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Research Report

Self-regulated analgesia in males but not females is mediated by endogenous opioids

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Abstract

Converging lines of preclinical and clinical research indicate that females, in stark contrast to males, display an increased prevalence of chronic pain. Females also demonstrate weaker analgesic efficacy in response to opioid therapies when compared with males. These sex-specific differences may be driven by dimorphic endogenous opioidergic responses. In rodent models, analgesia exhibited in males but not females was reversed by inhibiting endogenous opioidergic reception. In humans, the sex-specific endogenous system(s) supporting the direct attenuation of evoked pain has not been identified. To determine whether opioidergic blockade reverses self-regulated analgesia in males as compared to females, the present study combined two operationally analogous clinical trials ($n = 98$; 51 females and 47 males). In a double-blinded, counterbalanced study involving healthy ($n = 39$) and chronic low back pain ($n = 59$) populations, a high-dose naloxone (μ-, κ-, δ-opioid antagonist) vs. placebo-saline cross-over design (15 mg/kg bolus +0.1 mg/kg/h) tested the hypothesis that endogenous opioids mediate analgesia in males but not females. An 11-point visual analog scale (VAS) (0 = no pain; 10 = worst pain imaginable) evaluated pain ratings in response to noxious heat stimulation (49 °C; calf). After baseline pain testing, participants were randomized to a validated four-session mindfulness meditation or sham mindfulness meditation training intervention. Participants practiced their respective meditation during noxious heat, intravenous high-dose naloxone, and placebo saline, respectively. In males and females, meditation significantly lowered evoked pain during saline infusion. Intravenous naloxone inhibited analgesia in males, but pain relief was well preserved in females. The present findings indicate that endogenous opioids mediate self-regulated analgesia in males but not females and underscore the need to establish sex-specific pain therapeutics.

Significance Statement

It is well established that females exhibit dramatically higher chronic pain prevalence and opioid prescriptions than males. Opioids are also significantly less effective in females, which has led to greater opioid misuse and addiction. The endogenous opioid system is vital in facilitating analgesia and psychological resilience. Yet, whether endogenous opioids are differentially engaged during male as compared to female-based analgesia is unknown. To address this, two double-blinded, drug cross-over clinical trials in healthy and chronic pain populations were combined. Intravenous opioidergic blockade (i.e. naloxone), as compared to saline, was administered during noxious heat and meditation. Opioidergic antagonism inhibited self-regulated analgesia in males but not females. These findings suggest that pain therapies could be tailored by sex to target mechanisms supporting pain relief.

Introduction

Chronic pain disproportionately impacts females (1, 2). Compared with males, females are under-evaluated for chronic pain and are prescribed opioids at a higher rate (3). Among individuals with chronic back pain, females exhibit greater pain severity, morbidity, and weaker treatment efficacy than males (2). However, the mechanisms that can explain such discrepancies in treatment efficacy remain poorly characterized (4). There are several endogenous systems (5) that facilitate analgesia, including but not limited to the endocannabinoid (6), serotonergic, and dopaminergic systems. However, the endogenous opioidergic system is characterized as the primary driver of analgesia (7). That is, pain relief produced by placebo (8–12), distraction (13), and hypnosis (14, 15) is reversed by administration of the opioid antagonist, naloxone. However, naloxone-insensitive stress-induced (16), reappraisal-induced (17) and placebo-induced (6) analgesia have

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also been demonstrated. There are four known opioid receptor types including the mu (MOR), delta (DOR), kappa (KOR), and nociceptin (NOR) that exhibit divergent physiological and pain-relieving effects (5). MORs mediate analgesia and are associated with hedonia and reward-related processing (18). DORs are associated with increasing positive mood and affect (19). KORs, dense in brain regions such as the amygdala, locus coeruleus, and dorsal raphe, contribute to dysphoria and psychomimetic responses (20). NORs, predominantly situated in the cerebellum, play a role in modulating neurological processes related to motor coordination and cognition (21). Critically, sexually dimorphic endogenous processing supporting the direct attenuation of acute pain has not been identified in humans.

Converging lines of evidence across rodent and human studies in healthy and clinical pain populations demonstrate greater endogenous opioidergic involvement in males as compared to females (2, 22–24). Male rodents, when compared with female rodents, exhibit significantly greater (i) analgesia in response to intravenous morphine (25) and (ii) mu-opioid activation of the periaqueductal gray matter (PAG)–rostroventral medulla-based descending analgesic circuits following morphine administration (24). Studies in healthy humans have afforded mixed findings in identifying sex differences in nociceptive processing. In healthy human volunteers, females exhibit stronger mu-based (26) and kappa-based (27) opioid system activation during a pain-evoking stimulation when compared with males. Other work has contradicted said findings and revealed that healthy males exhibit greater mu-based opioid (28) and kappa-based opioid (20) system activation during pain evocation in contrast to females. These effects could be explained by, among other factors, sex-based differential primary afferent responses (29) and implicate sex-dependent differences in endogenous opioidergic signaling. Despite these dimorphic pain responses, sex-specific pain treatments are not employed likely due to, among other factors, the historical lack of female inclusion in mechanistically focused pain research (4) and inconsistency in research findings (2). Critically, the identification of a human sex-dependent endogenous system during the attenuation of acute pain has not been characterized. This is further complicated by differences between pain-free and chronic pain populations in opioid receptor availability (30–35).

To bridge these explanatory gaps, the present study combined data from two operationally analogous randomized clinical trials (NCT03419858; NCT04034004) that employed a double-blinded and naloxone vs. saline infusion cross-over design in pain-free participants (36) and patients with chronic low back pain (cLBP) (37) to test the role of endogenous opioids in meditation-based pain relief. Mindfulness meditation-based pain relief was found to be nonopioidergically mediated (36–39). Sex differences and the corresponding role of endogenous opioids in meditation-related analgesia by sex, respectively, have yet to be examined. The proposed study examined whether endogenous opioids are differentially engaged between males and females during the direct attenuation of evoked pain (49 °C) by two distinct pain modulatory meditation techniques (mindfulness and slow breathing).

Methods and materials

Study design, setting, and participants

In Studies 1 and 2, participants were instructed that the purpose of the study was to "test the potential role of endogenous opioids in mindfulness-based meditation." It was made clear to participants that they were not receiving a pain treatment. Participants self-reported their race/ethnicity. All participants ($n = 98$) were naïve to meditation and tested negative to a urine-based opiate/opioid screening. Study 1 included pain-free individuals ($n = 39$), and Study 2 included individuals diagnosed with cLBP($n = 59$).

Study 1 and 2 participant exclusion criteria

Study 1 exclusion criteria: The inclusion criteria included individuals 18–65 years of age, with no prior meditative experience, and with a cLBP diagnosis (medical record history confirmed). The exclusion criteria included those who tested positive for opioids, were pregnant, reported prior meditation experience, and/or had back surgery within a year of the enrollment.

Study 2 exclusion criteria: The inclusion criteria included individuals 18–65 years of age, no prior meditative experience, and pain-free. The exclusion criteria included those currently taking opioids, pregnant, and those with a history of syncope and a fear of needles and blood.

Participants

Study 1: The University of California San Diego Institutional Review Board (IRB190709) approved all procedures. Eighty-eight individuals diagnosed with cLBP for at least 3 months, had an average pain rating of >3 (out of 10), confirmed by medical records, provided consent, and were enrolled in the study. The study was preregistered (NCT04034004) prior to study initiation (January 2020 to December 2021) for a primary aim of determining whether mindfulness-based pain relief was mediated by endogenous opioids. Data collection occurred during the global COVID-19 pandemic. One participant was removed because she was inadvertently unblinded to her drug assignment by a study nurse. Participants were randomized into one of two (four-session; 20 min/session) mental training interventions ($n = 30$ mindfulness meditation and $n = 29$ sham mindfulness meditation. Thus, 60 participants (mean age = 47 years ± 7 years; 29 males [mean age = 49 ± 12 years]; 30 females [mean age = 44 ± 12 years]) completed all study procedures (45 were White, 4 were Black, 3 were Asian, and 7 were "mixed") and are included in the current study.

Study 2: The Wake Forest School of Medicine Institutional Review Board (IRB00040519) approved all study procedures. Eighty-seven healthy, pain-free, and meditation-naïve participants were recruited from the local community and provided informed consent. The study was preregistered (NCT03419858) prior to study initiation (2017 March 6to July 13) for a primary aim of determining whether mindfulness-based pain relief was mediated by endogenous opioids. Sixty participants (mean age = 27 years ± 12 years; 30 males [mean age = 26 ± 7 years]; 30 females [mean age = 27 ± 8 years]) completed all study procedures (1 = Asian, 13 = Black, 1 = Indigenous American, 45 = White). One participant (female; mindfulness meditation group) was removed from the final analysis because she was unblinded to her drug assignment (36). Participants were randomized into one of three (four-session; 20 min/session) mental training interventions ($n = 19$ mindfulness meditation; $n = 20$ sham mindfulness meditation; $n = 20$ slow breathing). To maintain consistency across the two studies, participants from the slow-breathing intervention were excluded from the present analysis. Thus, 39 participants (1 = Asian, 4 = Black, 1 = Indigenous American, 33 = White) were included in the present analysis.

Study 1 and 2 randomization procedures

Study 1: Cohort size included blocks of two and four. Fifteen males and 15 females in each group were administered saline in one

session and naloxone in the subsequent session, and vice versa. There were 15 females in each group. There were 15 males in the mindfulness meditation and 14 males in the sham mindfulness meditation group. Participants were randomized using an Excel-based random number generator by a study coordinator that did not collect any data into one of two (four-session; 20 min/session) mental training interventions (mindfulness meditation and sham mindfulness meditation).

Study 2: Cohort size included blocks of three and six. Ten males and 10 females in each group were administered saline in one session and naloxone in the subsequent session, and vice versa. Males and females were randomized without replacement across a block of 60 codes using an Excel-based random number generator by a study coordinator that did not collect any data. Participants were randomized into one of three (four-session; 20 min/session) mental training interventions ($n = 19$ mindfulness meditation; $n = 20$ sham mindfulness meditation; $n = 20$ slow breathing). To maintain consistency across the two studies, participants from the slow-breathing intervention ($n = 19$) were excluded from the present analysis. Thus, 39 participants were included from Study 2 in the present analysis.

Interventions

Mindfulness meditation training regimen: Participants in the meditation group participated in 4 separate sessions (20 min each) of mindfulness-based mental training. Interventions were performed remotely with Zoom technology. In Study 1, interventions were conducted in group settings unless "make-up" sessions were needed. Participants were informed that meditation training was secular and taught as the cognitive practice of mindfulness meditation. In each training session, mindfulness-based instructions (see intervention scripts) emphasized acknowledging arising thoughts, feelings, and/or emotions without judgment or emotional reaction and to "simply return their attention back to the breath" whenever such discursive events occurred. Participants were taught that perceived sensory/affective events were "momentary" and "fleeting" and did not require further evaluation. In the first meditation training session, participants were instructed to focus on the breath sensations occurring "at the tip of the nose." In the second meditation training session, participants were instructed to focus to the "full flow of the breath," including bodily sensations (rise and fall of the abdomen and chest), and to return attention back to breath sensations without further evaluation. On meditation training days 3 and 4, time spent providing guided instructions was reduced to allow practitioners to practice independently. Contrary to traditional meditation training programs, participants were instructed not to practice outside of training to reduce inter-individual variability in practice time.

Sham mindfulness meditation training regimen: The main purpose of this intervention was to *lead participants to believe they were practicing* mindfulness meditation without instructions related to mindfully attending to the breath in a nonevaluative manner. Participants were first told they were randomly assigned to a meditation group. Similar to the genuine mindfulness meditation group, participants were informed that meditation training was secular and taught as the cognitive practice of mindfulness meditation. In each of the four training sessions (20 min each), participants were instructed to sit with a straight posture, close their eyes, and to take a deep, slow breath "as we sit here in meditation" every 2–3 min. Importantly, there were no instructions related to attending to the breath sensations and/or to reduce judgments/reactions to arising sensory events. All aspects of the mindfulness meditation training were matched to the sham mindfulness meditation intervention including setting, posture, and facilitators. The primary difference between the mindfulness and sham mindfulness interventions corresponded to the explicit instructions to mindfully attend to the breath and arising thoughts during genuine mindfulness meditation training and not sham mindfulness meditation training.

Thermal stimulation: The same noxious heat device and stimulus was employed in the two clinical trials. Pain-evoking noxious *heat* series included 10, 12 s plateaus of 49 °C interspersed with 20 s of innocuous 35° delivered by a MEDOC TSA-II to the calf. In Study 1 (patients with cLBP), heat was applied to the unaffected calf (16 mm^2 thermal probe) before and after the cLBP-evoking straight leg raise (SLR) test. SLR was performed by a trained research technician, where participants' legs were lifted until a two-point pain increase was accomplished (pain data not presented here). In Study 2 (healthy individuals), noxious heat was applied to the right calf.

Drug administration: The naloxone dosages and procedures employed are well validated to comprehensively inhibit endogenous opioidergic signaling without unblinding of drug assignment (40). In both studies, a high dose (0.15 mg/kg bolus dose) of naloxone (Naloxone HCl; Amphastar Pharmaceuticals) in 25 mL of normal saline or saline alone was administered over 8 (Study 1) and 10 (Study 2) min via the intravenous line inserted into the antecubital vein of the nondominant arm. Onset of naloxone-induced opioidergic antagonism occurs after 2 min of intravenous initiation and has an average half-life of 64 min ("Summary of Product," Amphastar Pharmaceuticals). The duration of both experiments from the onset of naloxone infusion to completion was ~25 min. To ensure comprehensive opioidergic antagonism (40), a supplementary "maintenance" intravenous naloxone infusion (0.1 mg/kg/h) was administered immediately after the bolus ceased and continued until the end of the experiment (~15 min). Patients were allowed 2–7 days to complete the last infusion session after completing the first infusion session.

Primary outcome

Pain intensity ratings, in response to frankly noxious heat (49 °C), were collected by using a well-validated 15-cm, 11-point VAS (0 = no pain; 10 = worst pain imaginable) to quantify pain intensity ratings (Paresian Novelty (41–44)). The minimum rating was represented as "no pain sensation," and the maximum was characterized as "worst pain imaginable." Participants were instructed to slide the scale "to the right" to progressively expose more red on the algometer to reflect greater pain. The reverse side of the VAS depicted numbers from 0 to 10 that corresponded with each participant's response and were recorded by the experimenter.

Randomization and masking

Participants were randomized within 3 days of completing Experimental Session 1 in a double-blind fashion by a study coordinator not involved in any data collection. The treatment arms were permuted (Excel-based random number generation) with respect to drug assignment (mindfulness + naloxone, mindfulness + saline, sham mindfulness + naloxone, sham mindfulness + saline). Randomization was stratified by biological sex and drug order presentation. Patients, research nurses, and experimenters were blinded to drug assignment. Only the study physicians, research pharmacist, and research coordinator were aware of each participant's drug assignment, and none of those unblinded were involved in any data collection or analyses.

Study 1 experimental procedures

Session 1: After providing consent, participants were trained to use the VAS pain intensity (0 = no pain; 10 = worst pain imaginable) in response to a wide range of innocuous and noxious heat stimuli (32, 5-s stimuli ranging from 35 to 48 °C; left ventral forearm). Participants were then instructed to lie supine. The *heat* series was then administered, and VAS ratings were subsequently collected. The SLR test was then performed, and pain ratings were collected (data not reported here). This was followed by an 8-min rest period (control for the saline/naloxone boluses on subsequent testing sessions) and an additional SLR test. The second *heat* series was then administered, and VAS pain ratings were collected. Participants were then randomized to a four-session (20 min each) mindfulness or sham mindfulness intervention training after completing Session 1 (Fig. 1).

Sessions 2–5: Interventions (see Appendix for intervention scripts).

Experimental Sessions 6 and 7: Each subject participated in two separate experimental pain psychophysical sessions that included high-dose intravenous infusion of (i) naloxone (0.15 mg/kg bolus + 0.1 mg/kg/h) and (ii) placebo saline, respectively. In these sessions, ACTRI nurses first administered an opiate-focused urine drug screening to confirm that no participants were using opioids. The study nurse then inserted the intravenous catheter into the nondominant arm of each participant. Blood pressure, respiration rate, oxygen saturation, and heart rate data were systematically monitored and recorded throughout the entire experiment. Participants were then instructed to lie supine.

In each respective infusion session (Fig. 1), participants were first administered a noxious heat series and VAS pain ratings were collected. The SLR test was then performed, and pain ratings were again collected. A naloxone or saline bolus was then administered, and participants were instructed "to begin meditating until the end of the experiment." After the 8-min bolus, a maintenance infusion and the second SLR test was performed along with collection of VAS pain ratings. The second *heat* series was finally administered, and VAS pain ratings were collected again.

Study 2 experimental procedures

Experimental Session 1: Similar to Study 1 (Fig. 1), study volunteers were initially familiarized with 32, 5-s duration stimuli (35–49 °C) and use of the VAS on the ventral aspect of the left forearm while lying supine. Pain ratings in response to noxious heat were collected after each of a total of four *heat* series (right calf). The first two heats and the last two heats, respectively, were averaged. In the first two *heat* series, participants were instructed to reduce movement and rest. They were then instructed to lie quietly for 10 min. Two more *heat* series were then administered. Participants were then randomized to their respective intervention. Pain ratings from the first two and last heat series were averaged.

Experimental Sessions 2–5: Interventions: Instructions for mindfulness and sham mindfulness meditation training were matched to Study 1. However, in Study 2, interventions were conducted in-person in group settings unless "make-up" sessions were needed.

Experimental Sessions 6 and 7: After successful completion of each group's respective intervention—and similar to Study 1—participants reported to Wake Forest's Clinical Research Unit (CRU), within 2–7 days, to complete each infusion on separate days. In these sessions, CRU nurses first administered an opiate-

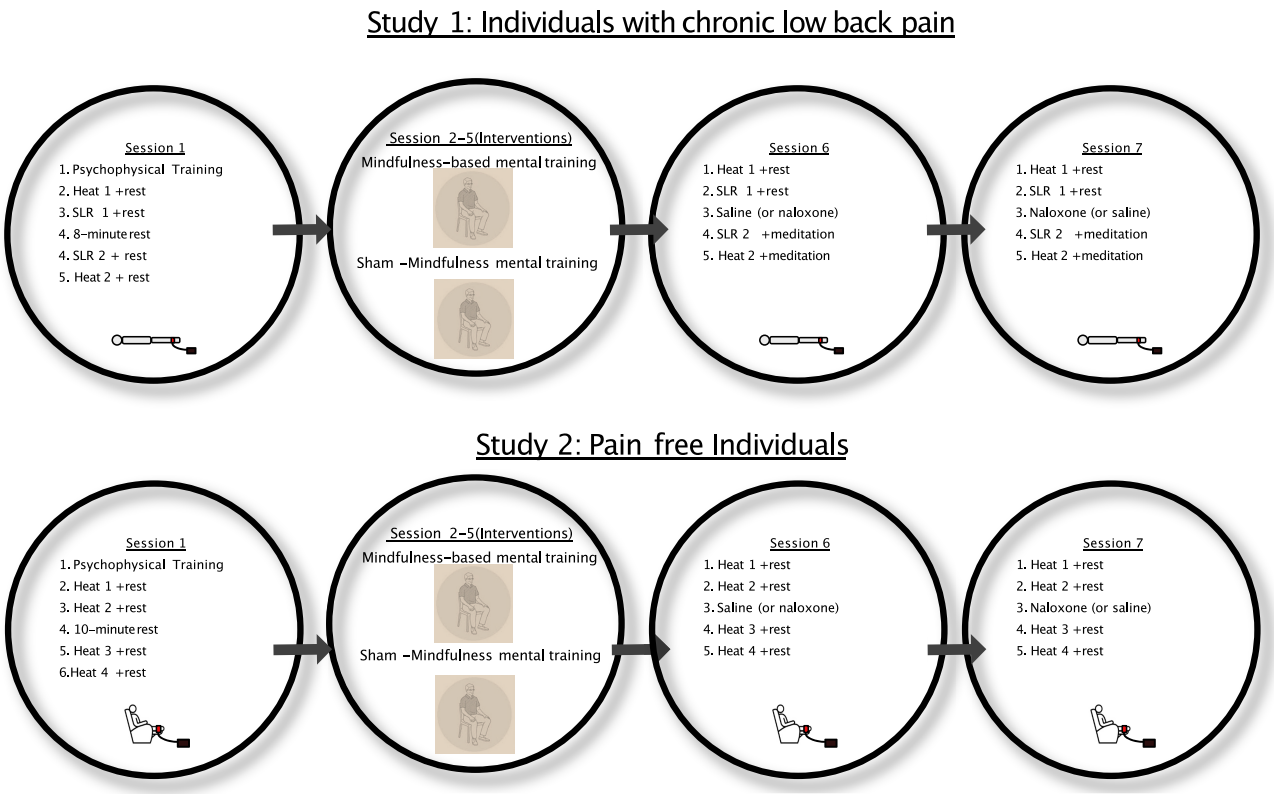

Fig. 1. Experimental procedures across the two clinical trials. The two randomized clinical trials employed paralleling experimental procedures including the same meditation interventions, noxious heat stimulation paradigm, and cross-over, drug dosages, double-blind drug administration. In Study 1, participants were positioned in the supine during heat stimulation and performed the SLR test before the after the first heat and before the second heat series. In Study 2, participants were positioned in a custom-made reclining chair during heat stimulation.

focused urine drug test. The study nurse inserted the intravenous catheter into the nondominant arm of each participant, blood pressure, respiration rate, oxygen saturation, and heart rate data were systematically monitored and recorded throughout the study. The first 2 *heat* series were administered, and VAS pain ratings were subsequently collected. Participants in the mindfulness and sham mindfulness meditation groups were instructed to "begin meditation and to continue until the end of the experiment." After the 10-min bolus, a maintenance infusion (0.1 mg/kg/h) was administered throughout the rest of the study. Two more *heat* series were administered during mindfulness meditation and sham mindfulness meditation. VAS pain ratings were collected after each heat series. Pain ratings from the first two and last heat series were averaged. Participants returned to the CRU to complete the final session and followed the same procedures as the prior infusion session (except drug assignment).

Statistical analyses

Primary: A 2 (group)×2 (sex)×2 (saline vs. naloxone infusion session)×2 (rest vs. meditation) ANOVA controlling for preintervention pain ratings was conducted to test the study hypothesis. A binary study-type dummy variable (Study 1 vs. Study 2) was also entered as a covariate in the model to control for potential differences between participants (pain-free vs. cLBP). Simple effects tests examined significant main effects and interactions. Significant interactions and main effects of participant type (pain-free vs. cLBP) were explored with an independent samples t test.

Exploratory: The following exploratory analyses were performed to determine whether potential dosage differences between males and females predicted pain changes. An independent samples t test examined whether naloxone dosage was significantly different between males and females. Further, the relationship between naloxone dosage and analgesia by sex was examined using multiple regression. The dependent variable was designated as change in naloxone-induced pain ratings from rest to meditation. Group and naloxone dosage were entered as the independent variables. A supplemental exploratory independent samples t test was performed to determine, in the present sample, whether patients living with cBLP differed in age when compared with pain-free individuals.

Results

Across both clinical trials, 59 (Study 1) were diagnosed with cLBP (medical record confirmation) and 39 were healthy and pain-free (Study 2). There were 51 (mean age [SD] = 37.6 [13.05]) females and 47 males (mean age [SD] = 39.40 years [15.6]). There were 78 White, 8 Black, 1 Indigenous American, 4 Asians, and 7 self-identified as "mixed" race.

Self-regulated analgesia in males but not females is dependent on endogenous opioidergic signaling

A three-way, sex × infusion session (saline vs. naloxone)×rest vs. meditation interaction ($F(1, 91) = 4.12$, $P = 0.04$, $\eta_p^2 = 0.04$; Table S1) was detected. In males, meditation significantly reduced pain ratings during saline infusion ($P < 0.001$; 95% CI = −1.20; −0.37; Fig. 2). However, during opioidergic blockade, meditation did not significantly reduce pain ($P = 0.23$; 95% CI = −0.74; 0.18) (Figs. 2 and S1; Tables 1 and S1). In contrast, females significantly reduced pain ratings during intravenous saline ($P = 0.08$, 95% CI = −0.76; 0.04) and during opiodergic blockade ($P = 0.008$, 95% CI = −1.04; −0.16). In males, meditation-induced analgesia was significantly greater during saline when compared with naloxone ($P = 0.01$, 95% CI = −1.00; −0.13). However, there were no significant differences in pain relief between saline and naloxone infusion sessions in females ($P = 0.78$, 95% CI = −0.36; 0.47). These findings demonstrate that self-regulated analgesia in males, but not in females, is opioidergically mediated. There were no other significant sex-based differences during rest and meditation in the saline and naloxone sessions (see Table S1; Supplementary Results).

Meditation-induced analgesia was significantly greater in individuals with chronic pain as compared to healthy volunteers

Primary: A two-way rest vs. meditation × study-type (pain-free vs. cLBP) interaction, $F(1, 91) = 6.81$, $P = 0.01$, $\eta_p^2 = 0.07$, also emerged. To interpret this effect, change in pain ratings, from meditation to rest, was averaged across the saline and naloxone sessions and entered into an independent samples t test to assess for differences in meditation-induced pain changes. Assumption for equal variance in pain ratings between healthy participants and

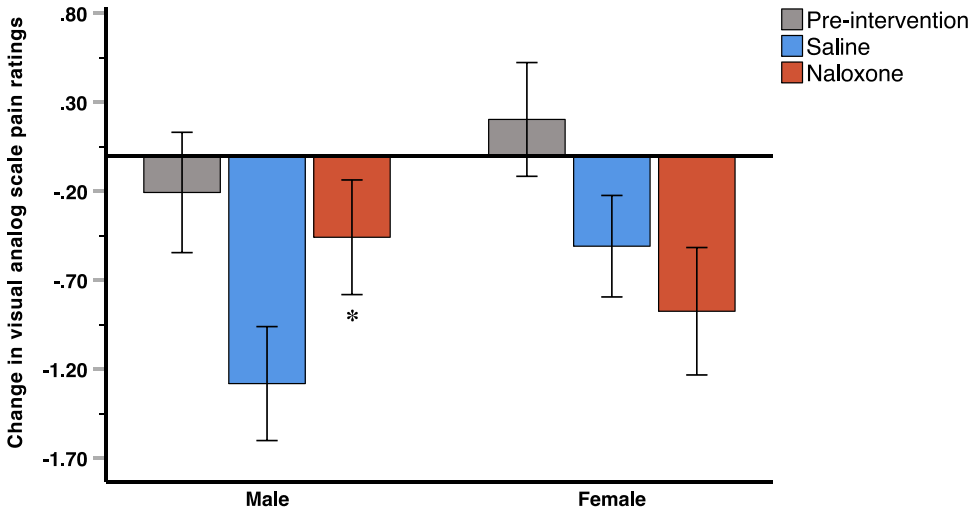

Fig. 2. Male and female change in pain the preintervention training baseline and postintervention training saline infusion and naloxone infusion sessions. * In males, naloxone was associated with weaker analgesia when compared with saline infusion ($P = 0.01$). There were no significant differences in pain relief during saline and naloxone infusion in females.

Table 1. Total sample pain ratings during each condition and study session.

Sex		Baseline heat 1: Rest	Baseline heat 2: Rest	Saline heat 1: Rest	Saline heat 2: Meditation	Naloxone heat 1: Rest	Naloxone heat 2: Meditation
Male	Mean	4.37	4.28	4.10	3.33	4.17	3.89
	SD	2.35	2.47	2.12	2.19	2.19	2.50
Female	Mean	4.90	5.05	4.60	4.23	4.89	4.29
	SD	2.55	2.49	2.29	2.19	2.56	2.50
Total	Mean	4.65	4.68	4.36	3.80	4.54	4.10
	SD	2.46	2.50	2.21	2.23	2.40	2.49

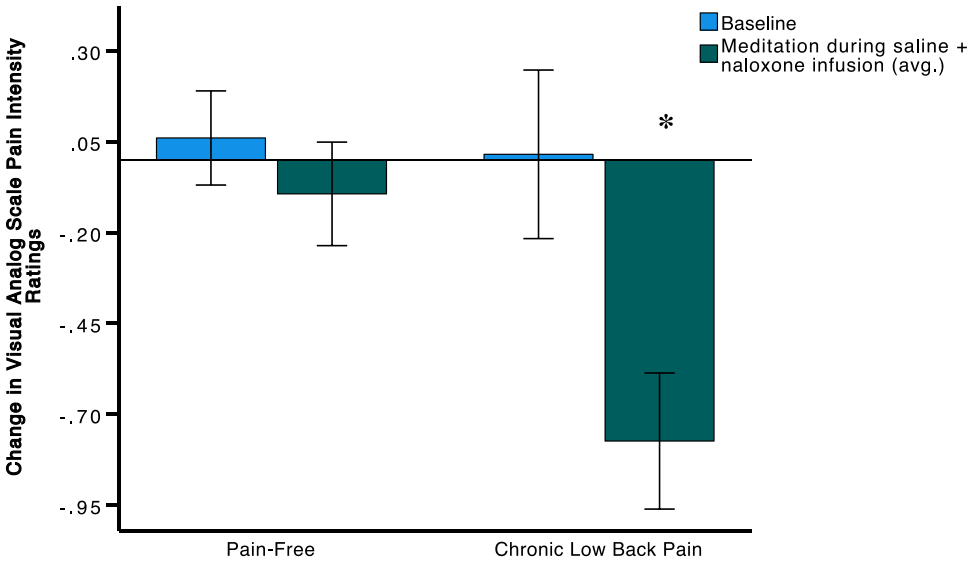

Fig. 3. Changes in pain ratings, in healthy individuals and those with chronic pain, in the preintervention session and the average (avg.) of the two postintervention sessions. Independent samples t test revealed that meditation-induced pain relief was significantly greater in individuals with cLBP than in pain-free individuals, *$P = 0.005$.

individuals with chronic pain was not met ($F = 8.58$, $P = 0.004$). The independent samples t test revealed that meditation-induced pain relief was significantly greater in individuals with cLBP than in pain-free individuals ($P = 0.005$, CI 95 = 0.21; 1.14; Fig. 3).

Exploratory: Males ($M = 90.11$; SEM = 2.37) weighed more than females ($M = 80.01$; SEM = 2.98). Consequently, males ($M = 15.50$; SEM = 0.40) were administered significantly higher dosages of naloxone ($P = 0.007$, 95% CI = 0.49; 3.05) to antagonize endogenous opioids than females ($M = 13.73$; SEM = 0.50). However, linear regression revealed that naloxone dosage ($B = 0.002$, $P = 0.97$) nor group ($B = -0.32$, $P = 0.35$) predicted meditation-induced pain changes during noxious heat stimulation (Table S2). In the present sample, patients living with cBLP (mean age [SD] = 46.25 [12.15]) were significantly ($P < 0.001$; 95% CI = −23.91; 15.22) older than pain-free (mean age [SD] = 26.69 [7.61]) individuals.

Discussion

The present study is the largest meditation-focused and intravenous naloxone, cross-over clinical trial conducted to date. The findings are the first to demonstrate sex-specific differences in the engagement of opioidergic processes during endogenously driven analgesia. We have previously shown that meditation is based analgesia not opioidergically meditated (37–39, 45). The present results indicate that this effect is differentially mediated by sex. Males and females significantly reduced noxious heat-induced pain during mindfulness (Table 2) and sham mindfulness meditation (Table 3) and saline infusion (Fig. 2). In males, but not females, endogenous opioidergic receptor blockade reduced the efficacy of meditation-related analgesia by threefold when compared with placebo-saline administration. In fact, males reported comparable pain reductions during meditation and naloxone infusion as the change exhibited in the preintervention training session, suggesting a male-specific and comprehensive reversal of the analgesic effects of meditation on pain by inhibiting opioid receptors. In contrast, females evinced enhanced analgesia during endogenous opioidergic antagonism, suggesting engagement of nonopioid endogenous pain modulatory systems. These results align with preclinical rodent work demonstrating that intrathecal antagonization of the PAG microglia in the opioid-binding, innate immune toll-like receptor 4 using naloxone, significantly enhanced morphine-induced analgesia in female rodents but reversed analgesia in males (23).

The present study employed naloxone dosages were reliable in comprehensively antagonizing endogenous opioid receptors (40). Males weighed more than females and thus required a significantly higher naloxone dosage to antagonize endogenous opioids. However, naloxone dosage did not explain the difference in pain relief during endogenous opioidergic blockade between sex. Although females generally rated higher pain ratings in response to noxious heat, there were no significant differences in pain during rest and meditation in the saline and naloxone infusion sessions, respectively. More research is needed to determine whether these findings are specific to meditation and/or other pain-alleviating techniques (5). Although speculative, sex-based differences in endogenous analgesic systems may be associated

Table 2. Pain ratings for males and females for each condition in the mindfulness group.

Sex		Baseline heat 1: Rest	Baseline heat 2: Rest	Saline heat 1: Rest	Saline heat 2: Meditation	Naloxone heat 1: Rest	Naloxone heat 2: Meditation
Male	Mean	3.94	4.06	4.09	3.10	3.92	3.77
	SD	2.10	2.41	2.25	2.25	2.16	2.55
Female	Mean	4.64	4.51	4.15	3.51	4.56	3.57
	SD	2.77	2.38	2.04	1.54	2.44	2.27
Total	Mean	4.30	4.29	4.12	3.30	4.25	3.67
	SD	2.46	2.38	2.12	1.91	2.31	2.39

Table 3. Pain ratings for males and females for each condition in the sham mindfulness group.

Sex		Baseline heat 1: Rest	Baseline heat 2: Rest	Saline heat 1: Rest	Saline heat 2: Meditation	Naloxone heat 1: Rest	Naloxone heat 2: Meditation
Male	Mean	4.81	4.50	4.12	3.57	4.42	4.02
	SD	2.56	2.57	2.02	2.16	2.24	2.49
Female	Mean	5.16	5.57	5.02	4.93	5.20	4.98
	SD	2.35	2.53	2.47	2.52	2.67	2.56
Total	Mean	4.99	5.07	4.60	4.29	4.83	4.52
	SD	2.43	2.58	2.29	2.43	2.49	2.55

with a distinct evolutionary-based analgesic mechanisms supporting pain relief. Endocannabinoids are differentially released during labor (46). A growing body of translational work indicates that the endocannabinoid system is engaged during analgesia in females at a greater capacity than male (47). Indeed, females display a heightened sensitivity to exogenously administered Δ^9-tetrahydrocannabinol and higher cannabinoid receptor density than males (48). Unfortunately, endocannabinoid concentrations, menstrual cycle, and gonadal hormone levels, mechanisms known to play a role in sex-based pain differences (2, 49), were not measured in the proposed work, and thus, the role of these factors on sex-based pain differences could not be deduced.

Importantly, the involvement of endogenous opioids was revealed in males but not females during self-regulated analgesia, in healthy adults and among those experiencing chronic pain to indicate a generalized between-sex effect across pain health status. Individuals with chronic pain also produced greater meditation-induced analgesia (−18%) than pain-free individuals (−3%), potentially reflecting the unique capacity for meditation to modify the comorbid and multifaceted exhibition of nociceptive processing in individuals with chronic pain. This effect could also be related to age, in our sample, since patients living with cBLP were significantly older than pain-free individuals. Although speculative, we provide novel evidence that older adults may report greater benefit in pain relief in meditation-related pain relief when compared with younger adults. This study also provides supplemental evidence that extensive mental training is not required to elicit the capacity to directly reduce acutely evoked pain (50), removing a significant barrier for the translation of mind-body approaches for chronic pain treatment. Together, the present data provide clear evidence that the acute attenuation of evoked pain engages dimorphic opioidergic mechanisms and highlights the critical need of establishing and promoting sex-specific pain therapeutics.

Supplementary Material

Supplementary material is available at *PNAS Nexus* online.

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Author Contributions

Conceptualization: M.R. and F.Z.; methodology, visualization, funding acquisition, supervision, and writing—original draft: F.Z.; investigation: J.G.D., V.O., L.K., G.R., N.G., G.P., J.C., J.B., K.C., and F.Z.; and writing—review and editing: J.G.D., M.R., R.E.W., B.G., R.F., and F.Z.

Data Availability

Data used in the present paper are available Open ICPSR at https://doi.org/10.3886/E209423V1.

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