



# Neural mechanisms underlying placebo and nocebo effects in tonic muscle pain

Min Chen<sup>a,1</sup>, Xiao Wu<sup>b,c,d,1</sup>, Libo Zhang<sup>c,d,1</sup>, Fengrui Zhang<sup>c,d</sup>, Linling Li<sup>e</sup>, Yingying Zhang<sup>f</sup>, Donglin Xiong<sup>g</sup>, Yunhai Qiu<sup>e</sup>, Li Hu<sup>c,d,\*</sup>, Weibo Xiao<sup>g,h,\*</sup>

<sup>a</sup> Department of Anesthesiology, Shenzhen Samii Medical Center, Guangdong Province, China

<sup>b</sup> CAS Key Laboratory of Behavioral Science, Institute of Psychology, Chinese Academy of Sciences, Beijing, China

<sup>c</sup> Department of Psychology, University of Chinese Academy of Sciences, Beijing, China

<sup>d</sup> CAS Key Laboratory of Mental Health, Institute of Psychology, Chinese Academy of Sciences, Beijing, China

<sup>e</sup> Research Center for Neural Engineering, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Guangdong Province, China

<sup>f</sup> Department of Neurology, Loudi Central Hospital, Hunan Province, China

<sup>g</sup> Department of Pain, Nanshan Hospital of Shenzhen City, Guangdong Medical College, Guangdong Province, China

<sup>h</sup> Department of Anesthesiology, Loudi Central Hospital, Hunan Province, China

## ARTICLE INFO

### Keywords:

Placebo analgesia

Nocebo hyperalgesia

Tonic muscle pain

Functional magnetic resonance imaging (fMRI)

Putamen

Caudate

## ABSTRACT

Pain is a highly subjective and multidimensional experience, significantly influenced by various psychological factors. Placebo analgesia and nocebo hyperalgesia exemplify this influence, where inert treatments result in pain relief or exacerbation, respectively. While extensive research has elucidated the psychological and neural mechanisms behind these effects, most studies have focused on transient pain stimuli. To explore these mechanisms in the context of tonic pain, we conducted a study using a 15-minute tonic muscle pain induction procedure, where hypertonic saline was infused into the left masseter of healthy participants. We collected real-time Visual Analogue Scale (VAS) scores and functional magnetic resonance imaging (fMRI) data during the induction of placebo analgesia and nocebo hyperalgesia via conditioned learning. Our findings revealed that placebo analgesia was more pronounced and lasted longer than nocebo hyperalgesia. Real-time pain ratings correlated significantly with neural activity in several brain regions. Notably, the putamen was implicated in both effects, while the caudate and other regions were differentially involved in placebo and nocebo effects. These findings confirm that the tonic muscle pain paradigm can be used to investigate the mechanisms of placebo and nocebo effects and indicate that placebo analgesia and nocebo hyperalgesia may have more distinct than common neural bases.

## 1. Introduction

Pain is a major public health issue. Chronic pain consistently ranks as one of the most burdensome non-fatal diseases (Vos et al., 2012) and incurs hundreds of billions of economic costs in major countries (Gaskin and Richard, 2012; Zhang et al., 2016). While few active treatments can cure chronic pain, placebos can alleviate pain in many chronic pain patients (Kaptchuk et al., 2020). This may be due to the fact that pain is a highly subjective, multidimensional experience significantly influenced by numerous psychological factors (Linton and Shaw, 2011). Two typical examples of how psychological states affect pain are placebo analgesia and nocebo hyperalgesia, where a pharmacologically inert

treatment leads to pain relief and pain exacerbation, respectively (Colloca and Barsky, 2020). Importantly, placebo effects play so important a role in pain management that they can explain nearly 50 % of treatment effects of analgesics (Bingel, 2020), even overshadowing the real effects of some treatments and leading to the failed development of analgesics (Raman, 2020; Rodrigues and Ferreira, 2020). Conversely, nocebo hyperalgesia is one of the reasons why some patients discontinue their pain treatment (Klinger et al., 2017). Therefore, revealing the neural bases of placebo/nocebo effects not only promotes a better understanding of placebo analgesia/nocebo hyperalgesia but also aids in alleviating pain and improving patients' well-being.

Decades of research have made remarkable advances in

\* Corresponding authors.

E-mail addresses: [huli@psych.ac.cn](mailto:huli@psych.ac.cn) (L. Hu), [xiaoweibo820901@163.com](mailto:xiaoweibo820901@163.com) (W. Xiao).

<sup>1</sup> These authors contributed equally

understanding the psychological and neural mechanisms of placebo and nocebo effects in pain. Expectations and learning are two major psychological mechanisms of both placebo and nocebo effects (Benedetti et al., 2022; Colloca and Barsky, 2020; Petrie and Rief, 2019; Tu et al., 2022). Generally speaking, positive expectations of pain relief lead to placebo analgesia, while negative expectations of pain exacerbation cause nocebo hyperalgesia (Zhang et al., 2019). For example, expectations established through direct verbal information about the efficacy or side effects of treatments have been shown to induce placebo or nocebo effects in pain (Colloca et al., 2008; van Laarhoven et al., 2011). Pairing cues with lower or higher pain stimuli, namely classical conditioning, also generates expectations and thus produces placebo or nocebo effects (Colloca and Benedetti, 2006; Reicherts et al., 2016; Tu et al., 2020). Classical conditioning itself also contributes to placebo and nocebo effects independent of expectations, as evidenced by implicit conditioning studies where participants are unaware of the coupling of cues and pain stimuli (Jensen et al., 2012; Liu et al., 2020; Tu et al., 2021a). Apart from psychological mechanisms, researchers have begun to unveil the neural bases of placebo analgesia and nocebo hyperalgesia. A meta-analysis has concluded that the endogenous opioid, endocannabinoid, and vasopressinergic systems are involved in placebo analgesia, while the cholecystikinin system is implicated in nocebo hyperalgesia (Skyt et al., 2020). Neuroimaging techniques, like functional magnetic resonance imaging (fMRI), have shown that placebo analgesia recruits areas such as the prefrontal cortex, cingulate cortex, precentral gyri, insula, putamen, caudate, thalamus, and periaqueductal gray (Benedetti et al., 2022). Conversely, nocebo hyperalgesia has been related to the dorsal anterior cingulate cortex, posterior insula, and parietal operculum (Fu et al., 2021).

These advances notwithstanding, two key issues remain not fully addressed as of now. One issue is that most basic research on placebo analgesia and nocebo hyperalgesia uses only transient painful stimuli like contact heat and electrical painful stimuli, which last at most several seconds (Fu et al., 2021). Clinically-relevant pain, however, often lasts much longer, ranging from minutes to months. Neural substrates of placebo and nocebo effects in clinically-relevant pain, such as tonic or chronic pain, may thus not be the same as those in transient pain. Indeed, different areas are involved in processing transient versus tonic/chronic pain (Reckziegel et al., 2019). For example, emotion-related areas play more important roles in tonic or chronic pain than in transient pain (Baliki and Apkarian, 2015). The other unaddressed issue is how similar or different neural mechanisms of placebo analgesia and nocebo effects are (Petrovic, 2008), particularly in the context of tonic pain. Placebo and nocebo effects seem to be two sides of the same coin: one arises from positive expectations and learning about treatments, while the other arises from negative expectations and learning. Consistent with this idea, placebo and nocebo effects have been found to show opposite opioid and dopamine responses (Scott et al., 2008). However, there are also different neural underpinnings for placebo and nocebo effects. For example, a meta-analysis has identified distinct neural networks for placebo and nocebo effects (Fu et al., 2021). It is even more unclear whether different or similar neural activities subserve placebo and nocebo effects in tonic pain.

To address these issues, the present study used a tonic muscle pain induction procedure, where hypertonic saline was infused into the left masseter of healthy participants for 15 min. In the meantime, we collected real-time Visual Analogue Scale (VAS) scores and fMRI data while placebo analgesia/nocebo hyperalgesia was induced via conditioned learning. To unveil and contrast the neural mechanisms of placebo and nocebo effects, we conducted a series of statistical analyses, e. g., general linear model, multilevel correlation and regression analyses, and multilevel mediation path analyses.

## 2. Methods

### 2.1. Participants

A total of 46 healthy, medication-free, right-handed male volunteers were recruited in the study, and 42 participants were included after excluding one participant for not being able to complete the whole experimental procedure, one for imaging artifacts, and two for excessive head motions ( $> 3.5 \text{ mm/}^\circ$ ) during fMRI scanning. Twenty-one participants were assigned to the placebo group (age:  $20.57 \pm 1.71$  years), while the remaining 21 were assigned to the nocebo group (age:  $21.09 \pm 2.04$  years). All participants met the safety criteria for MRI, had no history of neurological disorders, chronic pain disorders, or smoking, and had not experienced acute pain in the four weeks preceding the experiment. Each participant provided written informed consent and received monetary compensation for their participation. The study was approved by the Human Research Ethics Committee of the Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, China, and adhered to the principles of the Declaration of Helsinki.

### 2.2. Tonic pain induction

Deep sustained muscle pain was induced in the left masseter of participants using a closed-loop system designed for maintaining constant experimental muscle pain, as proposed by Zhang et al. (1993) and previously utilized in tonic pain studies (Scott et al., 2008; Zubieta et al., 2005). Initially, a bolus of hypertonic saline (5 % NaCl) was administered through a computer-controlled infusion pump within a 15-s timeframe. Then, hypertonic saline was delivered with a dynamic speed to ensure that the participant's pain intensity remained within a predefined range. The infusion speed was adjusted every 15 s by a proportional-integral-derivative (PID) controller, which took into account the participant's pain perception rating to the initial bolus and real-time ratings of the present pain intensity (Fig. 1A). Participants rated the present pain intensity using an electronic VAS ranging from 0 (no pain) to 10 (the most pain intensity imaginable). The VAS had 100 intervals in total, with each interval representing a score change of 0.1. Participants used two buttons located in their left hand to control the VAS scores: one button to increment the score by one unit with each press, and the other button to decrement the score. VAS scores were collected at a frequency of one score per second.

In this study, a run of tonic pain lasted for 25 min, consisting of a 5-min baseline period, followed by a 15-min phase of tonic pain induced by hypertonic saline infusion, and finished with a 5-min recovery period. Hypertonic saline infusion was not administered during the baseline and recovery phases. Please note that the 24-gauge needles were used in this study, with precise positioning guided by delineating the contour of the masseter muscle during jaw clenching. The needles were inserted to a depth of approximately 1 cm into the masseter muscle. After a successful infusion session (two runs), the metal needles were removed. It's important to highlight that all pain induction procedures were performed by licensed medical professionals. Noted that injection of hypertonic saline has been by far the most frequently used chemical stimulus in experimental muscle pain research because of its safety and reliability (Reddy et al., 2012).

### 2.3. Experimental design

The experiment was designed according to the concept of inducing expectations related to fictitious drug effects. It was conducted on two consecutive days for each participant to ensure that each day's experimental procedures could be completed within three hours and that participants remained in good condition throughout the experiment (Fig. 1B). On the first day (D1), participants' expectations regarding the effects of the pseudo "drug" were established, and on the second day (D2), the placebo and nocebo effects were evaluated during fMRI

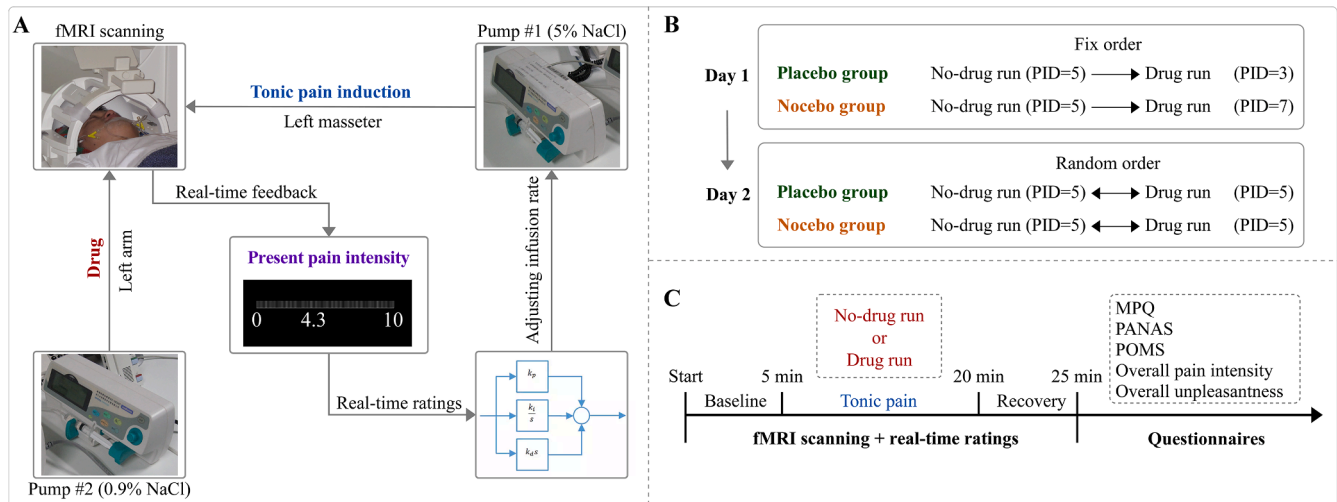


**Fig. 1.** Tonic pain induction and experimental procedures. (A) Tonic muscle pain was induced in the left masseter using hypertonic saline (5 % NaCl), with real-time feedback on the present pain intensity to adjust the infusion rate of hypertonic saline via a PID controller. This system aims to maintain the participant's pain intensity within a predefined range. (B) On day 1, participants in the placebo and nocebo groups were primed to anticipate respectively decreased and increased pain from the “drug” by manipulating the PID controller. On day 2, participants were informed to receive the same “drug” as on day 1, enabling the generation of placebo and nocebo effects based on their expectations. On day 1, the placebo group received lower pain (PID = 3), and the nocebo group received higher pain (PID = 7) during the drug run. Participants received moderate pain during the no-drug run (PID = 5). On day 2, the same stimulus intensity was applied in all conditions (PID = 5). (C) Both non-drug and drug runs lasted 25 min, comprising a 5-minute baseline, 15-minute tonic pain stimulation, and 5-minute recovery. Following recovery, participants were asked to complete several questionnaires. MPQ: the short form of McGill Pain Questionnaire, PANAS: Positive and Negative Schedule, POMS: the short form of Profile of Mood States, PID: a proportional-integral-derivative controller.

scanning. On D1, participants in the placebo group were primed to anticipate pain relief from the “drug”, while those in the nocebo group were primed to anticipate increased pain due to the “drug” (see details below). On D2, participants were informed that they would receive the same “drug” as on D1, enabling the generation of placebo and nocebo effects based on their expectations of the “drug”.

The experiment employed two infusion pumps. Pump #1 controlled the induction of tonic pain in the left masseter muscle, while pump #2 regulated the infusion purported to be the “drug” into the left arm. Importantly, the “drug” being referred to had no impact on pain modulation. Throughout the experiment, medication-grade isotonic saline solution (0.9 % NaCl) was administered into the left arm at a constant rate of 75  $\mu$ L/min on both days. Instead, the effect of the purported “drug” was generated by adjusting the intensity of the tonic pain administered to the left masseter muscle.

Each participant underwent two runs of tonic pain on both D1 and D2: one run with the “drug” infusion (drug run) and one without the “drug” infusion (no-drug run). The no-drug run served as the control condition, during which participants received a moderate level of tonic pain (0.25 mL bolus with PID = 5) without any specific instructions. In the drug run, participants were informed that the intravenous infusion (pump #2) would be switched from isotonic saline to a “drug” called “entacapone”, which is clinically used to treat Parkinson’s disease. They were also informed that the effects of the “drug” on pain relief were uncertain on D1. Then, participants in the placebo group received tonic pain with a lower intensity (0.15 mL bolus with PID = 3), while participants in the nocebo group received tonic pain with a higher intensity (0.3 mL bolus with PID = 7). On D2, all participants received the same stimuli in the drug run as in the no-drug run (0.25 mL bolus with PID = 5). However, it is possible that the placebo group anticipated the “drug” to alleviate pain, while the nocebo group anticipated the “drug” to exacerbate pain.

Additionally, participants always underwent the no-drug run followed by the drug run on D1, whereas the order was randomized on D2. There was a minimum time interval of 10 min between the two runs. Immediately after each run, the experimenter completed the short form of McGill Pain Questionnaire (MPQ; Melzack, 1987), the Positive and

Negative Affect Schedule (PANAS; Watson et al., 1988), and the short form of Profile of Mood States (POMS; Grove and Prapavessis, 1992). Additionally, the experimenter collected the overall pain intensity and unpleasantness during the run based on the participant’s oral responses. The MPQ assesses the present pain-related sensory and affect components, as well as the present pain intensity (PPI). The PANAS measures the positive and negative affect, and the POMS includes seven subscales to measure specific affect, including vigor, esteem-related affect, tension, anger, fatigue, depression, and confusion. Ratings for overall pain intensity and unpleasantness ranged from 0 (no pain/unpleasantness) to 10 (the most pain intensity/ unpleasantness imaginable).

#### 2.4. MRI data acquisition

Images were acquired using a 3.0 Tesla Siemens Trio scanner (Siemens Medical, Erlangen, Germany) at Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, China. High-resolution T1-weighted structural images were collected with 3D-Magnetization Prepared Rapid Gradient Echo Sequence (Repetition Time [TR] = 1900 ms, Echo Time [TE] = 2.55 ms, Field of View [FOV] = 240  $\times$  240 mm<sup>2</sup>, matrix size = 256  $\times$  256, flip angle = 9°, slice thickness = 1 mm, 176 slices). Functional images were acquired with echo planar imaging sequence (TR = 2000 ms, TE = 30 ms, FOV = 220  $\times$  220 mm<sup>2</sup>, matrix size = 64  $\times$  64, flip angle = 90°, slice thickness = 4 mm, spacing between slices = 5.2 mm, 31 slices, interleaved slice order). A total of 750 volumes were acquired for both the no-drug run and drug run, including 150 volumes for baseline, 450 volumes for tonic pain stimulation, and 150 volumes for recovery.

#### 2.5. MRI data preprocessing

MRI data were preprocessed using the SPM 12 toolbox (Statistical Parametric Mapping, <http://www.fil.ion.ucl.ac.uk/spm/>) following the preprocessing and quality control protocol proposed by Di and Biswal (2022). Specifically, structural images were segmented into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF), and deformation fields between the native and the Montreal Neurological

Institute (MNI) space were generated during the segmentation. For functional images, the initial ten volumes were discarded from each fMRI run to ensure the scanner signal stability. The remaining fMRI images were slice-timing corrected to the middle slice, head motion corrected to the mean image, coregistered to the skull-stripped bias-corrected structural images, normalized to the MNI space with the deformation field generated during structural image segmentation, and resampled to  $3 \times 3 \times 3 \text{ mm}^3$  isotropic voxels, and smoothed with a 6 mm full-width half maximum (FWHM) Gaussian kernel (termed smoothed fMRI images). These smoothed fMRI images were used for further fMRI General Linear Model (GLM) analyses. For timeseries analyses, an additional step to regress out covariates was conducted before smoothing. These covariates included the 24 Friston's head motion model (Friston et al., 1996) and the first principal component of the signals of the WM and CSF. The regressed then smoothed images, termed regressed fMRI images, were used for further timeseries extraction.

## 2.6. Statistical analyses

### 2.6.1. Behavioral data analyses

Statistical comparisons were conducted separately for D1 and D2 on each subscale of MPQ, PANAS, and POMS, as well as on the overall pain intensity and unpleasantness between the no-drug run and drug run as well as between placebo and nocebo groups. A two-way repeated-measures analysis of variance (ANOVA) was used to examine placebo and nocebo effects, with 'group' (placebo vs. nocebo) as the between-subjects factor and 'treatment' (drug vs. no-drug) as the within-subjects factor. When significant interactions were observed, post-hoc comparisons were performed between the no-drug run and drug run for placebo and nocebo groups, respectively. If the data were normally distributed, as determined by D'Agostino and Pearson's test that combines skew and kurtosis (D'Agostino, 1971), paired-sample *t*-tests were performed. Otherwise, Wilcoxon signed-rank tests were performed. To control for false positives, *p* values were adjusted using the False Discovery Rate (FDR) correction. Furthermore, only VAS scores indicating real-time pain intensity at odd time points (e.g., 1 s, 3 s, 5 s...) were retained for each participant to align with each TR (2 s), since each fMRI volume was slice-timing corrected to the middle slice. Mean VAS scores during the tonic pain phase (the middle 15 min) were computed for each participant and compared between no-drug run and drug run on D1 and D2 using two-way repeated-measures ANOVA with post-hoc paired-samples *t*-tests or Wilcoxon sign-rank tests, depending on the normality of the data. Additionally, to evaluate dynamic changes of VAS score differences, two-way repeated-measures ANOVA followed by paired-sample *t*-tests were performed between no-drug run and drug run at each timepoint on D2. All statistical analyses were conducted using the *scipy* package (Virtanen et al., 2020) and *pingouin* package (Vallat, 2018).

### 2.6.2. GLM analyses

For each participant and each run (i.e., no-drug run and drug run), first-level analyses were performed on smoothed fMRI images. Age and run order were included as covariates, along with six realignment parameters as regressors of no interest and real-time VAS scores (i.e., real-time pain intensity) as regressors of interest. A high-pass filter of 1000s was performed due to the prolonged duration of the tonic pain stimulation. T-contrasts were generated to identify brain regions exhibiting correlations with real-time VAS scores. At the group-level, the contrast maps were aggregated across all participants and both runs, and compared using one-sample *t*-tests against zero. The statistical analysis was constrained to a gray matter mask, which included voxels where the mean probability of the gray matter mask across all subjects was 0.2 or higher. To ensure robust and reliable results, a more stringent primary threshold (i.e.,  $p < 0.0001$ ) was applied at the voxel level. Cluster-level family-wise error (FWE) correction with a threshold of  $p < 0.05$  was also applied to correct for multiple comparisons. Brain regions showing

positive or negative correlations with real-time VAS scores were identified as regions of interest (ROIs), and ROI masks were generated using the *Marsbar* toolbox (Brett et al., 2010). Additionally, brain regions correlating with real-time VAS scores were examined separately for the no-drug run and drug run within the placebo and nocebo groups. In these analyses, a voxel-level threshold of  $p < 0.001$  and cluster-level FWE correction with a threshold of  $p < 0.05$  were applied to account for multiple comparisons.

### 2.6.3. Multilevel mediation path analyses

For further analyses, ROI timeseries were extracted from the regressed fMRI images. First, ROI timeseries during tonic pain stimulation (450 volumes) were normalized to the baseline by subtracting the mean value during the baseline period, separately for the no-drug run and drug run. Second, multilevel correlation analyses were performed between ROI timeseries and real-time VAS scores in the no-drug run and drug run for placebo and nocebo groups, respectively. This resulted in a total of four multilevel correlation analyses for each ROI. These correlation analyses were performed while clustered within each participant. Third, multilevel regression analyses were performed to explore whether significant differences existed in ROI timeseries between the no-drug run and drug run (the no-drug run was coded as 0, and the drug run was coded as 1) in placebo and nocebo groups, respectively. The ROI timeseries were treated as the dependent variable, and the run type was treated as the independent variable. Once again, the analyses were clustered within each participant. FDR correction was separately applied to the *p* values in each type of analysis (e.g., *p* values of the multilevel regression analyses for all ROIs in the placebo group). Time series extractions were conducted using the *nibabel* package (Brett et al., 2023), multilevel correlation analyses used the *correlation* package (Makowski et al., 2020, 2022), and multilevel regression analyses employed the *lme4* package (Bates et al., 2015).

Based on the second and third steps outlined above, ROIs that correlated with real-time VAS scores in both the no-drug run and drug run, while also demonstrating significant differences between the two runs were identified in the placebo and nocebo groups, respectively (e.g., ROIs with timeseries positively correlated with real-time VAS scores in both drug run and no-drug run, and showing significantly stronger activity in drug run compared to no-drug run in the placebo group). Furthermore, multilevel structural equation modeling (SEM) was built using run type as the independent variable (no-drug run was coded as 0, and drug run was coded as 1), timeseries from ROIs as mediators, and real-time VAS scores as the dependent variable. Please note that ROI timeseries were person-mean-centered before analyses. If multiple ROIs were involved, a latent variable was incorporated. A standard maximum likelihood estimator was used, and the SEM was built for positive and negative correlations separately. The estimate was considered statistically significant when the 95 % confidence interval (CI) excluded zero. Standardized path coefficients ( $\beta$ ), 95 % CIs, and *p* values were reported for both direct and indirect effects. The model fit was assessed using the following criteria if the model was not saturated ( $p = 1.000$ ,  $\chi^2 = 0$ ,  $df = 0$ ): the significance of chi-square test  $p > 0.05$ , the normed chi-square, i.e., the ratio of  $\chi^2$  to degree of freedom ( $df$ ),  $< 2$  (Ullman, 2001), the root mean square error of approximation (RMSEA)  $\leq 0.06$ , standardized root mean square residual (SRMR)  $\leq 0.08$ , both the comparative fit index (CFI) and the Tucker-Lewis index (TLI)  $\geq 0.95$  (Hu and Bentler, 1999). *Mplus* v8.3 (<https://www.statmodel.com/>) was used for the multilevel SEM analyses.

## 3. Results

### 3.1. Behavioral results

A post-hoc power calculation for the two-way repeated-measures ANOVA was performed using *G\*power* (Faul et al., 2009, 2007). Specifically, for overall pain intensity, the post-hoc power analysis was

conducted to verify the sample size used in the study (i.e., 42 participants). Using the G\*Power tool, an F-test was applied for two groups with two treatment conditions, a confidence level of 95 % ( $\alpha$  error probability = 0.05), and an effect size of 1.106 (derived from the partial  $\eta^2 = 0.55$  for the interaction effect). The results showed a power level ( $1-\beta$ ) of 1.0, which is above the 0.8 threshold typically considered adequate in the analysis (Kang, 2021). For mean VAS scores, the same post-hoc power analysis was performed to support the sample size of 42 participants. The F-test was applied with the same parameters, except for an effect size of 0.886 (derived from the partial  $\eta^2 = 0.44$  for the interaction effect). The results showed a power level ( $1-\beta$ ) of 1.0, also higher than the 0.8 threshold.

Two-way repeated-measures ANOVA revealed that overall pain intensity on D2 was not significantly modulated by the main effects of 'group' ( $F(1,40) = 3.07, p = 0.087$ ) or 'treatment' ( $F(1,40) = 1.99, p = 0.166$ ), but was significantly modulated by their interaction ( $F(1,40) = 49.72, p < 0.001, \eta^2 = 0.55$ ). Post-hoc paired-sample  $t$ -tests revealed significant placebo and nocebo effects (Fig. 2 left part; Table 1; Table S1). Specifically, overall pain intensity was significantly lower in the drug run than in the no-drug run in the placebo group ( $t(20) = 6.78, q_{\text{fdr}} < 0.001$ , Cohen's  $d = 1.69$ ), while it was significantly higher in the drug run than in the no-drug run in the nocebo group ( $t(20) = -3.61, q_{\text{fdr}} = 0.008$ , Cohen's  $d = -0.83$ ). In contrast, overall unpleasantness was neither significantly modulated by the main effects of 'group' ( $F(1,40) = 1.29, p = 0.262$ ) or 'treatment' ( $F(1,40) = 3.27, p = 0.078$ ), nor by their interaction ( $F(1,40) = 2.38, p = 0.131$ ).

Mean VAS scores were not significantly modulated by the main effects of 'group' ( $F(1,40) = 0.84, p = 0.365$ ) or 'treatment' ( $F(1,40) = 3.20, p = 0.081$ ), but were significantly modulated by their interaction ( $F(1,40) = 31.03, p < 0.001, \eta^2 = 0.44$ ). Post-hoc paired-sample  $t$ -tests also showed a significant difference in pain intensity between the no-drug run and drug run in both placebo ( $4.13 \pm 1.17$  vs.  $2.81 \pm 1.07, t$

(20) = 5.75,  $p < 0.001$ , Cohen's  $d = 1.14$ ) and nocebo groups ( $3.45 \pm 1.17$  vs.  $4.13 \pm 1.49, t(20) = -2.46, p = 0.023$ , Cohen's  $d = -0.49$ ). The real-time VAS scores depicted in Fig. 2 (right part) showed the dynamic changes in pain intensity difference over time. Point-by-point statistical analyses showed that real-time VAS scores were not significantly modulated by the main effects of 'group' or 'treatment' in most time intervals, but were significantly modulated by their interaction in most time intervals during tonic pain stimulation (Figure S1). Post-hoc paired-sample  $t$ -tests suggested that the placebo effect was presented throughout almost the entire tonic pain stimulation period, while the nocebo effect was observed primarily in the early part of the stimulation period (Fig. 2, right).

Significant interactions between the factors 'group' and 'treatment' were also observed in the sensory subscale of MPQ ( $F(1,40) = 12.36, p = 0.001, \eta^2 = 0.24$ ) and tension subscale of POMS ( $F(1,40) = 7.43, p = 0.009, \eta^2 = 0.16$ ). A significant main effect of 'treatment' was observed in the fatigue subscale of POMS ( $F(1,40) = 5.07, p = 0.03, \eta^2 = 0.11$ ). Post-hoc Wilcoxon sign-rank tests revealed significant decreases in negative affect from the no-drug run to the drug run in the placebo group on D2, i.e., tension:  $z$  based on negative ranks =  $-2.95, q_{\text{fdr}} = 0.011$ ; and fatigue:  $z$  based on negative ranks =  $-2.35, q_{\text{fdr}} = 0.049$ . However, no significant difference in affect was observed in the nocebo group on D2.

### 3.2. GLM results

Across all participants and both runs, positive correlations between BOLD responses and real-time VAS scores were observed in a series of brain regions, including the bilateral thalamus, bilateral insula, bilateral caudate, bilateral supracallosal anterior cingulate cortex (ACC), bilateral superior frontal gyrus (SFG), left orbital frontal cortex (OFC), right precentral gyrus, right postcentral gyrus, right supplementary motor area (SMA), right Rolandic operculum, right hippocampal gyrus, and

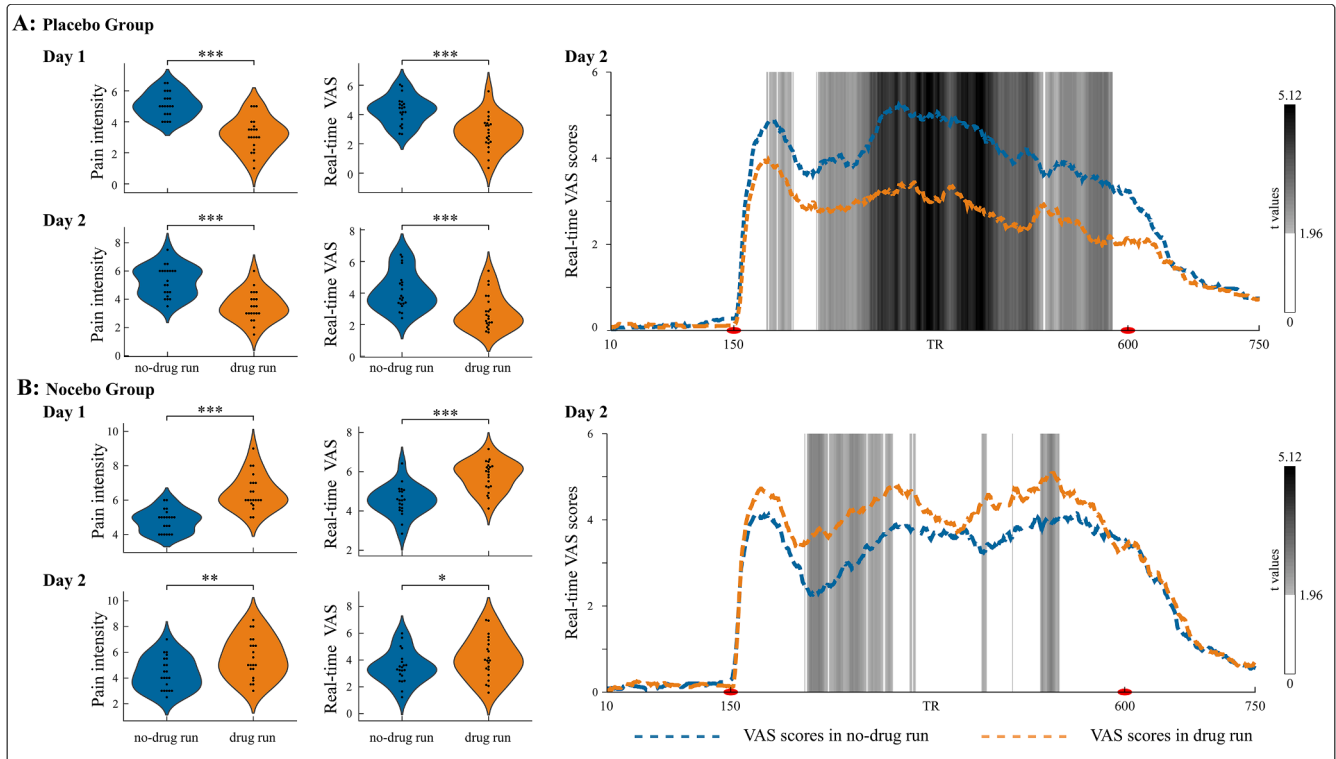


**Fig. 2.** Behavioral results in placebo (A) and nocebo groups (B). The left two columns illustrate overall pain intensity and real-time pain intensity averaged across time (i.e., VAS scores from 5 min to 20 min) on day 1 and day 2, respectively. The right column displays the time courses of real-time pain intensity on day 2, along with the statistical results (significant  $t$  values are marked in gray,  $t \leq 1.96$  or non-significant interaction effect was marked in white). VAS scores during the no-drug run and drug run are depicted in blue and orange, respectively. VAS: Visual Analogue Scale, TR: repetition time = 2 s.

**Table 1**  
Descriptive information (mean ± standard deviation) and statistical results for behavioral data on Day 2.

| Scale/Subscale         | Placebo Group (n = 21) |              |           |                  |           | Nocebo Group (n = 21) |              |           |                  |           |
|------------------------|------------------------|--------------|-----------|------------------|-----------|-----------------------|--------------|-----------|------------------|-----------|
|                        | no-drug                | drug         | t/z value | q <sub>fdr</sub> | Cohen's d | no-drug               | drug         | t/z value | q <sub>fdr</sub> | Cohen's d |
| MPQ/Sensory            | 7.76 ± 3.77            | 5.67 ± 2.88  | −3.08     | 0.008            | 0.61      | 8.33 ± 3.97           | 9.86 ± 5.56  | −1.79     | 0.142            |           |
| MPQ/Affective          | 1.86 ± 1.46            | 1.29 ± 1.61  | −1.97     | 0.091            |           | 2.10 ± 1.69           | 2.33 ± 2.03  | −0.74     | 0.537            |           |
| MPQ/PPI                | 0.33 ± 0.56            | 0.05 ± 0.21  | −2.12     | 0.073            |           | 0.33 ± 0.47           | 0.38 ± 0.58  | −0.38     | 0.745            |           |
| PANAS/Positive         | 21.19 ± 8.44           | 20.05 ± 7.74 | 1.24      | 0.313            |           | 19.81 ± 9.58          | 20.05 ± 8.43 | −0.38     | 0.745            |           |
| PANAS/Negative         | 14.48 ± 4.00           | 13.05 ± 4.06 | −3.29     | 0.007            | 0.35      | 15.33 ± 5.09          | 15.14 ± 4.72 | −0.03     | 0.979            |           |
| POMS/Vigor             | 6.24 ± 5.74            | 5.71 ± 5.62  | 0.97      | 0.425            |           | 5.48 ± 6.35           | 5.10 ± 5.81  | −0.51     | 0.668            |           |
| POMS/Esteem            | 4.62 ± 4.45            | 4.38 ± 4.21  | 0.79      | 0.520            |           | 4.29 ± 4.61           | 3.81 ± 3.87  | −0.74     | 0.537            |           |
| POMS/Tension           | 3.86 ± 3.14            | 2.62 ± 3.09  | −2.95     | 0.011            | 0.39      | 3.90 ± 3.57           | 4.81 ± 3.97  | −1.54     | 0.179            |           |
| POMS/Anger             | 1.19 ± 2.15            | 1.10 ± 3.42  | −0.87     | 0.468            |           | 1.33 ± 2.21           | 2.19 ± 3.38  | −1.76     | 0.132            |           |
| POMS/Fatigue           | 5.10 ± 3.64            | 3.62 ± 3.58  | −2.35     | 0.049            | 0.40      | 3.86 ± 4.11           | 3.43 ± 3.32  | 0.65      | 0.584            |           |
| POMS/Depression        | 1.76 ± 1.92            | 1.38 ± 3.09  | −1.48     | 0.196            |           | 2.71 ± 2.66           | 2.52 ± 2.95  | −0.12     | 0.922            |           |
| POMS/Confusion         | 2.57 ± 2.17            | 2.10 ± 2.16  | −1.63     | 0.161            |           | 2.67 ± 2.49           | 3.10 ± 2.65  | −1.16     | 0.329            |           |
| Overall Pain Intensity | 5.29 ± 1.04            | 3.50 ± 1.02  | 6.78      | < 0.001          | 1.69      | 4.37 ± 1.23           | 5.56 ± 1.56  | −3.61     | 0.008            | −0.83     |
| Overall Unpleasantness | 1.82 ± 1.51            | 1.17 ± 1.27  | −2.31     | 0.050            | 0.46      | 1.96 ± 1.39           | 1.91 ± 1.27  | 0.17      | 0.899            |           |

If the data were normally distributed, paired-sample *t*-tests were conducted and *t* values were provided; otherwise, Wilcoxon signed-rank tests were conducted and *z* values were provided. FDR correction was applied to the *p* values. MPQ: the short form of McGill Pain Questionnaire, PANAS: Positive and Negative Affect Schedule, POMS: short form of Profile of Mood States, PPI: Present Pain Intensity.

right pregenual ACC (Fig. 3 and Table 2). Conversely, negative correlations with real-time VAS scores were observed in the left amygdala extending into the hippocampal gyrus, and ventromedial prefrontal cortex (vmPFC) extending into the subgenual ACC, and right putamen (Table 2).

In the placebo group during the drug run, positive correlations with real-time VAS scores were mainly observed in the middle frontal gyrus (MFG) extending into the inferior frontal gyrus (IFG), while negative

correlations were observed in the vmPFC (Table S2). In the placebo group during the no-drug run, negative correlations with real-time VAS scores were mainly observed in the vmPFC extending into the subgenual ACC and fusiform gyrus extending into the parahippocampal gyrus (Table S2).

In the nocebo group during the drug run, positive correlations with real-time VAS scores were mainly observed in the left caudate, right insula, bilateral SFG, and right MFG/IFG extending into bilateral

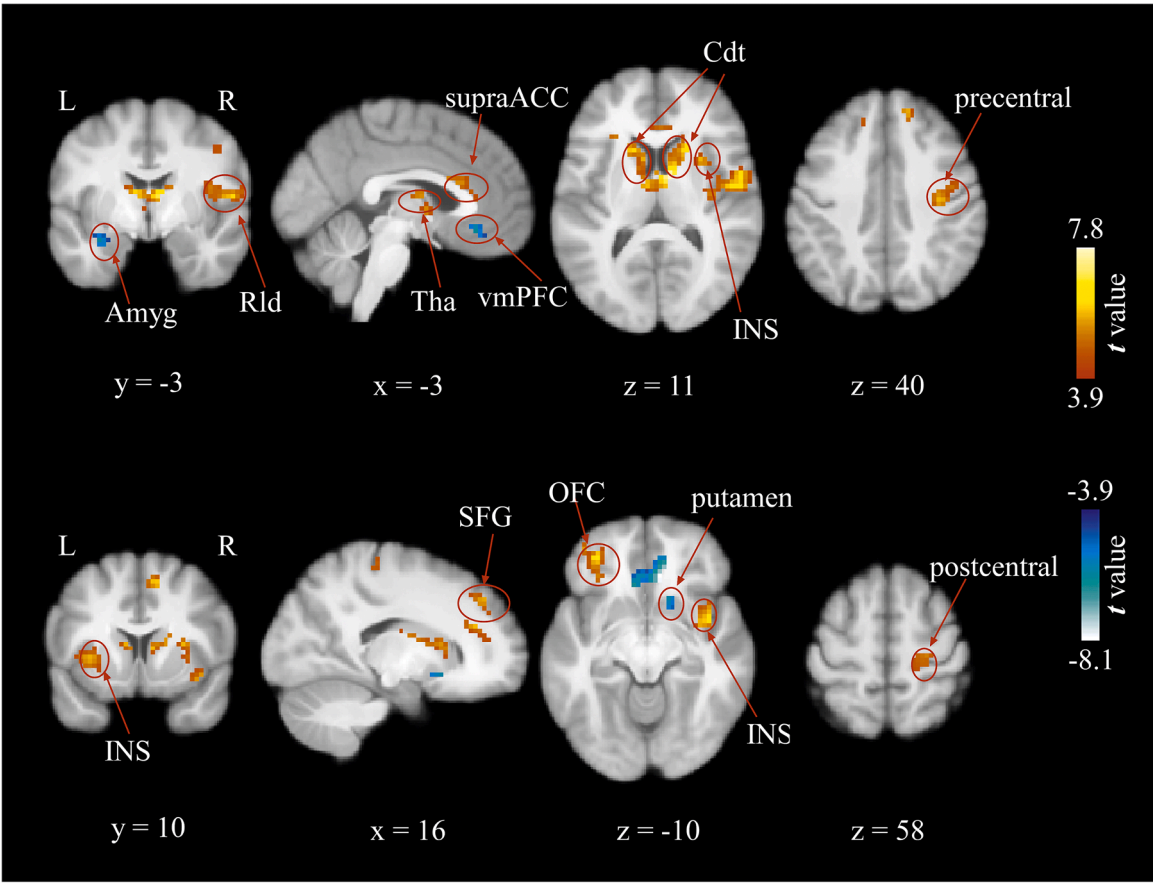


**Fig. 3.** Brain regions correlated with real-time pain intensity. L: left, R: right, Amyg: amygdala, Rld: Rolandic operculum, supraACC: supracallosal anterior cingulate cortex, Tha: thalamus, vmPFC: ventromedial prefrontal cortex, Cdt: caudate, INS: insula, precentral: precentral gyrus, SFG: superior frontal gyrus, OFC: orbital frontal cortex, postcentral: postcentral gyrus.

**Table 2**  
Brain regions showing positive or negative correlations with real-time pain intensity.

| Cluster size | Cluster <i>p</i><br>(FWE) | Hemisphere | Brain regions                                      | <i>t</i> value | Peak MNI coordinates |     |     |
|--------------|---------------------------|------------|----------------------------------------------------|----------------|----------------------|-----|-----|
|              |                           |            |                                                    |                | X                    | Y   | Z   |
| VAS (+)      |                           |            |                                                    |                |                      |     |     |
| 63           | < 0.001                   | B          | thalamus                                           | 7.17           | 6                    | −1  | 11  |
|              |                           |            |                                                    | 5.68           | −9                   | −4  | 14  |
|              |                           |            |                                                    | 5.50           | −6                   | −1  | −1  |
| 94           | < 0.001                   | L          | OFC                                                | 6.43           | −33                  | 44  | −10 |
|              |                           |            |                                                    | 5.43           | −33                  | 35  | −1  |
|              |                           |            |                                                    | 5.09           | −42                  | 50  | −10 |
| 175          | < 0.001                   | R          | Rolandic operculum extending into posterior insula | 6.12           | 57                   | −7  | 11  |
|              |                           |            |                                                    | 6.11           | 36                   | −13 | 20  |
| 49           | 0.001                     | R          | posterior insula                                   | 6.02           | 42                   | 2   | −10 |
|              |                           |            |                                                    | 5.24           | 45                   | 11  | −13 |
| 19           | 0.032                     | R          | SMA                                                | 5.68           | 12                   | 8   | 53  |
| 62           | < 0.001                   | B          | supracallosal ACC                                  | 5.52           | 0                    | 20  | 20  |
|              |                           |            |                                                    | 4.88           | 0                    | 32  | 8   |
|              |                           |            |                                                    | 4.39           | 9                    | 29  | 14  |
| 76           | < 0.001                   | R          | precentral gyrus                                   | 5.47           | 36                   | −16 | 44  |
|              |                           |            |                                                    | 4.57           | 45                   | −7  | 41  |
|              |                           |            |                                                    | 5.40           | 15                   | 38  | 23  |
| 26           | 0.013                     | R          | pregenual ACC                                      | 4.94           | 12                   | 29  | 38  |
|              |                           |            |                                                    | 5.35           | −30                  | 14  | 2   |
| 86           | < 0.001                   | L          | insula                                             | 4.91           | −27                  | 32  | 5   |
|              |                           |            |                                                    | 4.69           | −27                  | 26  | 11  |
|              |                           |            |                                                    | 5.35           | 33                   | −37 | −4  |
| 19           | 0.032                     | R          | hippocampal gyrus                                  | 5.33           | 30                   | 11  | 11  |
| 24           | 0.017                     | R          | insula                                             | 4.26           | 33                   | 11  | 2   |
|              |                           |            |                                                    | 5.23           | 15                   | 44  | 41  |
| 40           | 0.003                     | R          | SFG                                                | 4.61           | 12                   | 35  | 47  |
|              |                           |            |                                                    | 4.84           | −15                  | 35  | 44  |
| 23           | 0.019                     | L          | SFG                                                | 4.66           | −15                  | 38  | 35  |
|              |                           |            |                                                    | 4.22           | −9                   | 32  | 50  |
|              |                           |            |                                                    | 4.71           | 27                   | −22 | 59  |
| 57           | < 0.001                   | R          | postcentral gyrus extending into precentral gyrus  | 4.60           | 12                   | −37 | 62  |
|              |                           |            |                                                    | 7.44           | 9                    | 2   | 11  |
| 95           | < 0.001                   | R          | caudate                                            | 6.04           | 21                   | 17  | 14  |
|              |                           |            |                                                    | 5.50           | 21                   | 23  | 2   |
|              |                           |            |                                                    | 5.73           | −15                  | 17  | 11  |
| 43           | < 0.001                   | L          | caudate                                            | 5.68           | −9                   | −4  | 14  |
|              |                           |            |                                                    | 5.29           | −9                   | 11  | 11  |
|              |                           |            |                                                    | VAS (-)        |                      |     |     |
| 88           | < 0.001                   | B          | vmPFC extending into subgenual ACC                 | 8.12           | 9                    | 32  | −10 |
|              |                           |            |                                                    | 6.19           | −9                   | 26  | −10 |
|              |                           |            |                                                    | 5.89           | −3                   | 32  | −13 |
| 23           | 0.019                     | L          | amygdala extending into hippocampal gyrus          | 5.80           | −33                  | −1  | −19 |
|              |                           |            |                                                    | 4.42           | −27                  | −10 | −13 |
| 16           | 0.049                     | R          | putamen                                            | 5.63           | 15                   | 17  | −10 |
|              |                           |            |                                                    | 5.16           | 21                   | 29  | −13 |
|              |                           |            |                                                    | 4.35           | 27                   | 23  | −16 |

VAS (+): positive correlations with real-time VAS scores, VAS (-): negative correlations with real-time VAS scores. VAS: Visual Analogue Scale, OFC: orbital frontal cortex, SMA: supplementary motor area, ACC: anterior cingulate cortex, SFG: superior frontal gyrus, vmPFC: ventromedial prefrontal cortex, L: left, R: right, B: bilateral.

supracallosal ACC and right caudate (Table S2). Conversely, in the nocebo group during the no-drug run, positive correlations were observed in bilateral caudate extending into the thalamus (Table S2).

### 3.3. Multilevel mediation path analyses

Multilevel correlation and multilevel regression results were demonstrated in Fig. 4 and Table 3. The right putamen exhibited a negative correlation with real-time VAS scores in both the no-drug run and drug run for both placebo and nocebo groups ( $q_{\text{fdr}} < 0.05$  for all comparisons). It showed significantly greater activations in the drug run compared to the no-drug run in the placebo group, while showing significantly weaker activations in the drug run compared to the no-drug run in the nocebo group. The right caudate exhibited a positive correlation with real-time VAS scores in both the no-drug run and drug run for the nocebo group ( $q_{\text{fdr}} < 0.05$  for all comparisons). It showed significantly greater activations in the drug run compared to the no-drug run in the nocebo group. Further multilevel mediation path analysis showed a

significant partial mediation effect of the right putamen in the relationship between run type (no-drug run was coded as 0 and drug run was coded as 1) and real-time VAS scores with saturated models in both placebo and nocebo groups (Fig. 5; placebo group:  $\beta = -0.001$ , 95 % CI =  $[-0.002, -0.001]$ ,  $p = 0.001$ , nocebo group:  $\beta = 0.001$ , 95 % CI =  $[0.000, 0.002]$ ,  $p = 0.007$ ). Furthermore, the right caudate also showed a partial mediation effect in the relationship between run type and VAS scores in the nocebo group ( $\beta = 0.001$ , 95 % CI =  $[0.001, 0.005]$ ,  $p = 0.011$ ).

However, considering that the difference between the no-drug run and drug run was not always significant throughout the entire tonic pain stimulation phase (Fig. 2 bottom part), especially in the nocebo group, concerns arise regarding stringent thresholding on the difference of timeseries between the no-drug run and drug run, which could potentially lead to false negative errors. Thus, additional multilevel mediation path analyses were conducted, incorporating ROIs that showed significant activation differences between the no-drug run and drug run ( $p < 0.05$ ) without applying FDR correction. For the placebo group, all ROIs

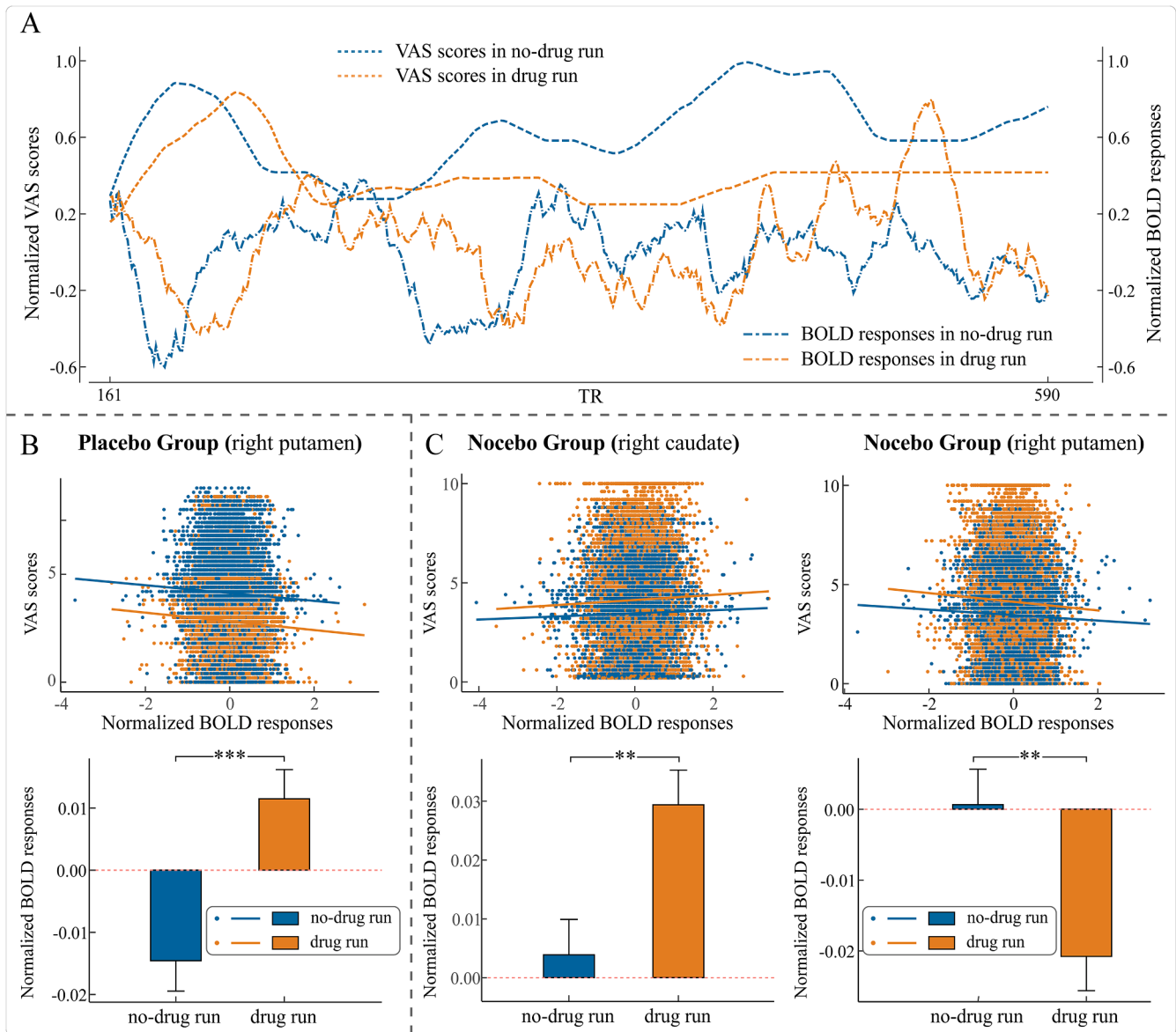


**Fig. 4.** Summarized multilevel correlation and multilevel regression results in the placebo (B) and nocebo groups (C), and an exemplary participant's smoothed time courses of VAS scores and BOLD responses (A). Multilevel correlations between ROI BOLD time courses and real-time VAS scores were performed separately in the no-drug run and drug run, clustered within each participant. Multilevel regression analyses were performed to explore the differences between the no-drug run and drug run, clustered within each participant. An exemplary participant from the placebo group is depicted, with the ROI being the right putamen (A). Please note that the time courses of BOLD responses and VAS scores were smoothed by replacing each time point with the mean value of the ten preceding and ten subsequent time points. The ROIs were the right putamen in the placebo group (B), the right caudate (C, left) and the right putamen in the nocebo group (C, right).

demonstrating negative correlations with real-time VAS scores, as well as significant differences between no-drug and drug runs, were included in the model, i.e., the left amygdala extending into the hippocampal gyrus, right putamen, vmPFC extending into the subgenual ACC. Multilevel SEM showed a good model fit ( $p = 0.463$ ,  $\chi^2/df = 0.899$ , RMSEA < 0.001, SRMR = 0.002, CFI = 1.000, TLI = 1.000), and the timeseries from these ROIs partially mediated the relationship between run type and VAS scores (Fig. 6A; indirect effect:  $\beta = -0.003$ , 95 % CI = [-0.005, -0.001],  $p < 0.001$ ). For the nocebo group, all ROIs demonstrating positive correlations with real-time VAS scores, as well as significant differences between no-drug and drug runs, were included in the model, i.e., the left SFG, left OFC, right pregenual ACC, and right caudate. Multilevel SEM showed a good model fit ( $p = 0.412$ ,  $\chi^2/df = 0.956$ , RMSEA < 0.001, SRMR = 0.002, CFI = 1.000, TLI = 1.000), and the timeseries from these ROIs partially mediated the relationship between run type and VAS scores (Fig. 6B; indirect effect:  $\beta = 0.003$ , 95 %

CI = [0.001, 0.004],  $p = 0.003$ ). For the nocebo group, only the right putamen, whose mediation result was presented in Fig. 5B, showed positive correlations with real-time VAS scores, as well as significant differences between no-drug and drug runs, after FDR correction.

#### 4. Discussion

Using a tonic muscle pain induction procedure, we investigated the neural mechanisms of placebo and nocebo effects in tonic pain. We obtained three main findings. First, placebo analgesia was more pronounced and lasted longer than nocebo hyperalgesia in our tonic pain paradigm, although both effects were successfully induced. Second, real-time pain ratings in this tonic pain paradigm were significantly correlated with neural activities in a series of brain regions. Third, the putamen was involved in both placebo and nocebo effects, while the caudate and other brain regions were differentially related to placebo

**Table 3**

Results of multilevel correlation and multilevel regression analyses.

|                        | Placebo Group |                          |          |                          |                          |                          |                | Nocebo Group |                          |          |                          |                          |                          |                |
|------------------------|---------------|--------------------------|----------|--------------------------|--------------------------|--------------------------|----------------|--------------|--------------------------|----------|--------------------------|--------------------------|--------------------------|----------------|
|                        | no-drug run   |                          | drug run |                          | drug run vs. no-drug run |                          |                | no-drug run  |                          | drug run |                          | drug run vs. no-drug run |                          |                |
|                        | <i>r</i>      | <i>q</i> <sub>fidr</sub> | <i>r</i> | <i>q</i> <sub>fidr</sub> | <i>t</i> value           | <i>q</i> <sub>fidr</sub> | <i>p</i> value | <i>r</i>     | <i>q</i> <sub>fidr</sub> | <i>r</i> | <i>q</i> <sub>fidr</sub> | <i>t</i> value           | <i>q</i> <sub>fidr</sub> | <i>p</i> value |
| (L) caudate            | 0.02          | 0.035                    | 0.02     | 0.040                    | −0.08                    | 0.937                    | 0.937          | 0.02         | 0.103                    | 0.05     | < 0.001                  | 2.56                     | 0.066                    | 0.010          |
| (L) SFG                | 0.02          | 0.032                    | 0.02     | 0.079                    | 0.60                     | 0.743                    | 0.547          | 0.02         | 0.038                    | 0.06     | < 0.001                  | 2.02                     | 0.117                    | 0.043          |
| (L) insula             | 0.04          | 0.001                    | 0.02     | 0.137                    | 1.99                     | 0.146                    | 0.046          | 0.02         | 0.103                    | 0.04     | < 0.001                  | 1.51                     | 0.208                    | 0.132          |
| (L) OFC                | 0.04          | 0.000                    | 0.03     | 0.012                    | 0.95                     | 0.544                    | 0.343          | 0.05         | < 0.001                  | 0.08     | < 0.001                  | 2.39                     | 0.070                    | 0.017          |
| (B) supracallosal ACC  | 0.03          | 0.015                    | 0.01     | 0.267                    | 2.43                     | 0.096                    | 0.015          | 0.04         | 0.001                    | 0.06     | < 0.001                  | 0.96                     | 0.376                    | 0.337          |
| (R) SMA                | 0.02          | 0.021                    | 0.02     | 0.141                    | 0.38                     | 0.833                    | 0.702          | 0.02         | 0.103                    | 0.04     | 0.001                    | −1.07                    | 0.362                    | 0.286          |
| (R) pregenual ACC      | 0.02          | 0.032                    | 0.02     | 0.154                    | 1.11                     | 0.513                    | 0.265          | 0.03         | 0.003                    | 0.03     | 0.004                    | 2.29                     | 0.070                    | 0.022          |
| (R) SFG                | 0.03          | 0.020                    | 0.03     | 0.005                    | −0.80                    | 0.622                    | 0.426          | 0.02         | 0.146                    | 0.07     | < 0.001                  | 2.36                     | 0.070                    | 0.019          |
| (R) postcentral gyrus  | 0.04          | 0.000                    | 0.01     | 0.437                    | 1.10                     | 0.513                    | 0.270          | 0.03         | 0.004                    | 0.04     | < 0.001                  | 1.62                     | 0.208                    | 0.106          |
| (R) insula             | 0.03          | 0.003                    | 0.02     | 0.154                    | 2.02                     | 0.146                    | 0.043          | 0.02         | 0.101                    | 0.04     | < 0.001                  | −1.36                    | 0.236                    | 0.174          |
| (R) hippocampal gyrus  | 0.03          | 0.017                    | 0.00     | 0.693                    | 1.27                     | 0.483                    | 0.203          | 0.00         | 0.870                    | 0.05     | < 0.001                  | −1.02                    | 0.365                    | 0.308          |
| (R) precentral gyrus   | 0.04          | 0.000                    | 0.02     | 0.137                    | −0.11                    | 0.937                    | 0.911          | 0.03         | 0.009                    | 0.06     | < 0.001                  | 0.39                     | 0.700                    | 0.700          |
| (R) posterior insula   | 0.02          | 0.032                    | 0.02     | 0.102                    | 1.02                     | 0.528                    | 0.306          | 0.04         | < 0.001                  | 0.06     | < 0.001                  | −1.60                    | 0.208                    | 0.110          |
| (R) Rolandic operculum | 0.04          | 0.001                    | 0.03     | 0.005                    | 0.24                     | 0.903                    | 0.808          | 0.05         | < 0.001                  | 0.08     | < 0.001                  | −1.51                    | 0.208                    | 0.131          |
| (B) thalamus           | 0.03          | 0.002                    | 0.02     | 0.079                    | 0.50                     | 0.784                    | 0.619          | 0.02         | 0.043                    | 0.04     | < 0.001                  | 1.46                     | 0.210                    | 0.144          |
| (R) caudate            | 0.02          | 0.072                    | 0.03     | 0.012                    | 1.45                     | 0.400                    | 0.147          | 0.02         | 0.045                    | 0.05     | < 0.001                  | 3.03                     | 0.024                    | 0.002          |
| (L) amygdala           | −0.03         | 0.002                    | −0.03    | 0.006                    | 2.02                     | 0.146                    | 0.043          | −0.07        | < 0.001                  | −0.01    | 0.275                    | −1.92                    | 0.132                    | 0.055          |
| (R) putamen            | −0.05         | 0.000                    | −0.04    | 0.001                    | 3.85                     | 0.002                    | < 0.001        | −0.05        | < 0.001                  | −0.04    | < 0.001                  | −3.08                    | 0.024                    | 0.002          |
| (B) vmPFC              | −0.06         | 0.000                    | −0.08    | < 0.001                  | 2.73                     | 0.059                    | 0.006          | −0.08        | < 0.001                  | −0.05    | < 0.001                  | −0.66                    | 0.539                    | 0.511          |

The first four columns in the “Placebo Group” and “Nocebo Group” sections present multilevel correlation results between ROI timeseries and real-time VAS scores during the no-drug run and drug run, respectively. The latter three columns display multilevel regression results, indicating the differences between the drug run and no-drug run. ROIs showing significant correlations with real-time VAS scores during both no-drug run and drug run (*q*<sub>fidr</sub> < 0.05), as well as significant differences between drug run and no-drug run (*p* < 0.05), were color-coded: green for the placebo group, red for the nocebo group, and purple for both groups. SFG: superior frontal gyrus, OFC: orbital frontal cortex, ACC: anterior cingulate cortex, vmPFC: ventromedial prefrontal cortex.

and nocebo effects. These findings confirm that the tonic muscle pain paradigm can be used to investigate the mechanisms of placebo and nocebo effects and indicate that placebo analgesia and nocebo hyperalgesia may have more distinct than common neural bases.

We successfully induced both placebo analgesia and nocebo hyperalgesia with tonic muscle pain stimuli. Interestingly, placebo had larger and longer effects on pain than nocebo did in the present study. The finding of larger placebo analgesia is in line with previous studies showing that placebo analgesia has a greater magnitude than nocebo hyperalgesia in both experimentally induced pain and chronic pain (Petersen et al., 2014; Porporatti et al., 2019; Vase et al., 2016). We also note that the placebo manipulation had larger effect on pain unpleasantness in Day 1 than the nocebo manipulation. This reduction of negative emotion is one form of reward and could lead to reinforcement learning (Flaten et al., 2011), which increases the magnitude of placebo analgesia. On the other hand, the observation that placebo analgesia last longer than placebo analgesia seems at odds with previous findings showing that nocebo hyperalgesia is more resistant to extinction (Au Yeung et al., 2014; Colagiuri and Quinn, 2018; Colagiuri et al., 2015; Thomaïdou et al., 2021). However, recent studies have demonstrated that nocebo hyperalgesia can be extinguished (Maeda et al., 2018; Meulders et al., 2015; Thomaïdou et al., 2020), which agrees with our finding that nocebo hyperalgesia diminishes over time. By contrast, once established, placebo effects can also persist due to self-reinforcing expectations (Jepma et al., 2018), making it difficult to extinguish. In addition, the duration of pain stimuli has been found to influence placebo effects, with longer pain stimuli amplifying placebo effects (Vase et al., 2009). It is thus also possible that long-lasting pain stimuli, like the one we used, further sustain placebo analgesia over a long period.

Directly correlating brain activations with real-time pain ratings, we found that activities in a series of brain areas covaried with pain experience. Many of these areas, e.g., the S1, ACC, insula, and thalamus, are consistently activated by various pain stimuli (Duerden and Albanese, 2013; Hu et al., 2015; Xu et al., 2020), and have been correlated with pain perception in previous studies (Bornhøvd et al., 2002; Buchel et al., 2002; Coghill et al., 1999; Derbyshire et al., 1997; Wilcox et al., 2015; Woo et al., 2017). However, our pain stimuli lasted for 15 min, much longer than the typical durations of pain stimuli used in most relevant

studies, whose duration ranges from several milliseconds like laser heat pain (Bornhøvd et al., 2002; Buchel et al., 2002; Zhang et al., 2022) to tens of seconds like contact heat pain (Horing et al., 2019; Wager et al., 2013; Woo et al., 2017). The consistent findings between our study and previous studies using short-lived stimuli thus highlight how transient and tonic pain perceptions are similarly represented in the human brain. However, there also seem to be distinctions between the perception of tonic pain and transient pain, and commonalities between tonic pain and chronic pain. Notably, the vmPFC, the ventral part of mPFC, was correlated with real-time pain ratings, while some studies have not found a significant correlation between neural activity in this region and pain perception induced by transient pain stimuli (Baliki et al., 2006; Coghill et al., 1999). Importantly, the mPFC has been shown to index spontaneous pain in chronic back pain patients (Baliki et al., 2006).

Using multilevel mediation analysis, we identified both shared and distinct neural underpinnings of placebo and nocebo effects. We found that the putamen was involved in placebo analgesia and nocebo hyperalgesia. This area exhibited greater activations in the drug run than in the no-drug run in the placebo group, while showing weaker activations in the drug run than in the no-drug run in the nocebo group. Multilevel mediation analysis further demonstrated that the putamen activity partially mediated both placebo and nocebo effects. This finding is consistent with previous studies showing the putamen’s involvement in placebo and nocebo effects (Freeman et al., 2015; Fu et al., 2021; Kong et al., 2008; Scott et al., 2008). A study using similar tonic muscle pain stimuli observed  $\mu$ -opioid and dopamine neurotransmission in the putamen and found opposite opioid and dopamine responses associated with placebo and nocebo effects (Scott et al., 2008). This shared neural basis of placebo and nocebo effects could be related to their partly shared psychological mechanisms. Associative learning like classical conditioning contributes to both placebo analgesia and nocebo hyperalgesia, and the putamen is implicated in the stimuli-outcome association (Grahn et al., 2008; Haruno and Kawato, 2006; Seger and Cincotta, 2005). Previous studies have also shown that the putamen is involved in pain perception (Li et al., 2014; Scott et al., 2006; Starr et al., 2011). The conditioned learning procedure in our study thus could modulate putamen activity, which then affected pain ratings in both the placebo and nocebo groups. Admittedly, associative learning also recruits areas other

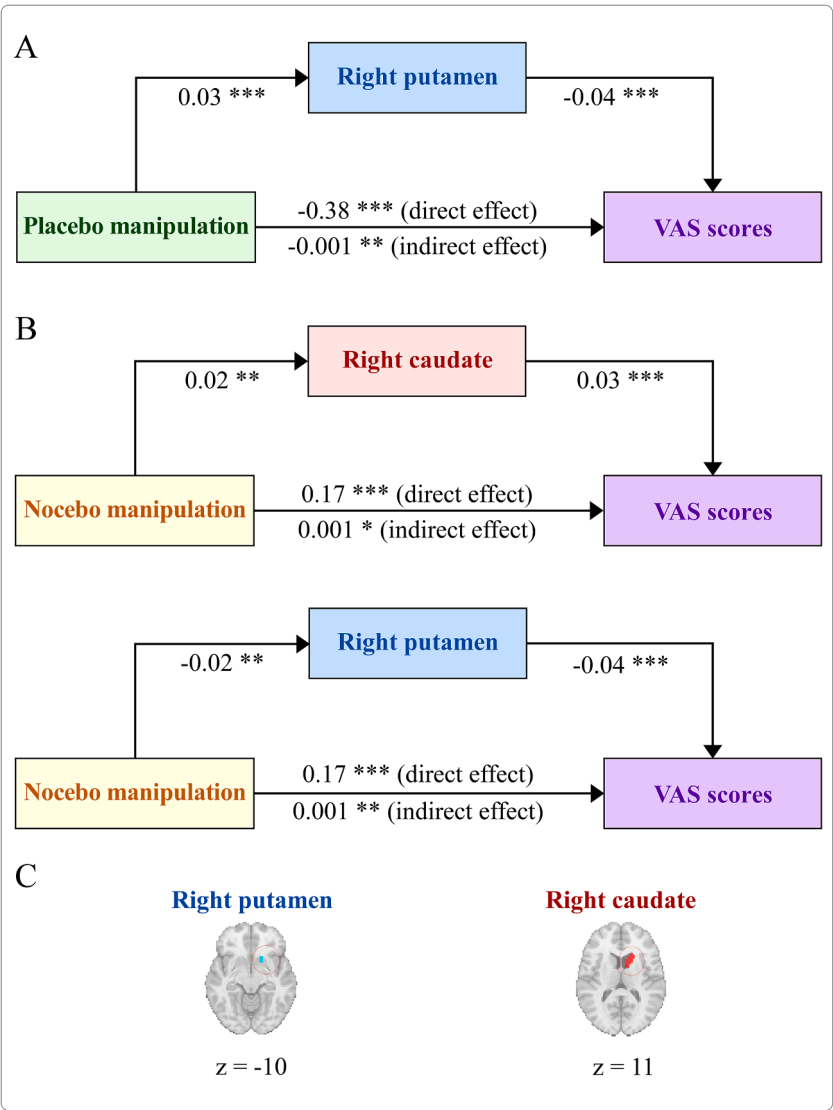


**Fig. 5.** Multilevel mediation path analyses results in the placebo (A) and nocebo groups (B). Only ROIs that passed the false discovery rate correction in both multilevel correlation and multilevel regression analyses were included, as displayed in (C). VAS: Visual Analogue Scale, i.e., real-time pain intensity.

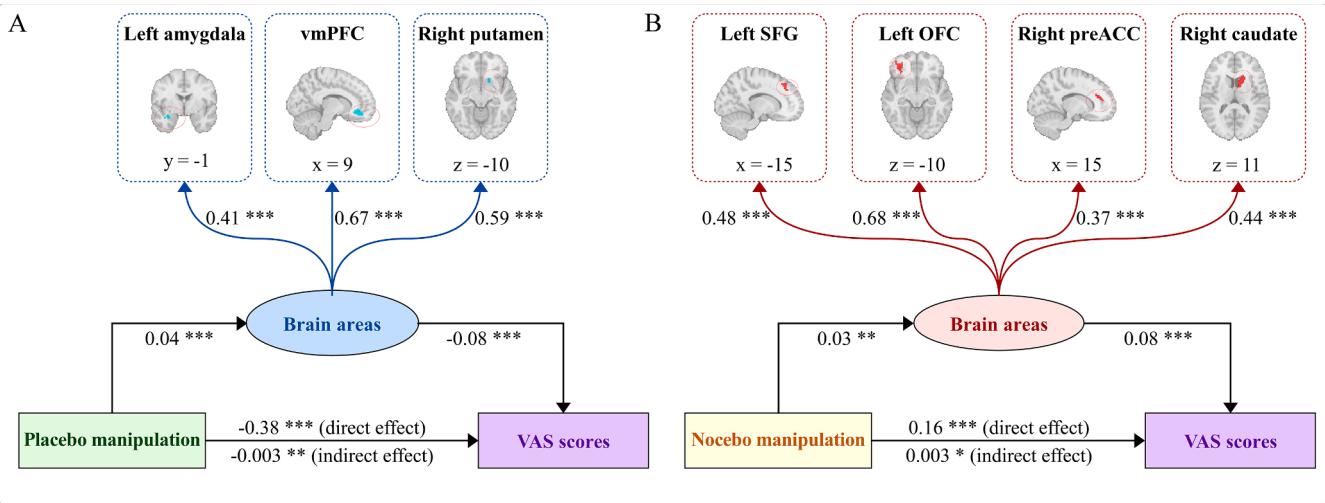


**Fig. 6.** Multilevel mediation path analyses results in the placebo (A) and nocebo groups (B). These models also include ROIs that showed significant differences in BOLD responses between the no-drug run and drug run but did not pass the false discovery rate correction. VAS: Visual Analogue Scale, vmPFC: ventromedial prefrontal cortex, SFG: superior frontal gyrus, OFC: orbital frontal cortex, preACC: pregenual anterior cingulate cortex.

than the putamen. The present study has a relatively small sample size, and thus may fail to detect mediation effects in other associative learning-related areas. Indeed, the indirect effect mediated by the putamen only accounted for a very small part of analgesia. Future studies may further examine the shared neural bases of placebo and nocebo effects in tonic pain.

Apart from shared neural substrates, we also found distinct neural bases for placebo and nocebo effects in pain, which resonate with previous meta-analysis showing that placebo analgesia and nocebo hyperalgesia are underpinned by distinct neural networks (Fu et al., 2021). A notable area we identified is the caudate. It showed significantly different activations between the drug and no-drug runs only in the nocebo group, which is in line with previous studies showing greater caudate activation in response to nocebo (Tu et al., 2021b). Importantly, the caudate further significantly mediated nocebo hyperalgesia, but not placebo analgesia. This finding may be explained by the caudate's role in anxiety (Guehl et al., 2008; Radua et al., 2010). Anxiety and the anxiety-related cholecystokininergic system have been repeatedly shown to mediate nocebo analgesia (Benedetti et al., 1997, 2006), but their roles in placebo analgesia are less clear (Skyt et al., 2020). These partly differential psychological and neurochemical mechanisms of nocebo and placebo effects in pain may be one of the reasons why the caudate seemed to only mediate nocebo hyperalgesia. The distinct neural mediators in our study could also be related to our finding that placebo analgesia and nocebo hyperalgesia had different magnitudes and durations, which suggests different underlying mechanisms. Future studies can adopt other tonic pain stimuli to examine if the caudate plays a greater role in nocebo effects than placebo effects. When adopting a more lenient statistical threshold, we also found that the amygdala and vmPFC mediated placebo analgesia, and that the SFG, OFC, and preACC mediated nocebo hyperalgesia. These regions were correlated with real-time pain ratings in the present study and have been reported in previous studies about placebo and nocebo effects (Benedetti et al., 2022; Fu et al., 2021; Zunhammer et al., 2021). While these findings need to be replicated in future studies due to a lenient thresholding, they indicate that placebo analgesia and nocebo hyperalgesia may have more distinct than common neural bases.

Our findings have implications for the clinical application of placebo analgesia and nocebo hyperalgesia in pain management. We found more long-lasting and pronounced placebo analgesia than nocebo hyperalgesia in our tonic pain paradigm, suggesting that more efforts should be made to specifically reduce nocebo hyperalgesia while also maximizing placebo analgesia in clinical practice. We also identified brain areas mediating placebo and nocebo effects in tonic pain, notably the putamen and caudate. Modulating the activity of these areas may boost placebo analgesia and dampen nocebo hyperalgesia, thus contributing to improved pain treatment. Previous studies using transient pain paradigms have successfully modulated placebo and nocebo effects with neuromodulation such as transcranial direct current stimulation (tDCS) (Egorova et al., 2015; Tu et al., 2021b). Our study further suggests targets for neuromodulation to harness placebo and nocebo effects in tonic pain, which may be more effective in helping chronic pain patients than targeting areas involved in placebo/nocebo effects in transient pain.

There are several limitations in the present study. First, our sample size was relatively small. Combined with the use of only one block for drug and non-drug runs, the small sample size could have led to a low signal-to-noise ratio of the neural responses, potentially leading to some false negatives. Future studies can recruit more participants to replicate and extend our findings, further elucidating the shared and distinct neural mechanisms of placebo analgesia and nocebo hyperalgesia. Second, we only used one type of tonic pain, namely tonic muscle pain. While this stimulus resembles certain forms of clinical or chronic pain like fibromyalgia in terms of subjective experience (Dunn et al., 2020), other types of tonic pain like capsaicin-induced pain can be employed in future studies to test whether our findings are generalizable across

different types of tonic pain stimuli. Third, results from our multilevel mediation analysis are correlational in nature (Bullock et al., 2010). The mediating roles of the areas we found such as the caudate and putamen need direct confirmation through neuromodulation studies (Li et al., 2023). Last, we focused solely on the relationship between the activation of single brain areas and placebo/nocebo effects. Future studies may investigate how dynamic interaction between multiple regions contributes to placebo/nocebo effects, since tonic pain has been shown to affect dynamic connectivity (Lee et al., 2021, 2022; Wang et al., 2023).

## 5. Conclusions

In conclusion, our study demonstrates that placebo analgesia and nocebo hyperalgesia can be effectively induced and measured using a tonic muscle pain paradigm. Our findings reveal that placebo analgesia is more pronounced and lasts longer than nocebo hyperalgesia, highlighting the differential impact of positive and negative expectations on pain perception. Furthermore, we identified distinct neural mechanisms underlying these effects, with the putamen playing a significant role in both placebo and nocebo responses, while the caudate was specifically implicated in nocebo hyperalgesia. These results suggest that placebo and nocebo effects have more distinct than common neural bases, providing valuable insights for future research and potential clinical applications in pain management.

## Funding

This work was supported by the National Key Research and Development Program of China (2023YFC2508702), National Natural Science Foundation of China (32071061), Beijing Natural Science Foundation (JQ22018), Medical Science and Technology Research Fund of Guangdong Province (A2021478), The Hundred Talents Program of Chinese Academy of Sciences (Y14408), Shenzhen Science and Technology Program (JC201005270293A), and Joint Laboratory for Basic Research of Neuropathic Pain (Y4Z050).

## CRediT authorship contribution statement

**Min Chen:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Xiao Wu:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Libo Zhang:** Writing – review & editing, Writing – original draft. **Fengrui Zhang:** Writing – review & editing, Visualization. **Linling Li:** Methodology, Investigation, Data curation. **Yingying Zhang:** Data curation. **Donglin Xiong:** Methodology, Conceptualization. **Yunhai Qiu:** Methodology, Funding acquisition, Conceptualization. **Li Hu:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Weibo Xiao:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.neuroimage.2024.120877](https://doi.org/10.1016/j.neuroimage.2024.120877).

## References

- Au Yeung, S.T., Colagiuri, B., Lovibond, P.F., Colloca, L., 2014. Partial reinforcement, extinction, and placebo analgesia. *Pain* 155, 1110–1117.
- Baliki, M.N., Apkarian, A.V., 2015. Nociception, pain, negative moods, and behavior selection. *Neuron* 87, 474–491.
- Baliki, M.N., Chialvo, D.R., Geha, P.Y., Levy, R.M., Harden, R.N., Parrish, T.B., Apkarian, A.V., 2006. Chronic pain and the emotional brain: specific brain activity associated with spontaneous fluctuations of intensity of chronic back pain. *J. Neurosci.* 26, 12165–12173.
- Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Soft.* 67, 1–48.
- Benedetti, F., Amanzio, M., Casadio, C., Oliaro, A., Maggi, G., 1997. Blockade of nocebo hyperalgesia by the cholecystokinin antagonist proglumide. *Pain* 71, 135–140.
- Benedetti, F., Amanzio, M., Vighetti, S., Asteggiano, G., 2006. The biochemical and neuroendocrine bases of the hyperalgesic nocebo effect. *J. Neurosci.* 26, 12014–12022.
- Benedetti, F., Frisaldi, E., Shaibani, A., 2022. Thirty years of neuroscientific investigation of placebo and nocebo: the interesting, the good, and the bad. *Annu. Rev. Pharmacol. Toxicol.* 62, 323–340.
- Bingel, U., 2020. Placebo 2.0: the impact of expectations on analgesic treatment outcome. *Pain* 161 (Suppl 1), S48–S56.
- Bornhovd, K., Quante, M., Glauche, V., Bromm, B., Weiller, C., Buchel, C., 2002. Painful stimuli evoke different stimulus-response functions in the amygdala, prefrontal, insula and somatosensory cortex: a single-trial fMRI study. *Brain* 125, 1326–1336.
- Brett, M., Anton, J.-L., Valabréque, R., Poline, J.B., 2010. Region of interest analysis using an SPM toolbox.
- Brett, M., Markiewicz, C.J., Hanke, M., Côté, M.-A., Cipollini, B., McCarthy, P., Jarecka, D., Cheng, C.P., Halchenko, Y.O., Cottaar, M., Larson, E., Ghosh, S., Wassermann, D., Gerhard, S., Lee, G.R., Baratz, Z., Wang, H.-T., Kastman, E., Kaczmarzyk, J., Guidotti, R., Daniel, J., Duek, O., Rokem, A., Madison, C., Papadopoulos Orfanos, D., Sólón, A., Moloney, B., Morency, F.C., Gonçalves, M., Markello, R., Riddell, C., Burns, C., Millman, J., Gramfort, A., Leppäkangas, J., van den Bosch, J.J.F., Vincent, R.D., Braun, H., Subramaniam, K., Van, A., Gorgolewski, K.J., Raamana, P.R., Klug, J., Nichols, B.N., Baker, E.M., Hayashi, S., Pinsard, B., Haselgrove, C., Hymers, M., Esteban, O., Koudoro, S., Pérez-García, F., Dockès, J., Oosterhof, N.N., Amirbekian, B., Christian, H., Nimmo-Smith, I., Nguyen, L., Reddigari, S., St-Jean, S., Panfilov, E., Garyfallidis, E., Varoquaux, G., Legarreta, J.H., Hahn, K.S., Waller, L., Hinds, O.P., Fauber, B., Perez, F., Roberts, J., Poline, J.-B., Stutters, J., Jordan, K., Cieslak, M., Moreno, M.E., Hrnčiar, T., Haenel, V., Schwartz, Y., Darwin, B.C., Thirion, B., Gauthier, C., Solovey, I., Gonzalez, I., Palasubramaniam, J., Lecher, J., Leinweber, K., Raktivan, K., Calábková, M., Fischer, P., Gervais, P., Gadde, S., Ballinger, T., Roos, T., Reddam, V.R., free84, 2023. nipy/nibabel: 5.1.0 Zenodo.
- Buchel, C., Bornhovd, K., Quante, M., Glauche, V., Bromm, B., Weiller, C., 2002. Dissociable neural responses related to pain intensity, stimulus intensity, and stimulus awareness within the anterior cingulate cortex: a parametric single-trial laser functional magnetic resonance imaging study. *J. Neurosci.* 22, 970–976.
- Bullock, J.G., Green, D.P., Ha, S.E., 2010. Yes, but what's the mechanism? (don't expect an easy answer). *J. Pers. Soc. Psychol.* 98, 550–558.
- Coghil, R.C., Sang, C.N., Maisog, J.M., Iadarola, M.J., 1999. Pain intensity processing within the human brain: a bilateral, distributed mechanism. *J. Neurophysiol.* 82, 1934–1943.
- Colagiuri, B., Quinn, V.F., 2018. Autonomic arousal as a mechanism of the persistence of nocebo hyperalgesia. *J. Pain* 19, 476–486.
- Colagiuri, B., Quinn, V.F., Colloca, L., 2015. Nocebo hyperalgesia, partial reinforcement, and extinction. *J. Pain* 16, 995–1004.
- Colloca, L., Barsky, A.J., 2020. Placebo and nocebo effects. *N. Engl. J. Med.* 382, 554–561.
- Colloca, L., Benedetti, F., 2006. How prior experience shapes placebo analgesia. *Pain* 124, 126–133.
- Colloca, L., Sigauco, M., Benedetti, F., 2008. The role of learning in nocebo and placebo effects. *Pain* 136, 211–218.
- D'Agostino, R.B., 1971. An omnibus test of normality for moderate and large size samples. *Biometrika* 58, 341–348.
- Derbyshire, S.W.G., Jones, A.K.P., Gyulai, F., Clark, S., Townsend, D., Firestone, L.L., 1997. Pain processing during three levels of noxious stimulation produces differential patterns of central activity. *Pain* 73, 431–445.
- Di, X., Biswal, B.B., 2022. A functional MRI pre-processing and quality control protocol based on statistical parametric mapping (SPM) and MATLAB. *Front. Neuroimaging* 1, 1070151.
- Duerden, E.G., Albanese, M.C., 2013. Localization of pain-related brain activation: a meta-analysis of neuroimaging data. *Hum. Brain Mapp.* 34, 109–149.
- Dunn, J.S., Mahns, D.A., Nagi, S.S., 2020. Modulation of muscle pain is not somatotopically restricted: an experimental model using concurrent hypertonic-normal saline infusions in humans. *Front. Pain. Res. (Lausanne)* 1, 601544.
- Egorova, N., Yu, R., Kaur, N., Vangel, M., Gollub, R.L., Dougherty, D.D., Kong, J., Camprodon, J.A., 2015. Neuromodulation of conditioned placebo/nocebo in heat pain: anodal vs cathodal transcranial direct current stimulation to the right dorsolateral prefrontal cortex. *Pain* 156, 1342–1347.
- Faul, F., Erdfelder, E., Buchner, A., Lang, A.-G., 2009. Statistical power analyses using G\*Power 3.1: tests for correlation and regression analyses. *Behav. Res. Methods* 41, 1149–1160.
- Faul, F., Erdfelder, E., Lang, A.G., Buchner, A., 2007. G\*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav. Res. Methods* 39, 175–191.
- Flaten, M.A., Aslaksen, P.M., Lyby, P.S., Bjorkedal, E., 2011. The relation of emotions to placebo responses. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 366, 1818–1827.
- Freeman, S., Yu, R., Egorova, N., Chen, X., Kirsch, I., Claggett, B., Kapchuk, T.J., Gollub, R.L., Kong, J., 2015. Distinct neural representations of placebo and nocebo effects. *Neuroimage* 112, 197–207.
- Friston, K.J., Williams, S., Howard, R., Frackowiak, R.S., Turner, R., 1996. Movement-related effects in fMRI time-series. *Magn. Reson. Med.* 35, 346–355.
- Fu, J., Wu, S., Liu, C., Camilleri, J.A., Eickhoff, S.B., Yu, R., 2021. Distinct neural networks subserve placebo analgesia and nocebo hyperalgesia. *Neuroimage* 231, 117833.
- Gaskin, D.J., Richard, P., 2012. The economic costs of pain in the United States. *J. Pain* 13, 715–724.
- Grahn, J.A., Parkinson, J.A., Owen, A.M., 2008. The cognitive functions of the caudate nucleus. *Prog. Neurobiol.* 86, 141–155.
- Grove, J.R., Prapavessis, H., 1992. Preliminary evidence for the reliability and validity of an abbreviated Profile of Mood States. *Int. J. Sport Psychol.* 23, 93–109.
- Guehl, D., Benazzouz, A., Aouizerate, B., Cuny, E., Rotge, J.Y., Rougier, A., Tignol, J., Bioulac, B., Burbaud, P., 2008. Neuronal correlates of obsessions in the caudate nucleus. *Biol. Psychiatry* 63, 557–562.
- Haruno, M., Kawato, M., 2006. Different neural correlates of reward expectation and reward expectation error in the putamen and caudate nucleus during stimulus-action-reward association learning. *J. Neurophysiol.* 95, 948–959.
- Horing, B., Sprenger, C., Buchel, C., 2019. The parietal operculum preferentially encodes heat pain and not salience. *PLoS Biol.* 17, e3000205.
- Hu, L., Zhang, L., Chen, R., Yu, H., Li, H., Mouraux, A., 2015. The primary somatosensory cortex and the insula contribute differently to the processing of transient and sustained nociceptive and non-nociceptive somatosensory inputs. *Hum. Brain Mapp.* 36, 4346–4360.
- Hu, L.T., Bentler, P.M., 1999. Cutoff criteria for fit indexes in covariance structure analysis: conventional criteria versus new alternatives. *Struct. Eq. Model.-a Multidiscip. J.* 6, 1–55.
- Jensen, K.B., Kapchuk, T.J., Kirsch, I., Raicek, J., Lindstrom, K.M., Berna, C., Gollub, R. L., Ingvar, M., Kong, J., 2012. Nonconscious activation of placebo and nocebo pain responses. *Proc. Natl. Acad. Sci. U. S. A.* 109, 15959–15964.
- Jepma, M., Koban, L., van Doorn, J., Jones, M., Wager, T.D., 2018. Behavioural and neural evidence for self-reinforcing expectancy effects on pain. *Nat. Hum. Behav.* 2, 838–855.
- Kang, H., 2021. Sample size determination and power analysis using the G\*Power software. *J. Educ. Eval. Health Prof.* 18, 17.
- Kapchuk, T.J., Hemond, C.C., Miller, F.G., 2020. Placebos in chronic pain: evidence, theory, ethics, and use in clinical practice. *BMJ* 370, m1668.
- Klinger, R., Blasini, M., Schmitz, J., Colloca, L., 2017. Nocebo effects in clinical studies: hints for pain therapy. *Pain. Rep.* 2.
- Kong, J., Gollub, R.L., Polich, G., Kirsch, I., Lavolette, P., Vangel, M., Rosen, B., Kapchuk, T.J., 2008. A functional magnetic resonance imaging study on the neural mechanisms of hyperalgesic nocebo effect. *J. Neurosci.* 28, 13354–13362.
- Lee, J.J., Kim, H.J., Ceko, M., Park, B.Y., Lee, S.A., Park, H., Roy, M., Kim, S.G., Wager, T. D., Woo, C.W., 2021. A neuroimaging biomarker for sustained experimental and clinical pain. *Nat. Med.* 27, 174–182.
- Lee, J.J., Lee, S., Lee, D.H., Woo, C.W., 2022. Functional brain reconfiguration during sustained pain. *Elife* 11.
- Li, C.M., Zhang, D.M., Yang, C.X., Ma, X., Gao, H.R., Zhang, D., Xu, M.Y., 2014. Acetylcholine plays an antinociceptive role by modulating pain-induced discharges of pain-related neurons in the caudate putamen of rats. *Neuroreport* 25, 164–170.
- Li, Z.J., Zhang, L.B., Chen, Y.X., Hu, L., 2023. Advancements and challenges in neuromodulation technology: interdisciplinary opportunities and collaborative endeavors. *Sci. Bull. (Beijing)* 68, 1978–1982.
- Linton, S.J., Shaw, W.S., 2011. Impact of psychological factors in the experience of pain. *Phys. Ther.* 91, 700–711.
- Liu, C., Pu, M., Lian, W., Hu, L., Mobbs, D., Yu, R., 2020. Conscious awareness differentially shapes analgesic and hyperalgesic pain responses. *J. Experiment. Psychol.-General* 149, 2007–2019.
- Maeda, Y., Kan, S., Fujino, Y., Shibata, M., 2018. Verbal instruction can induce extinction of fear of movement-related pain. *J. Pain* 19, 1063–1073.
- Makowski, D., Ben-Shachar, M.S., Patil, I., Lüdtke, D., 2020. Methods and algorithms for correlation analysis in R. *J. Open Source Softw.* 5, 2306–2309.
- Makowski, D., Wiernik, B.M., Patil, I., Lüdtke, D., Ben-Shachar, M.S., 2022. correlation: methods for correlation analysis [R package].
- Melzack, R., 1987. The short-form McGill pain questionnaire. *Pain* 30, 191–197.
- Meulders, A., Karsdorp, P.A., Claes, N., Vlaeyen, J.W.S., 2015. Comparing counterconditioning and extinction as methods to reduce fear of movement-related pain. *J. Pain* 16, 1353–1365.
- Petersen, G.L., Finnerup, N.B., Colloca, L., Amanzio, M., Price, D.D., Jensen, T.S., Vase, L., 2014. The magnitude of nocebo effects in pain: a meta-analysis. *Pain* 155, 1426–1434.
- Petrie, K.J., Rief, W., 2019. Psychobiological mechanisms of placebo and nocebo effects: pathways to improve treatments and reduce side effects. *Annu. Rev. Psychol.* 70, 599–625.
- Petrovic, P., 2008. Placebo analgesia and nocebo hyperalgesia—two sides of the same coin? *Pain* 136, 5–6.
- Porporatti, A.L., Costa, Y.M., Reus, J.C., Stuginski-Barbosa, J., Conti, P.C.R., Velly, A.M., De Luca Canto, G., 2019. Placebo and nocebo response magnitude on temporomandibular disorder-related pain: a systematic review and meta-analysis. *J. Oral Rehabil.* 46, 862–882.

- Radua, J., van den Heuvel, O.A., Surguladze, S., Mataix-Cols, D., 2010. Meta-analytical comparison of voxel-based morphometry studies in obsessive-compulsive disorder vs other anxiety disorders. *Arch. Gen. Psychiatry* 67, 701–711.
- Raman, R., 2020. Statistical methods in handling placebo effect. *Int. Rev. Neurobiol.* 153, 103–120.
- Reckziegel, D., Vachon-Presseau, E., Petre, B., Schnitzer, T.J., Baliki, M.N., Apkarian, A. V., 2019. Deconstructing biomarkers for chronic pain: context- and hypothesis-dependent biomarker types in relation to chronic pain. *Pain* 160 (Suppl 1), S37–S48.
- Reddy, K.S., Naidu, M.U., Rani, P.U., Rao, T.R., 2012. Human experimental pain models: a review of standardized methods in drug development. *J. Res. Med. Sci.* 17, 587–595.
- Reicherts, P., Gerdes, A.B., Pauli, P., Wieser, M.J., 2016. Psychological placebo and nocebo effects on pain rely on expectation and previous experience. *J. Pain* 17, 203–214.
- Rodrigues, F.B., Ferreira, J.J., 2020. Strategies to minimize placebo effects in research investigations. *Int. Rev. Neurobiol.* 153, 49–70.
- Scott, D.J., Heitzeg, M.M., Koeppe, R.A., Stohler, C.S., Zubieta, J.K., 2006. Variations in the human pain stress experience mediated by ventral and dorsal basal ganglia dopamine activity. *J. Neurosci.* 26, 10789–10795.
- Scott, D.J., Stohler, C.S., Egnatuk, C.M., Wang, H., Koeppe, R.A., Zubieta, J.K., 2008. Placebo and nocebo effects are defined by opposite opioid and dopaminergic responses. *Arch. Gen. Psychiatry* 65, 220–231.
- Seger, C.A., Cincotta, C.M., 2005. The roles of the caudate nucleus in human classification learning. *J. Neurosci.* 25, 2941–2951.
- Skytt, I., Lunde, S.J., Baastrop, C., Svensson, P., Jensen, T.S., Vase, L., 2020. Neurotransmitter systems involved in placebo and nocebo effects in healthy participants and patients with chronic pain: a systematic review. *Pain* 161, 11–23.
- Starr, C.J., Sawaki, L., Wittenberg, G.F., Burdette, J.H., Oshiro, Y., Quevedo, A.S., McHaffie, J.G., Coghill, R.C., 2011. The contribution of the putamen to sensory aspects of pain: insights from structural connectivity and brain lesions. *Brain* 134, 1987–2004.
- Thomaidou, M.A., Veldhuijzen, D.S., Meulders, A., Evers, A.W.M., 2021. An experimental investigation into the mediating role of pain-related fear in boosting nocebo hyperalgesia. *Pain* 162, 287–299.
- Thomaidou, M.A., Veldhuijzen, D.S., Peerdeman, K.J., Wiebing, N.Z.S., Blythe, J.S., Evers, A.W.M., 2020. Learning mechanisms in nocebo hyperalgesia: the role of conditioning and extinction processes. *Pain* 161, 1597–1608.
- Tu, Y., Bi, Y., Zhang, L., Wei, H., Hu, L., 2020. Mesocorticolimbic pathways encode cue-based expectancy effects on pain. *J. Neurosci.* 40, 382–394.
- Tu, Y., Pantazis, D., Wilson, G., Khan, S., Ahlfors, S., Kong, J., 2021a. How expectations of pain elicited by consciously and unconsciously perceived cues unfold over time. *Neuroimage* 235, 117985.
- Tu, Y., Wilson, G., Camprodon, J., Dougherty, D.D., Vangel, M., Benedetti, F., Kaptchuk, T.J., Gollub, R.L., Kong, J., 2021b. Manipulating placebo analgesia and nocebo hyperalgesia by changing brain excitability. *Proc. Natl. Acad. Sci. U. S. A.* 118.
- Tu, Y., Zhang, L., Kong, J., 2022. Placebo and nocebo effects: from observation to harnessing and clinical application. *Transl. Psychiatry* 12, 524.
- Ullman, J.B., 2001. Structural equation modeling. In: Tabachnick, B.G., Fidell, L.S. (Eds.), *Using Multivariate Statistics* Allyn & Bacon. Needham Heights, MA, pp. 653–771.
- Vallat, R., 2018. Pingouin: statistics in Python. *J. Open. Source Softw.* 3, 1026.
- van Laarhoven, A.I.M., Vogelaar, M.L., Wilder-Smith, O.H., van Riel, P., van de Kerkhof, P.C.M., Kraaijaat, F.W., Evers, A.W.M., 2011. Induction of nocebo and placebo effects on itch and pain by verbal suggestions. *Pain* 152, 1486–1494.
- 3rd Vase, L., Petersen, G.L., Riley, J.L., Price, D.D., 2009. Factors contributing to large analgesic effects in placebo mechanism studies conducted between 2002 and 2007. *Pain* 145, 36–44.
- Vase, L., Skytt, I., Hall, K.T., 2016. Placebo, nocebo, and neuropathic pain. *Pain* 157 (Suppl 1), S98–S105.
- Virtanen, P., Gommers, R., Oliphant, T.E., Haberland, M., Reddy, T., Cournapeau, D., Burovski, E., Peterson, P., Weckesser, W., Bright, J., van der Walt, S.J., Brett, M., Wilson, J., Millman, K.J., Mayorov, N., Nelson, A.R.J., Jones, E., Kern, R., Larson, E., Carey, C.J., Polat, I., Feng, Y., Moore, E.W., VanderPlas, J., Laxalde, D., Perktold, J., Cimrman, R., Henriksen, I., Quintero, E.A., Harris, C.R., Archibald, A.M., Ribeiro, A. H., Pedregosa, F., van Mulbregt, P., SciPy, C., 2020. SciPy 1.0: fundamental algorithms for scientific computing in Python. *Nat. Methods* 17, 261–272.
- 3rd Vos, T., Flaxman, A.D., Naghavi, M., Lozano, R., Michaud, C., Ezzati, M., Shibuya, K., Salomon, J.A., Abdalla, S., Aboyans, V., Abraham, J., Ackerman, I., Aggarwal, R., Ahn, S.Y., Ali, M.K., Alvarado, M., Anderson, H.R., Anderson, L.M., Andrews, K.G., Atkinson, C., Baddour, L.M., Bahalim, A.N., Barker-Collo, S., Barrero, L.H., Bartels, D.H., Basanez, M.G., Baxter, A., Bell, M.L., Benjamin, E.J., Bennett, D., Bernabe, E., Bhalla, K., Bhandari, B., Bikbov, B., Bin Abdulhak, A., Birbeck, G., Black, J.A., Blencowe, H., Blore, J.D., Blyth, F., Bolliger, I., Bonaventure, A., Boufous, S., Bourne, R., Boussinesq, M., Braithwaite, T., Brayne, C., Bridgett, L., Brooker, S., Brooks, P., Brugh, T.S., Bryan-Hancock, C., Bucello, C., Buchbinder, R., Buckle, G., Budke, C.M., Burch, M., Burney, P., Burstein, R., Calabria, B., Campbell, B., Canter, C.E., Carabin, H., Carapetis, J., Carmona, L., Cella, C., Charlson, F., Chen, H., Cheng, A.T., Chou, D., Chugh, S.S., Coffeng, L.E., Colan, S.D., Colquhoun, S., Colson, K.E., Condon, J., Connor, M.D., Cooper, L.T., Corriere, M., Cortinovis, M., de Vaccaro, K.C., Couser, W., Cowie, B.C., Criqui, M.H., Cross, M., Dabhadkar, K.C., Dahiya, M., Dahodwala, N., Damsere-Derry, J., Danaei, G., Davis, A., De Leo, D., Degenhardt, L., Dellavalle, R., Delossantos, A., Denenberg, J., Derrett, S., Des Jarlais, D.C., Dharmaratne, S.D., Dherani, M., Diaz-Torne, C., Dolk, H., Dorsey, E.R., Driscoll, T., Duber, H., Ebel, B., Edmond, K., Elbaz, A., Ali, S. E., Erskine, H., Erwin, P.J., Espindola, P., Ewoigbokhan, S.E., Farzadfar, F., Feigin, V., Felson, D.T., Ferrari, A., Ferri, C.P., Fevre, E.M., Finucane, M.M., Flaxman, S., Flood, L., Foreman, K., Forouzanfar, M.H., Fowkes, F.G., Franklin, R., Fransen, M., Freeman, M.K., Gabbe, B.J., Gabriel, S.E., Gakidou, E., Ganatra, H.A., Garcia, B., Gaspari, F., Gillum, R.F., Gmel, G., Gosselin, R., Grainger, R., Groeger, J., Guillemin, F., Gunnell, D., Gupta, R., Haagsma, J., Hagan, H., Halasa, Y.A., Hall, W., Haring, D., Haro, J.M., Harrison, J.E., Haymoeller, R., Hay, R.J., Higashi, H., Hill, C., Hoen, B., Hoffman, H., Hotez, P.J., Hoy, D., Huang, J.J., Ibeanusi, S.E., Jacobsen, K. H., James, S.L., Jarvis, D., Jasrasaria, R., Jayaraman, S., Johns, N., Jonas, J.B., Karthikeyan, G., Kassebaum, N., Kawakami, N., Keren, A., Khoo, J.P., King, C.H., Knowlton, L.M., Kobusingye, O., Koranteng, A., Krishnamurthi, R., Laloo, R., Laslett, L.L., Lathlean, T., Leasher, J.L., Lee, Y.Y., Leigh, J., Lim, S.S., Limb, E., Lin, J. K., Lipnick, M., Lipshultz, S.E., Liu, W., Loane, M., Ohno, S., Lyons, R., Ma, J., Mabweijano, J., MacIntyre, M.F., Malekzadeh, R., Mallinger, L., Manivannan, S., Marcenes, W., March, L., Margolis, D.J., Marks, G.B., Marks, R., Matsumori, A., Mazopoulous, R., Mayosi, B.M., McAnulty, J.H., McDermott, M.M., McGill, N., McGrath, J., Medina-Mora, M.E., Meltzer, M., Mensah, G.A., Merriman, T.R., Meyer, A.C., Miglioli, V., Miller, M., Miller, T.R., Mitchell, P.B., Mocumbi, A.O., Moffitt, T.E., Mokdad, A.A., Monasta, L., Montico, M., Moradi-Lakeh, M., Moran, A., Morawska, L., Mori, R., Murdoch, M.E., Mwaniki, M.K., Naidoo, K., Nair, M.N., Naldi, L., Narayan, K.M., Nelson, P.K., Nelson, R.G., Nevitt, M.C., Newton, C.R., Nolte, S., Norman, P., Norman, R., O'Donnell, J.H., O'Hanlon, S., Olives, C., Omer, S. B., Ortblad, K., Osborne, R., Ozgediz, D., Page, A., Pahari, B., Pandian, J.D., Rivero, A.P., Patten, S.B., Pearce, N., Padilla, R.P., Perez-Ruiz, F., Perico, N., Pesudovs, K., Phillips, D., Phillips, M.R., Pierce, K., Pion, S., Polanczyk, G.V., Polinder, S., Pope, C.A., Popova, S., Porrini, E., Pourmalek, F., Prince, M., Pullan, R. L., Ramaiah, K.D., Ranganathan, D., Ravahi, H., Regan, M., Rehm, J.T., Rein, D.B., Remuzzi, G., Richardson, K., Rivara, F.P., Roberts, T., Robinson, C., De Leon, F.R., Ronfani, L., Room, R., Rosenfeld, L.C., Rushton, L., Sacco, R.L., Saha, S., Sampson, U., Sanchez-Riera, L., Sanman, E., Schwebel, D.C., Scott, J.G., Segui-Gomez, M., Shahraz, S., Shepard, D.S., Shin, H., Shivakoti, R., Singh, D., Singh, G.M., Singh, J.A., Singleton, J., Sleet, D.A., Sliwa, K., Smith, E., Smith, J.L., Stapelberg, N. J., Steer, A., Steiner, T., Stolk, W.A., Stovner, L.J., Sudfeld, C., Syed, S., Tamburlini, G., Tavakkoli, M., Taylor, H.R., Taylor, J.A., Taylor, W.J., Thomas, B., Thomson, W.M., Thurston, G.D., Tleyjeh, I.M., Tonelli, M., Towbin, J.A., Truelsen, T., Tsilimbaris, M.K., Ubeda, C., Undurraga, E.A., van der Werf, M.J., van Os, J., Vavilala, M.S., Venketasubramanian, N., Wang, M., Wang, W., Watt, K., Weatherall, D.J., Weinstock, M.A., Weintraub, R., Weisskopf, M.G., Weissman, M.M., White, R.A., Whiteford, H., Wiersma, S.T., Wilkinson, J.D., Williams, H.C., Williams, S.R., Witt, E., Wolfe, F., Woolf, A.D., Wulf, S., Yeh, P.H., Zaidi, A.K., Zheng, Z.J., Zonies, D., Lopez, A.D., Murray, C.J., AlMazroa, M.A., Memish, Z.A., 2012. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the global burden of disease study 2010. *Lancet* 380, 2163–2196.
- Wager, T.D., Atlas, L.Y., Lindquist, M.A., Roy, M., Woo, C.W., Kross, E., 2013. An fMRI-based neurosignature of physical pain. *N. Engl. J. Med.* 368, 1388–1397.
- Wang, H., Guo, Y., Tu, Y., Peng, W., Lu, X., Bi, Y., Iannetti, G.D., Hu, L., 2023. Neural processes responsible for the translation of sustained nociceptive inputs into subjective pain experience. *Cereb. Cortex* 33, 634–650.
- Watson, D., Clark, L.A., Tellegen, A., 1988. Development and validation of brief measures of positive and negative affect: the PANAS scales. *J. Pers. Soc. Psychol.* 54, 1063–1070.
- Wilcox, C.E., Mayer, A.R., Teshiba, T.M., Ling, J., Smith, B.W., Wilcox, G.L., Mullins, P. G., 2015. The subjective experience of pain: an fMRI study of percept-related models and functional connectivity. *Pain. Med.* 16, 2121–2133.
- Woo, C.W., Schmidt, L., Krishnan, A., Jepma, M., Roy, M., Lindquist, M.A., Atlas, L.Y., Wager, T.D., 2017. Quantifying cerebral contributions to pain beyond nociception. *Nat. Commun.* 8, 14211.
- Xu, A., Larsen, B., Baller, E.B., Scott, J.C., Sharma, V., Adebimpe, A., Basbaum, A.I., Dworkin, R.H., Edwards, R.R., Woolf, C.J., Eickhoff, S.B., Eickhoff, C.R., Satterthwaite, T.D., 2020. Convergent neural representations of experimentally-induced acute pain in healthy volunteers: a large-scale fMRI meta-analysis. *Neurosci. Biobehav. Rev.* 112, 300–323.
- Zhang, F., Xiang, W., Li, C.Y., Li, S.C., 2016. Economic burden of irritable bowel syndrome in China. *World J. Gastroenterol.* 22, 10450–10460.
- Zhang, L., Lu, X., Bi, Y., Hu, L., 2019. Pavlov's pain: the effect of classical conditioning on pain perception and its clinical implications. *Curr. Pain. Headache Rep.* 23, 19.
- Zhang, L.B., Lu, X.J., Huang, G., Zhang, H.J., Tu, Y.H., Kong, Y.Z., Hu, L., 2022. Selective and replicable neuroimaging-based indicators of pain discriminability. *Cell Rep. Med.* 3, 100846.
- Zhang, X., Ashton-Miller, J.A., Stohler, C.S., 1993. A closed-loop system for maintaining constant experimental muscle pain in man. *IEEE Trans. Biomed. Eng.* 40, 344–352.
- Zubieta, J.K., Bueller, J.A., Jackson, L.R., Scott, D.J., Xu, Y., Koeppe, R.A., Nichols, T.E., Stohler, C.S., 2005. Placebo effects mediated by endogenous opioid activity on mu-opioid receptors. *J. Neurosci.* 25, 7754–7762.
- Zunhammer, M., Spisak, T., Wager, T.D., Bingel, U., Placebo Imaging, C., 2021. Meta-analysis of neural systems underlying placebo analgesia from individual participant fMRI data. *Nat. Commun.* 12, 1391.