.M., R.M.S. and M.N.H. participated in the research design. M.M., A.S.N., A.S. and M.N.H. conducted the experiments. B.F.C. contributed the drugs. M.M. and A.S.N. performed the data analyses. M.M., R.M.S. and M.N.H. wrote the manuscript. All authors contributed to the final manuscript and approved its submission.

CONFLICT OF INTEREST

M.N.H. is a member of the scientific advisory board for Sophren Therapeutics and Lundbeck. The rest of the authors declare no conflict of interest.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the BJP guidelines for Design and Analysis and Animal Experimentation, and as recommended by funding agencies, publishers and other organisations engaged with supporting research.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. Some data may not be made available because of privacy or ethical restrictions.

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REFERENCES

Alexander, S. P. H., Christopoulos, A., Davenport, A. P., Kelly, E., Mathie, A., Peters, J. A., … CGTP Collaborators. (2019a). the Concise Guide to PHARMACOLOGY 2019/20: G protein-coupled receptors. *British Journal of Pharmacology*, *176*, S57–S141.

Alexander, S. P. H., Fabbro, D., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., … CGTP Collaborators. (2019b). the Concise Guide to PHARMACOLOGY 2019/20: Enzymes. *British Journal of Pharmacology*, *176*, S297–S396.

Alexander, S. P. H., Mathie, A., Peters, J. A., Veale, E. L., Striessnig, J., Kelly, E., … CGTP Collaborators. (2019c). the Concise Guide to PHARMACOLOGY 2019/20: Ion channels. *British Journal of Pharmacology*, *176*, S142–S228.

Bandler, R., Keay, K. A., Floyd, N., & Price, J. (2000). Central circuits mediating patterned autonomic activity during active vs. passive emotional coping. *Brain Research Bulletin*, *53*, 95–104. https://doi.org/10.1016/S0361-9230(00)00313-0

Bialecki, J., Werner, A., Weilinger, N. L., Tucker, C. M., Vecchiarelli, H. A., Egaña, J., … Thompson, R. J. (2020). Suppression of presynaptic glutamate release by postsynaptic metabotropic NMDA receptor signalling to pannexin-1. *The Journal of Neuroscience*, *40*, 729–742. https://doi.org/10.1523/JNEUROSCI.0257-19.2019

Bitencourt, R. M., Pamplona, F. A., & Takahashi, R. N. (2008). Facilitation of contextual fear memory extinction and anti-anxiogenic effects of AM404 and cannabidiol in conditioned rats. *European Neuropsychopharmacology*, *18*, 849–859. https://doi.org/10.1016/j.euroneuro.2008.07.001

Blankman, J. L., & Cravatt, B. F. (2013). Chemical probes of endocannabinoid metabolism. *Pharmacological Reviews*, *65*, 849–871. https://doi.org/10.1124/pr.112.006387

Bradshaw, H. B., Rimmerman, N., Krey, J. F., & Walker, J. M. (2006). Sex and hormonal cycle differences in rat brain levels of pain-related cannabimimetic lipid mediators. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *291*, R349–R358. https://doi.org/10.1152/ajpregu.00933.2005

Breslau, N. (2009). The epidemiology of trauma, PTSD, and other posttrauma disorders. *Trauma, Violence & Abuse*, *10*, 198–210. https://doi.org/10.1177/1524838009334448

Campos, A. C., & Guimarães, F. S. (2009). Evidence for a potential role for TRPV1 receptors in the dorsolateral periaqueductal gray in the attenuation of the anxiolytic effects of cannabinoids. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *33*, 1517–1521. https://doi.org/10.1016/j.pnpbp.2009.08.017

Casarotto, P. C., Terzian, A. L. B., Aguiar, D. C., Zangrossi, H., Guimarães, F. S., Wotjak, C. T., & Moreira, F. A. (2012). Opposing roles for cannabinoid receptor type-1 (CB1) and transient receptor potential vanilloid type-1 channel (TRPV1) on the modulation of panic-like responses in rats. *Neuropsychopharmacology*, *37*, 478–486. https://doi.org/10.1038/npp.2011.207

Castelli, M., Fadda, P., Casu, A., Spano, M., Casti, A., Fratta, W., & Fattore, L. (2014). Male and female rats differ in brain cannabinoid CB1 receptor density and function and in Behavioural traits predisposing to drug addiction: Effect of ovarian hormones. *Current Pharmaceutical Design*, *20*, 2100–2113. https://doi.org/10.2174/13816128113199990430

Chávez, A. E., Chiu, C. Q., & Castillo, P. E. (2010). TRPV1 activation by endogenous anandamide triggers postsynaptic long-term depression in dentate gyrus. *Nature Neuroscience*, *13*, 1511–1518. https://doi.org/10.1038/nn.2684

Chhatwal, J. P., Davis, M., Maguschak, K. A., & Ressler, K. J. (2005). Enhancing cannabinoid neurotransmission augments the extinction of conditioned fear. *Neuropsychopharmacology*, *30*, 516–524. https://doi.org/10.1038/sj.npp.1300655

Colangeli, R., Di Maio, R., Pierucci, M., Deidda, G., Casarrubea, M., & Di Giovanni, G. (2019). Synergistic action of CB1 and 5-HT2B receptors in preventing pilocarpine-induced status epilepticus in rats. *Neurobiology of Disease*, *125*, 135–145. https://doi.org/10.1016/j.nbd.2019.01.026

Colangeli, R., Morena, M., Pittman, Q. J., Hill, M. N., & Teskey, G. C. (2020). Anandamide signaling augmentation rescues amygdala synaptic function and comorbid emotional alterations in a model of epilepsy. *The Journal of Neuroscience*, *40*, 6068–6081. https://doi.org/10.1523/JNEUROSCI.0068-20.2020

Colangeli, R., Pierucci, M., Benigno, A., Campiani, G., Butini, S., & Di Giovanni, G. (2017). The FAAH inhibitor URB597 suppresses hippocampal maximal dentate after discharges and restores seizure-induced impairment of short and long-term synaptic plasticity. *Scientific Reports*, *7*, 11152. https://doi.org/10.1038/s41598-017-11606-1

Colom-Lapetina, J., Li, A. J., Pelegrina-Perez, T. C., & Shansky, R. M. (2019). Behavioral diversity across classic rodent models is sex-dependent. *Frontiers in Behavioral Neuroscience*, *13*, 45. https://doi.org/10.3389/fnbeh.2019.00045

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

Craft, R. M., Wakley, A. A., Tsutsui, K. T., & Laggart, J. D. (2012). Sex differences in cannabinoid 1 vs. cannabinoid 2 receptor-selective antagonism of antinociception produced by Δ^9-tetrahydrocannabinol and CP55,940 in the rat. *The Journal of Pharmacology and Experimental Therapeutics*, *340*, 787–800. https://doi.org/10.1124/jpet.111.188540

Cristino, L., Starowicz, K., De Petrocellis, L., Morishita, J., Ueda, N., Guglielmotti, V., & Di Marzo, V. (2008). Immunohistochemical localization of anabolic and catabolic enzymes for anandamide and other putative endovanilloids in the hippocampus and cerebellar cortex of the mouse brain. *Neuroscience*, *151*, 955–968. https://doi.org/10.1016/j.neuroscience.2007.11.047

Curtis, M. J., Alexander, S., Cirino, G., Docherty, J. R., George, C. H., Giembycz, M. A., … Ahluwalia, A. (2018). Experimental design and analysis and their reporting II: Updated and simplified guidance for authors and peer reviewers. *British Journal of Pharmacology*, *175*, 987–993. https://doi.org/10.1111/bph.14153

de Fonseca, F. R., Cebeira, M., Ramos, J. A., Martín, M., & Fernández-Ruiz, J. J. (1994). Cannabinoid receptors in rat brain areas: Sexual differences, fluctuations during estrous cycle and changes after gonadectomy and sex steroid replacement. *Life Sciences*, *54*, 159–170. https://doi.org/10.1016/0024-3205(94)00585-0

Di Maio, R., Colangeli, R., & Di Giovanni, G. (2019). WIN 55,212-2 reverted pilocarpine-induced status epilepticus early changes of the interaction among 5-HT2C/NMDA/CB1 receptors in the rat hippocampus. *ACS Chemical Neuroscience*, *10*, 3296–3306. https://doi.org/10.1021/acschemneuro.9b00080

Di Marzo, V. (2008). Targeting the endocannabinoid system: To enhance or reduce? *Nature Reviews. Drug Discovery*, *7*, 438–455. https://doi.org/10.1038/nrd2553

Fanselow, M. S. (1980). Conditional and unconditional components of post-shock freezing. *The Pavlovian Journal of Biological Science: Official Journal of the Pavlovian*, *15*, 177–182.

Fattore, L., & Fratta, W. (2010). How important are sex differences in cannabinoid action. *British Journal of Pharmacology*, *160*, 544–548. https://doi.org/10.1111/j.1476-5381.2010.00776.x

González, S., Bisogno, T., Wenger, T., Manzanares, J., Milone, A., Berrendero, F., … Fernández-Ruiz, J. J. (2000). Sex steroid influence on cannabinoid CB1 receptor mRNA and endocannabinoid levels in the anterior pituitary gland. *Biochemical and Biophysical Research Communications*, *270*, 260–266. https://doi.org/10.1006/bbrc.2000.2406

Gruene, T. M., Flick, K., Stefano, A., Shea, S. D., & Shansky, R. M. (2015). Sexually divergent expression of active and passive conditioned fear responses in rats. *eLife*, *4*, 1–9.

Gruene, T. M., Roberts, E., Thomas, V., Ronzio, A., & Shansky, R. M. (2015). Sex-specific neuroanatomical correlates of fear expression in prefrontal-amygdala circuits. *Biological Psychiatry*, *78*, 186–193. https://doi.org/10.1016/j.biopsych.2014.11.014

Grueter, B. A., Brasnjo, G., & Malenka, R. C. (2010). Postsynaptic TRPV1 triggers cell type-specific long-term depression in the nucleus accumbens. *Nature Neuroscience*, *13*, 1519–1525. https://doi.org/10.1038/nn.2685

Gunduz-Cinar, O., MacPherson, K. P., Cinar, R., Gamble-George, J., Sugden, K., Williams, B., … Holmes, A. (2013). Convergent translational evidence of a role for anandamide in amygdala-mediated fear extinction, threat processing and stress-reactivity. *Molecular Psychiatry*, *18*, 813–823. https://doi.org/10.1038/mp.2012.72

Gupta, R. R., Sen, S., Diepenhorst, L. L., Rudick, C. N., & Maren, S. (2001). Estrogen modulates sexually dimorphic contextual fear conditioning and hippocampal long-term potentiation (LTP) in rats. *Brain Research*, *888*, 356–365. https://doi.org/10.1016/S0006-8993(00)03116-4

Hájos, N., Kathuria, S., Dinh, T., Piomelli, D., & Freund, T. F. (2004). Endocannabinoid transport tightly controls 2-arachidonoyl glycerol actions in the hippocampus: Effects of low temperature and the transport inhibitor AM404. *The European Journal of Neuroscience*, *19*, 2991–2996. https://doi.org/10.1111/j.0953-816X.2004.03433.x

Hartley, N. D., Gunduz-Cinar, O., Halladay, L., Bukalo, O., Holmes, A., & Patel, S. (2016). 2-arachidonoylglycerol signaling impairs short-term fear extinction. *Translational Psychiatry*, *6*, e749. https://doi.org/10.1038/tp.2016.26

Heinz, D. E., Genewsky, A., & Wotjak, C. T. (2017). Enhanced anandamide signaling reduces flight behavior elicited by an approaching robo-beetle. *Neuropharmacology*, *126*, 233–241. https://doi.org/10.1016/j.neuropharm.2017.09.010

Jovanovic, T., & Norrholm, S. D. (2011). Neural mechanisms of impaired fear inhibition in posttraumatic stress disorder. *Frontiers in Behavioral Neuroscience*, *5*, 44.

Kathuria, S., Gaetani, S., Fegley, D., Valiño, F., Duranti, A., Tontini, A., … Piomelli, D. (2003). Modulation of anxiety through blockade of anandamide hydrolysis. *Nature Medicine*, *9*, 76–81. https://doi.org/10.1038/nm803

Katona, I., & Freund, T. F. (2012). Multiple functions of endocannabinoid signaling in the brain. *Annual Review of Neuroscience*, *35*, 529–558. https://doi.org/10.1146/annurev-neuro-062111-150420

Lang, P. J., Davis, M., & Öhman, A. (2000). Fear and anxiety: Animal models and human cognitive psychophysiology. *Journal of Affective Disorders*, *61*, 137–159. https://doi.org/10.1016/S0165-0327(00)00343-8

Laricchiuta, D., Centonze, D., & Petrosini, L. (2013). Effects of endocannabinoid and endovanilloid systems on aversive memory extinction. *Behavioural Brain Research*, *256*, 101–107. https://doi.org/10.1016/j.bbr.2013.08.010

Lilley, E., Stanford, S. C., Kendall, D. E., Alexander, S. P., Cirino, G., Docherty, J. R., … Ahluwalia, A. (2020). ARRIVE 2.0 and the *British Journal of Pharmacology*: Updated guidance for 2020. *British Journal of Pharmacology*, *177*, 3611–3616. https://doi.org/10.1111/bph.15178

Llorente-Berzal, A., Terzian, A. L. B., Di Marzo, V., Micale, V., Viveros, M. P., & Wotjak, C. T. (2015). 2-AG promotes the expression of conditioned fear via cannabinoid receptor type 1 on GABAergic neurons. *Psychopharmacology*, *232*, 2811–2825. https://doi.org/10.1007/s00213-015-3917-y

Lutz, B., Marsicano, G., Maldonado, R., & Hillard, C. J. (2015). The endocannabinoid system in guarding against fear, anxiety and stress. *Nature Reviews. Neuroscience*, *16*, 705–718. https://doi.org/10.1038/nrn4036

Marcus, D. J., Bedse, G., Gaulden, A. D., Ryan, J. D., Kondev, V., Winters, N. D., … Patel, S. (2020). Endocannabinoid signaling collapse mediates stress-induced amygdalo-cortical strengthening. *Neuron*, *105*, 1062–1076.e6. https://doi.org/10.1016/j.neuron.2019.12.024

Maren, S. (2001). Neurobiology of Pavlovian fear conditioning. *Annual Review of Neuroscience*, *24*, 897–931. https://doi.org/10.1146/annurev.neuro.24.1.897

Maren, S., De Oca, B., & Fanselow, M. S. (1994). Sex differences in hippocampal long-term potentiation (LTP) and Pavlovian fear conditioning in rats: Positive correlation between LTP and contextual learning. *Brain Research*, *661*, 25–34. https://doi.org/10.1016/0006-8993(94)91176-2

Marinelli, S., Di Marzo, V., Berretta, N., Matias, I., Maccarrone, M., Bernardi, G., & Mercuri, N. B. (2003). Presynaptic facilitation of glutamatergic synapses to dopaminergic neurons of the rat substantia nigra by endogenous stimulation of vanilloid receptors. *The Journal of Neuroscience*, *23*, 3136–3144. https://doi.org/10.1523/JNEUROSCI.23-08-03136.2003

Marsch, R., Foeller, E., Rammes, G., Bunck, M., Kössl, M., Holsboer, F., … Wotjak, C. T. (2007). Reduced anxiety, conditioned fear, and hippocampal long-term potentiation in transient receptor potential vanilloid type 1 receptor-deficient mice. *The Journal of Neuroscience*, *27*, 832–839. https://doi.org/10.1523/JNEUROSCI.3303-06.2007

Marsicano, G., Wotjak, C. T., Azad, S. C., Bisogno, T., Rammes, G., Cascioll, M. G., … Di Marzo, V. (2002). The endogenous cannabinoid system controls extinction of aversive memories. *Nature*, *418*, 530–534. https://doi.org/10.1038/nature00839

Mayo, L. M., Asratian, A., Lindé, J., Morena, M., Haataja, R., Hammar, V., … Heilig, M. (2020). Elevated anandamide, enhanced recall of fear extinction, and attenuated stress responses following inhibition of fatty acid amide hydrolase: A randomized, controlled experimental medicine trial. *Biological Psychiatry*, *87*, 538–547. https://doi.org/10.1016/j.biopsych.2019.07.034

Metna-Laurent, M., Soria-Gómez, E., Verrier, D., Conforzi, M., Jégo, P., Lafenêtre, P., & Marsicano, G. (2012). Bimodal control of fear-coping strategies by CB cannabinoid receptors. *The Journal of Neuroscience*, *32*, 7109–7118. https://doi.org/10.1523/JNEUROSCI.1054-12.2012

Mezey, E., Tóth, Z. E., Cortright, D. N., Arzubi, M. K., Krause, J. E., Elde, R., … Szallasi, A. (2000). Distribution of mRNA for vanilloid receptor subtype 1 (VR1), and VR1-like immunoreactivity, in the central nervous system of the rat and human. *Proceedings of the National Academy of Sciences*, *97*, 3655–3660. https://doi.org/10.1073/pnas.97.7.3655

Milad, M. R., Pitman, R. K., Ellis, C. B., Gold, A. L., Shin, L. M., Lasko, N. B., … Rauch, S. L. (2009). Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. *Biological Psychiatry*, *66*, 1075–1082. https://doi.org/10.1016/j.biopsych.2009.06.026

Moreira, F. A., Aguiar, D. C., Terzian, A. L. B., Guimarães, F. S., & Wotjak, C. T. (2012). Cannabinoid type 1 receptors and transient receptor potential vanilloid type 1 channels in fear and anxiety-two sides of one coin? *Neuroscience*, *204*, 186–192. https://doi.org/10.1016/j.neuroscience.2011.08.046

Morena, M., Aukema, R. J., Leitl, K. D., Rashid, A. J., Vecchiarelli, H. A., Josselyn, S. A., & Hill, M. N. (2019). Upregulation of anandamide hydrolysis in the basolateral complex of amygdala reduces fear memory expression and indices of stress and anxiety. *The Journal of Neuroscience*, *39*, 1275–1292. https://doi.org/10.1523/JNEUROSCI.2251-18.2018

Morena, M., Berardi, A., Colucci, P., Palmery, M., Trezza, V., Hill, M. N., & Campolongo, P. (2018). Enhancing endocannabinoid neurotransmission augments the efficacy of extinction training and ameliorates traumatic stress-induced behavioral alterations in rats. *Neuropsychopharmacology*, *43*, 1284–1296. https://doi.org/10.1038/npp.2017.305

Morena, M., & Campolongo, P. (2014). The endocannabinoid system: An emotional buffer in the modulation of memory function. *Neurobiology of Learning and Memory*, *112*, 30–43. https://doi.org/10.1016/j.nlm.2013.12.010

Morena, M., De Castro, V., Gray, J. M., Palmery, M., Trezza, V., Roozendaal, B., … Campolongo, P. (2015). Training-associated emotional arousal shapes endocannabinoid modulation of spatial memory retrieval in rats. *The Journal of Neuroscience*, *35*, 13962–13974. https://doi.org/10.1523/JNEUROSCI.1983-15.2015

Morena, M., Leitl, K. D., Vecchiarelli, H. A., Gray, J. M., Campolongo, P., & Hill, M. N. (2016). Emotional arousal state influences the ability of amygdalar endocannabinoid signaling to modulate anxiety. *Neuropharmacology*, *111*, 59–69. https://doi.org/10.1016/j.neuropharm.2016.08.020

Morena, M., Patel, S., Bains, J. S., & Hill, M. N. (2016). Neurobiological interactions between stress and the endocannabinoid system. *Neuropsychopharmacology*, *41*, 80–102. https://doi.org/10.1038/npp.2015.166

Musella, A., De Chiara, V., Rossi, S., Prosperetti, C., Bernardi, G., Maccarrone, M., & Centonze, D. (2009). TRPV1 channels facilitate glutamate transmission in the striatum. *Molecular and Cellular Neurosciences*, *40*, 89–97. https://doi.org/10.1016/j.mcn.2008.09.001

Neumeister, A., Normandin, M. D., Pietrzak, R. H., Piomelli, D., Zheng, M. Q., Gujarro-Anton, A., … Huang, Y. (2013). Elevated brain cannabinoid CB 1 receptor availability in post-traumatic stress disorder: A positron emission tomography study. *Molecular Psychiatry*, *18*, 1034–1040. https://doi.org/10.1038/mp.2013.61

Nicholson, R. A., Liao, C., Zheng, J., David, L. S., Coyne, L., Errington, A. C., … Lees, G. (2003). Sodium channel inhibition by anandamide and synthetic cannabimimetics in brain. *Brain Research*, *978*, 194–204. https://doi.org/10.1016/S0006-8993(03)02808-7

Olango, W. M., Roche, M., Ford, G. K., Harhen, B., & Finn, D. P. (2012). The endocannabinoid system in the rat dorsolateral periaqueductal

grey mediates fear-conditioned analgesia and controls fear expression in the presence of nociceptive tone. *British Journal of Pharmacology*, *165*, 2549–2560. https://doi.org/10.1111/j.1476-5381.2011.01478.x

Pamplona, F. A., Bitencourt, R. M., & Takahashi, R. N. (2008). Short- and long-term effects of cannabinoids on the extinction of contextual fear memory in rats. *Neurobiology of Learning and Memory*, *90*, 290–293. https://doi.org/10.1016/j.nlm.2008.04.003

Patel, S., Hill, M. N., Cheer, J. F., Wotjak, C. T., & Holmes, A. (2017). The endocannabinoid system as a target for novel anxiolytic drugs. *Neuroscience and Biobehavioral Reviews*, *76*, 56–66. https://doi.org/10.1016/j.neubiorev.2016.12.033

Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M. T., Baker, M., … Würbel, H. (2020). The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *PLoS Biology*, *18*(7), e3000410. https://doi.org/10.1371/journal.pbio.3000410

Pryce, C. R., Lehmann, J., & Feldon, J. (1999). Effect of sex on fear conditioning is similar for context and discrete CS in Wistar, Lewis and Fischer rat strains. *Pharmacology, Biochemistry, and Behavior*, *64*, 753–759. https://doi.org/10.1016/S0091-3057(99)00147-1

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 neuromodulatory endocannabinoids. *Rapid Communications in Mass Spectrometry*, *29*, 1889–1897. https://doi.org/10.1002/rcm.7277

Ratano, P., Palmery, M., Trezza, V., & Campolongo, P. (2017). Cannabinoid modulation of memory consolidation in rats: Beyond the role of cannabinoid receptor subtype 1. *Frontiers in Pharmacology*, *8*, 200.

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. *Behavioural Brain Research*, *203*, 264–269. https://doi.org/10.1016/j.bbr.2009.05.013

Rey, A. A., Purrio, M., Viveros, M. P., & Lutz, B. (2012). Biphasic effects of cannabinoids in anxiety responses: CB1 and GABA B receptors in the balance of GABAergic and glutamatergic neurotransmission. *Neuropsychopharmacology*, *37*, 2624–2634. https://doi.org/10.1038/npp.2012.123

Riebe, C. J. N., Hill, M. N., Lee, T. T. Y., Hillard, C. J., & Gorzalka, B. B. (2010). Estrogenic regulation of limbic cannabinoid receptor binding. *Psychoneuroendocrinology*, *35*, 1265–1269. https://doi.org/10.1016/j.psyneuen.2010.02.008

Rubino, T., Realini, N., Castiglioni, C., Guidali, C., Viganó, D., Marras, E., … Parolaro, D. (2008). Role in anxiety behavior of the endocannabinoid system in the prefrontal cortex. *Cerebral Cortex*, *18*, 1292–1301. https://doi.org/10.1093/cercor/bhm161

Shansky, R. M. (2015). Sex differences in PTSD resilience and susceptibility: Challenges for animal models of fear learning. *Neurobiol Stress*, *1*, 60–65. https://doi.org/10.1016/j.ynstr.2014.09.005

Sticht, M. A., Lau, D. J., Keenan, C. M., Cavin, J.-B., Morena, M., Vemuri, V. K., … Hill, M. N. (2019). Endocannabinoid regulation of homeostatic feeding and stress-induced alterations in food intake in male rats. *British Journal of Pharmacology*, *176*, 1524–1540. https://doi.org/10.1111/bph.14453

Tsou, K., Mackie, K., Sañudo-Peña, M. C., & Walker, J. M. (1999). Cannabinoid CB1 receptors are localized primarily on cholecystokinin-containing GABAergic interneurons in the rat hippocampal formation. *Neuroscience*, *93*, 969–975. https://doi.org/10.1016/S0306-4522(99)00086-X

Uliana, D. L., Hott, S. C., Lisboa, S. F., & Resstel, L. B. M. (2016). Dorsolateral periaqueductal gray matter CB1 and TRPV1 receptors exert opposite modulation on expression of contextual fear conditioning. *Neuropharmacology*, *103*, 257–269. https://doi.org/10.1016/j.neuropharm.2015.12.020

Van Laere, K., Goffin, K., Casteels, C., Dupont, P., Mortelmans, L., de Hoon, J., & Bormans, G. (2008). Gender-dependent increases with healthy aging of the human cerebral cannabinoid-type 1 receptor binding using [18F]MK-9470 PET. *NeuroImage*, *39*, 1533–1541. https://doi.org/10.1016/j.neuroimage.2007.10.053

Velasco, E. R., Florido, A., Milad, M. R., & Andero, R. (2019). Sex differences in fear extinction. *Neuroscience and Biobehavioral Reviews*, *103*, 81–108. https://doi.org/10.1016/j.neubiorev.2019.05.020

Watson, T. C., Cerminara, N. L., Lumb, B. M., & Apps, R. (2016). Neural correlates of fear in the periaqueductal gray. *The Journal of Neuroscience*, *36*, 12707–12719. https://doi.org/10.1523/JNEUROSCI.1100-16.2016

Wiley, J. L., & Burston, J. J. (2014). Sex differences in Δ^9-tetrahydrocannabinol metabolism and in vivo pharmacology following acute and repeated dosing in adolescent rats. *Neuroscience Letters*, *576*, 51–55. https://doi.org/10.1016/j.neulet.2014.05.057

Wiskerke, J., Irimia, C., Cravatt, B. F., De Vries, T. J., Schoffelmeer, A. N. M., Pattij, T., & Parsons, L. H. (2012). Characterization of the effects of reuptake and hydrolysis inhibition on interstitial endocannabinoid levels in the brain: An in vivo microdialysis study. *ACS Chemical Neuroscience*, *3*, 407–417. https://doi.org/10.1021/cn300036b

Xing, G., Carlton, J., Jiang, X., Wen, J., Jia, M., & Li, H. (2014). Differential expression of brain cannabinoid receptors between repeatedly stressed males and females may play a role in age and gender-related difference in traumatic brain injury: Implications from animal studies. *Frontiers in Neurology*, *5*, 161.

Xing, J., & Li, J. (2007). TRPV1 receptor mediates glutamatergic synaptic input to dorsolateral periaqueductal gray (dl-PAG) neurons. *Journal of Neurophysiology*, *97*, 503–511. https://doi.org/10.1152/jn.01023.2006

Yasmin, F., Colangeli, R., Morena, M., Filipski, S., van der Stelt, M., Pittman, Q. J., … Chattarji, S. (2020). Stress-induced modulation of endocannabinoid signaling leads to delayed strengthening of synaptic connectivity in the amygdala. *Proceedings of the National Academy of Sciences of the United States of America*, *117*, 650–655. https://doi.org/10.1073/pnas.1910322116

Zeidan, M. A., Igoe, S. A., Linnman, C., Vitalo, A., Levine, J. B., Klibanski, A., … Milad, M. R. (2011). Estradiol modulates medial prefrontal cortex and amygdala activity during fear extinction in women and female rats. *Biological Psychiatry*, *70*, 920–927. https://doi.org/10.1016/j.biopsych.2011.05.016

Zer-Aviv, T. M., & Akirav, I. (2016). Sex differences in hippocampal response to endocannabinoids after exposure to severe stress. *Hippocampus*, *26*, 947–957. https://doi.org/10.1002/hipo.22577

Zygmunt, P. M., Chuang, H. H., Movahed, P., Julius, D., & Högestätt, E. D. (2000). The anandamide transport inhibitor AM404 activates vanilloid receptors. *European Journal of Pharmacology*, *396*, 39–42. https://doi.org/10.1016/S0014-2999(00)00207-7

Zygmunt, P. M., Petersson, J., Andersson, D. A., Chuang, H., Sørgård, M., Di Marzo, V., & Julius, D. (1999). Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature*, *400*, 452–457. https://doi.org/10.1038/22761

SUPPORTING INFORMATION

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How to cite this article: Morena M, Nastase AS, Santori A, Cravatt BF, Shansky RM, Hill MN. Sex-dependent effects of endocannabinoid modulation of conditioned fear extinction in rats. *Br J Pharmacol*. 2021;1–14. https://doi.org/10.1111/bph.15341