Exercise-induced pain modulation is sex, opioid, and fitness-dependent and mediated by the medial frontal cortex

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Abstract

Exercise leads to a release of endogenous opioids, potentially resulting in pain relief. However, the neurobiological underpinnings of this effect remain unclear, and studies in rodents and humans have mainly investigated this in male subjects. Using a pharmacological within-subject fMRI study with the opioid antagonist naloxone and different levels of exercise and pain we investigated exercise-induced hypoalgesia in a balanced sample (N = 39, 21 female). Overall, we were unable to detect exercise-induced pain modulation after exercise in heat pain. However, our data reveal a crucial interplay of drug, fitness level, and sex where males showed greater hypoalgesia through 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 exercise exerts a sex and fitness-dependent hypoalgesic effect mediated by endogenous opioids and the medial frontal cortex.

Keywords: Exercise, Pain, Fitness, Sex Differences, Opioids, fMRI, Hypoalgesia

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).

In particular, two factors are thought to potentially contribute to these diverging results on the pain-relieving effects of exercise. Firstly, only a limited number of studies could investigate potential sex differences in opioid or exercise-induced analgesia or studied 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 show a sex effect, whereas some (Koltyn, 2000; Sternberg et al., 2001; Vaegter et al., 2014) revealed a stronger effect in females, which contrasts to results in other domains, where opioid-dependent pain relief (e.g. 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.

Another potential factor influencing exercise-induced pain modulation is the individual fitness level (Sluka et al., 2018). 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). Intriguingly, 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 (Saanijoki et al., 2022; Sluka et al., 2018).

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)), limiting the interpretation of results (Naugle et al., 2012; Wewege and Jones, 2020). In addition, studies investigating the pharmacological underpinnings of exercise-induced pain modulation have employed very different interventions (i.e., different doses, 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).

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, p. 20), which is congruent with early imaging studies (Boecker et al., 2024, 2008; Geisler et al., 2019; Saanijoki et al., 2018; Scheef et al., 2012). Previous rodent studies have further emphasised sex differences in pain processing (for reviews see (Mogil, 2020, 2012)) and analgesic sensitivity (Martin et al., 2019; Mogil, 2020) warranting further investigation in humans (Mogil, 2020).

83 In this study, we examined exercise-induced pain modulation in a healthy, balanced
84 sample of both sexes and diverse fitness levels with a particular emphasis on the
85 opioidergic system. This preregistered study (drks.de: DRKS00029064) comprised an
86 initial calibration day and two experimental days (Figure 1). On the calibration day,
87 individual exercise intensities, pain intensities, as well as objectively assessed fitness
88 levels (functional threshold power; FTP_{20}) (40), were determined (Figure 1A). On each of
89 the two experimental days, participants cycled during four blocks at high (100% FTP) and
90 low (55% FTP; active recovery) intensity for 10 minutes. After each cycling block,
91 functional Magnetic Resonance Imaging (fMRI) commenced, where participants received
92 nine painful heat stimuli with a 15-second plateau each and rated their painfulness on a
93 visual analog scale (VAS) ranging between the calibrated pain threshold ('minimally
94 painful') and pain tolerance ('almost unbearably painful'). Furthermore, we employed a
95 double-blind cross-over pharmacological challenge across experimental days, where either
96 saline (placebo; SAL) or the μ-opioid antagonist naloxone (NLX) was administered
97 intravenously (see Methods for details) (Figure 1B).

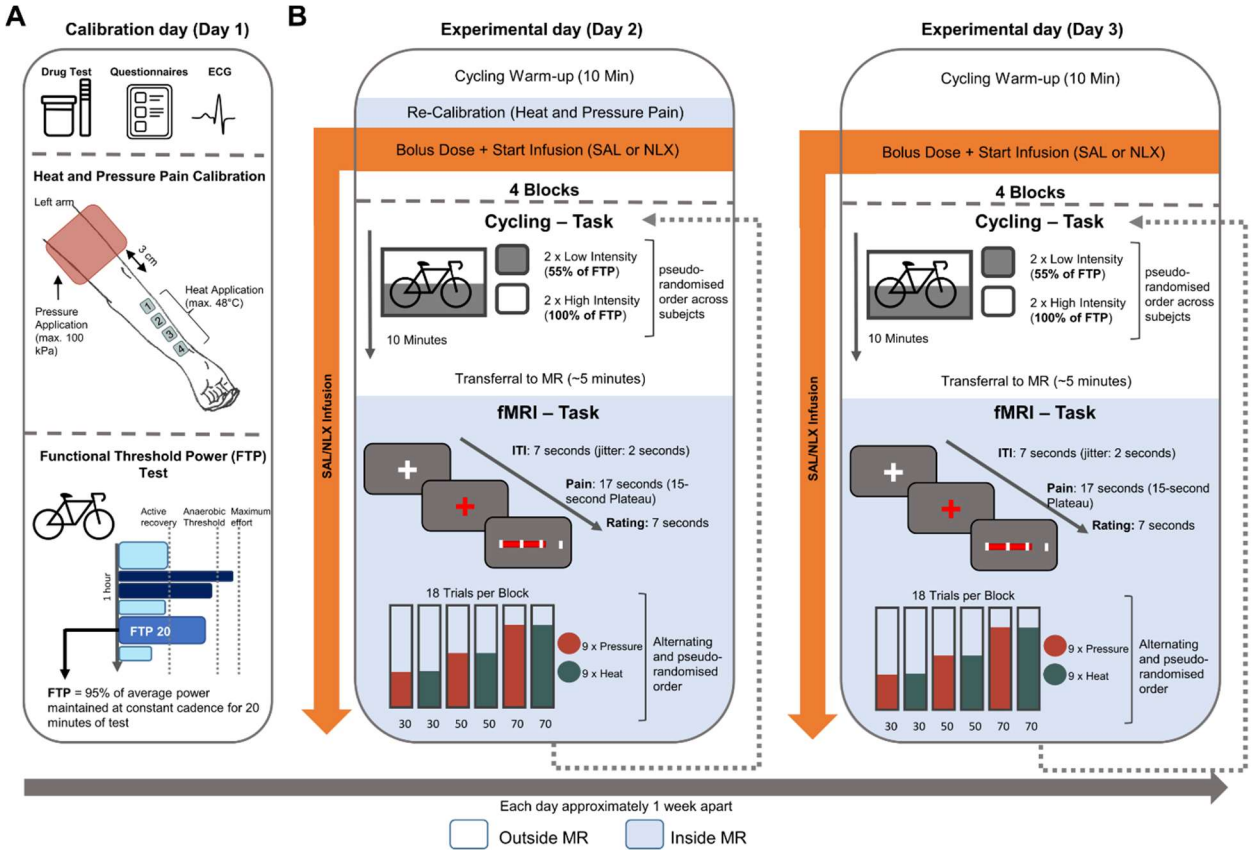

Figure 1. Experimental design. (**A**) Calibration day (Day 1) with heat and pressure pain calibration and Functional Threshold Power test (FTP) (see Table S1). (**B**) Experimental days (Day 2 and Day 3) with cycling task (outside MR) and fMRI task (inside MR) with the only difference in the drug treatment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

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 calibrated heat in the placebo (saline; SAL) condition. A significant main effect of stimulus intensity on heat pain ratings was observed (β = 1.42, CI [1.37,1.48], SE = 0.03, t(1358.65)= 51.84, $P < 2\times10^{-16}$; 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, $P_{corr\text{-}WB} < 0.001$), right dorsal posterior Insula (dpIns; Figure 2C; MNI_{xyz}: 39, -15, 18, T = 7.65, $P_{corr\text{-}WB} < 0.001$) and right middle cingulate cortex (MCC; Figure 2D; MNI_{xyz}: 6, 10, 39, T = 7.47, $P_{corr\text{-}WB} = 0.001$). The parameter estimates from the respective peak voxels mirrored the behavioural response pattern (Figure 2). 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.

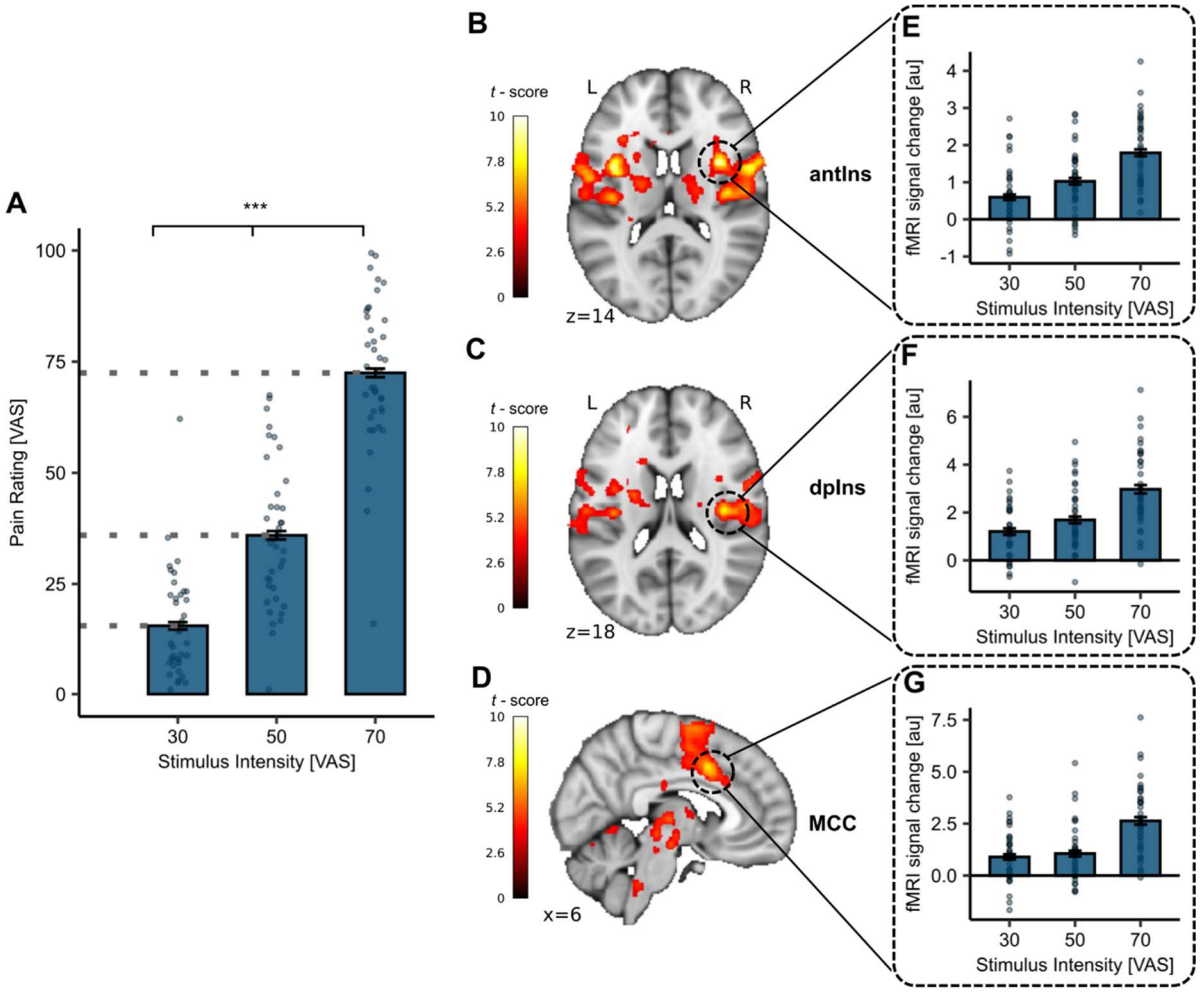

Figure 2. Behavioural and fMRI results for successful pain induction. (**A**) Heat pain ratings in the SAL condition for all stimulus intensities (VAS 30, 50, 70) showed a significant main effect of stimulus intensity ($P < 2\times10^{-16}$). The *P*-value reflects the significant main effect of stimulus 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, $P_{corr\text{-}WB} < 0.001$), (C) right dpIns ($MNI_{xyz}$: 39, -15, 18, T = 7.65, $P_{corr\text{-}WB} < 0.001$) and (D) right MCC ($MNI_{xyz}$: 6, 10, 39; T = 7.47, $P_{corr\text{-}WB} = 0.001$). 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 ($N = 39$).

146 **Implementation of exercise intervention**

147 As a next step, we aimed to confirm the successful implementation of our exercise
148 intervention. This intervention comprised previously calibrated high-intensity (HI) and
149 low-intensity (LI) exercise conditions, where the anticipated intensities were derived from
150 the individual functional threshold power (FTP) value determined on Day 1 (Figure 1A).
151 The anticipated power during HI power was set to 100% whereas LI power was set to 55%
152 of FTP. As intended, the maintained power during cycling was significantly higher in the
153 HI exercise condition compared to the LI exercise condition ($t(37) = 16.09$, $P < 2.2\times10^{-16}$,
154 mean $\text{power}_{\text{diff}} = 60.26$W, $d = 0.61$, $n = 38$; Figure 3A). Supporting this, the mean absolute
155 heart rate was significantly higher in the HI exercise condition compared to the LI exercise
156 condition ($t(33) = 20.48$, $P < 2.2\times10^{-16}$, mean $\text{bpm}_{\text{diff}} = 26.92$, $d = 1.85$, $n = 34$, Figure
157 3B). In one participant the power recording was incomplete ($n = 38$) and five participants
158 had faulty HR recordings in one session ($n = 34$) due to an instrumentation error.
159 Nevertheless, the actual power and HR maintained throughout the blocks were also
160 visually monitored. Furthermore, participants rated their perceived level of exertion (RPE)
161 on a BORG scale from 6 to 20 following each exercise block. The mean RPE across all HI
162 and LI exercise blocks was also significantly higher after HI compared to LI exercise
163 ($t(38) = 18.84$, $P < 2.2\times10^{-16}$, mean $\text{RPE}_{\text{diff}} = 6.46$, $d = 3.64$, $n = 39$; Figure 3C). Thus, the
164 calibration and implementation of the exercise intensities was successful.

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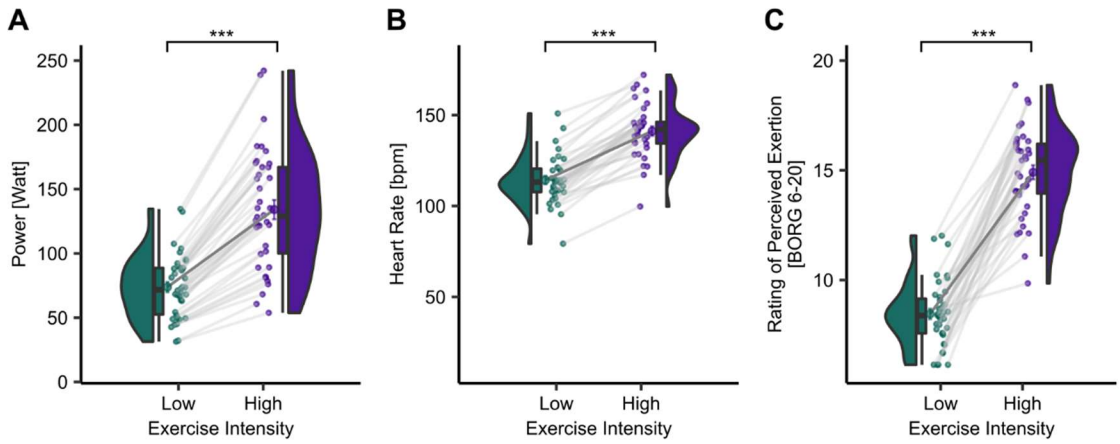

Figure 3. Successful implementation of high (HI) and low-intensity (LI) exercise. (**A**) Power output (Watt), (**B**) heart rate in beats per minute (bpm), and (**C**) rating of perceived exertion (RPE; BORG scale) during low (LI; green) and high intensity (HI; purple) cycling were all significantly 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.

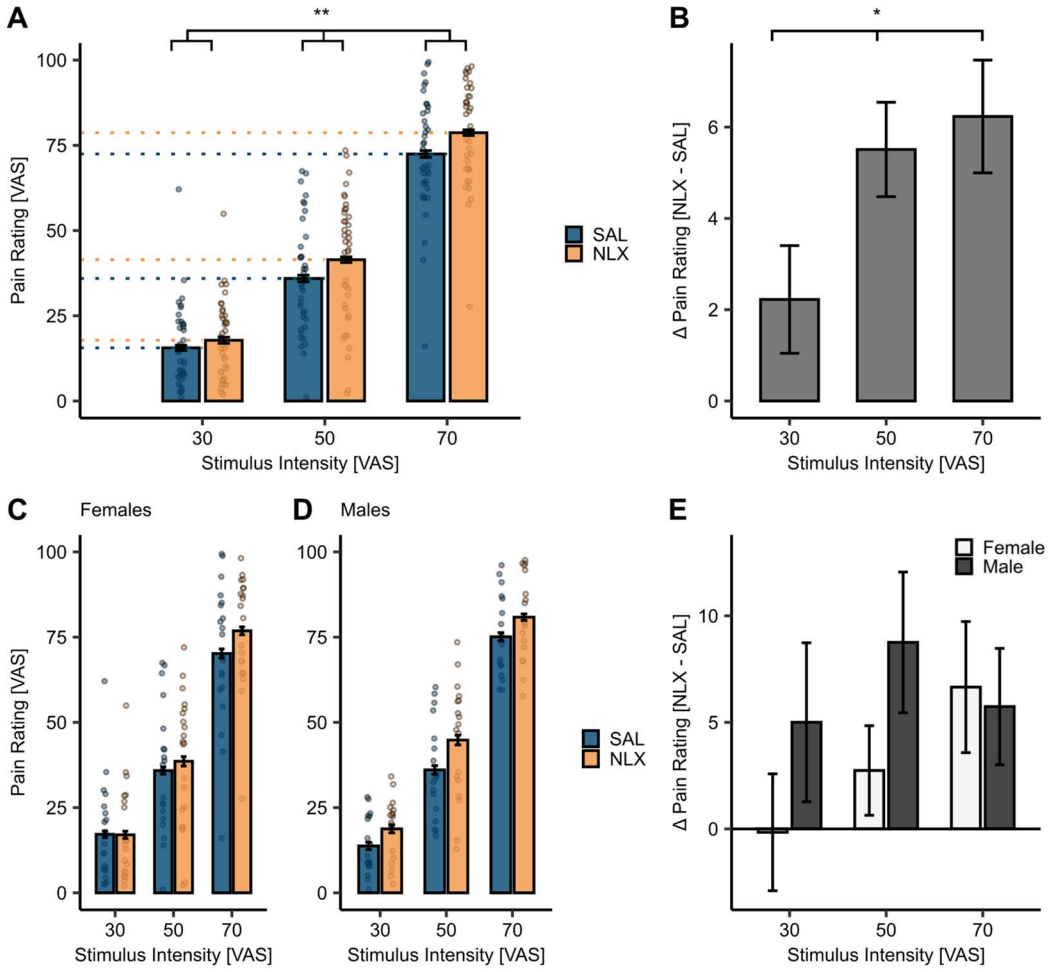

Figure 4. Behavioural results for the effect of drug treatment on pain. (**A**) Heat pain ratings revealed a significant interaction between drug and stimulus intensity ($P = 0.01$). Mean heat pain ratings were significantly higher under naloxone NLX treatment (orange) compared to placebo (SAL) (blue) across stimulus intensities. The *P*-value indicates a significant interaction effect of

198 stimulus intensity and drug. (**B**) Differences in heat pain ratings between NLX and SAL condition
199 (NLX – SAL) at each stimulus intensity revealed a significant main effect of stimulus intensity (P
200 = 0.02). (**C-D**) Heat pain ratings at all stimulus intensities in both drug treatment conditions are
201 significantly higher in NLX (orange) compared to SAL (blue) conditions for both sexes. (**E**)
202 Differences in heat pain ratings between NLX and SAL condition (NLX – SAL) at each stimulus
203 intensity for females (white) and males (dark grey) revealed a significant main effect of stimulus
204 intensity ($P = 0.003$) and sex ($P = 0.03$) with an interaction showing a trend ($P = 0.06$). P-values
205 were calculated using the LMER model for the interaction of stimulus intensity and drug. * $P <$
206 0.05, **, $P < 0.01$, *** $P < 0.001$. Error bars depict the SEM ($N = 39$).
207
208 In the next step, we identified brain regions associated with pain processing and
209 opioidergic pain modulation. We observed a significant activation (contrast interaction of
210 stimulus intensity and drug treatment) in the PAG (MNI_{xyz}: -2, -24, -8, T = 5.17, $P_{corr\text{-}SVC} =$
211 0.01; Figure 5A). The extracted parameter estimates from the peak voxel showed that
212 NLX increased activation compared to SAL, especially for the highest stimulus intensity
213 (Figure 5B and 5C). We used post-stimulus averaging (Finite Impulse Response or FIR
214 model) to extract the mean time course for the highest-intensity stimulus (VAS 70). A
215 significant difference in activation was evident seven seconds after stimulus onset (BOLD
216 response) for the remainder of the stimulus in the peak voxel in the PAG (Figure 5D; for
217 an uncorrected map at $P_{uncorr} < 0.001$ see Figure S2). We also investigated the contrast
218 heat NLX > heat SAL where activation in the right antIns showed a trend at whole-brain
219 correction (MNI_{xyz}: 46, 8, 6, T = 5.70, $P_{corr\text{-}WB} = 0.08$; Figure S3).
220

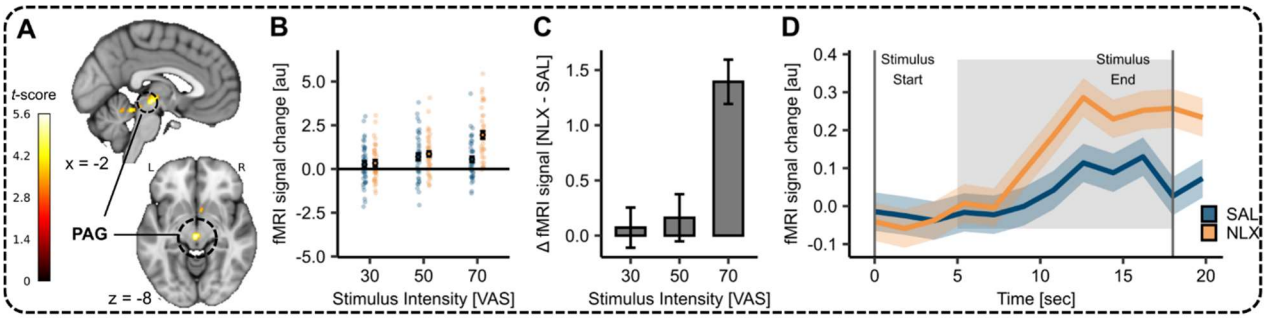

Figure 5. Effect of NLX in the periaqueductal grey (PAG). (**A**) Activation for the interaction of stimulus intensity and drug in the PAG (MNI_{xyz}: -2, -24, -8, T = 5.17, $P_{corr\text{-}SVC} = 0.01$) superimposed onto an MNI template brain. (**B**) Parameter estimates for SAL (blue) and NLX (orange) 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.

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

After we established a successful exercise and drug intervention, in the next step we investigated the modulating effect of exercise on pain and neuronal responses to pain regardless of the drug treatment. 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, $t(1354) = 0.77$, $P = 0.44$, Table S9). Furthermore, there were no significant differences in BOLD activation between both exercise conditions (contrast exercise high > low (SAL) or exercise low > exercise high (SAL). However, since participants in our study differed substantially in their fitness levels as indicated by their FTP (W/kg), we investigated this in our next analysis. For ease of interpretation, we used

the difference scores between pain ratings after LI and HI exercise bouts (LI – HI exercise pain ratings). Positive difference scores indicate hypoalgesia whereas negative difference scores indicate hyperalgesia. This analysis showed a significant main effect of fitness level on differences in pain ratings in the SAL condition (β = 6.45, *CI* [1.25, 11.65], *SE* = 2.56, $t(38) = 2.52$, $P = 0.02$; Table S10) indicating increased hypoalgesia with increasing fitness levels, pooled across all stimulus intensities (Figure 6A). 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, $P_{corr\text{-}SVC} = 0.05$; Figure 6B). 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 6C). These results suggest that the extent and direction of exercise-induced pain modulation depend on the individual fitness levels and is mediated by the mFC. (See Figure S4 for pain ratings and parameter estimates separately for HI and LI exercise and Figure S5 for an uncorrected activation map at $P_{uncorr} < 0.001$).

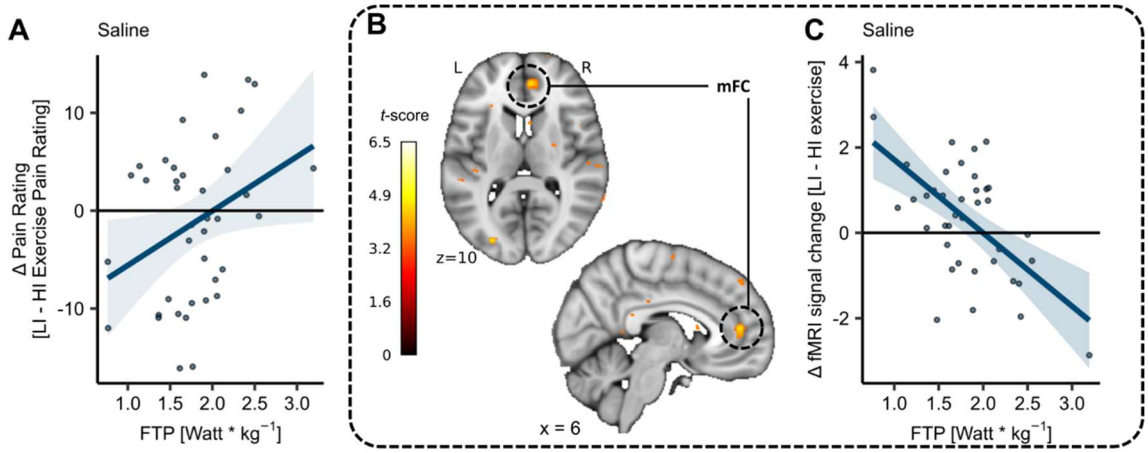

Figure 6. Fitness level on difference pain ratings (LI-HI exercise) and mFC activation. (**A**) Subject-specific differences in heat pain ratings (dots) between low-intensity (LI) and high-

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, $P_{corr\text{-}SVC} = 0.05$) 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 ($N = 39$).

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

Since our preceding analyses revealed that pain modulatory effects of exercise depend not only on fitness level but further suggest that males and females differ in their magnitude of pain perception in response to NLX administration at different stimulus intensities (Figure 4E), in the next step, we tested for sex effects in exercise-induced pain modulation. A significant interaction of sex, fitness level, and drug was observed ($\beta = -13.12$, *CI* [-23.69, -2.56], $SE = 5.42$, $t(190) = -2.43$, $P = 0.016$, Figure 7A and Figure 7B; Table S11). In the SAL condition, males showed larger hypoalgesia with increasing fitness levels (Figure 7A, red line) whereas females showed no hypoalgesic response regardless of fitness levels (Figure 7A, blue line). In the NLX condition, however, there was no significant association between fitness levels and difference in pain score in males (Figure 7B, red line) but a small positive correlation in females (Figure 7B, blue line). These results indicate 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 a significant activation in the mFC (MNI_{xyz}: 12, 64, 2; T = 4.78, $P_{corr\text{-}SVC} = 0.039$; Figure 7C). The parameter estimates from the peak voxel are shown for both drug treatment conditions and both sexes in Figures 7D and 7E. In the SAL condition, males showed a negative association of the difference (EIH) parameter estimates with fitness level (Figure 7D, red line), whereas females showed a positive association (Figure 7D, blue line). Importantly, this pattern was abolished under NLX (Figure 7E; see Figure S6 for an uncorrected activation map at $P_{uncorr} < 0.001$). Overall, these findings strongly demonstrate an interaction between sex and fitness level in opioidergic mechanisms that mediate exercise-induced pain modulation.

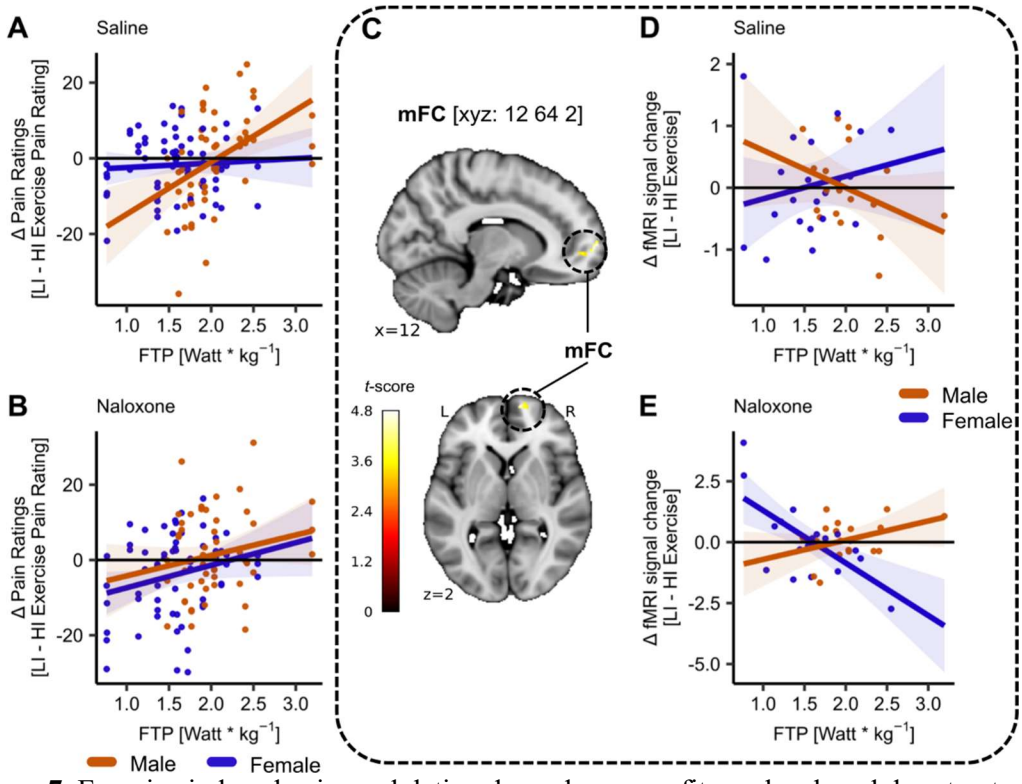

Figure 7. Exercise-induced pain modulation depends on sex, fitness level, and drug treatment in mFC. (**A**) Exercise-induced pain modulation in the SAL condition for males (red) and females (blue). Males showed larger hypoalgesic responses with increasing fitness levels as indicated by positive difference 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, $P_{corr\text{-}SVC} = 0.039$) 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**) 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: $N = 18$).

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 initial hypothesis of a main effect of exercise-induced pain modulation after high-intensity (HI) exercise compared to low-intensity (LI) exercise. However, we observed a significant interaction involving fitness level, sex, and drug administration. Males exhibited greater hypoalgesia after high-intensity compared to low-intensity exercise, particularly as fitness levels increased under the saline (SAL) condition. This effect was diminished upon administration of naloxone (NLX). This effect was not evident in females. This interaction was also apparent in opioidergic regions of the pain modulatory system, namely the mFC. Our results suggest that the endogenous opioid system plays a role in modulating behavioral and cortical pain responses to exercise through regions of the pain-modulatory system. Notably, our results emphasize the significance of individual fitness level and sex in influencing this modulation.

329 As expected, we were able to successfully induce heat pain at different stimulus
330 intensities, mirrored by different levels of activation in the right antIns, right dpIns, and
331 right MCC. As part of a highly distributed pain processing system (Coghill, 2020; Tracey
332 and Mantyh, 2007) the antIns has been established to encode pain intensity (Coghill, 2020)
333 and salience (Wiech et al., 2010) whereas the dpIns has been shown to encode stimulus
334 intensity and salience specific to heat pain (Horing et al., 2019; Segerdahl et al., 2015).
335 The function of the MCC has been more equivocal since some studies attributed a domain-
336 general function of integrating pain, negative affect, and cognitive control (Shackman et
337 al., 2011) whereas others argued in favour of a domain-specific representation of painful
338 stimulation (Kragel et al., 2018). Furthermore, we were able to successfully implement the
339 exercise intervention at the anticipated intensities. Our study utilised the FTP_{20} test as a
340 tool to determine the highest power output a cyclist can maintain in a quasi-steady state
341 (Allen and Coggan, 2006) and shows a high correlation with other instruments such as the
342 FTP_{60} test ($r = 0.61$) (Borszcz et al., 2018). It is frequently used in studies investigating
343 athletes (Borszcz et al., 2020; Mackey and Horner, 2021; Mc Grath et al., 2019).
344 Compellingly, the FTP_{20} test does not rely on approximations or self-reports but on the
345 actual power maintained (Allen and Coggan, 2006). Thus, we ensured the highest
346 accuracy in determining the individual exercise intensities at high and low-intensity.
347 Furthermore, previous reviews have shown that hypoalgesia following exercise is most
348 reliably induced by moderate-high-intensity exercise (Koltyn, 2002; Naugle et al., 2012;
349 Wewege and Jones, 2020). Thus, we decided to employ a high-intensity exercise protocol
350 using the FTP threshold as a reliable measure. What is more, previous studies (Vaegter et
351 al., 2015, 2019) have often employed a resting control condition, which somehow limits
352 the comparability of conditions since a resting baseline does not control for unspecific
353 factors such as attentional load or distraction (Brooks et al., 2017; Sprenger et al., 2012)

by exercise. Previous research has shown that high-intensity exercise produced greater hypoalgesia compared to low-intensity exercise (Jones et al., 2019; Vaegter et al., 2014), hence, 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 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. As the factors 'sex' and 'fitness level' were not explicitly mentioned in our preregistration, we further verified the suitability of our sample to investigate these factors. Therefore, we conducted an additional power analysis for the interaction of drug, fitness level, and sex on differential heat pain ratings. We employed the power analysis for mixed models implemented in R (powerCurve) with 1000 simulations. This reveales that with a power of 0.8, a sample size of $n = 27$ would have been sufficient to detect this effect (Figure S7).

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

379 modulation across stimulus intensities when NLX was administered. This finding suggests
380 two things: firstly, endogenous pain modulation through μ-opioid receptors increases with
381 increasing pain intensity and, secondly, the magnitude of this modulation differs between
382 sexes. With regards to the first aspect, the interaction of stimulus intensity and drug
383 treatment, a previous study found that the increased VAS ratings following NLX
384 administration were driven by lower intensity as opposed to high-intensity stimuli (Schoell
385 et al., 2010). Despite the sample in this study consisting of 50% men, this finding might
386 have been driven by a potential sex difference in opioidergic modulation at different
387 stimulus intensities. Concerning these potential sex differences in opioidergic modulation
388 in heat pain, previous meta-analyses found there to be a greater opioidergic effect in
389 women in experimental pain studies (Niesters et al., 2010). However, these studies do not
390 focus on heat pain and the direction and magnitude of the opioidergic effect depends on
391 interacting variables such as dose, pharmacokinetics, and types of pain (Niesters et al.,
392 2010). Since our study employed a within-subject cross-over design with an established
393 pharmacological intervention (Leknes and Atlas, 2020; Trøstheim et al., 2022a), we
394 ensured our results were unbiased and highly robust.
395

396 **Exercise-induced pain modulation and fitness level**

397 Behaviorally, we could show decreased pain perception following high-intensity
398 compared to low-intensity exercise to be associated with higher individual fitness levels.
399 This strongly suggests a dependence of exercise-induced pain modulation on individual
400 fitness. Recent studies indirectly support this notion and observed higher self-reported
401 physical activity levels to be associated with reduced temporal summation in heat pain
402 (Naugle and Riley, 2014) and increased heat pain thresholds to be associated with
403 individual fitness levels in males (Schmitt et al., 2020). Those findings are consistent with

404 the generally heightened pain tolerance reported in athletes compared to non-athletic
405 controls (Geva and Defrin, 2013; Tesarz et al., 2012). Along with the behavioural
406 findings, we identified increased activation in the mFC after high-intensity exercise to be
407 associated with higher fitness levels in the SAL condition. Previous research has reported
408 increased activation in the rACC to be associated with opioid analgesia (Petrovic et al.,
409 2002; Tinnermann et al., 2022), thus implying opioidergic involvement of this structure in
410 mediating exercise-induced pain modulation (Petrovic et al., 2002). Adding to this, the
411 pregenual ACC showed decreased activation in response to painful heat following a 2-h
412 run compared to before the run in trained male elite runners (Scheef et al., 2012).
413 Furthermore, larger decreases in μ-opioid receptor binding in regions including the ACC,
414 bilateral insula, and OFC in healthy males have been observed to be associated with higher
415 self-reported physical fitness levels (Saanijoki et al., 2022). In a further PET study
416 investigating exercise-induced euphoria as an opioidergic mechanism in male athletes, a
417 reduced opioid receptor binding in prefrontal brain regions including the ACC and OFC
418 was reported (Boecker et al., 2008). Our findings are in line with observations showing
419 that repeated exercise "trains" the mFC, which has a high μ-opioid receptor density
420 (Corder et al., 2018) and thus enables exercise-induced pain modulation even in the
421 context of brief bouts of high-intensity exercise.
422
423 **Sex, fitness level, and opioidergic mechanisms on exercise-induced pain modulation**
424 Crucially, we identified an interaction between sex, fitness level, and drug, where males
425 exhibited larger hypoalgesia following high-intensity exercise with increasing fitness
426 levels, which was attenuated by NLX. This pattern was not evident in females. Our brain
427 data also showed this three-way interaction in the mFC. More precisely, in the SAL
428 condition, males showed higher activation after high-intensity exercise compared to low-

429 intensity exercise in association with higher fitness levels, and this pattern was reversed in
430 the NLX condition. Females showed the reversed pattern to males in both, SAL and NLX,
431 conditions. Previous studies have shown opioidergic effects on pain after aerobic exercise
432 (Gurevich et al., 1994; Haier et al., 1981; Janal et al., 1984). For instance, an analgesic
433 response after long-distance running in male runners was shown to be reversed under NLX
434 (Janal et al., 1984). Even after a short-duration run, NLX has been shown to slow the
435 return of an analgesic effect in runners, and the magnitude of this effect was associated
436 with the individual fitness level (Haier et al., 1981). However, more recent human studies
437 reveal a more equivocal picture of the opioidergic mechanisms of exercise-induced pain
438 modulation (Koltyn, 2000; Wewege and Jones, 2020). One study observed that the
439 hypoalgesic effect of short-duration isometric exercise was unaffected by an orally
440 administered μ-antagonist (naltrexone) (Koltyn et al., 2014). Yet, in the same sample,
441 naltrexone was observed to block exercise-induced increases in the peripheral
442 endocannabinoids (eCB) N-arachidonylethanolamine (AEA) and 2-Arachidonoylglycerol
443 (2-AG) suggesting at least partial involvement of the endogenous opioid system in
444 exercise-induced pain modulation (Crombie et al., 2018). Despite that study being one of
445 the few studies linking eCB and the endogenous opioid system in exercise-induced pain
446 modulation it must be noted that it employed isometric exercise which might result in
447 different exercise-induced mechanisms after a single bout of exercise (Goldfarb and
448 Jamurtas, 1997; Wewege and Jones, 2020). Since rodent and early human research have
449 also predominantly employed male samples to avoid hormonal-related fluctuations
450 (Mogil, 2012; Mogil and Chanda, 2005), this might have led to an over-generalisation of
451 opioidergic effects (Koltyn et al., 2014; Mogil, 2012) whilst neglecting sex differences in
452 opioid analgesia (Bodnar and Kest, 2010; Niesters et al., 2010). Hence, it comes as no
453 surprise that findings in humans are heterogeneous with some studies showing no

difference in exercise-induced pain modulation between males and females (Koltyn et al., 2013, p. 203, 2014; Smith, 2004) whereas other studies showed hypoalgesia from exercise to be greater in women (Vaegter et al., 2014, p. 201) 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 (e.g. 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, p. 200). Those studies, however, did not specifically address the link between endogenous opioids, fitness levels, and sex-dependent differences in exercise-induced pain modulation. Our results imply that there is, in fact, an interaction between sex and fitness level in mediating exercise-induced pain modulation through opioidergic mechanisms in the mFC.

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 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

478 humans, however, the interpretability of results remains limited since only peripheral eCB
479 can be measured and central eCB blockage is difficult.
480
481 In conclusion, our study highlights the nuanced interplay of endogenous opioids, sex, and
482 fitness level in exercise-induced pain modulation along the mFC as part of the descending
483 pain modulatory system. Crucially, we show stronger exercise-induced hypoalgesia in
484 males, which was associated with fitness level and attenuated when NLX was
485 administered. These results provide a solid foundation for future research into the interplay
486 of these factors in persistent pain, which is essential to leverage these findings for the
487 benefit of chronic pain patients.
488

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. Afterwards, 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).

514 According to this, a sample size of $N = 39$ was sufficient to reveal results at effect size $f =$
515 0.3, (via G*Power 3.1, $\alpha = .05$, 1- $\alpha = 0.95$, 1 group, 2 measurements with correlation $r =$
516 0.5). Based on previous fMRI studies it can also be assumed that a sample size of $N = 30$
517 would be sufficient to detect changes in the brain (Mumford, 2012). In this study, the term
518 "sex" refers to "sex assigned at birth" as indicated by self-reports from the participants.
519 Multiple options ("male", "female", "other", and free text field) were provided. All
520 participants reported having been assigned the "female" of "male" sex at birth.
521

522 **Exclusion of participants**

523 Upon entering the study, participants were screened for anxiety (State and Trait
524 questionnaires of the German short version of the State-Trait Anxiety Inventory; STAI)
525 (Laux et al., 1981), depression (Beck Depression Inventory 2; BDI-II) (Beck et al., 2011)
526 as well as potential MR contraindications. Furthermore, participants' body mass index
527 (BMI) was required to range between 18 and 30. On the first day of the study, an
528 electrocardiogram (ECG; cardiofax, Electrocardiograph-1250, Nihon Koden) was
529 recorded to prove no cardiovascular irregularities. The age of participants was between 18
530 and 45 years. Eight participants were excluded from the study for the following reasons.
531 Two participants withdrew from the study after the first day due to circumstantial reasons
532 (moving, no appointment found). Two participants were excluded after day one due to an
533 unreliable thermal calibration and a BDI score below 18, respectively. Two participants
534 did not wish to continue the study after day two due to personal reasons. Two participants
535 were excluded after day two due to a syncope when administering the drug (in both cases
536 SAL has been administered). Upon data screening, one subject was excluded due to
537 excessive movement (> 0.6 mm difference between volumes within runs in more than 5%

538 of volumes in all runs) in the MR scanner. A total of thirty-nine participants (Age: *M* =
539 26.2, *SD* = 4.8, 21 female) were included in the final sample (Table S12).
540
541 **Ethics approval**
542 The study was approved by the Ethics committee of the medical board in Hamburg
543 ('Aerztekammer') and conducted following the Declaration of Helsinki (World Medical
544 Association, 2013). All subjects provided informed consent upon entering the study after
545 having been informed about the study procedures including thermal and pressure
546 stimulation, the MR procedure, the double-blind nature of the pharmacological
547 intervention, and potential adverse effects of NLX by the study investigator and study
548 physician. Upon completing or leaving the study, participants were informed of the order
549 of pharmacological treatment received by the unblinded study physician.
550
551 **Thermal and Pressure Stimuli**
552 Thermal stimulation was applied using a thermode (TSA-2, Medoc, Israel) attached to the
553 left lower arm where four 2.5x2.5 cm squares were drawn below each other and numbered
554 (Figure 1a). For the calibration, the thermode head was positioned in the second square.
555 Each thermal stimulus lasted up to 17 seconds in total with a plateau of 15 seconds and a
556 ramp speed of 13°C/s (ramp-up, ramp-down). For the pressure calibration, a computer-
557 controlled cuff pressure algometer (CPAR; NociTech, Denmark, and Aalborg University,
558 Denmark) consisting of a compressor tube and 13 cm wide tourniquet cuff (VBM medical,
559 Sulz, Germany, 61 cm length) was used. The cuff was mounted to the left upper arm with
560 a 3 cm distance to the cubital fossa to exert pressure on the upper arm tissue (Graven-
561 Nielsen et al., 2015). Additionally, a protective tubular elastic dressing (Tricofix, D/5, 6
562 cmx20 cm) was positioned underneath the cuff to protect the bare skin from potential

563 harm. The pressure was applied by inflating the chambers of the cuff with a maximal
564 pressure limit of 100 kilopascal (kPa) and a rise speed dependent on the target pressure.
565 The stimulus length of the pressure stimuli was also up to 17 seconds (ramp-up, 15s
566 plateau, ramp-down).
567
568 **Calibration Procedure (Day 1)**
569 The first day of the study took place outside the MR scanner and served as a calibration
570 day (Figure 1a). Blood pressure, baseline heart rate (HR), and oxygen saturation (SPO2)
571 were measured using a blood pressure cuff (boso medicus uno, bosch+sohn) and a pulse
572 oximeter (pulox® Pusloximeter, Novidion GmbH), respectively. The thermal and pressure
573 stimuli were calibrated individually but with an identical protocol in a pseudo-randomized
574 order across participants. During calibration, participants remained in a supine position
575 with the blinds down to emulate the inside of the MR scanner bore. As an initial step,
576 participants received two low stimuli (10/20 kPa or 41°C/42°C) to get acquainted with the
577 stimuli. Following this, the pain threshold was estimated by applying six adaptive stimuli
578 which were rated on a binary scale (painful vs. not painful). The resulting pain threshold
579 was used as a basis for the following linear regression algorithm aiming to collect ratings
580 across different intensities on a Visual Analog Scale (10, 30, 50, 70, 90 VAS). After each
581 stimulus, participants had to rate its painfulness ('How painful was the last stimulus?") on
582 a VAS ranging from 0 (minimally painful) to 100 (almost unbearably painful) using the
583 left and right key of a pointer as a button box (Logitech) with their right hand. The
584 calibration was finished once sufficient rating coverage for all target intensities was
585 reached. The thermal and pressure calibration aimed to identify super threshold intensities
586 (°C and kPa) corresponding to 30, 50, and 70 on the VAS scale using this linear regression
587 algorithm yielding comparable intensities across subjects.

588

589 **Functional Threshold Power Test (Day 1)**

590 As a final step of the calibration day, a functional threshold power (FTP) test was
591 conducted on a stationary cycle ergometer (KICKR Bike, Wahoo, Atlanta, United States).
592 The FTP test was first described by Allen and Coggan (2006) and determines the
593 maximum average power output that can be maintained for one hour which serves as an
594 approximation of the anaerobic threshold (Allen and Coggan, 2012). The FTP_{20} test is
595 derived from the original FTP test but estimates 95% of FTP based on 20 minutes of
596 steady cycling at maximum possible power output. The test is divided into six stages
597 lasting one hour in total (Figure 1a and Table S1). Within this test protocol, the average
598 power output measured for the last 20-minute interval is taken as 95% of the actual FTP
599 (Allen and Coggan, 2012). The FTP_{20} Test allows the identification of individually
600 calibrated power zones. In this present study, we used high-intensity exercise (Zone 4:
601 Threshold, 91 -106% of FTP) and low-intensity exercise (Zone 1: Active recovery, ~55%
602 of FTP) as intensities. During the FTP_{20} test, participants were free to adjust the resistance
603 of the ergometer whilst maintaining a constant cadence. Power output in Watt, heart rate
604 (HR belt; Garmin® HRM-PRO™ PLUS) in beats per minute (bpm), and cadence (in
605 RPM) were monitored throughout the FTP_{20} test. The current power and HR as well as the
606 remaining time of each section were displayed on a 50-by-30 cm screen in front of the
607 participants to allow for self-monitoring. Participants were asked to refrain from any
608 exhausting physical activities the day of and before the calibration day. The FTP value
609 from the FTP_{20} test was corrected for participants' weight (FTP/weight) and served as an
610 indicator of individual fitness level in the analyses. The distribution of the weight-
611 corrected FTP for males and females is visualized in Figure S8. There was no significant
612 association between heat pain thresholds and weight-corrected FTP (r = -0.23, P = 0.16;

613 Figure S9). On the bike, the knee angle was adjusted to a 5-degree bend when the pedal
614 stroke was at the bottom (Vaegter et al., 2019). The knee was also set to be over the pedal
615 axle when the cranks were parallel to the ground. The upper body was adjusted to a
616 forward lean with a slight bend of the elbows to maintain a neutral spine position with a
617 natural lumbar flexion.
618
619 **Experimental Paradigm (Day 2 and Day 3)**
620 The two experimental days were at least three days apart (*Median* = 7 days, *Mean* = 12.35
621 days, *SD* = 14.5 days) and identical with the only difference being the pharmacological
622 treatment (NLX vs. SAL) received (Figure 1b). Each visit took place in the morning with a
623 starting time of 8:15 a.m. and varied by a maximum of four hours between participants to
624 account for potential circadian influences. Blood pressure and SPO_2 were measured before
625 each cycling block and monitored throughout the investigation. On each experimental day,
626 a urine sample was tested for amphetamines, opiates, marijuana, methamphetamines,
627 morphine (Surestep, Multidrug Test, Innovacon Inc., San Diego, USA), and potential
628 pregnancies (only female participants; hCG Ultra Test, Serum/Urine 10mIU, Mexacare
629 GmbH, Germany). Each experimental day consisted of 4 runs. Each run included a ten-
630 minute cycling block at a high (100% FTP) or low intensity (55% FTP) in a pseudo-
631 randomised order across participants which was immediately followed by an MR scan. On
632 the second experimental day, an anatomical scan (T1) was acquired alongside conducting
633 a re-calibration of thermal and pressure stimuli. The re-calibration was based on the
634 previously calibrated intensities from the calibration day (Day 1) and was adjusted based
635 on the ratings provided in the scanner corresponding to 30, 50, and 70 VAS. This
636 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., 2022b). 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., 2022b) 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., 2022b) with an effective central opioid block throughout the study (Eippert et al., 2009; Trøstheim et al., 2022b). 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

687 they suspected to have received on which experimental day to capture potential
688 expectations about the pharmacological treatment.
689
690 **Data Acquisition**
691 **Behavioural Data Acquisition**
692 During the cycling blocks, heart rate, power output (Watt), and cadence (RPM) were
693 recorded using MATLAB 2021b. For each cycling block the required power as well as the
694 actual power maintained by the participant were displayed on a screen to allow for self-
695 monitoring. During cycling, participants' HR and SPO_2 were constantly monitored and
696 recorded. In one participant recording of the power output failed and in five participants
697 HR recordings in one session failed due to equipment issues. However, power output and
698 HR maintained throughout the runs were visible and accessible at all times for the
699 participant. Upon completion of the ten-minute cycling, participants were asked to rate
700 their perceived exertion (PRE) on the BORG scale (no exertion (6) – maximal exertion
701 (20)) (Borg, 1998) as well as to indicate their current mood (sad (0) – happy (10)). The
702 transfer to the MR scanner commenced as quickly as possible (Time: *Mean* = 5 min, *SD* =
703 1 min) whilst maintaining the participants' safety at all times. Inside the MR scanner, all
704 stimulus presentation was realized using MATLAB R2016b and Psychophysics Toolbox
705 (Version 3.0.19). Physiological data, including respiration and heart rate, was recorded at
706 1000 Hz with the Expression System (In Vivo, Gainesville, USA) using the spike2
707 software and a CED1401 system (Cambridge Electronic Design). A fixation cross and
708 VAS scale were displayed on a screen inside the scanner bore. Thermal stimuli were
709 applied using a TSA-2 Thermode with a 2.5x2.5 cm probe attached to the left volar
710 forearm. Pressure stimuli were applied using a pressure cuff attached to the left upper arm
711 and a CPAR outside the MR. The infusion pump was positioned outside the scanner with a

712 ten-meter tube reaching into the scanner room to maintain a constant dose of NLX or
713 SAL. After two pre-exposure stimuli of each modality, thermal and pressure stimuli were
714 applied in an alternating fashion and at different calibrated intensities (randomized at 30,
715 50, 70 VAS). Each stimulus lasted 17 s in total (ramp-up, plateau 15 s, ramp-down)
716 indicated by a visual cue (red fixation cross). Following each stimulus, the painfulness was
717 rated on a VAS from 'minimally painful' (converted to 0 for analyses) to 'almost
718 unbearably painful' (converted to 100 for analyses) by using a button box. The rating took
719 place within 7 seconds after which an ITI of 7 seconds (jittered by 2 seconds) was
720 introduced before commencing with the next trial. One MRI block consisted of nine
721 thermal and nine pressure stimuli at three intensity levels (30, 50, 70 VAS). In total,
722 participants spent 15 minutes per block inside the MR scanner.
723

724 **MRI Data Acquisition**

725 Functional magnetic resonance imaging (fMRI) data was acquired using a 3 Tesla
726 Siemens system (Magnetom PRISMA; Siemens Healthcare, Erlangen, Germany) with a
727 64–channel head coil. Sixty slices were acquired (2 mm slice thickness) using T2*-
728 weighted echo-planar imaging (EPI) with multiband factor 2 (repetition time (TR): 1.8
729 seconds; echo time (TE): 26ms; flip angle: 70°). The Field of View (FOV) was 240 mm
730 and positioned to include the upper part of the medulla and the brainstem. The voxel size
731 was thus 2 x 2 x 2 mm^3. Four volumes at the beginning of each run were discarded.
732 Furthermore, T1-weighted images were acquired for each subject using an MPRAGE
733 sequence on the first experimental day (day 2). Due to the nature of the study where
734 participants cycled outside the MR scanner and had to be repositioned for each run,
735 shimming and auto-align took place at the beginning of each run and before acquiring EPI
736 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

787 respiratory-related motion (Brooks et al., 2008). This technique determines cardiac- and
788 respiratory-related noise by allotting cardiac and respiratory phases to individual volumes
789 within a time series. Subsequently, these assigned phases are utilized in a low-order
790 Fourier expansion to generate time-course regressors elucidating signal variations
791 attributed to cardiac or respiratory activities. A refined physiological noise model was
792 applied, computing three cardiac and four respiratory harmonics, along with a
793 multiplicative term incorporating interactions between cardiac and respiratory noise
794 resulting in 18 regressors (Harvey et al., 2008). These resulting noise regressors were
795 included in the first-level analyses. The contrast images from the first level analysis were
796 then spatially normalized to MNI space using individual deformation fields (i.e.
797 combining nonlinear coregistration and DARTEL spatial normalization) and finally
798 smoothed using a 6mm full width at half maximum (FWHM) Gaussian smoothing kernel.
799 In addition to a hemodynamic response function (HRF) model, we also used a Finite
800 Impulse Response model (FIR). This model was used to visualize the time course of
801 BOLD responses after the onset of the pain stimulus. In this analysis, the stimulus duration
802 was divided into 12 bins, each covering the duration of one TR spanning a total of 21.6
803 seconds after stimulus onset.
804
805 **Second-level analyses**
806 Subsequently, the spatially normalized and smoothed contrast images from the first-level
807 analysis were used for random-effects group-level statistics. The successful application of
808 heat pain was established by investigating the parametric contrast heat VAS 70 > 50 > 30,
809 where higher stimulus intensities would recruit a more widespread (sub-)cortical pain
810 network than lower intensities. To explore the effect of drug treatment in the brain, the
811 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 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 contrasts: 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 $P_{corr\text{-}WB}$ = x.xx. Furthermore, we used a small volume correction mask (SVC) to correct for multiple comparisons, and the corrected *P*-values are reported as $P_{corr\text{-}SVC}$ = x.xx. This mask was based on the preregistered ROIs (rACC, vmPFC, PAG, RVM with an additional 1 mm FWHM Gaussian smoothing kernel (Figure S10 and Table S13). 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.

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841

842 **Author Contributions**

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

846

847 **Declaration of Interests**

848 The other authors declare no competing interests.

849

850 **Ethics**

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

854

855 **Additional Files**

856 Supplemental Information: Supplemental Methods, Figures S1–S10 and Tables S1-S15

857

858 **References**

859 Allen H, Coggan A. 2012. Training and Racing with a Power Meter, 2nd Ed. VeloPress.

860 Allen H, Coggan A. 2006. Racing and training with a power meter. Boulder, CO: VeloPress.

861 Aslaksen PM, Bystad M, Vambheim SM, Flaten MA. 2011. Gender differences in placebo analgesia: event-related
862 potentials and emotional modulation. *Psychosom Med* **73**:193–199. doi:10.1097/PSY.0b013e3182080d73

863 Atlas LY, Wager TD. 2014. A Meta-analysis of Brain Mechanisms of Placebo Analgesia: Consistent Findings and
864 Unanswered Questions. *Handb Exp Pharmacol* **225**:37–69. doi:10.1007/978-3-662-44519-8_3

865 Bates D, Mächler M, Bolker B, Walker S. 2014. Fitting Linear Mixed-Effects Models using lme4.

866 Beck AT, Steer RA, Brown G. 2011. Beck Depression Inventory–II. doi:10.1037/t00742-000

867 Bodnar RJ, Kest B. 2010. Sex differences in opioid analgesia, hyperalgesia, tolerance and withdrawal: Central
868 mechanisms of action and roles of gonadal hormones. *Horm Behav*, Sex and drugs: Sex differences and
869 hormonal effects on drug abuse. **58**:72–81. doi:10.1016/j.yhbeh.2009.09.012

870 Boecker H, Daamen M, Maurer A, Bodensohn L, Werkhausen J, Lohaus M, Manunzio C, Manunzio U, Radbruch A,
871 Attenberger U, Dukart J, Upadhyay N. 2024. Fractional amplitude of low-frequency fluctuations associated
872 with μ-opioid and dopamine receptor distributions in the central nervous system after high-intensity exercise
873 bouts. *Front Neuroimaging* **3**:1332384. doi:10.3389/fnimg.2024.1332384

874 Boecker H, Sprenger T, Spilker ME, Henriksen G, Koppenhoefer M, Wagner KJ, Valet M, Berthele A, Tolle TR.
875 2008. The Runner's High: Opioidergic Mechanisms in the Human Brain. *Cereb Cortex* **18**:2523–2531.
876 doi:10.1093/cercor/bhn013

877 Borg G. 1998. Borg's perceived exertion and pain scales, Borg's perceived exertion and pain scales. Champaign, IL,
878 US: Human Kinetics.

879 Borras MC, Becerra L, Ploghaus A, Gostic JM, DaSilva A, Gonzalez RG, Borsook D. 2004. FMRI Measurement of
880 CNS Responses to Naloxone Infusion and Subsequent Mild Noxious Thermal Stimuli in Healthy
881 Volunteers. *J Neurophysiol* **91**:2723–2733. doi:10.1152/jn.00249.2003

882 Borszcz FK, Tramontin AF, Bossi AH, Carminatti LJ, Costa VP. 2018. Functional Threshold Power in Cyclists:
883 Validity of the Concept and Physiological Responses. *Int J Sports Med* **39**:737–742. doi:10.1055/s-0044-
884 101546

885 Borszcz FK, Tramontin AF, Costa VP. 2020. Reliability of the Functional Threshold Power in Competitive Cyclists.
886 *Int J Sports Med* **41**:175–181. doi:10.1055/a-1018-1965

887 Brellenthin AG, Crombie KM, Cook DB, Sehgal N, Koltyn KF. 2017. Psychosocial Influences on Exercise-Induced
888 Hypoalgesia. *Pain Med* **18**:538–550. doi:10.1093/pm/pnw275

889 Brooks JCW, Beckmann CF, Miller KL, Wise RG, Porro CA, Tracey I, Jenkinson M. 2008. Physiological noise
890 modelling for spinal functional magnetic resonance imaging studies. *NeuroImage* **39**:680–692.
891 doi:10.1016/j.neuroimage.2007.09.018

892 Brooks JCW, Davies W-E, Pickering AE. 2017. Resolving the Brainstem Contributions to Attentional Analgesia. *J*
893 *Neurosci Off J Soc Neurosci* **37**:2279–2291. doi:10.1523/JNEUROSCI.2193-16.2016

894 Bulls HW, Freeman EL, Anderson AJ, Robbins MT, Ness TJ, Goodin BR. 2015. Sex differences in experimental
895 measures of pain sensitivity and endogenous pain inhibition. *J Pain Res* **8**:311–320.
896 doi:10.2147/JPR.S84607

Coghill RC. 2020. The Distributed Nociceptive System: A Framework for Understanding Pain. *Trends Neurosci* **43**:780–794. doi:10.1016/j.tins.2020.07.004

Corder G, Castro DC, Bruchas MR, Scherrer G. 2018. Endogenous and Exogenous Opioids in Pain. *Annu Rev Neurosci* **41**:453–473. doi:10.1146/annurev-neuro-080317-061522

Crombie KM, Brellenthin AG, Hillard CJ, Koltyn KF. 2018. Endocannabinoid and opioid system interactions in exercise-induced hypoalgesia. *Pain Med* **19**:118–123. doi:10.1093/pm/pnx058

Curran SL, Andrykowski MA, Studts JL. 1995. Short Form of the Profile of Mood States (POMS-SF): Psychometric information. *Psychol Assess* **7**:80–83. doi:10.1037/1040-3590.7.1.80

Desai S, Borg B, Cuttler C, Crombie KM, Rabinak CA, Hill MN, Marusak HA. 2022. A Systematic Review and Meta-Analysis on the Effects of Exercise on the Endocannabinoid System. *Cannabis Cannabinoid Res* **7**:388–408. doi:10.1089/can.2021.0113

Eippert F, Bingel U, Schoell ED, Yacubian J, Klinger R, Lorenz J, Büchel C. 2009. Activation of the Opioidergic Descending Pain Control System Underlies Placebo Analgesia. *Neuron* **63**:533–543. doi:10.1016/j.neuron.2009.07.014

Friston KJ, Williams S, Howard R, Frackowiak RSJ, Turner R. 1996. Movement-Related effects in fMRI time-series. *Magn Reson Med* **35**:346–355. doi:10.1002/mrm.1910350312

Fuss J, Steinle J, Bindila L, Auer MK, Kirchherr H, Lutz B, Gass P. 2015. A runner's high depends on cannabinoid receptors in mice. *Proc Natl Acad Sci* **112**:13105–13108. doi:10.1073/pnas.1514996112

Ge H-Y, Madeleine P, Arendt-Nielsen L. 2004. Sex differences in temporal characteristics of descending inhibitory control: an evaluation using repeated bilateral experimental induction of muscle pain. *PAIN* **110**:72. doi:10.1016/j.pain.2004.03.005

Geisler M, Eichelkraut L, Miltner WHR, Weiss T. 2019. Expectation of exercise in trained athletes results in a reduction of central processing to nociceptive stimulation. *Behav Brain Res* **356**:314–321. doi:10.1016/j.bbr.2018.08.036

Geva N, Defrin R. 2013. Enhanced pain modulation among triathletes: A possible explanation for their exceptional capabilities. *PAIN*® **154**:2317–2323. doi:10.1016/j.pain.2013.06.031

Glover GH, Li T-Q, Ress D. 2000. Image-based method for retrospective correction of physiological motion effects in fMRI: RETROICOR. *Magn Reson Med* **44**:162–167. doi:10.1002/1522-2594(200007)44:1<162::AID-MRM23>3.0.CO;2-E

Goldfarb AH, Jamurtas AZ. 1997. β-Endorphin Response to Exercise. *Sports Med* **24**:8–16. doi:10.2165/00007256-199724010-00002

Gorgolewski KJ, Auer T, Calhoun VD, Craddock RC, Das S, Duff EP, Flandin G, Ghosh SS, Glatard T, Halchenko YO, Handwerker DA, Hanke M, Keator D, Li X, Michael Z, Maumet C, Nichols BN, Nichols TE, Pellman J, Poline J-B, Rokem A, Schaefer G, Sochat V, Triplett W, Turner JA, Varoquaux G, Poldrack RA. 2016. The brain imaging data structure, a format for organizing and describing outputs of neuroimaging experiments. *Sci Data* **3**:160044. doi:10.1038/sdata.2016.44

Granot M, Weissman-Fogel I, Crispel Y, Pud D, Granovsky Y, Sprecher E, Yarnitsky D. 2008. Determinants of endogenous analgesia magnitude in a diffuse noxious inhibitory control (DNIC) paradigm: Do conditioning stimulus painfulness, gender and personality variables matter? *Pain* **136**:142–149. doi:10.1016/j.pain.2007.06.029

Graven-Nielsen T, Vaegter HB, Finocchietti S, Handberg G, Arendt-Nielsen L. 2015. Assessment of musculoskeletal pain sensitivity and temporal summation by cuff pressure algometry: a reliability study. *Pain* **156**:2193–2202. doi:10.1097/j.pain.0000000000000294

Gurevich M, Kohn PM, Davis C. 1994. Exercise-induced analgesia and the role of reactivity in pain sensitivity. *J Sports Sci* **12**:549–559. doi:10.1080/02640419408732205

Haier RJ, Quaid K, Mills JSC. 1981. Naloxone Alters Pain Perception After Jogging. *Psychiatry Res* **5**:231–232. doi:10.1016/0165-1781(81)90052-4

Harvey AK, Pattinson KTS, Brooks JCW, Mayhew SD, Jenkinson M, Wise RG. 2008. Brainstem functional magnetic resonance imaging: Disentangling signal from physiological noise. *J Magn Reson Imaging* **28**:1337–1344. doi:10.1002/jmri.21623

Horing B, Sprenger C, Büchel C. 2019. The parietal operculum preferentially encodes heat pain and not salience. *PLoS Biol* **17**:e3000205. doi:10.1371/journal.pbio.3000205

Hox JJ, Moerbeek M, Schoot R van de. 2010. Multilevel Analysis: Techniques and Applications, Second Edition, 2nd ed. New York: Routledge.

Janal MN, Colt EWD, Crawford W, Glusman M. 1984. Pain Sensitivity, Mood and Plasma Endocrine Levels in Man Following Long-Distance Running: Effects of Naloxone. *Pain* **19**:13–25. doi:10.1016/0304-3959(84)90061-7

Jones MD, Nuzzo JL, Taylor JL, Barry BK. 2019. Aerobic Exercise Reduces Pressure More Than Heat Pain Sensitivity in Healthy Adults. *Pain Med* **20**:1534–1546. doi:10.1093/pm/pny289

Klich S, Krymski I, Michalik K, Kawczyński A. 2018. Effect of short-term cold-water immersion on muscle pain sensitivity in elite track cyclists. *Phys Ther Sport Off J Assoc Chart Physiother Sports Med* **32**:42–47. doi:10.1016/j.ptsp.2018.04.022

Koltyn K f., Knauf M t., Brellenthin A g. 2013. Temporal summation of heat pain modulated by isometric exercise. *Eur J Pain* **17**:1005–1011. doi:10.1002/j.1532-2149.2012.00264.x

Koltyn KF. 2002. Exercise-Induced Hypoalgesia and Intensity of Exercise. *Sports Med* **32**:477–487. doi:10.2165/00007256-200232080-00001

Koltyn KF. 2000. Analgesia Following Exercise. *Sports Med* **29**:85–98. doi:10.2165/00007256-200029020-00002

Koltyn KF, Brellenthin AG, Cook DB, Sehgal N, Hillard C. 2014. Mechanisms of exercise-induced hypoalgesia. *J Pain* **15**:1294–1304. doi:10.1016/j.jpain.2014.09.006

Kragel PA, Kano M, Van Oudenhove L, Ly HG, Dupont P, Rubio A, Delon-Martin C, Bonaz BL, Manuck SB, Gianaros PJ, Ceko M, Reynolds Losin EA, Woo C-W, Nichols TE, Wager TD. 2018. Generalizable representations of pain, cognitive control, and negative emotion in medial frontal cortex. *Nat Neurosci* **21**:283–289. doi:10.1038/s41593-017-0051-7

Kut E, Candia V, Overbeck J von, Pok J, Fink D, Folkers G. 2011. Pleasure-Related Analgesia Activates Opioid-Insensitive Circuits. *J Neurosci* **31**:4148–4153. doi:10.1523/JNEUROSCI.3736-10.2011

Laux L, Glanzmann P, Schaffner P, Spielberger CD. 1981. Das State-Trait Angstinventar. Theoretische Grundlagen und Handanweisung.

Leknes S, Atlas LY. 2020. Flawed methodology undermines conclusions about opioid-induced pleasure: implications for psychopharmacology. *Br J Anaesth* **124**:e29–e33. doi:10.1016/j.bja.2019.10.006

Lesnak JB, Sluka KA. 2020. Mechanism of exercise-induced analgesia: what we can learn from physically active animals. *PAIN Rep* **5**:e850. doi:10.1097/PR9.0000000000000850

Mackey J, Horner K. 2021. What is known about the FTP [20] test related to cycling? A scoping review. *J Sports Sci* **39**:2735–2745. doi:10.1080/02640414.2021.1955515

Martin LJ, Acland EL, Cho C, Gandhi W, Chen D, Corley E, Kadoura B, Levy T, Mirali S, Tohyama S, Khan S, MacIntyre LC, Carlson EN, Schweinhardt P, Mogil JS. 2019. Male-Specific Conditioned Pain Hypersensitivity in Mice and Humans. *Curr Biol* **29**:192-201.e4. doi:10.1016/j.cub.2018.11.030

Mc Grath E, Mahony N, Fleming N, Donne B. 2019. Is the FTP Test a Reliable, Reproducible and Functional Assessment Tool in Highly-Trained Athletes? *Int J Exerc Sci* **12**:1334–1345.

Mogil JS. 2020. Qualitative sex differences in pain processing: emerging evidence of a biased literature. *Nat Rev Neurosci* **21**:353–365. doi:10.1038/s41583-020-0310-6

Mogil JS. 2012. Sex differences in pain and pain inhibition: multiple explanations of a controversial phenomenon. *Nat Rev Neurosci* **13**:859–866. doi:10.1038/nrn3360

Mogil JS, Chanda ML. 2005. The case for the inclusion of female subjects in basic science studies of pain. *PAIN* **117**:1. doi:10.1016/j.pain.2005.06.020

Mumford J. 2012. A power calculation guide for FMRI studies. *Soc Cogn Affect Neurosci* **7**:738–42. doi:10.1093/scan/nss059

Naugle KM, Fillingim RB, Riley JL. 2012. A meta-analytic review of the hypoalgesic effects of exercise. *J Pain* **13**:1139–1150. doi:10.1016/j.jpain.2012.09.006

Naugle KM, Naugle KE, Fillingim RB, Samuels B, Riley JLI. 2014. Intensity Thresholds for Aerobic Exercise-Induced Hypoalgesia. *Med Sci Sports Exerc* **46**:817–825. doi:10.1249/MSS.0000000000000143

Naugle KM, Riley JL. 2014. Self-reported Physical Activity Predicts Pain Inhibitory and Facilitatory Function. *Med Sci Sports Exerc* **46**:622–629. doi:10.1249/MSS.0b013e3182a69cf1

Niesters M, Dahan A, Kest B, Zacny J, Stijnen T, Aarts L, Sarton E. 2010. Do sex differences exist in opioid analgesia? A systematic review and meta-analysis of human experimental and clinical studies. *PAIN* **151**:61. doi:10.1016/j.pain.2010.06.012

Padawer WJ, Levine FM. 1992. Exercise-induced analgesia: fact or artifact? *Pain* **48**:131–135. doi:10.1016/0304-3959(92)90048-G

Petrovic P, Kalso E, Petersson KM, Ingvar M. 2002. Placebo and Opioid Analgesia-- Imaging a Shared Neuronal Network. *Science* **295**:1737–1740. doi:10.1126/science.1067176

R: The R Project for Statistical Computing. n.d. https://www.r-project.org/

Raudenbush SW, Bryk AS. 2001. Hierarchical Linear Models: Applications and Data Analysis Methods, 2nd Edition. ed. Thousand Oaks: SAGE Publications, Inc.

Ruble SB, Hoffman MD, Shepanski MA, Valic Z, Buckwalter JB, Clifford PS. 2005. Thermal Pain Perception After Aerobic Exercise. *Arch Phys Med Rehabil* **86**:1019–1023. doi:10.1016/j.apmr.2004.09.024

Saanijoki T, Kantonen T, Pekkarinen L, Kalliokoski K, Hirvonen J, Malén T, Tuominen L, Tuulari JJ, Arponen E, Nuutila P, Nummenmaa L. 2022. Aerobic Fitness is Associated with Cerebral mu-Opioid Receptor Activation in Healthy Humans. *Med Sci Sports Exerc*. doi:10.1249/MSS.0000000000002895

Saanijoki T, Tuominen L, Tuulari JJ, Nummenmaa L, Arponen E, Kalliokoski K, Hirvonen J. 2018. Opioid Release after High-Intensity Interval Training in Healthy Human Subjects. *Neuropsychopharmacology* **43**:246–254. doi:10.1038/npp.2017.148

1017 Scheef L, Jankowski J, Daamen M, Weyer G, Klingenberg M, Renner J, Mueckter S, Schürmann B, Musshoff F,
1018 Wagner M, Schild HH, Zimmer A, Boecker H. 2012. An fMRI study on the acute effects of exercise on pain
1019 processing in trained athletes. *Pain* **153**:1702–1714. doi:10.1016/j.pain.2012.05.008
1020 Schmitt A, Wallat D, Stangier C, Martin JA, Schlesinger-Irsch U, Boecker H. 2020. Effects of fitness level and
1021 exercise intensity on pain and mood responses. *Eur J Pain* **24**:568–579. doi:10.1002/ejp.1508
1022 Schoell ED, Bingel U, Eippert F, Yacubian J, Christiansen K, Andresen H, May A, Buechel C. 2010. The Effect of
1023 Opioid Receptor Blockade on the Neural Processing of Thermal Stimuli. *PLoS ONE* **5**:e12344.
1024 doi:10.1371/journal.pone.0012344
1025 Segerdahl AR, Mezue M, Okell TW, Farrar JT, Tracey I. 2015. The dorsal posterior insula subserves a fundamental
1026 role in human pain. *Nat Neurosci* **18**:499–500. doi:10.1038/nn.3969
1027 Shackman AJ, Salomons TV, Slagter HA, Fox AS, Winter JJ, Davidson RJ. 2011. The integration of negative affect,
1028 pain and cognitive control in the cingulate cortex. *Nat Rev Neurosci* **12**:154–167. doi:10.1038/nrn2994
1029 Siebers M, Biedermann SV, Bindila L, Lutz B, Fuss J. 2021. Exercise-induced euphoria and anxiolysis do not depend
1030 on endogenous opioids in humans. *Psychoneuroendocrinology* **126**:105173.
1031 doi:10.1016/j.psyneuen.2021.105173
1032 Sluka KA, Frey-Law L, Hoeger Bement M. 2018. Exercise-induced pain and analgesia? Underlying mechanisms and
1033 clinical translation. *Pain* **159**:S91–S97. doi:10.1097/j.pain.0000000000001235
1034 Smith LD. 2004. The effects of competition and exercise on pain perception.
1035 Sprenger C, Eippert F, Finsterbusch J, Bingel U, Rose M, Büchel C. 2012. Attention modulates spinal cord responses
1036 to pain. *Curr Biol CB* **22**:1019–1022. doi:10.1016/j.cub.2012.04.006
1037 Stagg NJ, Mata HP, Ibrahim MM, Henriksen EJ, Porreca F, Vanderah TW, Malan TP. 2011. Regular exercise
1038 reverses sensory hypersensitivity in a rat neuropathic model: role of endogenous opioids. *Anesthesiology*
1039 **114**:940–948. doi:10.1097/ALN.0b013e318210f880
1040 Sternberg WF, Boka C, Kas L, Alboyadjia A, Gracely RH. 2001. Sex-Dependent Components of the Analgesia
1041 Produced by Athletic Competition. *J Pain* **2**:65–74. doi:10.1054/jpai.2001.18236
1042 Tesarz J, Schuster AK, Hartmann M, Gerhardt A, Eich W. 2012. Pain perception in athletes compared to normally
1043 active controls: a systematic review with meta-analysis. *Pain* **153**:1253–1262.
1044 doi:10.1016/j.pain.2012.03.005
1045 Tinnermann A, Sprenger C, Büchel C. 2022. Opioid analgesia alters corticospinal coupling along the descending pain
1046 system in healthy participants. *eLife*. doi:10.7554/eLife.74293
1047 Tracey I, Mantyh PW. 2007. The Cerebral Signature for Pain Perception and Its Modulation. *Neuron* **55**:377–391.
1048 doi:10.1016/j.neuron.2007.07.012
1049 Tran TD, Wang H, Tandon A, Hernandez-Garica L, Casey KL. 2010. Temporal summation of heat pain in humans:
1050 Evidence supporting thalamocortical modulation. *Pain* **150**:93–102. doi:10.1016/j.pain.2010.04.001
1051 Trøstheim M, Eikemo M, Haaker J, Frost JJ, Leknes S. 2022a. Opioid antagonism in humans: a primer on optimal
1052 dose and timing for central mu-opioid receptor blockade. *Neuropsychopharmacology* 1–9.
1053 doi:10.1038/s41386-022-01416-z
1054 Trøstheim M, Eikemo M, Haaker J, Frost JJ, Leknes S. 2022b. Opioid Antagonism in Humans: A Primer on Optimal
1055 Dose and Timing for Central Mu-Opioid Receptor Blockade. doi:10.1101/2022.02.25.481943
1056 Vaegter H, Handberg G, Jorgensen M, Kinly A, Graven-Nielsen T. 2015. Aerobic exercise and cold pressor test
1057 induce hypoalgesia in active and inactive men and women. *Pain Med*. doi:10.1111/pme.12641

1058 Vaegter HB, Bjerregaard LK, Redin M-M, Rasmussen SH, Graven-Nielsen T. 2019. Hypoalgesia after bicycling at
1059 lactate threshold is reliable between sessions. *Eur J Appl Physiol* **119**:91–102. doi:10.1007/s00421-018-
1060 4002-0
1061 Vaegter HB, Handberg G, Graven-Nielsen T. 2014. Similarities between exercise-induced hypoalgesia and
1062 conditioned pain modulation in humans. *Pain* **155**:158–167. doi:10.1016/j.pain.2013.09.023
1063 Vaegter HB, Jones MD. 2020. Exercise-induced hypoalgesia after acute and regular exercise: experimental and
1064 clinical manifestations and possible mechanisms in individuals with and without pain. *Pain Rep* **5**:e823.
1065 doi:10.1097/PR9.0000000000000823
1066 Wewege MA, Jones MD. 2020. Exercise-Induced Hypoalgesia in Healthy Individuals and People With Chronic
1067 Musculoskeletal Pain: A Systematic Review and Meta-Analysis. *J Pain*. doi:10.1016/j.jpain.2020.04.003
1068 Wiech K, Lin C, Brodersen KH, Bingel U, Ploner M, Tracey I. 2010. Anterior Insula Integrates Information about
1069 Salience into Perceptual Decisions about Pain. *J Neurosci* **30**:16324–16331.
1070 doi:10.1523/JNEUROSCI.2087-10.2010
1071 Wilson EB. 1927. Probable Inference, the Law of Succession, and Statistical Inference. *J Am Stat Assoc* **22**:209–212.
1072 doi:10.1080/01621459.1927.10502953
1073 World Medical Association. 2013. World Medical Association Declaration of Helsinki: ethical principles for medical
1074 research involving human subjects. *JAMA* **310**:2191–2194. doi:10.1001/jama.2013.281053
1075 Wu B, Zhou L, Chen C, Wang J, Hu L, Wang X. 2022. Effects of Exercise-induced Hypoalgesia and Its Neural
1076 Mechanisms. *Med Sci Sports Exerc* **54**:220–231. doi:10.1249/MSS.0000000000002781
1077