

Short communication

Sex-specific differences in nociceptive behaviour and the amygdala endocannabinoid system in the rat monoiodoacetate-induced knee osteoarthritis model

Mehnaz I. Ferdousi^{a,c,d,1}, Rosmara Infantino^{a,c,d,1}, Maria C. Redmond^{a,c,d},
 Santhosh D. Krishnan^{a,c,d}, Canny K. Adjei^{a,c,d}, Barry McDermott^e, Alison Liddy^f,
 Leo Quinlan^{b,c,d}, Martin O'Halloran^e, David P. Finn^{a,c,d,*}

^a Pharmacology and Therapeutics, School of Pharmacy and Medical Sciences, Institute for Health Discovery and Innovation, University of Galway, Galway City, Ireland

^b Physiology, School of Pharmacy and Medical Sciences, University of Galway, Galway City, Ireland

^c Galway Neuroscience Centre, University of Galway, Galway City, Ireland

^d Centre for Pain Research, University of Galway, Galway City, Ireland

^e Translational Medical Device Lab, University of Galway, Galway City, Ireland

^f Relevium Medical, Business Innovation Centre, Upper Newcastle, Galway City, Ireland

ARTICLE INFO

Keywords:

Osteoarthritis
 Chronic pain
 Sex differences
 Oestrous cycle
 MIA model
 Endocannabinoid system
 Amygdala

ABSTRACT

Introduction: Osteoarthritis (OA) is a chronic, progressive joint disorder with higher prevalence and pain severity in women than men. The endocannabinoid system (ECS) modulates pain and shows sexual dimorphism, yet sex-specific alterations in central ECS signalling in OA pain remain under-investigated.

Objectives: We investigated sex-dependent differences in pain-related behaviours and central ECS signalling in the rat monoiodoacetate (MIA) model of knee OA pain.

Methods: Adult male and female Sprague-Dawley rats (8–9 weeks old) received an intra-articular injection of either MIA (2 mg/50 µL) or saline (Sham control) in the left knee. Pain-related behaviours were assessed over 63 days post-injection. The influence of oestrous cycle stage on pain-related behaviours was examined in 100 additional MIA-injected female rats. Endocannabinoids [anandamide (AEA), 2-arachidonoylglycerol (2-AG)] and *N*-acylethanolamines [palmitoylethanolamide (PEA), oleoylethanolamide (OEA)] were quantified in brain and spinal cord regions (LC-MS/MS), and ECS-related gene expression (*Faah*, *Mgl1*, *Cnr1*, *Cnr2*) was assessed (RT-qPCR).

Results: MIA females exhibited greater weight-bearing deficits and hind paw mechanical hypersensitivity than males throughout the study period. Oestrous cycle stage did not influence MIA-induced pain-related behaviours. MIA males showed lateralised ECS signalling in the amygdala, with elevated endocannabinoid and *N*-acylethanolamine levels in the ipsilateral/left amygdala, a pattern absent in females. A bilateral reduction of *Mgl1* expression in the amygdala was observed in MIA female rats compared to Sham controls.

Conclusions: In the MIA model, female rats demonstrate greater nociceptive behaviour and a different amygdala ECS profile, compared with males, paving the way for further investigation of ECS-mediated mechanisms underlying sex differences in OA pain.

1. Introduction

Osteoarthritis (OA) is a disabling condition affecting over 500 million people worldwide (Tang et al., 2025). Defined as a chronic

disorder of movable joints, and characterised by cellular stress and extracellular matrix degradation (Kraus et al., 2015), OA is now recognised as a complex and multifactorial condition involving all joint tissues (Tang et al., 2025). Women show a higher prevalence of OA

* Corresponding author at: Room 3004, Pharmacology and Therapeutics, School of Pharmacy and Medical Sciences, Human Biology Building, University of Galway, Galway City, H91 W5P7, Ireland.

E-mail address: David.Finn@universityofgalway.ie (D.P. Finn).

¹ Contributed equally.

<https://doi.org/10.1016/j.ynpai.2026.100227>

Received 23 May 2026; Received in revised form 4 July 2026; Accepted 15 July 2026

Available online 19 July 2026

2452-073X/© 2026 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

compared to men, and greater OA-related pain severity, especially for knee OA (Di et al., 2024). Anatomical, biomechanical, and hormonal factors have been suggested to account for these sex differences (Segal et al., 2024), alongside the recognised sexual dimorphism in pain sensitivity observed across multiple chronic pain conditions (Bartley and Fillingim, 2013; Fillingim et al., 2009; Finn et al., 2026; Mogil, 2020).

Notably, clinical and preclinical studies demonstrate that females display distinct features compared to males in diverse aspects related to OA pain pathophysiology (Segal et al., 2024), including inflammatory responses (Mun et al., 2020; Perruccio et al., 2019), neuroimmune interactions (Luo et al., 2021; Tavares-Ferreira et al., 2022; Valdrighi et al., 2022), peripheral and central sensitisation mechanisms (Bartley et al., 2016; Chen et al., 2020; Diogenes et al., 2006; Sannajust et al., 2019), and hormonal influence on pain perception (Aloisi and Bonifazi, 2006; Hellström and Anderberg, 2003). Despite this growing evidence of sex differences in OA pain, comprehensive behavioural characterisation of chronic OA-related pain in male and female rodent models remains limited, particularly in long-term experimental settings.

The endocannabinoid system (ECS) plays a pivotal role in pain perception and modulation (Starowicz et al., 2017; Woodhams et al., 2017), and exhibits sexual dimorphism, with physiological, pathological, and pharmacological implications (Blanton et al., 2021; Boullon et al., 2023, 2021; Craft et al., 2013; Henderson-Redmond et al., 2021; Llorente-Berzal et al., 2022). To date, ECS involvement in monoiodoacetate (MIA)-induced OA pain and related depressive behaviour has been demonstrated (Burston et al., 2013; Kędziora et al., 2023; La Porta et al., 2013). However, the role of the ECS in sex-related OA pain differences remains largely unexplored.

Here, we provide a longitudinal behavioural assessment of MIA-induced knee OA pain in male and female rats across an extended chronic phase (up to 63 days post-MIA), and examine the influence of the oestrous cycle on behavioural outcomes in female MIA rats. We further investigated any sex-related alterations of the central ECS in spinal cord and various brain regions involved in pain processing and modulation.

2. Methods

Detailed methodology is reported in **Supplementary Methods and Results**.

Briefly, adult Sprague-Dawley rats ($n = 24$, 3–9 per experimental group) were used in the male versus female comparative study. Data from an additional 100 MIA-injected female rats were analysed to assess oestrous cycle influence on knee OA pain-related behaviours, allowing for appropriate statistical power. The sample size was determined based on similar studies reported in the literature (Ro et al., 2020). All procedures were approved by the Animal Care and Research Ethics Committee, University of Galway, and licensed by the Health Products Regulatory Authority, Ireland, in compliance with EU Directive 2010/63. The study is reported in line with the ARRIVE 2.0 Essential 10 guidelines (Percie du Sert et al., 2020). All in vivo work was carried out by female experimenters.

After baseline behavioural assessments, MIA (2 mg/50 μ L) was injected intra-articularly into the left knee under anaesthesia to induce an OA-like state (Pomonis et al., 2005). Sham controls received an equivalent volume of saline under similar conditions. Pain-related behaviours – static weight-bearing (SWB) (Bove et al., 2003), mechanical hypersensitivity (electronic von Frey, eVF) (Di Marino et al., 2024), and cold hypersensitivity (acetone drop, AD) (Yoon et al., 1994) – were assessed for 63 days post-injection. On Day 64, rats were euthanised, brains and spinal cords harvested, and the amygdala, periaqueductal grey, rostral ventral medulla, prefrontal cortex, thalamus, and dorsal lumbar spinal cord (L3–L6) were gross-dissected. Endocannabinoid [anandamide (AEA), 2-arachidonoylglycerol (2-AG)] and *N*-acylethanolamine [palmitoylethanolamide (PEA), oleoylethanolamide (OEA)] levels were quantified using LC-MS/MS (Boullon et al., 2021; Corcoran

et al., 2020; Kerr et al., 2012), and ECS-related gene expression [fatty acid amide hydrolase (*Faah*), monoacylglycerol lipase (*MglI*), cannabinoid receptor type 1 (*Cnr1*), and cannabinoid receptor type 2 (*Cnr2*)] was assessed using RT-qPCR (Di Marino et al., 2024), as described previously.

The additional female cohort ($n = 100$) underwent the same MIA induction and then behavioural assessments at baseline and on Days 7 and 14 only. Oestrous stage was determined by vaginal cytology (McLean et al., 2012). Representative images of the different stages of the oestrous cycle are shown in **Suppl. Fig. 1**.

Data are presented as mean $\pm$ SEM. Normality and homogeneity of variance were assessed using the Shapiro-Wilk and Levene's tests, respectively. Outliers were identified using the ROUT method ($Q = 1\%$) (Motulsky and Brown, 2006), and excluded from biochemical analyses only. No behavioural data were excluded. Behavioural time-course data were analysed using three-way repeated measures (RM) ANOVA (within-subjects factor: time, between-subjects factors: sex and model). Neurochemical and gene expression data were analysed using two-way (factors: sex and model) or three-way ANOVA (factors: sex, model, and side), or the corresponding mixed-effects model when missing values resulted from outlier removal. Tukey's post hoc test was used for multiple comparisons. Unpaired *t*-tests with Welch's correction, Fisher's exact test, Wilcoxon one-sample *t*-test, or binomial logistic regression were applied, where appropriate (see **Suppl. Material**). Statistical significance was set at $p < 0.05$. Analyses were performed using GraphPad Prism 10.6.1.

3. Results

3.1. Female rats display greater MIA-induced nociceptive behaviours than male rats

A time-course analysis of pain-related behaviours was conducted in male and female MIA rats to characterise any sex differences.

In the SWB test, three-way RM ANOVA revealed significant main effects of time ($F_{(8,191)} = 11.17$, $p < 0.0001$), sex ($F_{(1,24)} = 100.80$, $p = 0.001$), and model ($F_{(1,24)} = 235.00$, $p < 0.0001$) with significant interaction effects of time $\times$ model ($F_{(8,191)} = 11.42$, $p < 0.0001$), and sex $\times$ model ($F_{(1,24)} = 14.48$, $p = 0.001$) on ipsilateral hind limb weight-bearing deficits, expressed relative to the contralateral hind limb (% WB). From Day 5 onward, MIA-injected animals of both sexes displayed persistent ipsilateral %WB reduction compared with baseline ($p < 0.01$) and compared with Sham controls ($p < 0.0001$) (Fig. 1a). MIA Females consistently exhibited greater deficits than MIA males throughout the observation period, with the largest differences observed on Day 21 (Day 21: $32.62 \pm 2.06\%$ vs $42.42 \pm 1.40\%$, $p < 0.02$; Fig. 1a). In the eVF test, ipsilateral and contralateral PWTs were analysed separately. Analysis of ipsilateral PWTs revealed significant main effects of time ($F_{(5,535,132.8)} = 19.10$, $p < 0.0001$), sex ($F_{(1,24)} = 36.24$, $p < 0.0001$), and model ($F_{(1,24)} = 482.60$, $p < 0.0001$), with significant interaction effects of time $\times$ sex ($F_{(5,535,132.8)} = 2.31$, $p < 0.0001$) and time $\times$ model ($F_{(5,535,132.8)} = 31.36$, $p < 0.0001$). Both sexes developed long-lasting MIA-induced mechanical hypersensitivity of the ipsilateral hind paw from Day 5, with reduced PWT versus baseline ($p < 0.01$) and versus Sham controls ($p < 0.01$) (Fig. 1b). MIA females showed greater ipsilateral hind paw mechanical hypersensitivity than MIA males at both early and late time points throughout the observation period (Day 14: 16.73 ± 1.11 g vs 26.92 ± 1.62 g, $p = 0.0008$; Day 63: 15.99 ± 1.54 g vs 24.93 ± 2.17 g, $p = 0.022$; Fig. 1b). No differences were observed in contralateral PWTs or in male or female Sham controls relative to baseline. Percentage change indices ($\Delta\%$) for post-MIA nociceptive behaviours were therefore calculated only in MIA rats, either relative to baseline (BL) or by comparing the ipsilateral with the contralateral side. Females showed significantly higher WB% Δ (BL) than males ($31.21 \pm 2.55\%$ vs $18.55 \pm 1.19\%$; $t = 4.49$, $df = 8.57$, $p = 0.002$; unpaired *t*-test with Welch's correction; Fig. 1a.I) and larger PWT% Δ (contra), ($66.02 \pm$

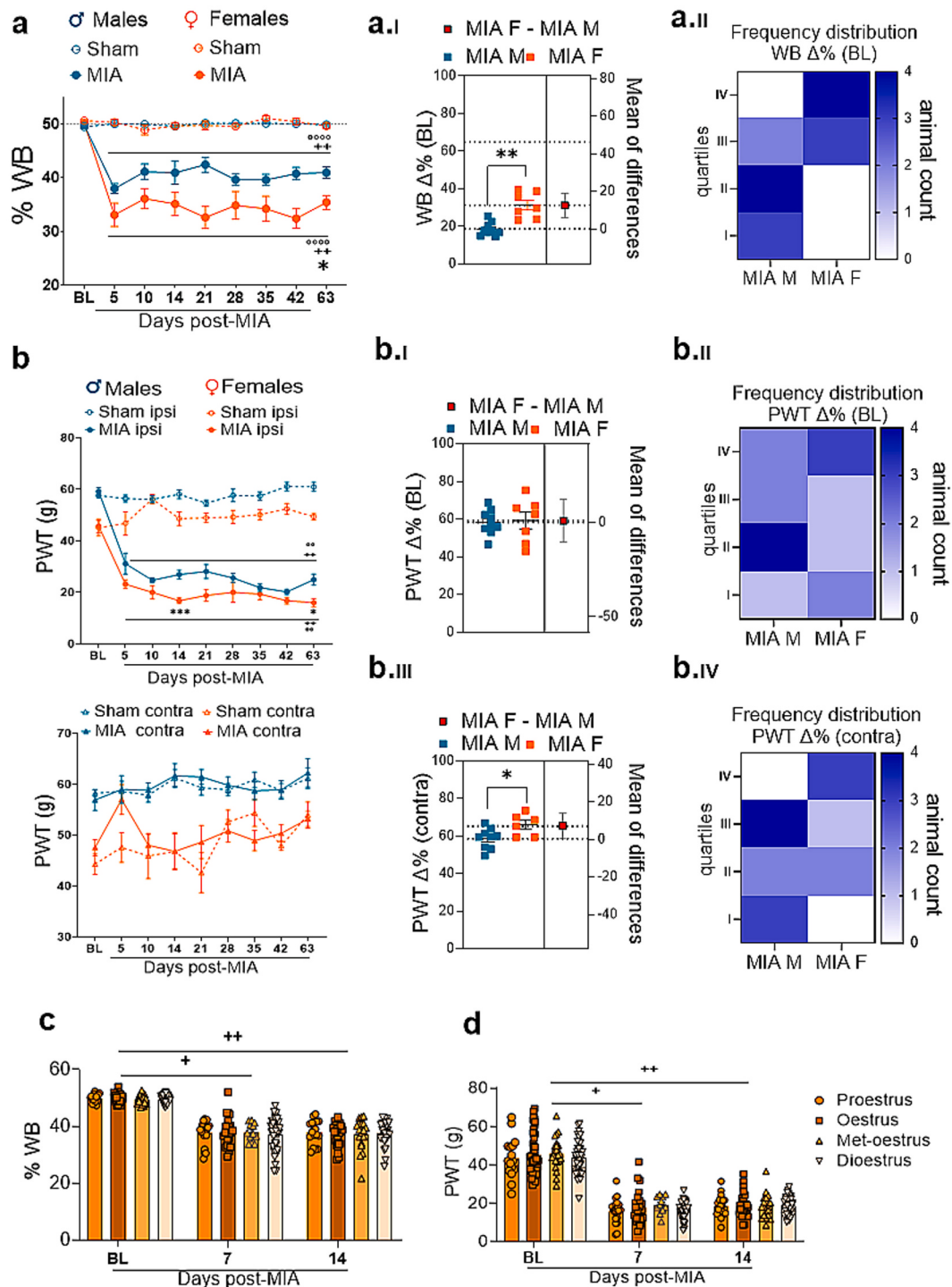


Fig. 1. MIA-induced knee OA pain-related behaviours in male and female rats and across oestrous cycle stages in female rats. (a) Static weight-bearing test (%WB on ipsilateral/left hind limb) in male and female MIA or Sham rats; (a.I) Estimation plot of WBΔ% relative to baseline (BL) in MIA rats; (a.II) Heatmap of WBΔ%(BL) frequency distribution in males vs females MIA rats. (b) Electronic von Frey test (PWT, g) on ipsilateral (upper panel) and contralateral (lower panel) hind paws in male and female MIA or Sham rats; (b.I) Estimation plot of ipsilateral PWTΔ% relative to BL in MIA rats; (b.II) Heatmap of PWTΔ%(BL) frequency in males vs females MIA rats; (b.III) Estimation plot of ipsilateral PWTΔ% relative to contralateral hind paw (contra) in MIA rats; (b.IV) Heatmap of ipsilateral PWTΔ%(contra) frequency in male vs female MIA rats. Data are expressed as mean ± SEM (MIA: $n = 8$ males, $n = 7$ females; Sham: $n = 6$ males, $n = 3$ females). Time-course data were analysed by three-way repeated measures ANOVA with Tukey's (a and b) post hoc test. Mean differences (a.I, b.I, b.III) were analysed by unpaired t -test with Welch's correction. (c and d) Behavioural responses of female MIA rats across the four oestrous cycle stages in static weight-bearing test (ipsilateral %WB) (c) and electronic von Frey test (PWT, g) (d) at baseline, Day 7, and Day 14 post-injection. Data are expressed as mean ± SEM ($n = 100$) and analysed using two-way ANOVA followed by Tukey's post hoc test (c-d). Symbols: $^{\circ}p < 0.01$, $^{\circ\circ\circ}p < 0.0001$ vs respective Sham controls; $^*p < 0.05$, $^{**}p < 0.01$ vs MIA males; $^{++}p < 0.01$ vs BL. Abbreviation: M = males, F = females.

2.36% vs $58.65 \pm 1.83\%$; $t = 2.47$, $df = 10.45$, $p = 0.032$; unpaired t -test with Welch's correction; Fig. 1b.III), whereas PWT% Δ (BL) did not differ between sexes (Fig. 1b.I). Frequency distribution analysis of these indices (Fig. 1a.II, 1b.II, 1b.IV) revealed a significant difference in WB % Δ relative to BL (Fisher's exact test, $p = 0.004$; Fig. 1a.II), and eVF% Δ relative to contralateral side (Fisher's exact test, $p = 0.024$; Fig. 1b.IV), with most males distributed in lower quartiles (I-II) and females in the upper quartiles (III-IV). No sex-related differences were observed in cold hypersensitivity (AD test, Suppl. Fig. 2).

3.2. The oestrous cycle did not influence knee OA-related pain phenotype in female MIA rats

Given the observed sex differences in MIA-induced pain-related behaviours, we investigated whether oestrous cycling could account for variability in female responses. The influence of oestrous stage on ipsilateral %WB and PWT was assessed in a separate cohort of 100 female MIA rats at baseline and on Days 7 and 14 post-MIA (Fig. 1c and d). Two-way ANOVA revealed a significant effect of time on both %WB ($F_{(11, 318)} = 77.49$, $p < 0.0001$) (Fig. 1c) and PWT ($F_{(11, 318)} = 90.18$, $p < 0.0001$) (Fig. 1d), but no significant effect of oestrous stage or time $\times$ oestrous stage interaction was found. Binomial logistic regression confirmed that the oestrous cycle stage did not predict responder status for %WB or PWT at any of the three timepoints (all $p > 0.05$; Suppl. Tables 1 and 2).

3.3. MIA rats exhibit sex-dependent differences in endocannabinoid levels and *Mgl* expression in the amygdala

To explore neurobiological mechanisms underlying sex differences, we next examined ECS signalling in key pain-related brain regions and dorsal lumbar spinal cord.

Analysis of endocannabinoid and *N*-acylethanolamine levels in the left versus right amygdala revealed sex-dependent lateralisation. MIA males consistently showed higher ipsilateral/left amygdala levels than contralateral/right side across all analytes: 2-AG (side: $F_{(1,26)} = 4.68$, $p = 0.04$; 10.66 ± 2.05 nmol/g tissue vs 4.50 ± 0.63 nmol/g tissue, $p = 0.046$; Fig. 2a), AEA (side: $F_{(1,26)} = 9.08$, $p = 0.005$, model: $F_{(1,14)} = 5.71$, $p = 0.03$; 0.088 ± 0.009 nmol/g tissue vs 0.035 ± 0.005 nmol/g tissue, $p = 0.006$; Fig. 2b), PEA (side: $F_{(1,26)} = 7.36$, $p = 0.006$; model: $F_{(1,12)} = 10.48$, $p = 0.007$; 0.31 ± 0.03 nmol/g tissue vs 0.15 ± 0.03 nmol/g tissue, $p = 0.006$; Fig. 2c), and OEA (side: $F_{(1,26)} = 10.42$, $p = 0.003$; model: $F_{(1,12)} = 11.46$, $p = 0.005$; 0.73 ± 0.06 nmol/g tissue vs 0.39 ± 0.043 nmol/g tissue, $p = 0.008$; Fig. 2d). This lateralisation pattern in the analytes was absent in the MIA females, and in Sham controls of both sexes (Fig. 2a-d). Lateralisation indices (LI) were calculated for all analytes (Fig. 2a.I-d.I), as described in Suppl. Methods, and analysed with Wilcoxon one-sample t -test against a theoretical LI = 0 (i.e. no lateralisation). Lateralisation was significant for all analytes in MIA males (2-AG: $t = 4.43$, $df = 7$, $p = 0.0034$; AEA: $t = 5.58$, $df = 6$, $p = 0.0014$; PEA: $t = 8.19$, $df = 7$, $p < 0.0001$; OEA: $t = 7.13$, $df = 7$, $p = 0.0002$). Endocannabinoid and *N*-acylethanolamine levels in other regions did not differ between sexes (Suppl. Fig. 3).

Finally, gene expression analysis in the amygdala revealed that *Faah*, *Cnr1*, and *Cnr2* transcripts showed no lateralisation and no sex differences (Fig. 2e, g and h). In contrast, there was a significant effect of model on *Mgl* expression ($F_{(1,39)} = 11.70$, $p = 0.0015$; Fig. 2f), with lower levels in MIA females than Sham controls in contralateral/right ($70.12 \pm 8.98\%$ vs $245.82 \pm 149.21\%$, $p = 0.036$) amygdala, consistent with a trend toward higher 2-AG levels in contralateral/right amygdala of MIA females compared to Sham females.

4. Discussion

The present study provides evidence for sex differences in pain-related behaviours in the MIA-induced knee OA rat model. Previous

studies reported sex- and age-dependent differences over shorter time frames (up to 21 days post-MIA), showing that aged female rats display greater weight-bearing deficits and hind paw mechanical hypersensitivity (Ro et al., 2020). Our findings extend this observation demonstrating that sex differences in OA-related pain phenotype persist long-term up to 63 days post-MIA. Specifically, female MIA rats showed lower ipsilateral %WB, suggestive of greater spontaneous pain-related behaviour at the knee OA-like lesion site. This finding aligns with clinical observations that women with knee OA report more severe pain than men (Segal et al., 2024). Additionally, female MIA rats displayed greater hind paw mechanical hypersensitivity than males. This observation is consistent with reports that female rodents are more prone to central sensitisation and developing secondary hypersensitivity, not only in MIA-induced knee OA (Ro et al., 2020), but also in other MIA-induced arthropathy models, including a temporomandibular joint disorder model (Sannajust et al., 2019).

Notably, the oestrous cycle stage did not affect MIA-induced pain-related behaviours. Although higher oestrogen levels have been associated with reduced pain sensitivity in chronic pain conditions (Aloisi and Bonifazi, 2006; Hellström and Anderberg, 2003) and protection of joint structures in humans (Boyan et al., 2013; Jin et al., 2017; Roman-Blas et al., 2009; Welhaven et al., 2024) and animal models (Calvo et al., 2007; Høegh-Andersen et al., 2004; Roman-Blas et al., 2009; Sniekers et al., 2008; Yang et al., 2012), findings in OA remain equivocal (Cirillo et al., 2006; Segal et al., 2024). In our study, oestrous stage at the time of MIA injection did not influence the development of the OA-like pain phenotype, suggesting that physiological higher oestrogen levels in oestrus and proestrus phases are not sufficient to exert protective effects. Nonetheless, dose-dependent effects of oestrogen replacement therapy have been described (Sahyoun et al., 1999). Moreover, there is well-documented dissociation between structural joint damage and pain severity (Bedson and Croft, 2008; Dieppe and Lohmander, 2005; Fukui et al., 2010; Jin et al., 2017), suggesting additional neurobiological mechanisms that could modulate pain in a sex-dependent manner (Bartley and Fillingim, 2013; Mogil, 2020; Segal et al., 2024), and reinforcing the necessity of adopting sex-inclusive approaches in both clinical and preclinical research (Finn et al., 2026).

In this context, the present study identified, for the first time, sex-specific alterations in the MIA model for the ECS within the amygdala, a key region involved in both sensory and affective dimensions of pain processing (Finn et al., 2003; Neugebauer, 2015; Thompson and Neugebauer, 2017). The ECS is sexually dimorphic and plays a critical role in central sensitisation and maladaptive plasticity in chronic pain (Finn et al., 2021; Woodhams et al., 2017). While CB₂ receptor involvement in spinal sensitisation has been reported in male MIA rats (Burstion et al., 2013; La Porta et al., 2013), and CB₁ receptors have been implicated in MIA-induced knee OA pain and associated depressive-like behaviours in male rats (Kędziora et al., 2023), sex-specific ECS alterations in supraspinal regions remain under explored. Our neurochemical analyses revealed lateralised amygdala ECS signalling in MIA males, a pattern not seen in MIA females. Previous studies in other rat chronic pain models, including spared nerve injury (Bouillon et al., 2021) and chemotherapy-induced peripheral neuropathy (Di Marino et al., 2024), found no sex-specific differences in amygdala endocannabinoid content under either pathological or control/sham conditions. This study reveals, for the first time, a sex-specific divergence in this pattern in the MIA model.

Amygdala lateralisation in pain processing is well-recognised (Neugebauer, 2015), with the right central amygdala (CeA) associated with maladaptive pronociceptive plasticity (Allen et al., 2021; Ji and Neugebauer, 2009; Neugebauer, 2015), whereas the left CeA may serve protective, non-pronociceptive functions (Allen et al., 2021; Sadler et al., 2017). In models of OA and formalin-induced persistent inflammatory pain, male rodents showed central sensitisation within the right CeA, dependent on protein kinase A (PKA) (Bird et al., 2005; Fu et al., 2008; Han et al., 2005) and extracellular signal-regulated kinase

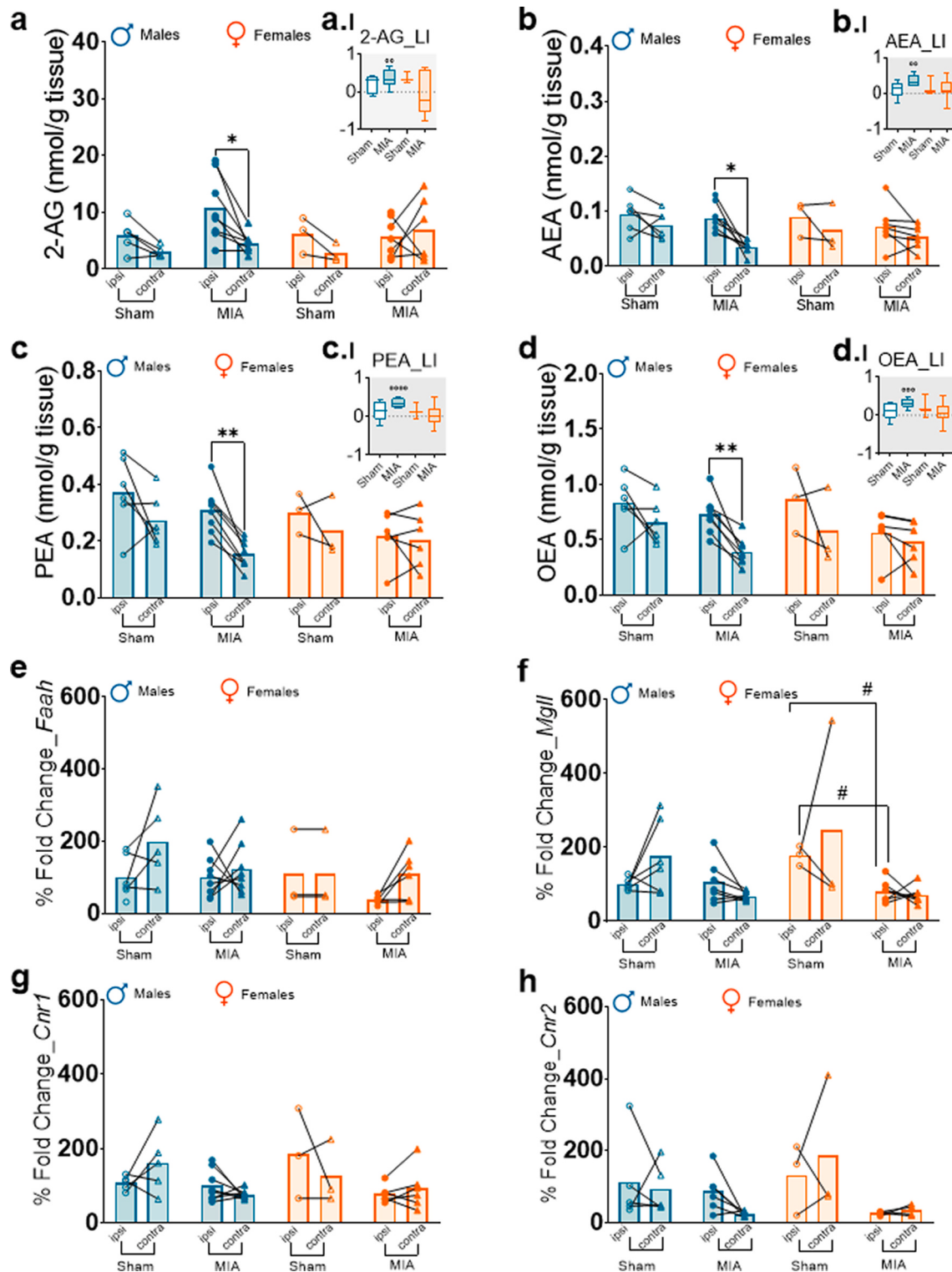


Fig. 2. Levels of endocannabinoids and *N*-acyl ethanolamines and expression of ECS-related genes in the ipsilateral (left) and contralateral (right) amygdala of male and female MIA or Sham rats. (a–d) Endocannabinoid (2-AG, AEA) and *N*-acyl ethanolamine (PEA, OEA) levels in the left and right amygdala of male and female MIA or Sham rats; (a.I–d.I) Lateralisation index (LI) of respective analytes. (e–h) Expression of *Faah*, *Mgl*, *Cnr1*, and *Cnr2* transcripts in the left and right amygdala of male and female MIA or Sham rats as a percentage of levels in Sham male ipsi/left samples. Data are expressed as mean ± SEM (n = 3–9 per group) and analysed using three-way ANOVA (or corresponding mixed effects model due to outlier removal) with Tukey's post hoc test (a–h) or Wilcoxon one-sample t-test (a.I–d.I). Symbols: *p < 0.05, **p < 0.01 vs contra/right amygdala; °°p < 0.01, °°°p < 0.001, °°°°p < 0.0001 vs LI = 0; #p < 0.05 vs Sham control. Abbreviation: M = males, F = females.

(ERK) activation (Carrasquillo and Gereau 4th, 2007, 2008), which was not observed in the left amygdala (Ji and Neugebauer, 2009). Interestingly, PKA and ERK are modulated by CB₁ and CB₂ downstream signalling (Han et al., 2022; Howlett et al., 2002; Leo and Abood, 2021), influencing neural transmission patterns. Our finding of elevated 2-AG and AEA in the left/ipsilateral amygdala of MIA males, but not females, suggests a possible sex-dependent role for ECS signalling in hemispheric lateralisation related to OA pain. The concomitant increase in PEA and OEA levels in the left/ipsilateral amygdala of MIA males further suggest potential sex-specific ECS-mediated anti-inflammatory mechanisms. Both PEA and OEA exert anti-inflammatory effects via PPAR- α activation (Guida et al., 2017; Li et al., 2023; Luo et al., 2019; Pérez-Martín et al., 2023; Re et al., 2007) and can compete for endocannabinoid-degrading enzymes, thereby sustaining elevated endocannabinoid levels (Iannotti et al., 2016; Iannotti and Vitale, 2021), consistent with our findings. The present study also revealed a bilateral reduction in *Mgll* expression in the amygdala of MIA females compared to Sham controls. This sex-specific alteration in amygdala *Mgll* transcript levels appears to be distinct from that reported in other chronic pain models, such as the hind limb ischemia-reperfusion injury model (Healy et al., 2026), suggesting that OA pain may engage the ECS in a manner that differs from other persistent pain states. Collectively, these findings further highlight sex-dependent adaptations of the amygdala ECS in chronic OA pain.

Altogether, these findings demonstrate that female rats, independent of oestrous cycle stage, exhibit greater nociceptive behaviours than males in the MIA model of knee OA pain. We identify, for the first time, sex-specific patterns in the amygdala ECS signalling in this model, with MIA males showing left-sided lateralisation of ECS tone that is absent in female counterparts. Although the functional significance of this lateralisation remains to be established, these findings suggest that distinct neurobiological mechanisms may underlie sex differences in OA pain. This initial evidence thus calls for further investigation, potentially opening new paths toward mechanism-based and sex-informed personalised therapeutic approaches for OA pain.

CRediT authorship contribution statement

Mehnaz I. Ferdousi: Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rosmara Infantino:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Maria C. Redmond:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Santhosh D. Krishnan:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Canny K. Adjei:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Barry McDermott:** Writing – review & editing, Methodology, Conceptualization. **Alison Liddy:** Writing – review & editing, Supervision, Funding acquisition. **Leo Quinlan:** Writing – review & editing, Supervision, Funding acquisition. **Martin O'Halloran:** Writing – review & editing, Supervision, Funding acquisition. **David P. Finn:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Martin O'Halloran, Alison Liddy, Leo Quinlan, David Finn reports financial support was provided by European Innovation Council (EIC). Martin O'Halloran reports financial support was provided by European Research Council (ERC). Alison Liddy and Mehnaz Ferdousi reports a relationship with Relevium Medical that includes: employment. Martin O'Halloran, Alison Liddy, David Finn reports a relationship with Relevium Medical that includes: equity or stocks. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work

reported in this paper.

Acknowledgements

Our sincere thanks to Prof Katarzyna Starowicz and Dr. Marta Bryk of the Institute of Pharmacology, Polish Academy of Sciences, and Prof Victoria Chapman and Dr. Sara Goncalves of the University of Nottingham, for their valuable assistance with training in the MIA model and MIA-related behavioural testing procedures.

Funding sources

This work was supported by the Horizon Europe European Innovation Council (EIC) Transition grant ARTRIGEL project (project# 101058000) and Horizon Europe European Research Council (ERC) Arth-Alleve: Disruptive therapies for the long-term relief of osteoarthritis pain (grant ID: 101045226).

Appendix. Supplementary Methods and Results

Supplementary Methods and Results to this article can be found online at <https://doi.org/10.1016/j.jnypai.2026.100227>.

Data availability

Data will be made available on request.

References

- Allen, H.N., Bobnar, H.J., Kolber, B.J., 2021. Left and right hemispheric lateralization of the amygdala in pain. *Prog. Neurobiol.* 196, 101891. <https://doi.org/10.1016/j.pneurobio.2020.101891>.
- Aloisi, A.M., Bonifazi, M., 2006. Sex hormones, central nervous system and pain. *Horm. Behav.* 50, 1–7. <https://doi.org/10.1016/j.yhbeh.2005.12.002>.
- Bartley, E.J., Fillingim, R.B., 2013. Sex differences in pain: a brief review of clinical and experimental findings. *Br. J. Anaesth.* 111, 52–58. <https://doi.org/10.1093/bja/aet127>.
- Bartley, E.J., King, C.D., Sibille, K.T., Cruz-Almeida, Y., Riley 3rd, J.L., Glover, T.L., Goodin, B.R., Sotolongo, A.S., Herbert, M.S., Bulls, H.W., Staud, R., Fessler, B.J., Redden, D.T., Bradley, L.A., Fillingim, R.B., 2016. Enhanced pain sensitivity among individuals with symptomatic knee osteoarthritis: potential sex differences in central sensitization. *Arthritis Care Res.* 68, 472–480. <https://doi.org/10.1002/acr.22712>.
- Bedson, J., Croft, P.R., 2008. The discordance between clinical and radiographic knee osteoarthritis: a systematic search and summary of the literature. *BMC Musculoskelet. Disord.* 9, 116. <https://doi.org/10.1186/1471-2474-9-116>.
- Bird, G.C., Lash, L.L., Han, J.S., Zou, X., Willis, W.D., Neugebauer, V., 2005. Protein kinase A-dependent enhanced NMDA receptor function in pain-related synaptic plasticity in rat amygdala neurons. *J. Physiol.* 564, 907–921. <https://doi.org/10.1113/jphysiol.2005.084780>.
- Blanton, H.L., Barnes, R.C., McHann, M.C., Bilbrey, J.A., Wilkerson, J.L., Guindon, J., 2021. Sex differences and the endocannabinoid system in pain. *Pharmacol. Biochem. Behav.* 202, 173107. <https://doi.org/10.1016/j.pbb.2021.173107>.
- Bouillon, L., Finn, D.P., Llorente-Berzal, A., 2021. Sex differences in a rat model of peripheral neuropathic pain and associated levels of endogenous cannabinoid ligands. *Front. Pain Res. (Lausanne, Switzerland)* 2, 673638. <https://doi.org/10.3389/fpain.2021.673638>.
- Bouillon, L., Llorente-Berzal, A., Finn, D.P., 2023. Investigation of sexual dimorphism in an animal model of neuropathic pain: role of the endocannabinoid system.
- Bove, S.E., Calcaterra, S.L., Brooker, R.M., Huber, C.M., Guzman, R.E., Juneau, P.L., Schrier, D.J., Kilgore, K.S., 2003. Weight bearing as a measure of disease progression and efficacy of anti-inflammatory compounds in a model of monosodium iodoacetate-induced osteoarthritis. *Osteoarthr. Cartil.* 11, 821–830. [https://doi.org/10.1016/s1063-4584\(03\)00163-8](https://doi.org/10.1016/s1063-4584(03)00163-8).
- Boyan, B.D., Hart, D.A., Enoka, R.M., Nicoletta, D.P., Resnick, E., Berkley, K.J., Sluka, K.A., Kwok, C.K., Tosi, L.L., O'Connor, M.I., Coutts, R.D., Kohrt, W.M., 2013. Hormonal modulation of connective tissue homeostasis and sex differences in risk for osteoarthritis of the knee. *Biol. Sex Differ.* 4, 3. <https://doi.org/10.1186/2042-6410-4-3>.
- Burston, J.J., Sagar, D.R., Shao, P., Bai, M., King, E., Brailsford, L., Turner, J.M., Hathway, G.J., Bennett, A.J., Walsh, D.A., Kendall, D.A., Lichtman, A., Chapman, V., 2013. Cannabinoid CB2 receptors regulate central sensitization and pain responses associated with osteoarthritis of the knee joint. *PLoS One* 8, e80440. <https://doi.org/10.1371/journal.pone.0080440>.
- Calvo, E., Castañeda, S., Largo, R., Fernández-Valle, M.E., Rodríguez-Salvanes, F., Herrero-Beaumont, G., 2007. Osteoporosis increases the severity of cartilage damage in an experimental model of osteoarthritis in rabbits. *Osteoarthr. Cartil.* 15, 69–77. <https://doi.org/10.1016/j.joca.2006.06.006>.

- Carrasquillo, Y., Gereau 4th, R.W., 2007. Activation of the extracellular signal-regulated kinase in the amygdala modulates pain perception. *J. Neurosci.* 27, 1543–1551. <https://doi.org/10.1523/JNEUROSCI.3536-06.2007>.
- Carrasquillo, Y., Gereau 4th, R.W., 2008. Hemispheric lateralization of a molecular signal for pain modulation in the amygdala. *Mol. Pain* 4, 24. <https://doi.org/10.1186/1744-8069-4-24>.
- Chen, Y., Moutal, A., Navratilova, E., Kopruszinski, C., Yue, X., Ikegami, M., Chow, M., Kanazawa, I., Bellampalli, S.S., Xie, J., Patwardhan, A., Rice, K., Fields, H., Akopian, A., Neugebauer, V., Dodick, D., Khanna, R., Porreca, F., 2020. The prolactin receptor long isoform regulates nociceptor sensitization and opioid-induced hyperalgesia selectively in females. *Sci. Transl. Med.* 12. <https://doi.org/10.1126/scitranslmed.aay7550>.
- Cirillo, D.J., Wallace, R.B., Wu, L., Yood, R.A., 2006. Effect of hormone therapy on risk of hip and knee joint replacement in the Women's Health Initiative. *Arthritis Rheum.* 54, 3194–3204. <https://doi.org/10.1002/art.22138>.
- Corcoran, L., Mattimoe, D., Roche, M., Finn, D.P., 2020. Attenuation of fear-conditioned analgesia in rats by monoacylglycerol lipase inhibition in the anterior cingulate cortex: potential role for CB(2) receptors. *Br. J. Pharmacol.* 177, 2240–2255. <https://doi.org/10.1111/bph.14976>.
- Craft, R.M., Marusch, J.A., Wiley, J.L., 2013. Sex differences in cannabinoid pharmacology: a reflection of differences in the endocannabinoid system? *Life Sci.* 92, 476–481. <https://doi.org/10.1016/j.lfs.2012.06.009>.
- Di Marino, C., Llorente-Berzal, A., Diego, A.M., Bella, A., Bouillon, L., Berrococo, E., Roche, M., Finn, D.P., 2024. Characterisation of the effects of the chemotherapeutic agent paclitaxel on neuropathic pain-related behaviour, anxiodepressive behaviour, cognition, and the endocannabinoid system in male and female rats. *Front. Pharmacol.* 15, 1505980. <https://doi.org/10.3389/fphar.2024.1505980>.
- Di, J., Bai, Jiang, Zhang, J., Chen, J., Hao, Y., Bai, Jiaqi, Xiang, C., 2024. Regional disparities, age-related changes and sex-related differences in knee osteoarthritis. *BMC Musculoskelet. Disord.* 25, 66. <https://doi.org/10.1186/s12891-024-07191-w>.
- Dieppe, P.A., Lohmander, L.S., 2005. Pathogenesis and management of pain in osteoarthritis. *Lancet (London, England)* 365, 965–973. [https://doi.org/10.1016/S0140-6736\(05\)71086-2](https://doi.org/10.1016/S0140-6736(05)71086-2).
- Diogenes, A., Patwardhan, A.M., Jeske, N.A., Ruparel, N.B., Goffin, V., Akopian, A.N., Hargreaves, K.M., 2006. Prolactin modulates TRPV1 in female rat trigeminal sensory neurons. *J. Neurosci.* 26, 8126–8136. <https://doi.org/10.1523/JNEUROSCI.0793-06.2006>.
- Fillingim, R.B., King, C.D., Ribeiro-Dasilva, M.C., Rahim-Williams, B., Riley 3rd, J.L., 2009. Sex, gender, and pain: a review of recent clinical and experimental findings. *J. Pain* 10, 447–485. <https://doi.org/10.1016/j.jpain.2008.12.001>.
- Finn, D.P., Chapman, V., Jhaveri, M.D., Samanta, S., Manders, T., Bowden, J., Matthews, L., Marsden, C.A., Beckett, S.R., 2003. The role of the central nucleus of the amygdala in nociception and aversion. *Neuroreport* 14, 981–984. <https://doi.org/10.1097/01.wnr.0000069062.85441.29>.
- Finn, D.P., Haroutounian, S., Hohmann, A.G., Krane, E., Soliman, N., Rice, A.S.C., 2021. Cannabinoids, the endocannabinoid system, and pain: a review of preclinical studies. *Pain* 162, S5–S25. <https://doi.org/10.1097/j.pain.0000000000002268>.
- Finn, D.P., McGuire, B.E., Beggs, S., Boerner, K.E., Davis, K.D., Defrin, R., De Koninck, Y., Devan, H., Donovan, R., Fetter, E., Flor, H., Fullen, B.M., Healy, C.R., Keogh, E., Kuner, R., Kunz, M., Lane, R.M., Lautenbacher, S., Mayer, E.A., Mogil, J.S., O'Mahony, S.M., O'Sullivan, K., Riodan, L., Salter, M.W., Scarlatti, F., Shorten, G., Sluka, K.A., Stinson, J.N., Vowles, K.E., Walker, S.M., Yalcin, I., Roche, M., 2026. Recommendations for the inclusion and study of sex and gender in research. *Nat. Neurosci.* 29, 256–266. <https://doi.org/10.1038/s41593-025-02164-1>.
- Fu, Y., Han, J., Ishola, T., Scerbo, M., Adwanikar, H., Ramsey, C., Neugebauer, V., 2008. PKA and ERK, but not PKC, in the amygdala contribute to pain-related synaptic plasticity and behavior. *Mol. Pain* 4, 26. <https://doi.org/10.1186/1744-8069-4-26>.
- Fukui, N., Yamane, S., Ishida, S., Tanaka, K., Masuda, R., Tanaka, N., Katsuragawa, Y., Fukui, S., 2010. Relationship between radiographic changes and symptoms or physical examination findings in subjects with symptomatic medial knee osteoarthritis: a three-year prospective study. *BMC Musculoskelet. Disord.* 11, 269. <https://doi.org/10.1186/1471-2474-11-269>.
- Guida, F., Luongo, L., Bocella, S., Giordano, M.E., Romano, R., Bellini, G., Manzo, I., Furiano, A., Rizzo, A., Imperatore, R., Iannotti, F.A., D'Aniello, E., Piscitelli, F., Sca Rossi, F., Cristino, L., Di Marzo, V., de Novellis, V., Maione, S., 2017. Palmitoylethanolamide induces microglia changes associated with increased migration and phagocytic activity: involvement of the CB2 receptor. *Sci. Rep.* 7, 375. <https://doi.org/10.1038/s41598-017-00342-1>.
- Han, J.S., Li, W., Neugebauer, V., 2005. Critical role of calcitonin gene-related peptide 1 receptors in the amygdala in synaptic plasticity and pain behavior. *J. Neurosci.* 25, 10717–10728. <https://doi.org/10.1523/JNEUROSCI.4112-05.2005>.
- Han, Q.-W., Shao, Q.-H., Wang, X.-T., Ma, K.-L., Chen, N.-H., Yuan, Y.-H., 2022. CB2 receptor activation inhibits the phagocytic function of microglia through activating ERK/AKT-Nurr1 signal pathways. *Acta Pharmacol. Sin.* 43, 2253–2266. <https://doi.org/10.1038/s41401-021-00853-8>.
- Healy, C.R., Redmond, M.C., Gethin, G., Pandit, A., Finn, D.P., 2026. Sex differences in pain-related behaviour and the endocannabinoid system following hind limb ischemia-reperfusion injury. *J. Pain* 40, 106197. <https://doi.org/10.1016/j.jpain.2026.106197>.
- Hellström, B., Anderberg, U.M., 2003. Pain perception across the menstrual cycle phases in women with chronic pain. *Percept. Mot. Skills* 96, 201–211. <https://doi.org/10.2466/pms.2003.96.1.201>.
- Henderson-Redmond, A.N., Crawford, L.C., Sepulveda, D.E., Hale, D.E., Lesperance, J.J., Morgan, D.J., 2021. Sex differences in tolerance to Delta-9-tetrahydrocannabinol in mice with cisplatin-evoked chronic neuropathic pain. *Front. Mol. Biosci.* 8, 684115. <https://doi.org/10.3389/fmolb.2021.684115>.
- Høegh-Andersen, P., Tankó, L.B., Andersen, T.L., Lundberg, C.V., Mo, J.A., Heegaard, A.-M., Delaissé, J.-M., Christgau, S., 2004. Ovariectomized rats as a model of postmenopausal osteoarthritis: validation and application. *Arthritis Res. Ther.* 6, R169–R180. <https://doi.org/10.1186/ar1152>.
- Howlett, A.C., Barth, F., Bonner, T.I., Cabral, G., Casellas, P., Devane, W.A., Felder, C.C., Herkenham, M., Mackie, K., Martin, B.R., Mechoulam, R., Pertwee, R.G., 2002. International union of pharmacology. XXVII. classification of cannabinoid receptors. *Pharmacol. Rev.* 54, 161–202. <https://doi.org/10.1124/pr.54.2.161>.
- Iannotti, F.A., Vitale, R.M., 2021. The endocannabinoid system and PPARs: focus on their signalling crosstalk, action and transcriptional regulation. *Cells* 10. <https://doi.org/10.3390/cells10030586>.
- Iannotti, F.A., Di Marzo, V., Petrosino, S., 2016. Endocannabinoids and endocannabinoid-related mediators: targets, metabolism and role in neurological disorders. *Prog. Lipid Res.* 62, 107–128. <https://doi.org/10.1016/j.plipres.2016.02.002>.
- Ji, G., Neugebauer, V., 2009. Hemispheric lateralization of pain processing by amygdala neurons. *J. Neurophysiol.* 102, 2253–2264. <https://doi.org/10.1152/jn.00166.2009>.
- Jin, X., Wang, B.H., Wang, X., Antony, B., Zhu, Z., Han, W., Cicuttini, F., Wluka, A.E., Winzenberg, T., Blizzard, L., Jones, G., Ding, C., 2017. Associations between endogenous sex hormones and MRI structural changes in patients with symptomatic knee osteoarthritis. *Osteoarthritis Cartil.* 25, 1100–1106. <https://doi.org/10.1016/j.joca.2017.01.015>.
- Kędziora, M., Bocella, S., Marabese, I., Mlost, J., Infantino, R., Maione, S., Starowicz, K., 2023. Inhibition of anandamide breakdown reduces pain and restores LTP and monoamine levels in the rat hippocampus via the CB(1) receptor following osteoarthritis. *Neuropharmacology* 222, 109304. <https://doi.org/10.1016/j.neuropharm.2022.109304>.
- Kerr, D.M., Burke, N.N., Ford, G.K., Connor, T.J., Harhen, B., Egan, L.J., Finn, D.P., Roche, M., 2012. Pharmacological inhibition of endocannabinoid degradation modulates the expression of inflammatory mediators in the hypothalamus following an immunological stressor. *Neuroscience* 204, 53–63. <https://doi.org/10.1016/j.neuroscience.2011.09.032>.
- Kraus, V.B., Blanco, F.J., Englund, M., Karsdal, M.A., Lohmander, L.S., 2015. Call for standardized definitions of osteoarthritis and risk stratification for clinical trials and clinical use. *Osteoarthritis Cartil.* 23, 1233–1241. <https://doi.org/10.1016/j.joca.2015.03.036>.
- La Porta, C., Bura, S.A., Aracil-Fernández, A., Manzanares, J., Maldonado, R., 2013. Role of CB1 and CB2 cannabinoid receptors in the development of joint pain induced by monosodium iodoacetate. *Pain* 154, 160–174. <https://doi.org/10.1016/j.pain.2012.10.009>.
- Leo, L.M., Abood, M.E., 2021. CB1 cannabinoid receptor Signaling and biased Signaling. *Molecules* 26. <https://doi.org/10.3390/molecules26175413>.
- Li, Y., Zhang, Y., Wang, Q., Wu, C., Du, G., Yang, L., 2023. Oleylethanolamide protects against acute ischemic stroke by promoting PPARα-mediated microglia/macrophage M2 polarization. *Pharmaceuticals (Basel)* 16. <https://doi.org/10.3390/ph16040621>.
- Llorente-Berzal, A., McGowan, F., Gaspar, J.C., Rea, K., Roche, M., Finn, D.P., 2022. Sexually dimorphic expression of fear-conditioned analgesia in rats and associated alterations in the endocannabinoid system in the periaqueductal Grey. *Neuroscience* 480, 117–130. <https://doi.org/10.1016/j.neuroscience.2021.11.005>.
- Luo, D., Zhang, Y., Yuan, X., Pan, Y., Yang, L., Zhao, Y., Zhuo, R., Chen, C., Peng, L., Li, W., Jin, X., Zhou, Y., 2019. Oleylethanolamide inhibits glial activation via modulating PPARα and promotes motor function recovery after brain ischemia. *Pharmacol. Res.* 141, 530–540. <https://doi.org/10.1016/j.phrs.2019.01.027>.
- Luo, X., Chen, O., Wang, Z., Bang, S., Ji, J., Lee, S.H., Huh, Y., Furutani, K., He, Q., Tao, X., Ko, M.-C., Bortsov, A., Donnelly, C.R., Chen, Y., Nackley, A., Berta, T., Ji, R.-R., 2021. IL-23/IL-17A/TRPV1 axis produces mechanical pain via macrophage-sensory neuron crosstalk in female mice. *Neuron* 109, 2691–2706.e5. <https://doi.org/10.1016/j.neuron.2021.06.015>.
- McLean, A.C., Valenzuela, N., Fai, S., Bennett, S.A.L., 2012. Performing vaginal lavage, crystal violet staining, and vaginal cytological evaluation for mouse estrous cycle staging identification. *JoVE*, e4389. <https://doi.org/10.3791/4389>.
- Mogil, J.S., 2020. Qualitative sex differences in pain processing: emerging evidence of a biased literature. *Nat. Rev. Neurosci.* 21, 353–365. <https://doi.org/10.1038/s41583-020-0310-6>.
- Motulsky, H.J., Brown, R.E., 2006. Detecting outliers when fitting data with nonlinear regression - a new method based on robust nonlinear regression and the false discovery rate. *BMC Bioinformatics* 7, 123. <https://doi.org/10.1186/1471-2105-7-123>.
- Mun, C.J., Letzen, J.E., Nance, S., Smith, M.T., Khanuja, H.S., Sterling, R.S., Bicket, M.C., Haythornthwaite, J.A., Jamison, R.N., Edwards, R.R., Campbell, C.M., 2020. Sex differences in Interleukin-6 responses over time following laboratory pain testing among patients with knee osteoarthritis. *J. Pain* 21, 731–741. <https://doi.org/10.1016/j.jpain.2019.11.003>.
- Neugebauer, V., 2015. Amygdala pain mechanisms. *Handb. Exp. Pharmacol.* 227, 261–284. https://doi.org/10.1007/978-3-662-46450-2_13.
- Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M.T., Baker, M., Browne, W. J., Clark, A., Cuthill, I.C., Dirnagl, I.C., Emerson, M., Garner, P., Holgate, S.T., Howells, D.W., Karp, N.A., Lazic, S.E., Lidster, K., MacCallum, C.J., Macleod, M., Pearl, E.J., Petersen, O.H., Rawle, F., Reynolds, P., Rooney, K., Sena, E.S., Silberberg, S.D., Steckler, T., Würbel, H., 2020. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *Br. J. Pharmacol.* 177, 3617–3624. <https://doi.org/10.1111/bph.15193>.
- Pérez-Martín, E., Pérez-Revuelta, L., Barahona-López, C., Pérez-Boyoero, D., Alonso, J.R., Díaz, D., Weruaga, E., 2023. Oleylethanolamide treatment modulates both neuroinflammation and microgliosis, and prevents massive leukocyte infiltration to

- the cerebellum in a mouse model of neuronal degeneration. *Int. J. Mol. Sci.* 24. <https://doi.org/10.3390/ijms24119691>.
- Perruccio, A.V., Badley, E.M., Power, J.D., Canizares, M., Kapoor, M., Rockel, J., Chandran, V., Gandhi, R., Mahomed, N.M., Davey, J.R., Syed, K., Veillette, C., Rampersaud, Y.R., 2019. Sex differences in the relationship between individual systemic markers of inflammation and pain in knee osteoarthritis. *Osteoarthritis Cartilage* 29, 100004. <https://doi.org/10.1016/j.jocarto.2019.100004>.
- Pomonis, J.D., Boulet, J.M., Gottshall, S.L., Phillips, S., Sellers, R., Bunton, T., Walker, K., 2005. Development and pharmacological characterization of a rat model of osteoarthritis pain. *Pain* 114, 339–346. <https://doi.org/10.1016/j.pain.2004.11.008>.
- Re, G., Barbero, R., Miolo, A., Di Marzo, V., 2007. Palmitoylethanolamide, endocannabinoids and related cannabimimetic compounds in protection against tissue inflammation and pain: potential use in companion animals. *Vet. J.* 173, 21–30. <https://doi.org/10.1016/j.tvjl.2005.10.003>.
- Ro, J.Y., Zhang, Y., Tricou, C., Yang, D., da Silva, J.T., Zhang, R., 2020. Age and sex differences in acute and osteoarthritis-like pain responses in rats. *J. Gerontol. A Biol. Sci. Med. Sci.* 75, 1465–1472. <https://doi.org/10.1093/gerona/glz186>.
- Roman-Blas, J.A., Castañeda, S., Largo, R., Herrero-Beaumont, G., 2009. Osteoarthritis associated with estrogen deficiency. *Arthritis Res. Ther.* 11, 241. <https://doi.org/10.1186/ar2791>.
- Sadler, K.E., McQuaid, N.A., Cox, A.C., Behun, M.N., Trouten, A.M., Kolber, B.J., 2017. Divergent functions of the left and right central amygdala in visceral nociception. *Pain* 158, 747–759. <https://doi.org/10.1097/j.pain.0000000000000830>.
- Sahyoun, N.R., Brett, K.M., Hochberg, M.C., Pamuk, E.R., 1999. Estrogen replacement therapy and incidence of self-reported physician-diagnosed arthritis. *Prev. Med. (Baltim)*. 28, 458–464. <https://doi.org/10.1006/pmed.1998.0440>.
- Sannajust, S., Imbert, I., Eaton, V., Henderson, T., Liaw, L., May, M., Barbe, M.F., King, T., 2019. Females have greater susceptibility to develop ongoing pain and central sensitization in a rat model of temporomandibular joint pain. *Pain* 160, 2036–2049. <https://doi.org/10.1097/j.pain.0000000000001598>.
- Segal, N.A., Nilges, J.M., Oo, W.M., 2024. Sex differences in osteoarthritis prevalence, pain perception, physical function and therapeutics. *Osteoarthritis Cartilage* 32, 1045–1053. <https://doi.org/10.1016/j.joca.2024.04.002>.
- Sniers, Y.H., Weinans, H., Bierma-Zeinstra, S.M., van Leeuwen, J.P.T.M., van Osch, G. J.V.M., 2008. Animal models for osteoarthritis: the effect of ovariectomy and estrogen treatment - a systematic approach. *Osteoarthritis Cartilage* 16, 533–541. <https://doi.org/10.1016/j.joca.2008.01.002>.
- Starowicz, K., Finn, D.P., Academy, P., Pathophysiology, P., Starowicz, K., Finn, D.P., 2017. Cannabinoids and pain: sites and mechanisms of action. *Adv. Pharmacol.* 80, 437–475. <https://doi.org/10.1016/bs.apha.2017.05.003>.
- Tang, S., Zhang, C., Oo, W.M., Fu, K., Risberg, M.A., Bierma-Zeinstra, S.M., Neogi, T., Atukorala, I., Malfait, A.-M., Ding, C., Hunter, D.J., 2025. Osteoarthritis. *Nat. Rev. Dis. Primers* 11, 10. <https://doi.org/10.1038/s41572-025-00594-6>.
- Tavares-Ferreira, D., Ray, P.R., Sankaranarayanan, I., Mejia, G.L., Wangzhou, A., Shiers, S., Uttarkar, R., Megat, S., Barragan-Iglesias, P., Dussor, G., Akopian, A.N., Price, T.J., 2022. Sex differences in nociceptor Translatomes contribute to divergent prostaglandin Signaling in male and female mice. *Biol. Psychiatry* 91, 129–140. <https://doi.org/10.1016/j.biopsych.2020.09.022>.
- Thompson, J.M., Neugebauer, V., 2017. Amygdala plasticity and pain. *Pain Res Manag* 2017, 8296501. <https://doi.org/10.1155/2017/8296501>.
- Valdighi, N., Vago, J.P., Blom, A.B., van de Loo, F.A.J., Blaney Davidson, E.N., 2022. Innate immunity at the Core of sex differences in osteoarthritic pain? *Front. Pharmacol.* 13, 881500. <https://doi.org/10.3389/fphar.2022.881500>.
- Welhaven, H.D., Welfley, A.H., Pershad, P., Satalich, J., O'Connell, R., Bothner, B., Vap, A.R., June, R.K., 2024. Metabolic phenotypes reflect patient sex and injury status: a cross-sectional analysis of human synovial fluid. *Osteoarthritis Cartilage* 32, 1074–1083. <https://doi.org/10.1016/j.joca.2023.09.004>.
- Woodhams, S.G., Chapman, V., Finn, D.P., Hohmann, A.G., Neugebauer, V., 2017. The cannabinoid system and pain. *Neuropharmacology* 124, 105–120. <https://doi.org/10.1016/j.neuropharm.2017.06.015>.
- Yang, J.-H., Kim, J.-H., Lim, D.-S., Oh, K.-J., 2012. Effect of combined sex hormone replacement on bone/cartilage turnover in a murine model of osteoarthritis. *Clin. Orthop. Surg.* 4, 234–241. <https://doi.org/10.4055/cios.2012.4.3.234>.
- Yoon, C., Wook, Y.Y., Sik, N.H., Ho, K.S., Mo, C.J., 1994. Behavioral signs of ongoing pain and cold allodynia in a rat model of neuropathic pain. *Pain* 59, 369–376. [https://doi.org/10.1016/0304-3959\(94\)90023-X](https://doi.org/10.1016/0304-3959(94)90023-X).