

Acute aerobic exercise does not modulate pain potentially due to differences in fitness levels and sex effects – results from a pharmacological fMRI study

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eLife Assessment

Nold et al. examined exercise-induced pain modulation in a pharmacological within-subject fMRI study using the opioid-antagonist naloxone and different levels of aerobic exercise and pain. This study provides **valuable** findings showing that the intensity of exercise does not seem to impact the hypoalgesic effect; however, the evidence is **incomplete** for the claims relating to the overall effect of exercise on pain, since they cannot be tested with the proposed experimental design.

<https://doi.org/10.7554/eLife.102392.1.sa2>

Abstract

Exercise might lead to a release of endogenous opioids, potentially resulting in pain relief. However, the neurobiological underpinnings of this effect remain unclear. Using a pharmacological within-subject fMRI study with the opioid antagonist naloxone and different levels of aerobic exercise and pain we investigated exercise-induced hypoalgesia ($N = 39$, 21 female). Overall, we were unable to detect reduced pain after high-intensity aerobic exercise as compared to low-intensity aerobic exercise. Accordingly, we observed no significant changes in the descending pain modulatory system. The μ -opioid antagonist naloxone significantly increased overall pain ratings but showed no interaction with exercise intensity. An exploratory analysis suggested an influence of fitness level and sex where males showed greater hypoalgesia after high-intensity exercise with increasing fitness levels. This effect was attenuated by naloxone and mirrored by fMRI signal changes in the medial frontal cortex, where activation also varied with fitness level and sex, and was reversed by naloxone. These results indicate that aerobic exercise has no overall effect on pain in a mixed population sample, but individual factors such as fitness level and sex might play a role.

Introduction

Exercise can release endogenous opioids resulting in an attenuation of pain, termed exercise-induced hypoalgesia. However, the underlying neural (Vaegter and Jones, 2020; Wu et al., 2022) and pharmacological mechanisms remain controversial (Koltyn, 2000; Naugle et al., 2012; Wewege and Jones, 2020) and results on pain modulation induced by exercise are heterogeneous (Klich et al., 2018; Koltyn et al., 2014; Padawer and Levine, 1992; Ruble et al., 2005; Sternberg et al., 2001; Vaegter et al., 2015, 2019).

Previous rodent research has implicated the descending pain modulatory system, including the ACC, medial frontal cortex including the ventromedial prefrontal cortex (vmPFC), and the PAG in exercise-induced pain modulation (Lesnak and Sluka, 2020; Stagg et al., 2011). This is partly congruent with early imaging studies in humans (Boecker et al., 2008; Ellingson et al., 2016; Geisler et al., 2019; Saanijoki et al., 2018; Scheef et al., 2012), where the descending pain modulatory system, including the periaqueductal grey (PAG) and insula, seems to be involved in modulating pain perception following a 2-h run (Scheef et al., 2012) along with the fusiform gyrus and hippocampus (Geisler et al., 2019).

The activation of the endogenous opioid system during exercise has been the most widely discussed mechanism in exercise-induced pain modulation (Naugle et al., 2012; Sluka et al., 2018). Still, the pharmacological underpinnings in humans have remained equivocal (Koltyn, 2000), where administering a μ -opioid antagonist in some studies decreased (Droste et al., 1988; Haier et al., 1981; Janal et al., 1984; Olausson et al., 1986) or did not affect exercise-induced pain modulation (Droste et al., 1991; Koltyn et al., 2014; Olausson et al., 1986). Earlier studies found an analgesic response to pain after long-distance running in male runners that was reversed after naloxone administration (Janal et al., 1984). Even after a short-duration run, naloxone slowed the return of the analgesic effect (Haier et al., 1981). Previous imaging studies (Boecker et al., 2008; Saanijoki et al., 2018) further supported that exercise elicits the release of endogenous opioids in crucial opioidergic structures i.e. the insula, ACC, and prefrontal regions. However, most studies investigating the pharmacological underpinnings of exercise-induced pain modulation have employed very different interventions (i.e., different doses and different routes of administration such as oral or intravenous), making it difficult to draw clear conclusions about the extent of central μ -opioid modulation through exercise in humans (Koltyn, 2000; Leknes and Atlas, 2020; Wewege and Jones, 2020).

Furthermore, studies on exercise-induced pain modulation have been criticized (Padawer and Levine, 1992) for using single-arm pre-post measurements instead of randomized trials (for reviews see Naugle et al., 2012; Vaegter and Jones, 2020), as well as heterogeneous study designs (Vaegter and Jones, 2020), further limiting the interpretation of results (Naugle et al., 2012; Wewege and Jones, 2020).

In this study, we examined exercise-induced pain modulation in a healthy sample with a particular emphasis on the opioidergic system. This preregistered study (drks.de: DRKS00029064) comprised an initial calibration day and two experimental days (**Figure 1**). On the calibration day, individual exercise intensities, pain intensities, as well as objectively assessed fitness levels (functional threshold power; FTP₂₀) (40), were determined (**Figure 1A**). On each of the two experimental days, participants cycled during four blocks at high (100% FTP) and low (55% FTP; active recovery) intensity for 10 minutes. After each cycling block, functional Magnetic Resonance Imaging (fMRI) commenced, where participants received nine painful heat stimuli with a 15-second plateau each and rated their painfulness on a visual analog scale (VAS) ranging between the calibrated pain threshold ('minimally painful') and pain tolerance ('almost unbearably

painful'). Furthermore, we employed a double-blind cross-over pharmacological challenge across experimental days, where either saline (placebo; SAL) or the μ -opioid antagonist naloxone (NLX) was administered intravenously (see Methods for details) (**Figure 1B** [↗](#)).

Results

In our preregistration, we also included pressure pain to investigate exercise-induced pain modulation across different pain modalities. However, pressure pain did not show a significant effect of exercise in any of the analyses performed; therefore, this report focuses on heat pain. As the inter-trial interval (ITI) between heat pain stimuli needs to be about 30 seconds due to the stimulus length (Tran et al., 2010 [↗](#)), the interspersed pressure pain stimuli allowed us to additionally (i) characterize the brain responses to pressure as compared to heat pain and (ii) investigate the effect of a μ -opioid antagonist on these responses. However, these results are beyond the scope of this paper and will be reported separately. The final sample included healthy female ($n = 21$) and male ($n = 18$) participants of varying fitness levels. The equal distribution of menstrual phases (based on self-report and divided into three phases: follicular, ovulatory, and luteal) for the experimental days was estimated using a χ^2 -test (Wilson, 1927 [↗](#)) and showed neither significant differences on each experimental day nor between days (see Table S2).

Effective induction of heat pain

As a first step, we verified the successful application of the calibrated heat pain intensities in the placebo (saline; SAL) condition. A significant main effect of stimulus intensity on heat pain ratings was observed ($\beta = 1.42$, $CI [1.37, 1.48]$, $SE = 0.03$, $t(1358.65) = 51.84$, $P < 2 \times 10^{-16}$; **Figure 2A** [↗](#), Table S3, and S4). Corroborating this, our fMRI data showed blood oxygenation level-dependent (BOLD) activation changes in key brain regions associated with pain processing such as the right anterior Insula (antIns; **Figure 2B** [↗](#); MNI_{xyz}: 36, 6, 14, $T = 10.35$, $P_{corr-WB} < 0.001$), right dorsal posterior Insula (dpIns; **Figure 2C** [↗](#); MNI_{xyz}: 39, -15, 18, $T = 7.65$, $P_{corr-WB} < 0.001$) and right middle cingulate cortex (MCC; **Figure 2D** [↗](#); MNI_{xyz}: 6, 10, 39, $T = 7.47$, $P_{corr-WB} = 0.001$). The parameter estimates from the respective peak voxels mirrored the behavioural response pattern (**Figure 2E-G** [↗](#)). Overall, this confirms the successful administration of thermal heat stimuli at the intended intensities. For an uncorrected activation map at $P_{uncorr} < 0.001$ of the entire brain see Figure S1.

Implementation of exercise intervention

As a next step, we aimed to confirm the successful implementation of our exercise intervention. This intervention comprised previously calibrated high-intensity (HI) and low-intensity (LI) exercise conditions, where the anticipated intensities were derived from the individual functional threshold power (FTP) value determined on Day 1 (**Figure 1A** [↗](#)). The anticipated power during HI power was set to 100% whereas LI power was set to 55% of FTP. As intended, the maintained power during cycling was significantly higher in the HI exercise condition compared to the LI exercise condition ($t(37) = 16.09$, $P < 2.2 \times 10^{-16}$, mean power_{diff} = 60.26W, $d = 0.61$, $n = 38$; **Figure 3A** [↗](#)). Supporting this, the mean absolute heart rate was significantly higher in the HI exercise condition compared to the LI exercise condition ($t(33) = 20.48$, $P < 2.2 \times 10^{-16}$, mean bpm_{diff} = 26.92, $d = 1.85$, $n = 34$, **Figure 3B** [↗](#)). In one participant the power recording was incomplete ($n = 38$) and five participants had faulty HR recordings in one session ($n = 34$) due to an instrumentation error. Nevertheless, the actual power and HR maintained throughout the blocks were also visually monitored. Furthermore, participants rated their perceived level of exertion (RPE) on a BORG scale from 6 to 20 following each exercise block. The mean RPE across all HI and LI exercise blocks was also significantly higher after HI compared to LI exercise ($t(38) = 18.84$, $P < 2.2 \times 10^{-16}$, mean RPE_{diff} = 6.46, $d = 3.64$, $n = 39$; **Figure 3C** [↗](#)). Thus, the calibration and implementation of the exercise intensities were successful.

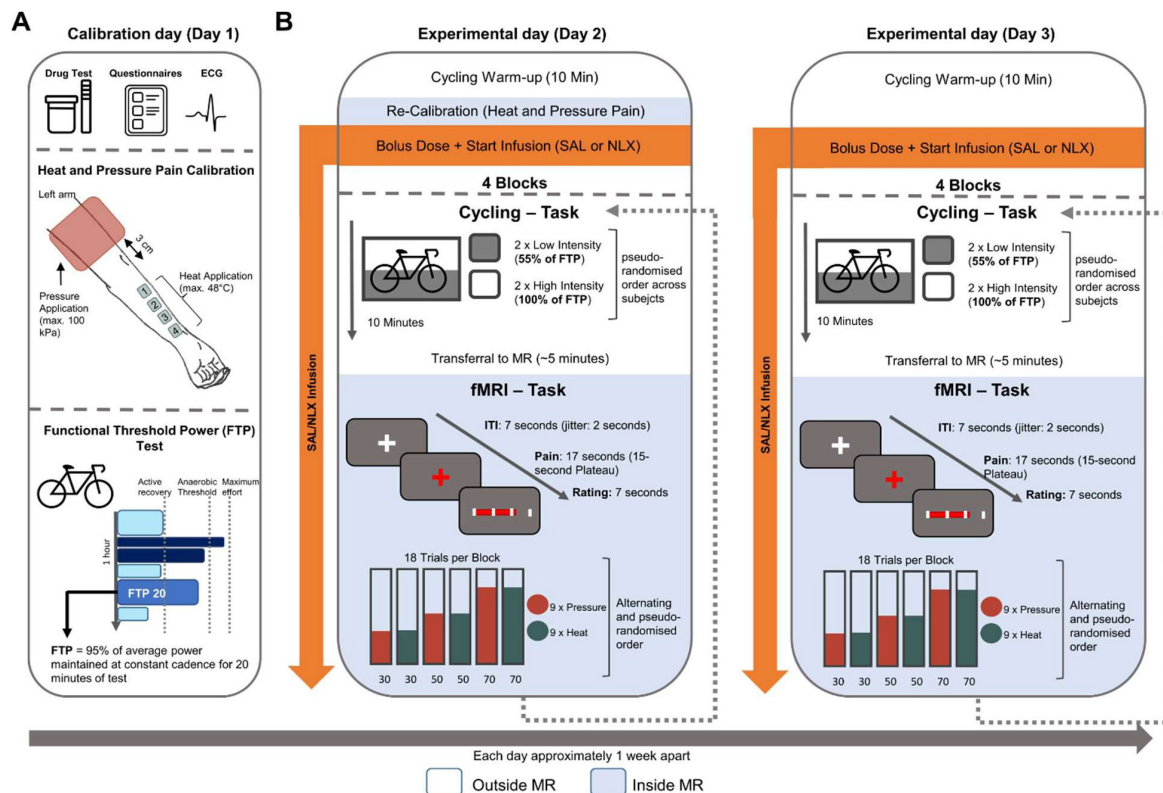


Figure 1.

Experimental design.

(A) Calibration day (Day 1) with heat and pressure pain calibration and Functional Threshold Power test (FTP) (see Table S1).
 (B) Experimental days (Day 2 and Day 3) with cycling task (outside MR) and fMRI task (inside MR) with the only difference in the drug treatment administered (SAL or NLX).

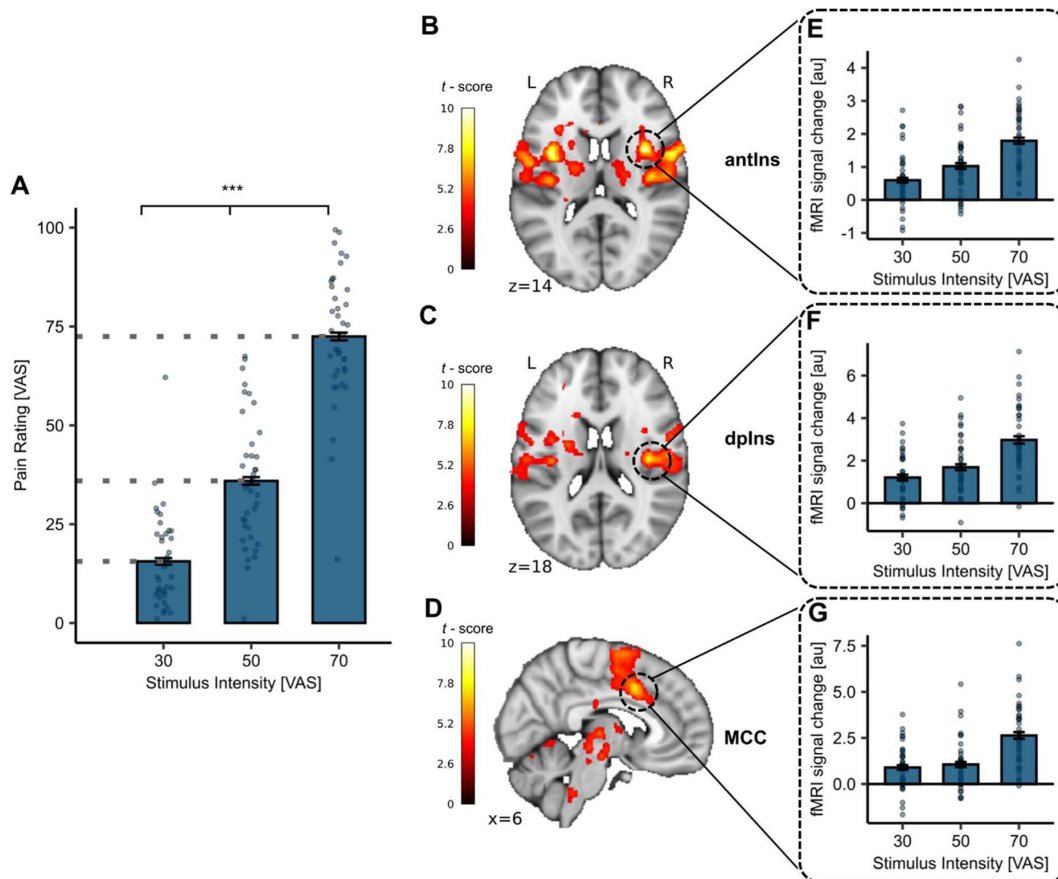


Figure 2.

Behavioural and fMRI results for successful pain induction.

(**A**) Heat pain ratings in the SAL condition for all stimulus intensities (VAS 30, 50, 70) showed a significant main effect of stimulus intensity ($P < 2 \times 10^{-16}$). The P -value reflects the significant main effect of stimulus intensity from the linear mixed effect model (LMER). (**B-D**) Significant activation for the parametric effect (Heat VAS 70 > 50 > 30) in the SAL condition in the (B) right antIns (MNI_{xyz}: 36, 6, 14; $T = 10.35$, $P_{corr-WB} < 0.001$), (C) right dpIns (MNI_{xyz}: 39, -15, 18; $T = 7.65$, $P_{corr-WB} < 0.001$) and (D) right MCC (MNI_{xyz}: 6, 10, 39; $T = 7.47$, $P_{corr-WB} = 0.001$). Displayed are the uncorrected activation maps ($P_{uncorr} < 0.001$) for visualisation purposes. (**E-G**) Bars depict mean parameter estimates from the respective peak voxels for all stimulus intensities across participants whereas dots display subject-specific mean parameter estimates at the respective stimulus intensity. P -values were calculated using the LMER model for the fixed effect of stimulus intensity. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Error bars depict SEM ($N = 39$).

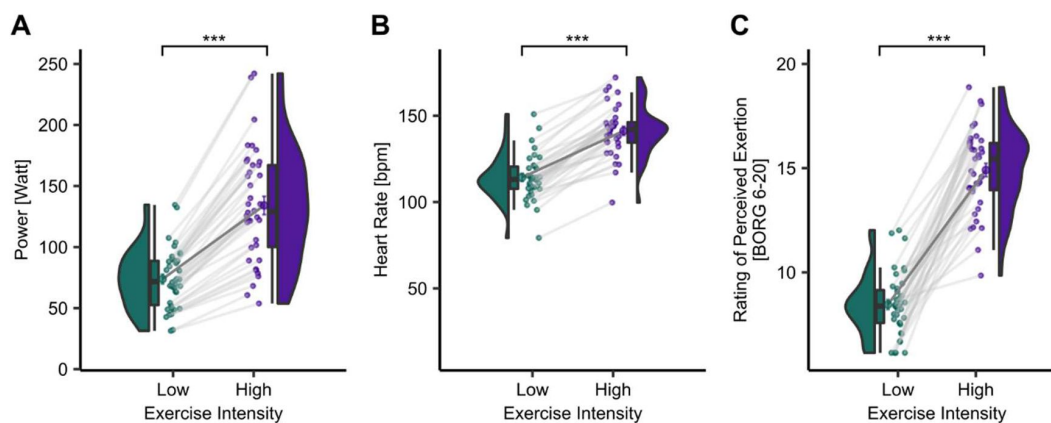


Figure 3.

Successful implementation of high (HI) and low-intensity (LI) exercise.

(A) Power output (Watt), (B) heart rate in beats per minute (bpm), and (C) rating of perceived exertion (RPE; BORG scale) during low (LI; green) and high intensity (HI; purple) cycling were all significantly different. *P*-values were calculated using a paired *t*-test (two-tailed, power: *N* = 38, hear rate: *N* = 34, BORG: *N* = 39). * *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001.

The effect of drug treatment in response to stimulus intensity

Next, we investigated the effect of our drug treatment with the μ -opioid antagonist naloxone (NLX). Previous studies have shown that NLX can increase perceived pain by blocking the effect of endogenous opioids (37, 38). In line with those findings, we observed a significant interaction of drug and stimulus intensity on heat pain ratings ($\beta = 0.10$, CI [0.02, 0.18], $SE = 0.04$, $t(2755) = 2.46$, $P = 0.01$; **Figure 4A**, Table S5, and S6), where, with increasing stimulus intensities the differences between ratings in the NLX and SAL condition increased ($\beta = 2.00$, CI [0.40, 3.61], $SE = 0.81$, $t(77) = 2.46$, $P = 0.02$; **Figure 4B**, Table S7). We also investigated this interaction of drug and stimulus intensity between sexes and observed this interaction in females ($\beta = 0.17$, CI [0.06, 0.28], $SE = 0.06$, $t(1471) = 3.00$, $P = 0.003$; **Figure 4C**) but not in males ($\beta = 0.02$, CI [-0.09, 0.13], $SE = 0.06$, $t(1255) = 0.32$, $P = 0.75$; **Figure 4D**). The difference in pain ratings between the drug treatment conditions revealed a significant main effect of stimulus intensity ($\beta = 3.41$, CI [1.27, 5.54], $SE = 1.09$, $t(76) = 3.12$, $P = 0.003$) and sex ($\beta = 9.75$, CI [1.23, 18.23], $SE = 4.42$, $t(101.60) = 2.21$, $P = 0.03$; **Figure 4E** and Table S8). In contrast to females, males showed an overall large drug effect at every stimulus intensity. These findings demonstrate a robust drug effect, where pain ratings increased by blocking μ -opioid receptors. The increasing magnitude of the drug effect with increasing stimulus intensities indicates that the opioidergic system might be more extensively employed by more intense noxious stimuli. Since this magnitude differs for lower intensities between males and females it also indicates a possible sex-dependent effect regarding endogenous opioids.

In the next step, we identified brain regions associated with pain processing and opioidergic pain modulation. We observed a significant activation (contrast interaction of stimulus intensity and drug treatment) in the PAG (MNI_{xyz}: -2, -24, -8, $T = 5.17$, $P_{corr-SVC} = 0.01$; **Figure 5A**). The extracted parameter estimates from the peak voxel showed that NLX increased activation compared to SAL, especially for the highest stimulus intensity (**Figure 5B** and **5C**). We used post-stimulus averaging (Finite Impulse Response or FIR model) to extract the mean time course for the highest-intensity stimulus (VAS 70). A significant difference in activation was evident seven seconds after stimulus onset (BOLD response) for the remainder of the stimulus in the peak voxel in the PAG (**Figure 5D**; for an uncorrected map at $P_{uncorr} < 0.001$ see Figure S2). We also investigated the contrast heat NLX > heat SAL where activation in the right antIns showed a trend at whole-brain correction (MNI_{xyz}: 46, 8, 6, $T = 5.70$, $P_{corr-WB} = 0.08$; Figure S3).

No overall effect of aerobic exercise on pain modulation

After we established a successful exercise and drug intervention, we investigated the modulating effect of exercise on pain ratings and neuronal responses in the SAL condition. We were unable to support our preregistered hypothesis of a main effect of exercise on pain as there was no hypoalgesic effect evident in the behavioural pain ratings comparing HI to LI exercise in the SAL condition ($\beta = 1.19$, CI [-1.85, 4.22], $SE = 1.55$, $t(1354) = 0.77$, $P = 0.44$; **Figure 6A**, blue bars and Table S9). Furthermore, there were no significant differences in BOLD activation between both exercise conditions (contrasts: exercise high > low (SAL) or exercise low > high (SAL)). For the uncorrected activation maps at $P_{uncorr} < 0.01$ please refer to Figures S4 and S5. Additionally, we extracted the parameter estimates from the preregistered ROIs (RVM, PAG, and frontal midline; **Figure 6B-D**) and estimated the LMER models with exercise intensity on parameter estimates in the SAL condition (**Figure 6E-G**, blue bars). None of these models yielded a significant main effect of exercise intensity (Tables S10-S12).

Despite not detecting a hypoalgesic effect of exercise intensity in the SAL condition, we investigated whether naloxone might alter the pain ratings depending on the exercise intensity. We estimated an LMER model with exercise intensity and drug treatment which yielded a significant main effect of drug treatment ($\beta = 4.50$, CI [1.36, 7.64], $SE = 1.60$, $t(2755) = 2.81$, $P = 0.005$) but no significant interaction effect of exercise intensity and drug treatment ($\beta = 0.27$, CI [-4.17,

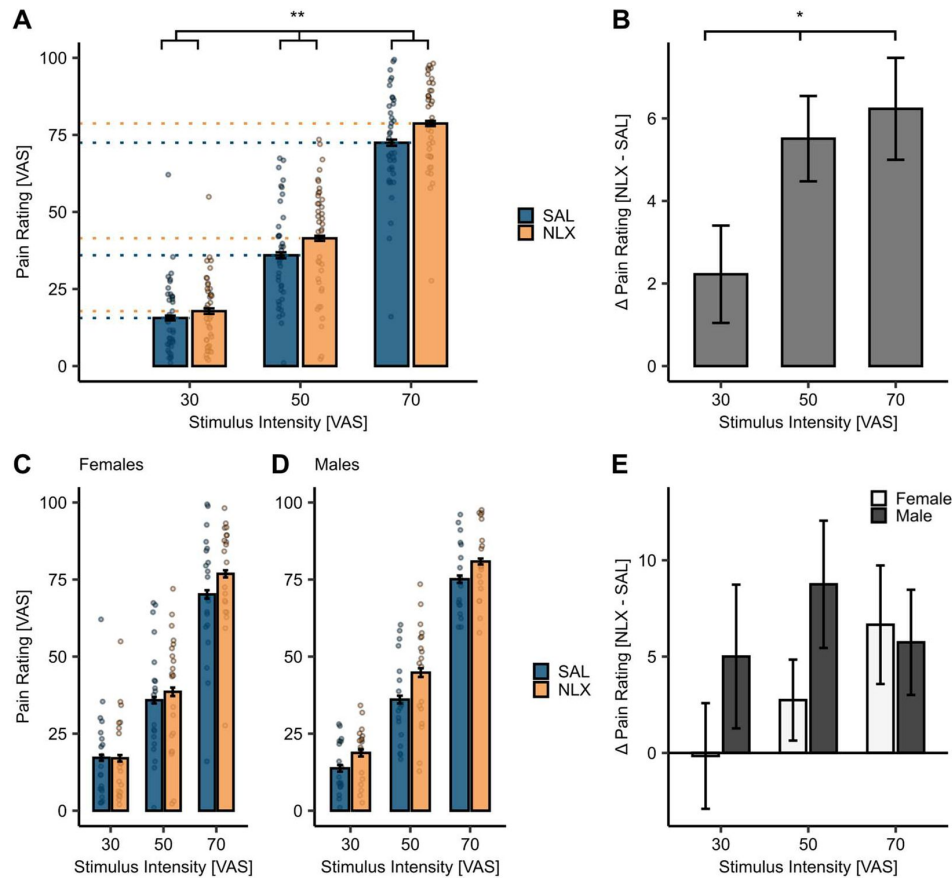


Figure 4.

Behavioural results for the effect of drug treatment on pain.

(A) Heat pain ratings revealed a significant interaction between drug and stimulus intensity ($P = 0.01$). Mean heat pain ratings were significantly higher under naloxone NLX treatment (orange) compared to placebo (SAL) (blue) across stimulus intensities. The P -value indicates a significant interaction effect of stimulus intensity and drug. (B) Differences in heat pain ratings between NLX and SAL condition (NLX - SAL) at each stimulus intensity revealed a significant main effect of stimulus intensity ($P = 0.02$). (C-D) Heat pain ratings at all stimulus intensities in both drug treatment conditions are significantly higher in NLX (orange) compared to SAL (blue) conditions for both sexes. (E) Differences in heat pain ratings between NLX and SAL condition (NLX - SAL) at each stimulus intensity for females (white) and males (dark grey) revealed a significant main effect of stimulus intensity ($P = 0.003$) and sex ($P = 0.03$) with an interaction showing a trend ($P = 0.06$). P -values were calculated using the LMER model for the interaction of stimulus intensity and drug. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Error bars depict the SEM ($N = 39$).

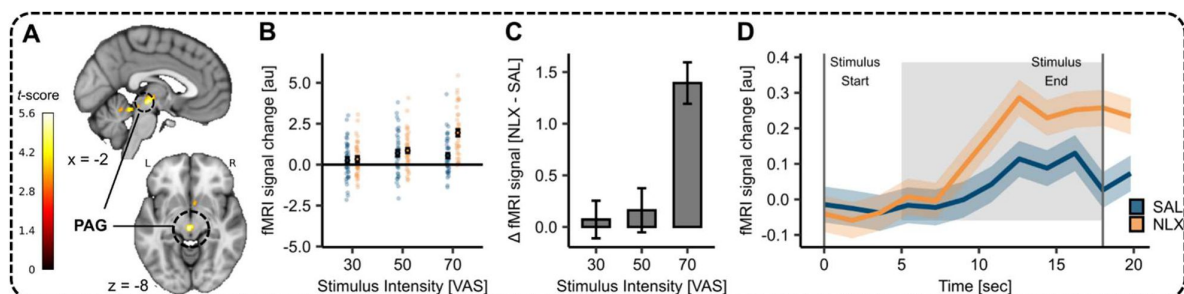


Figure 5.

Effect of NLX in the periaqueductal grey (PAG).

(A) Activation for the interaction of stimulus intensity and drug in the PAG (MNI_{xyz} : -2, -24, -8, $T = 5.17$, $P_{corr-SVC} = 0.01$) superimposed onto an MNI template brain. (B) Parameter estimates for SAL (blue) and NLX (orange) conditions for the respective peak voxel in the PAG (MNI_{xyz} : -2, -24, -8). (C) Difference between parameter estimates of the peak voxel between NLX and SAL condition at each stimulus intensity. (D) Time course of BOLD responses for SAL (blue) and NLX (orange) during high pain (VAS 70) in this voxel. The shaded areas depict the SEM. The grey solid lines indicate the start and end of the painful stimulus. The shaded grey area displays the approximate time window for BOLD response taking into account a 5s delay of the hemodynamic response function.

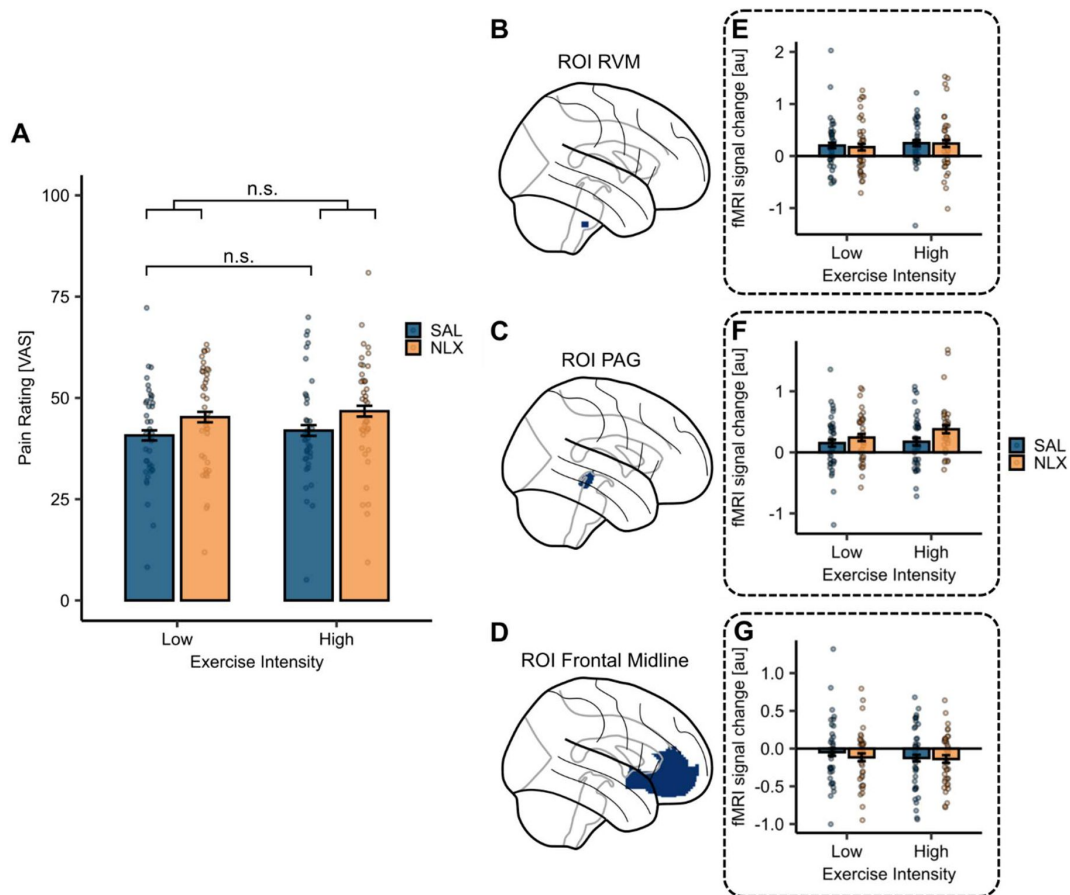


Figure 6.

Effect of exercise intensity and drug treatment on pain modulation.

(A) No significant main effect of exercise intensity on pain ratings in the SAL condition ($P = 0.44$, blue bars. The P -value was calculated using the LMER model with exercise intensity. In a separate LMER model for the interaction of exercise intensity and drug treatment, this interaction effect was not significant ($P = 0.91$) but a significant main effect of drug treatment ($P = 0.005$) was evident. Bars depict the average pain ratings in the SAL (blue) and NLX (orange) conditions in both exercise conditions and the dots represent the subject-specific average ratings across all stimulus intensities. Error bars depict the SEM ($N = 39$). (B-D) Regions of interest (ROIs) in the (B) RVM, (C) PAG, and (D) frontal midline (comprised of ACC and vmPFC). (E-G) Parameter estimates extracted from both exercise and treatment conditions for the respective ROIs showed no significant effect of exercise intensity in the SAL condition (Table S10 – S12) as well as no interaction of stimulus intensity with drug treatment (Tables S14 – S16). n.s. = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

4.71], $SE = 2.27$, $t(2755) = 0.12$, $P = 0.91$; **Figure 6A** and Table S13). Corroborating this, there were no significant differences in BOLD activation for the contrasts interaction drug treatment x exercise intensity (positive and negative weights). For completeness, we visualised the uncorrected BOLD activation maps at $P_{uncorr} < 0.01$ in Figures S6 and S7. We again extracted the parameter estimates from the preregistered ROIs (**Figure 6B-D**) across both treatment conditions and estimated the LMER models with the interaction of exercise intensity and drug treatment on parameter estimates from the respective ROIs (**Figure 6E-G**). None of these models yielded a significant interaction effect of stimulus intensity and drug treatment (Tables S14-S16). Since we could not establish an overall hypoalgesic effect of exercise, our preregistered hypothesis of reducing the hypoalgesic effect through naloxone administration behaviourally and neuronally could not be confirmed.

Potential association of exercise-induced pain modulation with fitness level in the medial frontal cortex (mFC)

Although not preregistered, we additionally explored whether accounting for the different fitness levels of our participants in the statistical model can yield an exercise-induced analgesia effect. As a measure of fitness levels, we utilised the FTP (W/kg), and for ease of interpretation, we further used the difference scores between pain ratings after low (LI) and high (HI) exercise bouts (LI – HI exercise pain ratings). Positive difference scores indicate hypoalgesia (i.e. higher pain ratings after LI exercise) whereas negative difference scores indicate hyperalgesia (i.e. higher pain ratings after HI exercise). This exploratory analysis showed a significant main effect of fitness level on differences in pain ratings in the SAL condition ($\beta = 6.45$, $CI [1.25, 11.65]$, $SE = 2.56$, $t(38) = 2.52$, $P = 0.02$; Table S17) suggesting increased hypoalgesia with increasing fitness levels, pooled across all stimulus intensities (**Figure 7A**). In the brain, the corresponding contrast (exercise high > exercise low with the mean-centered covariate FTP (W/kg)) was also investigated in the SAL condition. Here we observed a significant activation of the medial frontal cortex (mFC; MNI_{xyz} : 6, 45, 10; $T = 4.59$, $P_{corr-SVC} = 0.05$; **Figure 7B**). The parameter estimates from this voxel revealed that an increase in fitness level was negatively associated with the difference scores between parameter estimates from the drug treatment conditions (**Figure 7C**). These results suggest that the extent and direction of exercise-induced pain modulation potentially depend on the individual fitness levels and might be mediated by the mFC. (See Figure S8 for pain ratings and parameter estimates separately for HI and LI exercise and Figure S9 for an uncorrected activation map at $P_{uncorr} < 0.001$).

Exploring the effect of sex, fitness level, and endogenous opioids on exercise-induced pain modulation along the mFC

As a second, not preregistered, exploratory analysis we tested for sex effects in exercise-induced pain modulation. This was motivated by our preceding analyses suggesting that males and females differ in the modulation of pain by NLX administration at different stimulus intensities (**Figure 4E**). We observed a significant interaction of sex, fitness level, and drug ($\beta = -13.12$, $CI [-23.69, -2.56]$, $SE = 5.42$, $t(190) = -2.43$, $P = 0.016$, **Figure 8A** and **Figure 8B**; Table S18). In the SAL condition, males showed larger hypoalgesia with increasing fitness levels (**Figure 8A**, red line) whereas females showed no hypoalgesic response regardless of fitness levels (**Figure 8A**, blue line). In the NLX condition, however, there was no significant association between fitness levels and difference in pain score in males (**Figure 8B**, red line) but a small positive correlation in females (**Figure 8B**, blue line). These results possibly hint at diverging pain modulatory mechanisms in males and females depending on their fitness level and drug treatment. In the next step, we investigated this interaction of fitness level, sex, and drug in the neuroimaging data, formally implemented as a two-sample (male vs. female) t -test for the interaction contrast between exercise intensity and drug with the subject covariate of individual FTP. This revealed significant activation in the mFC (MNI_{xyz} : 12, 64, 2; $T = 4.78$, $P_{corr-SVC} = 0.039$; **Figure 8C**). The parameter

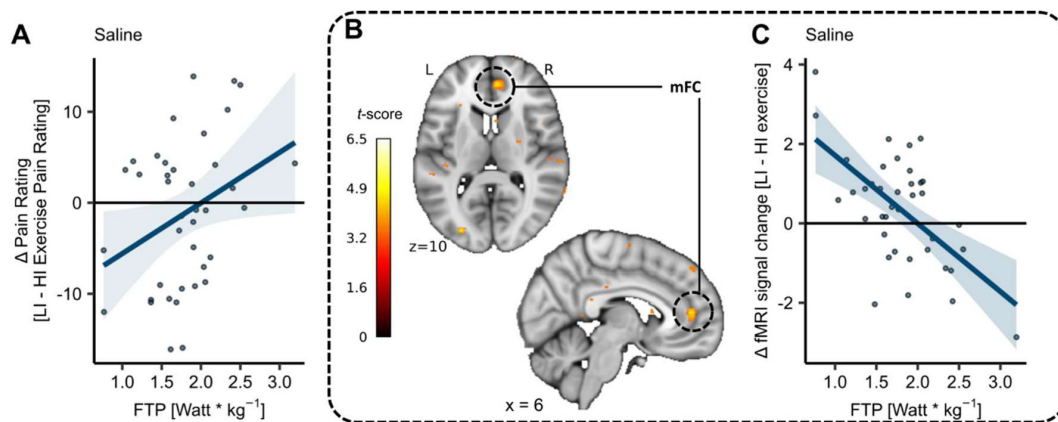


Figure 7.

Fitness level on difference pain ratings (LI-HI exercise) and mFC activation.

(A) Subject-specific differences in heat pain ratings (dots) between low-intensity (LI) and high-intensity (HI) exercise conditions (LI - HI exercise pain ratings) and corresponding regression line pooled across all stimulus intensities in the SAL condition. Fitness level (FTP) showed a significant positive relation to heat pain ratings with a significant main effect of FTP ($P = 0.02$) on difference ratings. (B) Cortical activation for contrast: exercise high > exercise low with mean-centred covariate FTP (weight-corrected) in the right mFC (MNI_{xyz}: 6, 45, 10; $T = 4.59$, $P_{corr-SVC} = 0.05$) across all stimulus intensities in the SAL condition superimposed onto an MNI template brain. (C) Differences between parameter estimates of LI and HI exercise conditions (LI - HI exercise parameter estimates) from respective peak voxel, plotted for each subject as a function of fitness level (FTP). Regression lines are visualised and shaded areas represent the SEM ($N = 39$).

estimates from the peak voxel are shown for both drug treatment conditions and both sexes in **Figures 8D** and **8E**. In the SAL condition, males showed a negative association of the difference (EIH) parameter estimates with fitness level (**Figure 8D**, red line), whereas females showed a positive association (**Figure 8D**, blue line). Importantly, this pattern was abolished under NLX (**Figure 8E**; see Figure S10 for an uncorrected activation map at $P_{uncorr} < 0.001$). Despite being an exploratory analysis, these findings suggest an interaction between sex and fitness level in opioidergic mechanisms that might mediate exercise-induced pain modulation.

Discussion

This double-blind cross-over study aimed to disentangle the neural and pharmacological mechanisms of exercise-induced pain modulation in healthy individuals of varying fitness levels. Our data did not support our preregistered hypothesis of a main effect of exercise-induced pain modulation after high-intensity exercise compared to low-intensity exercise in the saline (SAL) condition. What is more, naloxone (NLX) administration resulted in increased pain ratings across both exercise conditions but showed no interaction with exercise intensity. These results suggest that there is no overall effect of aerobic exercise on pain and, further, no direct involvement of the endogenous opioid system. In an exploratory analysis, we observed a significant interaction involving fitness level, sex, and drug administration where males exhibited greater hypoalgesia, particularly as fitness levels increased under the saline (SAL) condition. This effect was diminished upon administration of naloxone (NLX) and was also apparent in opioidergic regions of the pain modulatory system, namely the medial frontal cortex (mFC).

As expected, we were able to successfully induce heat pain at different stimulus intensities, mirrored by different levels of activation in the right anterior insula (antIns), right dorsal posterior insula (dpIns), and right midcingulate cortex (MCC). As part of a highly distributed pain processing system (Coghill, 2020; Tracey and Mantyh, 2007) the antIns has been established to encode pain intensity (Coghill, 2020) and salience (Wiech et al., 2010) whereas the dpIns has been shown to encode stimulus intensity and salience specific to heat pain (Horing et al., 2019; Segerdahl et al., 2015). The function of the MCC has been more equivocal since some studies attributed a domain-general function of integrating pain, negative affect, and cognitive control (Shackman et al., 2011) whereas others argued in favour of a domain-specific representation of painful stimulation (Kragel et al., 2018).

Opioidergic mechanisms in heat pain

Previous research has shown that heat pain is modulated by the descending pain modulatory system including the PAG (Borras et al., 2004; Petrovic et al., 2002) mainly through opioidergic mechanisms such as placebo analgesia (Eippert et al., 2009; Petrovic et al., 2002; Tinnermann et al., 2022). Accordingly, studies showed VAS ratings of intense heat stimuli to be increased after NLX administration (Borras et al., 2004; Kut et al., 2011), along with a significant modulation of BOLD responses in the PAG (Eippert et al., 2009). In line with this, we could show that NLX significantly increased perceived painfulness mirrored by activation in the PAG (Corder et al., 2018). An important aspect of our study is that the magnitude of opioidergic pain modulation increased with pain intensity in females, whereas males showed a constant, but high magnitude of pain modulation across stimulus intensities when NLX was administered. This finding suggests two things: firstly, endogenous pain modulation through μ -opioid receptors increases with increasing pain intensity and, secondly, the magnitude of this modulation differs between sexes. Since our study employed a within-subject cross-over design with an established pharmacological intervention (Leknes and Atlas, 2020; Trøstheim et al., 2022), we ensured our results were unbiased and highly robust.

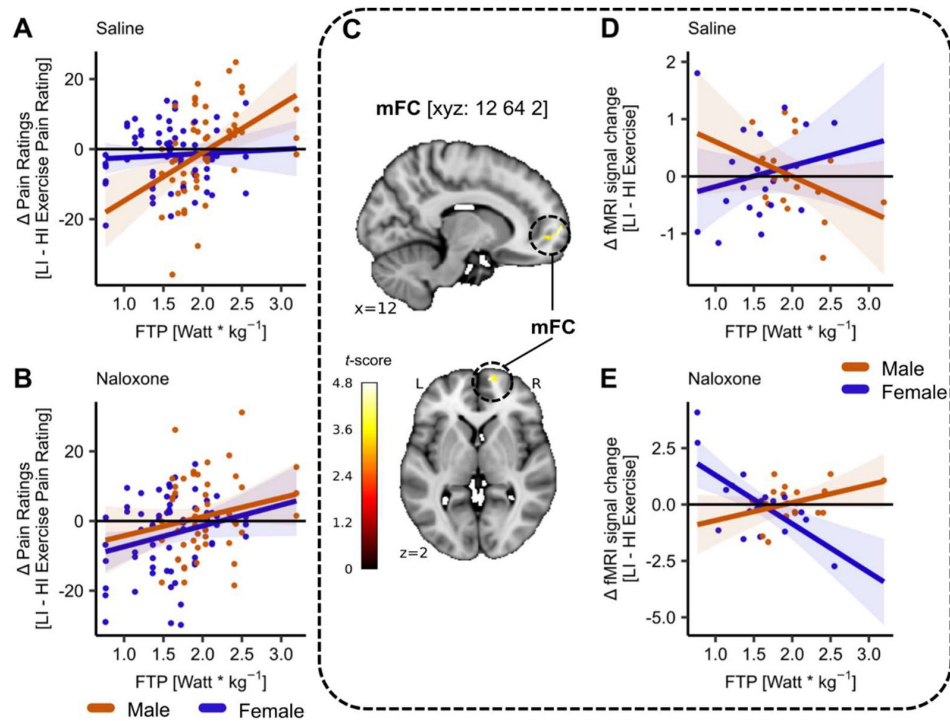


Figure 8.

Exercise-induced pain modulation potentially depends on sex, fitness level, and drug treatment in mFC.

(A) Exercise-induced pain modulation in the SAL condition for males (red) and females (blue). Males showed larger hypoalgesic responses with increasing fitness levels as indicated by positive difference ratings in the SAL condition. Females showed no association between fitness levels and difference ratings. (B) No exercise-induced pain modulation after blocking μ -opioid receptors with NLX in males (red) and females (blue). (C) The activation pattern in the mFC (MNI_{xyz}: 12, 64, 2; T = 4.78, $P_{corr-SVC}$ = 0.039) resulting from a two-sample t-test (two-tailed) between males and females for contrast interaction of exercise and drug with covariate FTP superimposed onto an MNI template brain. (D-E) The difference in parameter estimates from the peak voxel in the mFC in (D) SAL and (E) NLX condition for males (red) and females (blue). Each dot represents the difference in parameter estimates between LI and HI exercise conditions (LI - HI exercise) for each subject averaged across all stimulus intensities. Shaded areas represent the SEM (female: $N = 21$, male: $N = 18$).

No overall effect of aerobic exercise on pain modulation

We were unable to detect significant differences in pain ratings and BOLD signal between the exercise conditions (high- and low-intensity) in the SAL condition. Several factors might contribute to this null finding. For one, the exercise intensities employed in this study might not have been suitable to induce pain modulation. Previous research has suggested that high- intensity exercise produces greater hypoalgesia compared to low-intensity exercise (Jones et al., 2019 [↗](#); Vaegter et al., 2014 [↗](#)). What is more, previous studies (Vaegter et al., 2015 [↗](#), 2019 [↗](#)) have often employed a resting control condition, which somehow limits the comparability of exercise conditions since a resting baseline does not control for unspecific factors such as attentional load or distraction (Brooks et al., 2017 [↗](#); Sprenger et al., 2012 [↗](#)) through exercise. Thus, we opted for the control condition of low-intensity exercise instead of rest to also add to the knowledge of a dose-response relationship of exercise intensity needed to induce a hypoalgesic effect. We confirmed the intensities of both exercise conditions by physiological (heart rate (HR)) and behavioural measures (rating of perceived exertion (RPE)). Participants indicated significant differences in HR and RPE between both exercise conditions, confirming the actual and perceived distinction between the exercise conditions.

Another factor to consider is that our study utilised the FTP₂₀ test as a tool to determine the highest power output a cyclist can maintain in a quasi-steady state (Allen and Coggan, 2006 [↗](#)). It is frequently used in studies investigating athletes (Borszcz et al., 2020 [↗](#); Mackey and Horner, 2021 [↗](#); Mc Grath et al., 2019) and shows a high correlation with other measures such as the FTP₆₀ test ($r = 0.61$) (Borszcz et al., 2018 [↗](#)). Compellingly, the FTP₂₀ test does not rely on approximations or self-reports but on the actual power maintained (Allen and Coggan, 2006 [↗](#)). Previous reviews have shown that hypoalgesia following exercise is most reliably induced by moderate-high-intensity exercise (Koltyn, 2002 [↗](#); Naugle et al., 2012 [↗](#); Weweg and Jones, 2020 [↗](#)). Thus, we decided to employ a high-intensity exercise protocol using the FTP threshold as a reliable measure. Nevertheless, since our study is one of the first studies to employ this FTP₂₀ test with a non-athlete, balanced sample of varying fitness levels, one could argue that the FTP₂₀ test is not a reliable estimation of the high-intensity exercise condition. As mentioned above, the RPE confirmed a successful implementation of the exercise intervention at the anticipated intensities suggesting the FTP₂₀ test to be a reliable estimation of functional threshold power in our sample. Overall, we ensured the highest accuracy in determining the individual exercise intensities at high and low-intensity.

Furthermore, the composition of the sample, which included non-athletes with varying fitness levels and of both sexes, might have contributed to the missing overall effect of aerobic exercise on pain modulation. In human studies, many findings on exercise-induced pain modulation are derived from studies in athletes or physically active individuals showing increased hypoalgesia following exercise compared to subjects with lower fitness levels (Crombie et al., 2018 [↗](#); Geva and Defrin, 2013 [↗](#); Naugle and Riley, 2014 [↗](#); Schmitt et al., 2020 [↗](#)). This observation might be related to greater exercise-induced opioid release in structures such as the periaqueductal grey (PAG) or the frontal medial cortex (including the anterior cingulate cortex (ACC)) in fitter individuals (Saaniijoki et al., 2022 [↗](#); Sluka et al., 2018 [↗](#)). Furthermore, exercise-induced analgesia in rodents and humans has predominantly been studied in all-male samples (Koltyn et al., 2014 [↗](#); Mogil, 2012 [↗](#); Niesters et al., 2010 [↗](#)). Of those studies that have considered potential sex differences in exercise-induced pain modulation, some (Brellenthin et al., 2017 [↗](#); Koltyn et al., 2013 [↗](#); Naugle et al., 2014 [↗](#)) could not reveal a sex effect, whereas others (Koltyn, 2000 [↗](#); Sternberg et al., 2001 [↗](#); Vaegter et al., 2014 [↗](#)) revealed a stronger effect in females. This is in contrast to results in other domains, where opioid-dependent pain relief through conditioned pain modulation (Bulls et al., 2015 [↗](#); Ge et al., 2004 [↗](#); Granot et al., 2008 [↗](#)) or placebo analgesia (Aslaksen et al., 2011 [↗](#); Desai et al., 2022 [↗](#)) has been observed to be stronger in males. We deliberately did not employ professional athletes in this study but rather individuals of varying fitness levels of both sexes to make our results more applicable to the general population.

In the context of exercise-induced pain modulation, different types of pain have been investigated (i.e. thermal, pressure, and electrical) as well as different measurements of such (threshold, intensity, and tolerance) (for reviews see Koltyn, 2000 [\[1\]](#); Naugle et al., 2012 [\[2\]](#); Vaegter & Jones, 2020 [\[3\]](#)). For aerobic exercise, the highest effect sizes were reported for pressure pain (pain intensity: Cohen's $d = 0.69$) closely followed by heat pain (pain intensity: $d = 0.59$) (Naugle et al., 2012 [\[2\]](#)). Despite having successfully induced pressure pain using computer-controlled cuff pressure algometry, we were unable to detect significant changes in pain ratings for different exercise intensities. However, most of the studies employing pressure pain used handheld algometry exerting local pressure which can be prone to experimenter bias (Vaegter and Jones, 2020 [\[3\]](#)) and those results obtained might not apply to cuff pressure pain. Thermal heat pain was the other chosen method to induce pain and, as mentioned above, we were able to successfully induce heat pain at different stimulus intensities, mirrored by different levels of activation in the right antIns, right dpIns, and right MCC. Since our method to induce thermal heat pain corresponds to the method used in previous studies, the results of this pain modality might be more comparable to those of existing studies.

Exploring the effect of fitness level and sex on exercise-induced pain modulation

Although not preregistered, we identified pain decreases following high-intensity compared to low-intensity exercise to be associated with higher individual fitness levels in the SAL condition. In agreement with previous studies (Geva and Defrin, 2013 [\[4\]](#); Saanijoki et al., 2022 [\[5\]](#); Schmitt et al., 2020 [\[6\]](#); Tesarz et al., 2012 [\[7\]](#)), this suggests a dependence of exercise-induced pain modulation on individual fitness. Recent studies indirectly support this notion and observed higher self-reported physical activity levels to be associated with reduced temporal summation in heat pain (Naugle and Riley, 2014 [\[8\]](#)) and increased heat pain thresholds to be associated with individual fitness levels in males (Schmitt et al., 2020 [\[6\]](#)). In a larger cohort, an association between physical activity levels and pressure pain tolerance (Årnes et al., 2023 [\[9\]](#)) as well as cold pressor pain tolerance (Årnes et al., 2021 [\[10\]](#)) was observed. Along with our behavioural findings, we identified increased activation in the mFC after high-intensity exercise to be associated with higher fitness levels in the SAL condition. Previous research has reported increased activation in the rACC to be associated with opioid analgesia (Petrovic et al., 2002 [\[11\]](#); Tinnermann et al., 2022 [\[12\]](#)), thus implying opioidergic involvement of this structure in mediating exercise-induced pain modulation (Petrovic et al., 2002 [\[11\]](#)). Adding to this, the pregenual ACC showed decreased activation in response to painful heat following a 2-h run compared to before the run in trained male elite runners (Scheef et al., 2012 [\[13\]](#)). Furthermore, larger decreases in μ -opioid receptor binding in regions including the ACC, bilateral insula, and OFC in healthy males have been observed to be associated with higher self-reported physical fitness levels (Saanijoki et al., 2022 [\[5\]](#)). This finding could support observations showing that repeated exercise “trains” the mFC, which has a high μ -opioid receptor density (Corder et al., 2018 [\[14\]](#)) and thus enables exercise-induced pain modulation even in the context of brief bouts of high-intensity exercise.

Furthermore, we explored the interaction between sex, fitness level, and drug, where males exhibited larger hypoalgesia following high-intensity exercise with increasing fitness levels, which was attenuated by NLX. This pattern was not evident in females. Our brain data also showed this three-way interaction in the mFC. More precisely, in the SAL condition, males showed higher activation after high-intensity exercise compared to low-intensity exercise in association with higher fitness levels, and this pattern was reversed in the NLX condition. Females showed the reversed pattern to males in both, SAL and NLX, conditions. Since rodent and early human research have also predominantly employed male samples to avoid hormonal-related fluctuations (Mogil, 2012 [\[15\]](#); Mogil and Chanda, 2005 [\[16\]](#)), this might have led to an over-generalisation of opioidergic effects (Koltyn et al., 2014 [\[17\]](#); Mogil, 2012 [\[15\]](#)) whilst neglecting sex differences in opioid analgesia (Bodnar and Kest, 2010 [\[18\]](#); Niesters et al., 2010 [\[19\]](#)). Hence, it comes as no surprise that findings in humans are heterogeneous (Koltyn, 2000 [\[1\]](#); Wewege and Jones, 2020 [\[20\]](#)) with some

studies showing no difference in exercise-induced pain modulation between males and females (Koltyn et al., 2014 [↗](#), 2013 [↗](#); Smith, 2004 [↗](#)) whereas other studies showed hypoalgesia from exercise to be greater in women (Vaegter et al., 2014 [↗](#)) but most research on exercise-induced pain modulation has been conducted in men. The few existing imaging studies show that frontal midline structures (ACC, OFC) with high opioid receptor concentration are involved in exercise-induced (pain) modulation (Boecker et al., 2008 [↗](#); Saanijoki et al., 2022 [↗](#); Scheef et al., 2012 [↗](#)). A recent meta-analysis has shown that increases in the medial frontal cortex (including the vmPFC) activity along with reduced activation in regions associated with noxious stimuli (i.e. ACC) could be linked to an expectation of reduced pain, in this case, after high-intensity exercise (Atlas and Wager, 2014 [↗](#)). When comparing athletes and non-athletes, one study identified an interaction of sex and athletic status where female athletes displayed significantly lower cold pressor ratings than female non-athletes, but there was no difference in men (Smith, 2004 [↗](#)). These exploratory findings suggest sex-dependent and fitness level-dependent effects in exercise-induced pain modulation but do not provide a definite answer. Future research should further investigate the role of sex and fitness levels concerning exercise-induced hypoalgesia.

Limitations

One limitation that should be considered when interpreting the results is the influence of other mediating factors in exercise-induced pain modulation such as endocannabinoids (eCB; Crombie et al., 2018 [↗](#); Siebers et al., 2021 [↗](#)). Previous animal studies have established their crucial influence in modulating mood and promoting anxiolysis following exercise (Fuss et al., 2015 [↗](#)). In humans, however, the interpretability of results remains limited since only peripheral eCB can be measured and central eCB blockage is difficult.

In conclusion, our study showed no overall pain modulatory effects of aerobic exercise, neither behaviourally nor in brain responses. However, explorative analyses suggested that there is, in fact, an interaction between sex and fitness level in mediating exercise-induced pain modulation through opioidergic mechanisms in the mFC. To thoroughly investigate this, future research should specifically address the link between endogenous opioids, fitness levels, and sex-dependent differences in exercise-induced pain modulation.

Materials and Methods

The study was preregistered with the WHO-accredited *Deutsches Register für Klinische Studien* (DRKS; Study ID: DRKS00029064; German Registry for clinical studies) before data acquisition.

Experimental Design

This randomized control fMRI study employed a pharmacological intervention with the μ -opioid antagonist NLX in a within-subject design and was divided into a calibration and two experimental days (**Figure 1** [↗](#)). On the experimental days, participants cycled outside the MR for 10 minutes at high or low-intensity on a stationary bike. Afterward, they rated the perceived painfulness of heat and pressure stimuli inside the MR. This procedure was repeated for four runs per experimental day. The objective of this study was to identify the behavioural, pharmacological, and neuronal underpinnings of exercise-induced pain modulation.

Participants

Forty-eight healthy, right-handed participants were invited to take part in the study. In the framework of the within-subject design, all participants underwent all conditions which included an exercise factor (high vs. low exercise intensity) and a pharmacological intervention (NLX vs. SAL). All participants received the pharmacological intervention in a randomized double-blind fashion on separate study days, where the μ -opioid antagonist NLX or placebo SAL was

administered i.v. Our final sample size was determined based on previous behavioural studies investigating exercise-induced pain modulation after aerobic exercise in a pre-post measurement design across different pain modalities ($d = 0.59$ for heat pain intensity, $d = 0.69$ for pressure pain intensity) (Naugle et al., 2012 [↗](#)). According to this, a sample size of $N = 39$ was sufficient to reveal results at effect size $f = 0.3$, (via G*Power 3.1, $\alpha = .05$, $1 - \alpha = 0.95$, 1 group, 2 measurements with correlation $r = 0.5$). Based on previous fMRI studies it can also be assumed that a sample size of $N = 30$ would be sufficient to detect changes in the brain (Mumford, 2012 [↗](#)). In this study, the term “sex” refers to “sex assigned at birth” as indicated by self-reports from the participants. Multiple options (“male”, “female”, “other”, and free text field) were provided. All participants reported having been assigned the “female” or “male” sex at birth.

Exclusion of participants

Upon entering the study, participants were screened for anxiety (State and Trait questionnaires of the German short version of the State-Trait Anxiety Inventory; STAI) (Laux et al., 1981 [↗](#)), depression (Beck Depression Inventory 2; BDI-II) (Beck et al., 2011 [↗](#)) as well as potential MR contraindications. Furthermore, participants’ body mass index (BMI) was required to range between 18 and 30. On the first day of the study, an electrocardiogram (ECG; cardiofax, Electrocardiograph-1250, Nihon Koden) was recorded to prove no cardiovascular irregularities. The age of participants was between 18 and 45 years. Eight participants were excluded from the study for the following reasons. Two participants withdrew from the study after the first day due to circumstantial reasons (moving, no appointment found). Two participants were excluded after day one due to an unreliable thermal calibration and a BDI score below 18, respectively. Two participants did not wish to continue the study after day two due to personal reasons. Two participants were excluded after day two due to a syncope when administering the drug (in both cases SAL has been administered). Upon data screening, one subject was excluded due to excessive movement (> 0.6 mm difference between volumes within runs in more than 5% of volumes in all runs) in the MR scanner. A total of thirty-nine participants (Age: $M = 26.2$, $SD = 4.8$, 21 female) were included in the final sample (Table S19).

Ethics approval

The study was approved by the Ethics committee of the medical board in Hamburg (‘Aerztekammer’) and conducted following the Declaration of Helsinki (World Medical Association, 2013 [↗](#)). All subjects provided informed consent upon entering the study after having been informed about the study procedures including thermal and pressure stimulation, the MR procedure, the double-blind nature of the pharmacological intervention, and potential adverse effects of NLX by the study investigator and study physician. Upon completing or leaving the study, participants were informed of the order of pharmacological treatment received by the unblinded study physician.

Thermal and Pressure Stimuli

Thermal stimulation was applied using a thermode (TSA-2, Medoc, Israel) attached to the left lower arm where four 2.5×2.5 cm squares were drawn below each other and numbered (Figure 1A [↗](#)). For the calibration, the thermode head was positioned in the second square. Each thermal stimulus lasted up to 17 seconds in total with a plateau of 15 seconds and a ramp speed of 13°C/s (ramp-up, ramp-down). For the pressure calibration, a computer- controlled cuff pressure algometer (CPAR; NociTech, Denmark, and Aalborg University, Denmark) consisting of a compressor tube and 13 cm wide tourniquet cuff (VBM medical, Sulz, Germany, 61 cm length) was used. The cuff was mounted to the left upper arm with a 3 cm distance to the cubital fossa to exert pressure on the upper arm tissue (Graven-Nielsen et al., 2015 [↗](#)). Additionally, a protective tubular elastic dressing (Tricofix, D/5, $6 \text{ cm} \times 20 \text{ cm}$) was positioned underneath the cuff to protect the bare skin from potential harm. The pressure was applied by inflating the chambers of the cuff with a

maximal pressure limit of 100 kilopascal (kPa) and a rise speed dependent on the target pressure. The stimulus length of the pressure stimuli was also up to 17 seconds (ramp-up, 15s plateau, ramp-down).

Calibration Procedure (Day 1)

The first day of the study took place outside the MR scanner and served as a calibration day (**Figure 1A** [↗](#)). Blood pressure, baseline heart rate (HR), and oxygen saturation (SPO₂) were measured using a blood pressure cuff (boso medicus uno, bosch+sohn) and a pulse oximeter (pulox® Pusloximeter, Novidion GmbH), respectively. The thermal and pressure stimuli were calibrated individually but with an identical protocol in a pseudo-randomized order across participants. During calibration, participants remained in a supine position with the blinds down to emulate the inside of the MR scanner bore. As an initial step, participants received two low stimuli (10/20 kPa or 41°C/42°C) to get acquainted with the stimuli. Following this, the pain threshold was estimated by applying six adaptive stimuli which were rated on a binary scale (painful vs. not painful). The resulting pain threshold was used as a basis for the following linear regression algorithm aiming to collect ratings across different intensities on a Visual Analog Scale (10, 30, 50, 70, 90 VAS). After each stimulus, participants had to rate its painfulness ('How painful was the last stimulus?') on a VAS ranging from 0 (minimally painful) to 100 (almost unbearably painful) using the left and right key of a pointer as a button box (Logitech) with their right hand. The calibration was finished once sufficient rating coverage for all target intensities was reached. The thermal and pressure calibration aimed to identify super threshold intensities (°C and kPa) corresponding to 30, 50, and 70 on the VAS scale using this linear regression algorithm yielding comparable intensities across subjects.

Functional Threshold Power Test (Day 1)

As a final step of the calibration day, a functional threshold power (FTP) test was conducted on a stationary cycle ergometer (KICKR Bike, Wahoo, Atlanta, United States). The FTP test was first described by Allen and Coggan (2006) [↗](#) and determines the maximum average power output that can be maintained for one hour which serves as an approximation of the anaerobic threshold (Allen and Coggan, 2012 [↗](#)). The FTP₂₀ test is derived from the original FTP test but estimates 95% of FTP based on 20 minutes of steady cycling at maximum possible power output. The test is divided into six stages lasting one hour in total (**Figure 1A** [↗](#) and Table S1). Within this test protocol, the average power output measured for the last 20-minute interval is taken as 95% of the actual FTP (Allen and Coggan, 2012 [↗](#)). The FTP₂₀ Test allows the identification of individually calibrated power zones. In this present study, we used high-intensity exercise (Zone 4: Threshold, 91 -106% of FTP) and low-intensity exercise (Zone 1: Active recovery, ~55% of FTP) as intensities. During the FTP₂₀ test, participants were free to adjust the resistance of the ergometer whilst maintaining a constant cadence. Power output in Watt, heart rate (HR belt; Garmin® HRM-PRO™ PLUS) in beats per minute (bpm), and cadence (in RPM) were monitored throughout the FTP₂₀ test. The current power and HR as well as the remaining time of each section were displayed on a 50-by-30 cm screen in front of the participants to allow for self-monitoring. Participants were asked to refrain from any exhausting physical activities the day of and before the calibration day. The FTP value from the FTP₂₀ test was corrected for participants' weight (FTP/weight) and served as an indicator of individual fitness level in the analyses. The distribution of the weight-corrected FTP for males and females is visualized in Figure S11. There was no significant association between heat pain thresholds and weight-corrected FTP ($r = -0.23$, $P = 0.16$; Figure S12). On the bike, the knee angle was adjusted to a 5-degree bend when the pedal stroke was at the bottom (Vaegter et al., 2019 [↗](#)). The knee was also set to be over the pedal axle when the cranks were parallel to the ground. The upper body was adjusted to a forward lean with a slight bend of the elbows to maintain a neutral spine position with a natural lumbar flexion.

Experimental Paradigm (Day 2 and Day 3)

The two experimental days were at least three days apart (*Median* = 7 days, *Mean* = 12.35 days, *SD* = 14.5 days) and identical with the only difference being the pharmacological treatment (NLX vs. SAL) received (**Figure 1B**). Each visit took place in the morning with a starting time of 8:15 a.m. and varied by a maximum of four hours between participants to account for potential circadian influences. Blood pressure and SPO₂ were measured before each cycling block and monitored throughout the investigation. On each experimental day, a urine sample was tested for amphetamines, opiates, marijuana, methamphetamines, morphine (Surestep, Multidrug Test, Innovacon Inc., San Diego, USA), and potential pregnancies (only female participants; hCG Ultra Test, Serum/Urine 10mIU, Mexacare GmbH, Germany). Each experimental day consisted of 4 runs. Each run included a ten-minute cycling block at a high (100% FTP) or low intensity (55% FTP) in a pseudo-randomised order across participants which was immediately followed by an MR scan. On the second experimental day, an anatomical scan (T1) was acquired alongside conducting a re-calibration of thermal and pressure stimuli. The re-calibration was based on the previously calibrated intensities from the calibration day (Day 1) and was adjusted based on the ratings provided in the scanner corresponding to 30, 50, and 70 VAS. This procedure served to account for potential differences in perception due to the different environment of the MR scanner but also to acquaint participants with the procedure inside the MR to minimize the time of transition between the cycle ergometer and MR scanner.

Drug administration

The procedure of drug administration and dosage was based on a previous study using NLX (Eippert et al., 2009) as well as a web-based application for detailed planning of μ -opioid antagonist administration (Trøstheim et al., 2022). Participants were positioned in a supine position before administering a bolus dose of NLX (0.15 mg/kg; Naloxon-ratiopharm® 0.4 mg/ml injection solution, Ratiopharm, Ulm, Germany) or SAL (Isotone Kochsalz-Lösung 0.9% Braun) via peripheral venous access (PVA) in the right antecubital vein. To ensure correct administration, two participants received intravenous access on the back of their right hand upon giving verbal consent and ensuring no pain was caused by the location of the intravenous line. Shortly after administering the bolus dose, the intravenous infusion dose of NLX (0.2 mg/kg/h diluted in SAL) or SAL was started using an infusion pump (Perfusor® Space, Braun, Munich, Germany). After ensuring that no pain was caused by the intravenous line and that participants felt comfortable, the first cycling block commenced. The first 10-minute cycling block also served to ensure that NLX reached a peak level in the blood plasma (Trøstheim et al., 2022) before positioning participants in the MR scanner. The infusion dose was constantly running throughout the whole duration of the study, including cycling blocks outside as well as pain stimulation inside the MR, providing a constant supply of NLX or SAL. Considering the relatively short half-life of NLX (~70 minutes in blood plasma; Summary of Product Characteristics, Ratiopharm), this procedure ensures a steady-state concentration of NLX for the duration of the study (Trøstheim et al., 2022) with an effective central opioid block throughout the study (Eippert et al., 2009; Trøstheim et al., 2022). The experimenter (J.N.) and research assistants who interacted with the participants were blinded to the pharmacological intervention and were at no point in the study unblinded. The research assistant administering the drug treatment was also blind to the treatment. Only the study physician (T. F.), who did not interact with the participants on the experimental days of the study was unblinded as to what drug was administered. Unblinding of the participants took place after the experiment by the study physician without anyone else present to prevent expectation induction in the experimenter and student assistants. Before unblinding the participants, they were asked what treatment they received on which experimental day (incorrect: *n* = 4, unsure/identical: *n* = 19, correct: *n* = 16).

Questionnaires

On the calibration day (Day 1) participants completed the short form of the Profile of Mood States (POMS) (Curran et al., 1995 [↗](#)), STAI (Laux et al., 1981 [↗](#)), Becks Depression Inventory 2 (BDI-2) (Beck et al., 2011 [↗](#)), and a questionnaire on the expectation of exercise in psychological and physiological domains. Furthermore, participants' sleep quality, food intake, menstrual cycle phase, smoking, alcohol, and caffeine consumption on the study day and before the study days were assessed. The menstrual cycle phase was assessed by self-report on each experimental day where female participants indicated which phase applied to them (three phases: follicular, ovulatory, and luteal or hormonal contraceptives). Four female participants indicated they were on hormonal contraceptives. On both experimental days (Day 2 and Day 3) participants filled out the POMS before and after the study to monitor potential mood changes. Furthermore, participants were asked to fill out questionnaires including BDI, STAI, and a questionnaire concerning the side effects of NLX/SAL after both experimental days (Day 2 and Day 3). On the last experimental day (Day 3) participants had to indicate which pharmacological treatment they suspected to have received on which experimental day to capture potential expectations about the pharmacological treatment.

Data Acquisition

Behavioural Data Acquisition

During the cycling blocks, heart rate, power output (Watt), and cadence (RPM) were recorded using MATLAB 2021b. For each cycling block the required power as well as the actual power maintained by the participant were displayed on a screen to allow for self-monitoring. During cycling, participants' HR and SPO₂ were constantly monitored and recorded. In one participant recording of the power output failed and in five participants HR recordings in one session failed due to equipment issues. However, power output and HR maintained throughout the runs were visible and accessible at all times for the participant. Upon completion of the ten-minute cycling, participants were asked to rate their perceived exertion (PRE) on the BORG scale (no exertion (6) – maximal exertion (20)) (Borg, 1998 [↗](#)) as well as to indicate their current mood (sad (0) – happy (10)). The transfer to the MR scanner commenced as quickly as possible (Time: *Mean* = 5 min, *SD* = 1 min) whilst maintaining the participants' safety at all times. Inside the MR scanner, all stimulus presentation was realized using MATLAB R2016b and Psychophysics Toolbox (Version 3.0.19). Physiological data, including respiration and heart rate, was recorded at 1000 Hz with the Expression System (In Vivo, Gainesville, USA) using the spike2 software and a CED1401 system (Cambridge Electronic Design). A fixation cross and VAS scale were displayed on a screen inside the scanner bore. Thermal stimuli were applied using a TSA-2 Thermode with a 2.5x2.5 cm probe attached to the left volar forearm. Pressure stimuli were applied using a pressure cuff attached to the left upper arm and a CPAR outside the MR. The infusion pump was positioned outside the scanner with a ten-meter tube reaching into the scanner room to maintain a constant dose of NLX or SAL. After two pre-exposure stimuli of each modality, thermal and pressure stimuli were applied in an alternating fashion and at different calibrated intensities (randomized at 30, 50, 70 VAS). Each stimulus lasted 17 s in total (ramp-up, plateau 15 s, ramp-down) indicated by a visual cue (red fixation cross). Following each stimulus, the painfulness was rated on a VAS from 'minimally painful' (converted to 0 for analyses) to 'almost unbearably painful' (converted to 100 for analyses) by using a button box. The rating took place within 7 seconds after which an ITI of 7 seconds (jittered by 2 seconds) was introduced before commencing with the next trial. One MRI block consisted of nine thermal and nine pressure stimuli at three intensity levels (30, 50, 70 VAS). In total, participants spent 15 minutes per block inside the MR scanner.

MRI Data Acquisition

Functional magnetic resonance imaging (fMRI) data was acquired using a 3 Tesla Siemens system (Magnetom PRISMA; Siemens Healthcare, Erlangen, Germany) with a 64-channel head coil. Sixty slices were acquired (2 mm slice thickness) using T2*-weighted echo-planar imaging (EPI) with multiband factor 2 (repetition time (TR): 1.8 seconds; echo time (TE): 26ms; flip angle: 70°). The Field of View (FOV) was 240 mm and positioned to include the upper part of the medulla and the brainstem. The voxel size was thus 2 x 2 x 2 mm³. Four volumes at the beginning of each run were discarded. Furthermore, T1-weighted images were acquired for each subject using an MPRAGE sequence on the first experimental day (day 2). Due to the nature of the study where participants cycled outside the MR scanner and had to be repositioned for each run, shimming and auto-align took place at the beginning of each run and before acquiring EPI images.

Statistical Analyses

Behavioural Statistical Analyses

Behavioural statistical analyses were performed using MATLAB 2021b and RStudio (Version 2021.09.1). We used the *lmer* function from the *lme4* package (Version 1.1-35.1) (Bates et al., 2014) and the *lm* function from the *stats* package (Version 3.6.2) (*R: The R Project for Statistical Computing*) to conduct linear mixed effect (LMER) and linear models (LM) in R, respectively (Hox et al., 2010; Raudenbush and Bryk, 2001). See supplemental information (supplemental methods) for a detailed description of the statistical models used.

MRI Analyses Preprocessing

All MRI data was analysed using SPM12 (Wellcome Trust Centre for Neuroimaging, London, UK) and MATLAB 2021b (The Mathworks Inc. 2021b). The brain data was processed using a standardized SPM12-based pipeline (https://github.com/ChristianBuechel/spm_bids_pipeline.git) in BIDS format (Gorgolewski et al., 2016) to ensure data accessibility and reproducibility. The T1-weighted anatomical image was acquired on the first of the experimental days. The functional images from both experimental days were pre-processed together. After discarding the first four images of each run (dummy scans), the functional images were slice time corrected and realigned using rigid-body motion correction with six degrees of freedom. Next, a non-linear coregistration was performed using the mean EPI from the realignment and the T1 image. After this, the T1 image was normalized to MNI space (MNI152NLin2009cAsym as provided by CAT12 toolbox) using DARTEL. After this, transformation fields were created by combining the deformation field from the non-linear coregistration with the DARTEL flow fields to map the EPI images to T1 space and template space. A mask for the 1st-level GLMs was created based on the GM and WM from the T1 image and then warped into the EPI space and finally smoothed with a 3mm FWHM Gaussian kernel. After this, a second realignment took place but was constrained by a brain mask to minimize the effects of the eye movements. Finally, all EPI images were resliced in their individual space. Noise regressors for the first level analysis were created using six principal components of white matter responses and six principal components of CSF responses as well as a ROI at the posterior tip of the lateral ventricle (as used in (Horing et al., 2019)). As a final step, careful quality checks took place of all functional images generated after realignment, nonlinear co-registration, and normalization through visual inspection, focusing especially on the successful alignment across both fMRI sessions to allow for subsequent analyses.

First-level analyses

The single-subject analyses of the brain data were performed in native space using a General Linear model (GLM) approach. The design matrices for each subject comprised eight runs in total, each containing the stimulus onsets for heat and pressure stimuli at all three intensity levels (VAS

30, 50, 70) and a session constant. Additionally, the six motion parameters were augmented by their derivatives (12 parameters) and the squares of parameters and derivatives resulting in a total of 24 motion parameters (Friston et al., 1996 [↗](#)). An additional spike correction was performed, where individual volumes with voxels with a deviation of 0.6 mm between each volume with its preceding volume of each run were flagged and individually modeled. In addition, we included RETROICOR (Glover et al., 2000 [↗](#)) based physiological noise regressors to account for cardiac and respiratory-related motion (Brooks et al., 2008 [↗](#)). This technique determines cardiac and respiratory-related noise by allotting cardiac and respiratory phases to individual volumes within a time series. Subsequently, these assigned phases are utilized in a low-order Fourier expansion to generate time-course regressors elucidating signal variations attributed to cardiac or respiratory activities. A refined physiological noise model was applied, computing three cardiac and four respiratory harmonics, along with a multiplicative term incorporating interactions between cardiac and respiratory noise resulting in 18 regressors (Harvey et al., 2008 [↗](#)). These resulting noise regressors were included in the first-level analyses. The contrast images from the first level analysis were then spatially normalized to MNI space using individual deformation fields (i.e. combining nonlinear coregistration and DARTEL spatial normalization) and finally smoothed using a 6mm full width at half maximum (FWHM) Gaussian smoothing kernel. In addition to a hemodynamic response function (HRF) model, we also used a Finite Impulse Response model (FIR). This model was used to visualize the time course of BOLD responses after the onset of the pain stimulus. In this analysis, the stimulus duration was divided into 12 bins, each covering the duration of one TR spanning a total of 21.6 seconds after stimulus onset.

Second-level analyses

Subsequently, the spatially normalized and smoothed contrast images from the first-level analysis were used for random-effects group-level statistics. The successful application of heat pain was established by investigating the parametric contrast heat VAS 70 > 50 > 30, where higher stimulus intensities would recruit a more widespread (sub-)cortical pain network than lower intensities. To explore the effect of drug treatment in the brain, the contrast for the interaction of drug (SAL and NLX) and stimulus intensity (VAS 30, 50, 70) was calculated. Furthermore, we estimated the contrast heat NLX > heat SAL. To investigate the pain modulatory effect of exercise intensity in the SAL condition, the contrasts exercise high (SAL) > exercise low (SAL) and exercise low > high (SAL) were estimated. Furthermore, we extracted the parameter estimates from preregistered opioidergic ROIs (RVM, PAG, frontal midline) and estimated the LMER model corresponding to the behavioural analyses in the SAL condition. To confirm if NLX reduced the hypoalgesic effect, we estimated the contrasts interaction exercise intensity and drug treatment (positive and negative weight) as well as extracting the parameter estimates from the ROIs to conduct LMER models. To investigate the pain modulatory effect of exercise and fitness level in the SAL condition, the contrast exercise high (SAL) > exercise low (SAL) with the mean-centered covariate fitness level (weight-corrected FTP) was estimated. The parameter estimates from each condition were subtracted (LI exercise – HI exercise) and the difference in parameter estimates between both exercise conditions was visualised. To test the three-way interaction of fitness level, sex, and drug in the brain, a two-sample *t*-test of the contrast interaction exercise intensity and drug with the covariate fitness level has been conducted between males and females.

Correction for multiple comparisons

For whole-brain analyses, we used family-wise-error (FWE) rate as correction for multiple comparisons, and the according *P*-values are reported as $P_{corr-WB} = x.xx$. Furthermore, we used a small volume correction mask (SVC) to correct for multiple comparisons, and the corrected *P*-values are reported as $P_{corr-SVC} = x.xx$. This mask was based on the preregistered ROIs (rACC, vmPFC, PAG, RVM with an additional 1 mm FWHM Gaussian smoothing kernel (Figure S13 and Table S20). All thresholds for statistical significance were set to $P < 0.05$. Activation maps at an uncorrected level ($P_{uncorr} < 0.001$) for each contrast are reported in the supplement.

Data availability

The datasets for the raw behavioural and fMRI data generated in the current study are available from the corresponding author on reasonable request and after publication. All necessary data to evaluate the results of the study are included in the manuscript and supplementary materials.

Acknowledgements

We thank Marilyn Mintah and Marie-Sophie Morgenroth for their support with data acquisition. We also thank Alexandra Tinnermann for her helpful comments. Further, we thank the radiographers at the Department of Systems Neuroscience and Jürgen Finsterbusch for providing the MR protocol.

Additional information

Funding

C.B. and J.N. are supported by ERC-AdG-883892-PainPersist. C.B. is supported by DFG SFB 289 Project A02 (Project-ID 422744262–TRR 289).

Author Contributions

Conceptualization, C.B. and J.N.; Methodology, C.B. and J.N.; Investigation, C.B., J.N. and T.F.; Visualisation, J.N.; Writing – Original Draft, C.B. and J.N.; Funding Acquisition, C.B.; Resources, C.B.; Supervision, C.B.

Ethics

Human subjects: All participants gave informed written consent. The study was approved by the Ethics board of the Hamburg Medical Association (PV4932). We support inclusive, diverse, and equitable conduct of research.

Code availability

This study was programmed using MATLAB 2021b and Psychophysics Toolbox (Version 3.0.19). A custom fMRI preprocessing pipeline was used (https://github.com/ChristianBuechel/spm_bids_pipeline.git). The custom behavioural and fMRI analyses pipelines are available on the public repository https://github.com/jannenold/peep_analyses_mri_behavioural. For fMRI data visualisation, we used python-based software Nilearn (<https://nilearn.github.io/dev/index.html>) and an MNI template with 1mm smoothing (MNI152_T1_1mm_brain).

Additional files

Supplemental Information. *Supplemental Methods, Figures S1–S13, Tables S1–S22, and Supplemental References.*

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Reviewer #1 (Public review):**Summary:**

Participants in this study completed three visits. In the first, participants received experimental thermal stimulations which were calibrated to elicit three specific pain responses (30, 50, 70) on a 0-100 visual analogue scale (VAS). Experimental pressure stimulations were also calibrated at an intensity to the same three pain intensity responses. In the subsequent two visits, participants completed another pre-calibration check (Visit 2 of 3 only). Then, prior to the exercise NALOXONE or a SALINE placebo-control was administered intravenously. Participants then completed 1 of 4 blocks of HIGH (100%) or LOW (55%) intensity cycling which was tailored according to a functional threshold power (FTP) test completed in Visit 1. After each block of cycling lasting 10 minutes, participants entered an MRI scanner and were stimulated with the same thermal and pressure stimulations that corresponded to 30, 50, and 70 pain intensity ratings from the calibration stage. Therefore, this study ultimately sought to investigate whether aerobic exercise does indeed incur a hypoalgesia effect. More specifically, researchers tested the validity of the proposed endogenous pain modulation mechanism. Further investigation into whether the intensity of exercise had an effect on pain and the neurological activation of pain-related brain centres were also explored.

Results show that in the experimental visits (Visit 2 and 3), when participants exercised at two distinct intensities as intended. Power output, heart rate, and perceived effort ratings were higher during the HIGH versus LOW-intensity cycling. In particular, HIGH intensity exercise was perceived as "hard" / ~15 on the Borg (1974, 1998) scale, whereas LOW intensity exercise was perceived as "very light" / ~9 on the same scale.

The fMRI data from Figure 1 indicates that the anterior insula, dorsal posterior insula, and middle cingulate cortex show pronounced activation as stimulation intensity and subsequent pain responses increased, thus linking these brain regions with pain intensity and corroborating what many studies have shown before.

Results also showed that participants rated a higher pain intensity in the NALOXONE condition at all three stimulation intensities compared to the SALINE condition. Therefore, the expected effect of NALOXONE in this study seemed to occur whereby opioid receptors were "blocked" and thus resulted in higher pain ratings compared to a SALINE condition where opioid receptors were "not blocked". When accounting for participant sex, NALOXONE had negligible effects at lower experimental nociceptive stimulations for females compared to males who showed a hyperalgesia effect to NALOXONE at all stimulation intensities (peak effect at 50 VAS). Females did show a hyperalgesia effect at stimulation intensities corresponding to 50 and 70 VAS pain ratings. The fMRI data showed that the periaqueductal gray (PAG) showed increased activation in the NALOXONE versus SALINE condition at higher thermal stimulation intensities. The PAG is well-linked to endogenous pain modulation.

When assessing the effects of NALOXONE and SALINE after exercise, results showed no significant differences in subsequent pain intensity ratings.

When assessing the effect of aerobic exercise intensity on subsequent pain intensity ratings, authors suggested that aerobic exercise in the form of a continuous cycling exercise tailored to an individual's FTP is not effective at eliciting an exercise-induced hypoalgesia response - irrespective of exercise intensity. This is because results showed that pain responses did not differ significantly between HIGH and LOW intensity exercise with (NALOXONE) and without (SALINE) an opioid antagonist. Therefore, authors have also questioned the mechanisms (endogenous opioids) behind this effect.

Strengths:

Altogether, the paper is a great piece of work that has provided some truly useful insight into the neurological and perceptual mechanisms associated with pain and exercise-induced hypoalgesia. The authors have gone to great lengths to delve into their research question(s) and their methodological approach is relatively sound. The study has incorporated effective pseudo-randomisation and conducted a rigorous set of statistical analyses to account for as many confounds as possible. I will particularly credit the authors on their analysis which explores the impact of sex and female participants' stage of menses on the study outcomes. It would be particularly interesting for future work to pursue some of these lines of research which investigate the differences in the endogenous opioid mechanism between sexes and the added interaction of stage of menses or training status.

There are certainly many other areas that this article contributes to the literature due to the depth of methods the research team has used. For example, the authors provide much insight into: the impact of exercise intensity on the exercise-induced hypoalgesia effect; the impact of sex on the endogenous opioid modulation mechanism; and the impact of exercise intensity on the neurological indices associated with endogenous pain modulation and pain processing. All of which, the researchers should be credited for due to the time and effort they have spent completing this study. Indeed, their in-depth analysis of many of these areas provides ample support for the claims they make in relation to these specific questions. As such, I consider their evidence concerning the fMRI data to be very convincing (and interesting).

Weaknesses:

Although the authors have their own view of their results, I do however, have a slightly different take on what the post-exercise pain ratings seem to show and its implications for judging whether an exercise-induced hypoalgesia effect is present or not. From what I have read, I cannot seem to find whether the authors have compared the post-exercise pain ratings against any data that was collected pre-exercise/at rest or as part of the calibration. Instead, I believe the authors have only compared post-exercise pain ratings against one another (i.e., HIGH versus LOW, NALOXONE versus SALINE). In doing so, I think the authors cannot fully assume that there is no exercise-induced hypoalgesia effect as there is no true control comparison (a no-exercise condition).

In more detail, Figure 6A appears to show an average of all pain ratings combined per participant (is this correct?). As participants were exposed to stimulations expected to elicit a 30, 50, or 70 VAS rating based on pre-calibration values, therefore the average rating would be expected to be around 50. What Figure 6A shows is that in the SALINE condition, average pain ratings are in fact ~10-15 units lower (~35) and then in the NALOXONE condition, average pain ratings are ~5 units lower (~45) for both exercise intensities. From this, I would surmise the following:

It appears there is an exercise-induced hypoalgesia effect as average pain ratings are ~30% lower than pre-calibrated/resting pain ratings within the SALINE condition at the same temperature of stimulation (it would also be interesting to see if this effect occurred for the pressure pain).

It appears there is evidence for the endogenous opioid mechanism as the NALOXONE condition demonstrates a minimal hypoalgesia effect after exercise. I.e., NALOXONE indeed blocked the opioid receptors, and such inhibition prevented the endogenous opioid system from taking effect.

It appears there is no effect of exercise intensity on the exercise-induced hypoalgesia effect. That is, participants can cycle at a moderate intensity (55% FTP) and incur the same hypoalgesia benefits as cycling at an intensity that demarcates the boundary between heavy and severe intensity exercise (100%FTP). This is a great finding in my mind as anyone

wishing to reduce pain can do so without having to engage in exercise that is too effortful/intense and therefore aversive - great news! This likely has many applications within the field of public health.

I will very slightly caveat my summaries with the fact that a more ideal comparison here would be a control condition whereby participants did the same experimental visit but without any exercise prior to entering the MRI scanner. I consider the overall strength of the evidence to be solid, with the answer to the primary research question still a little ambiguous.

<https://doi.org/10.7554/eLife.102392.1.sa1>

Reviewer #2 (Public review):

Summary:

This interesting study compared two different intensities of aerobic exercise (low-intensity, high-intensity) and their efficacy in inducing a hypoalgesic reaction (i.e. exercise-induced hypoalgesia; EIH). fMRI was used to identify signal changes in the brain, with the infusion of naloxone used to identify hypoalgesia mechanisms. No differences were found in post-exercise pain perception between the high-intensity and low-intensity conditions, with naloxone infusion causing increased pain perception across both conditions which was mirrored by activation in the medial frontal cortex (identified by fMRI). However, the primary conclusion made in this manuscript (i.e. that aerobic exercise has no overall effect on pain in a mixed population sample) cannot be supported by this study design, because the methodology did not include a baseline (i.e. pain perception following no exercise) to compare high/low-intensity exercise against. Therefore, some of the statements/implications of the findings made in this manuscript need to be very carefully assessed.

Strengths:

- (1) The use of fMRI and naloxone provides a strong approach by which to identify possible mechanisms of EIH.
- (2) The infusion of naloxone to maintain a stable concentration helps to ensure a consistent effect and that the time course of the protocol won't affect the consistency of changes in pain perception.
- (3) The manipulation checks (differences in intensity of exercise, appropriate pain induction) are approached in a systematic way.
- (4) Whilst the exploratory analyses relating to the interactions for fitness level and sex were not reported in the study pre-registration, they do provide some interesting findings which should be explored further.

Weaknesses:

- (1) Given that there is no baseline/control condition, it cannot be concluded that aerobic exercise has no effect on pain modulation because that comparison has not been made (i.e. pain perception at 'baseline' has not been compared with pain perception after high/low-intensity exercise). Some of the primary findings/conclusions throughout the manuscript state that there is 'No overall effect of aerobic exercise on pain modulation', but this cannot be concluded.
- (2) Across the manuscript, a number of terms are used interchangeably (and applied, it seems, incorrectly) which makes the interpretation of the manuscript difficult (e.g. how the author's use the term 'exercise-induced pain').

(3) There is a lack of clarity on the interventions used in the methods, for example, it is not exactly clear the time and order in which the exercise tasks were implemented.

(4) The exercise test (functional threshold power) used to set the intensity of the low/high exercise bouts is not an accurate means of demarcating steady state and non-steady state exercise. As a result, at the intensity selected for the high-intensity exercise in this study, it is likely that the challenge presented for the high-intensity exercise would have been very different between participants (e.g. some would have been in the 'heavy' domain, whereas others would be in the 'severe' domain).

(5) It is likely that participants did not properly understand how to use the 6-20 Borg scale to rate their perceived effort, and so caution must be taken in how this RPE data is used/interpreted.

(6) Although interesting, the secondary analyses (relating to the interaction effects of fitness level and sex) were not included in the study pre-registration, and so the study was not designed to undertake this analysis. These findings should be taken with caution.

<https://doi.org/10.7554/eLife.102392.1.sa0>