



The role of negative emotions in sex differences in pain sensitivity

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ABSTRACT

Pain perception varies widely among individuals due to the varying degrees of biological, psychological, and social factors. Notably, sex differences in pain sensitivity have been consistently observed in various experimental and clinical investigations. However, the neuropsychological mechanism underlying sex differences in pain sensitivity remains unclear. To address this issue, we quantified pain sensitivity (i.e., pain threshold and tolerance) using the cold pressure test and negative emotions (i.e., pain-related fear, pain-related anxiety, trait anxiety, and depression) using well-established questionnaires and collected magnetic resonance imaging (MRI) data (i.e., high-resolution T1 structural images and resting-state functional images) from 450 healthy subjects. We observed that, as compared to males, females exhibited lower pain threshold and tolerance. Notably, sex differences in pain sensitivity were mediated by pain-related fear and anxiety. Specifically, pain-related fear and anxiety were the complementary mediators of the relationship between sex and pain threshold, and they were the indirect-only mediators of the relationship between sex and pain tolerance. Besides, structural MRI data revealed that the amygdala subnuclei (i.e., the lateral and basal nuclei in the left hemisphere) volumes were the complementary mediators of the relationship between sex and pain-related fear, which further influenced pain sensitivity. Altogether, our results provided a comprehensive picture of how negative emotions (especially pain-related negative emotions) and related brain structures (especially the amygdala) contribute to sex differences in pain sensitivity. These results deepen our understanding of the neuropsychological underpinnings of sex differences in pain sensitivity, which is important to tailor a personalized method for treating pain according to sex and the level of pain-related negative emotions for patients with painful conditions.

1. Introduction

Pain is an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage (Williams and Craig, 2016). Due to the varying degrees of biological, psychological, and social factors, the sensitivity of pain perception varies widely among individuals (Hu and Iannetti, 2019; Schulz et al., 2011). Notably, sex differences in pain sensitivity have been consistently observed in various experimental and clinical investigations (Fillingim, 2017; Hashmi and Davis, 2014; Keogh, 2020; Mogil, 2021). Using well-controlled experimental designs, researchers observed robust findings that females report higher pain sensitivity (e.g., lower pain threshold, lower pain tolerance, and higher pain perception rating to nociceptive stimuli of a fixed intensity) in response to noxious stimuli of different modalities (e.g., mechanical, electrical, cold, heat, and ischemic stimuli) than males (Bulls et al., 2015; Goodin et al., 2009; Hunter et al., 2019; Kim et al., 2017; Pud et al., 2009; Racine et al., 2012; Sarlani et al., 2003; Tartar et al., 2020; Tashani et al., 2017). Epidemiological data also demonstrated that females are at higher risk for

many chronic pain conditions (Holley et al., 2017; Meints et al., 2018; Vetvik and MacGregor, 2017; von Deneen et al., 2019) and are more likely to report higher pain intensity (Meints et al., 2018; Ruau et al., 2012) and more pain-related disability compared to males (Vetvik and MacGregor, 2017).

Interestingly, sex differences were also observed in the processing of negative emotions that are highly associated with pain experiences (Wiech and Tracey, 2009). Sex differences in negative emotions, especially fear, anxiety, and depression, during pain processing could represent plausible contributors to sex differences in pain sensitivity (Fillingim, 2017). Previous studies reported that females typically experience more negative emotions, such as pain-related fear (Hirsh et al., 2008), pain-related anxiety (Thibodeau et al., 2013), trait anxiety (Goffaux et al., 2011), and depression (Altemus et al., 2014). Notably, these negative emotions could modulate sex differences in pain sensitivity. For example, trait anxiety can explain sex differences in pain sensitivity elicited by electrical stimuli (Goffaux et al., 2011), pain-related fear was associated with sex differences of pain responses to thermal stimuli (Horn et al., 2014), and pain-related anxiety influences

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pain perception to thermal and cold stimuli differently in males and females (Tashani et al., 2010; Thibodeau et al., 2013).

Three types of pain sensitivity measurements are widely used in both basic and clinical studies. There are (1) pain threshold, which is the minimal stimulus intensity required to elicit pain, (2) pain tolerance, which is the maximal stimulus intensity that an individual can withstand, and (3) pain perception rating to a standardized stimulus intensity (Chapman et al., 1985). Notably, pain threshold and pain tolerance differ considerably in their loading on physiological and psychological components (Harris and Rollman, 1983), and pain tolerance probably has a greater psychological component and seems to be more amenable to experimental manipulation through psychological variables (Gelfand, 1964). Therefore, we hypothesized that females have higher pain sensitivity than males (represented as lower pain threshold and pain tolerance), and sex differences in pain sensitivity might be mediated by negative emotions, especially for pain tolerance that is more psychologically relevant.

Neuroimaging studies, particularly those using magnetic resonance imaging (MRI), have substantially increased our knowledge of how the brain processes pain (Davis and Moayed, 2013; Hu et al., 2015; Su et al., 2019) and negative emotions (Gainotti, 2019). Sex differences in brain structure and function related to pain processing have been extensively investigated, which might have the potential to uncover the neural underpinnings of sex differences in pain sensitivity (Fillingim et al., 2009). In light of the modulatory effect of negative emotions on pain perception, Wiech and Tracey reviewed the relevant literature and summarized the possible neural mechanisms underlying this modulatory influence (Wiech and Tracey, 2009). The periaqueductal gray, amygdala, anterior cingulate cortex, and anterior insula are key brain areas in both pain and emotional processing (Wiech and Tracey, 2009). Accordingly, we also hypothesized that sex differences in brain structure and function might have the potential to uncover the neural underpinnings of sex differences in pain sensitivity, either directly or through the modulation of negative emotions.

To test the aforementioned hypotheses, we quantified pain sensitivity (i.e., pain threshold and tolerance) using the cold pressure test (CPT) that is considered a good model to mimic clinical pain (Rainville et al., 1992) and negative emotions (i.e., pain-related fear, pain-related anxiety, trait anxiety, and depression) using well-established questionnaires, and collected MRI data (i.e., high-resolution T1 structural images and resting-state functional images) from 450 healthy participants (290 females and 160 males). We assessed: (1) sex differences in pain threshold and pain tolerance, (2) the role of negative emotions in sex differences in pain threshold and pain tolerance, respectively, (3) the roles of brain structural and functional features in sex differences in pain sensitivity or negative emotions. Finally, we built a theoretical model to quantify the mediation relationship among sex, negative emotions, structural and functional brain features, and pain sensitivity using path analyses and structural equation modeling. The present study could deepen our understanding of the possible neuropsychological underpinnings of sex differences in pain sensitivity.

2. Materials and methods

2.1. Participants

A total of 450 healthy participants were recruited in this study. Twelve left-handed participants were excluded due to the brain morphology and function differences between left-handed and right-handed participants (Amunts et al., 2000; Andersen and Siebner, 2018; Pool et al., 2015), and seven right-handed participants were excluded from the analysis due to incomplete MRI data collection, yielding a total sample of 431 right-handed participants (152 males: mean age = 20.96 years, standard deviation = 2.09, ranging from 18 to 26 years; 279 females: mean age = 20.66 years, standard deviation = 2.01, ranging from 18 to 26 years). All included participants had no safety contraindications

for MRI, no history of major medical, psychiatric illness, or pain-related diseases, and no alcohol or drug abuse. The experiment was approved by the local Ethics Committee. All procedures were performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from each participant before the experiment, and all participants received monetary compensation after their participation.

2.2. Experimental procedures

The whole experiment is composed of three parts: (1) completing several emotion-related questionnaires at the beginning of the experiment; (2) taking a CPT to assess their pain sensitivity five minutes later; and (3) participating in a scanning session to collect the structural and resting-state functional MRI (fMRI) data four hours to one week later after the CPT.

2.3. Emotion-related questionnaires

Negative emotions, such as fear (Hirsh et al., 2008), anxiety (Goffaux et al., 2011; Thibodeau et al., 2013), and depression (Altemus et al., 2014), may represent plausible contributors to sex differences in pain sensitivity. Therefore, questionnaires that were used to assess these negative emotions were considered in the present study, including the Chinese version of Fear of Pain Questionnaire (FPQ) (McNeil and Rainwater, 1998), Pain Anxiety Symptoms Scale-20 (PASS-20) (Coons et al., 2004), the Trait version of State-Trait Anxiety Inventory (STAI-T) (Shek, 1988), and Beck Depression Inventory (BDI) (Beck et al., 1988). Details of these questionnaires are described as follows.

The FPQ is a 30-item questionnaire scored using a 4-point Likert scale, which is used to assess situationally-specific fears of painful stimuli as a trait-like phenomenon (McNeil and Rainwater, 1998). The PASS-20 is a short version of the PASS with 20 items on a 6-point Likert Scale, the PASS is used to measure latent, nonspecific pain-related anxiety under daily pain events (McCracken et al., 1992). The STAI-T is a subscale of State-Trait Anxiety Inventory scored using a 4-point Likert scale, which is a 20-item scale adopted to assess the degree of one's dispositional anxiety (Spielberger, 2010). The BDI is a 21-item scale used to measure one's characteristic attitudes and symptoms of depression in the last week (Beck et al., 1988). The reliability and validity of the Chinese version questionnaires have been well established in Chinese populations: FPQ (Yang et al., 2012), PASS (Zhou et al., 2017), STAI-T (Shek, 1988), and BDI (Shek, 1990). In the current sample, reliabilities of the scales were satisfactory: Cronbach's alpha = 0.93, 0.88, 0.87, and 0.83 for FPQ, PASS, STAI-T, and BDI, respectively. The total score of each questionnaire was used in the following statistical analyses.

2.4. Pain sensitivity assessment

Since previous studies demonstrated that the CPT had good reliability and validity for measuring individuals' pain sensitivity (Birnie et al., 2012; Koenig et al., 2014), this test was adopted in the present study to assess pain threshold and pain tolerance, which are two main indices to represent pain sensitivity. Cold pain was induced by placing participants' left hand into the cold water at a temperature of 2 ± 0.1 °C, using a circulating water bath (DX-208 water bath, Beijing Changliu Scientific Instruments Co., China) with continuously circulating water at a flow speed of 15 L/min. To eliminate the difference in baseline skin temperature across participants before CPT, all participants were asked to put their left hand into water at a temperature of ~ 22 °C for 30 s. Subsequently, participants were instructed to immerse their left hand into the cold water at a depth of about 3 cm above the wrist as long as possible. During this period, participants were required to focus their attention on the perception of the left hand, report their pain once they began to feel it, and keep their hand in the water until the pain became intolerable. At this time, participants could remove their left hand from

the cold water immediately. For safety reasons, a cutoff time of 300 s was enforced when participants did not withdraw their left hand until 300 s. Pain threshold was operationalized as the duration of immersion from the time that the left hand was placed in the water to the time that the participant began to feel pain. Pain tolerance was defined as the total time from placing the left hand in the water to actively or passively withdrew the hand from the water. The time was recorded by the experimenter standing a few feet behind the participants at an angle of 45° using a stopwatch.

2.5. MRI data acquisition

MRI data were collected using a 3.0-Tesla MRI system (Discovery MR 750; General Electric Healthcare, Milwaukee, WI, USA) at the Brain and Cognitive Neuroscience Research Center, Liaoning Normal University, Dalian, China. A standard birdcage with an 8-channel head coil, along with restraining foam pads, was used to minimize head motion and scanner noise. High resolution T1-weighted structural images were acquired using a gradient echo (3D SPGR) sequence with the following parameters: flip angle = 8°; field of view = 256 × 256 mm²; data matrix = 256 × 256; in-plane resolution = 1 × 1 mm²; slices = 176; slice thickness = 1 mm. Ten-minute resting-state functional images were acquired using an echo-planar-imaging sequence with the following parameters: repetition time = 2000 ms; echo time = 29 ms; flip angle = 90°; field of view = 192 × 192 mm²; data matrix = 64 × 64; in-plane resolution = 3 × 3 mm²; slice thickness = 3 mm. During the resting-state fMRI data acquisition, the screen presented a white fixation ‘+’ in the center of the black background. Participants were instructed to relax and remain still with their eyes open, not to fall asleep, and not to think about anything.

2.6. Structural MRI: cortical thickness and subcortical volume analysis

Structural MRI images were analyzed using the FreeSurfer software (v7.0.0, <http://surfer.nmr.mgh.harvard.edu>). According to previous studies (Dale et al., 1999; Fischl and Dale, 2000; Fischl et al., 1999), the pre-processing steps included motion correction, removal of non-brain tissue using a hybrid watershed/surface deformation procedure, automated Talairach transformation of each participant's native brain, segmentation of subcortical white matter and deep gray matter volumetric structures, intensity normalization, tessellation of the gray/white matter boundary, automated topology correction, surface deformation following intensity gradients to optimally place the gray/white and gray/cerebrospinal fluid borders at the location where the largest shift in intensity defines the transition to the other tissue class, and registration of the participant's brain to a common spherical atlas. All FreeSurfer outputs were visually inspected to assure the accuracy of the segmentation, and manual corrections were made, if necessary. To obtain cortical thickness difference maps, the cortical surface data was smoothed with a 10-mm full-width at half maximum Gaussian kernel to reduce local variation in the measurements (Li et al., 2015).

Cortical thickness was estimated based on the shortest distance of equivalent vertex distance between pial and gray-white matter surfaces with a surface-based group analysis using the FreeSurfer's statistical tool Qdec. Subcortical volumes were calculated from the automated procedure for volumetric measures of the brain structures implemented in FreeSurfer. Specifically, the subcortical volumes of the nucleus accumbens, caudate nucleus, putamen, pallidum, thalamus, amygdala, and hippocampus in each hemisphere were extracted from all participants.

In addition, due to the heterogeneous functions associated with emotions in different amygdala subnuclei (Bzdok et al., 2013; Roy et al., 2009), the amygdala was automatically segmented into nine nuclei (the lateral nucleus, basal nucleus, accessory basal nucleus, cortical nucleus, central nucleus, medial nucleus, anterior amygdala area, cortico-amygdaloid transition area, and paralaminar nucleus) in each hemisphere using a validated atlas building algorithm available in FreeSurfer

(Saygin et al., 2017). The volumes of these subnuclei in the amygdala in each hemisphere were extracted for further statistical analyses.

2.7. Functional MRI: fractional amplitude of low frequency fluctuations (fALFF) analysis

Resting-state fMRI data were analyzed using Data Processing and Analysis for (Resting-State) Brain Imaging (DPABI) software (Yan et al., 2016). The first ten volumes were discarded for the spin saturation effects and subjects' adaptation to their immediate environment. Images were slice timing corrected and spatially realigned. Subjects with head motion more than 2.0 mm of maximal translation (in any direction of x, y, or z) or 2.0° of maximal rotation throughout scanning were excluded from further analysis (six males and eight females were excluded). The structural images were co-registered to their corresponding realigned images, and then were segmented into gray matter, white matter, and cerebrospinal fluid using the Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL) tool (Ashburner, 2007) to compute transformations from individual native space to Montreal Neurological Institute (MNI) space. White matter signal, cerebrospinal fluid signal, and Friston's-24 motion parameters (Friston et al., 1996) were regressed as nuisance covariates from the pre-processed resting-state fMRI data. The processed fMRI data were normalized to the MNI space (voxel size = 3 × 3 × 3 mm³) using DARTEL, and then spatially smoothed with a Gaussian kernel of 6-mm full width at half maximum to reduce spatial noise.

Since the fALFF could be used to detect the intensity of regional spontaneous brain activity with high sensitivity and specificity (Zou et al., 2008), this analysis was adopted in the present study to characterize functional features in resting-state fMRI data. Specifically, the time series of each voxel was converted into the frequency domain (ranging from 0 to 0.25 Hz), and the power spectrum was obtained using the fast Fourier transform. The square root of the power spectrum was computed at each frequency, and for each voxel, and the averaged square root of the power spectrum across the frequencies ranging from 0.01 to 0.1 Hz was obtained. The fALFF value was calculated as the ratio of the power spectrum of low frequency fluctuations (0.01–0.1 Hz) to that of the entire frequency range (0–0.25 Hz).

2.8. Statistical analyses

Demographic information, such as age and education level, was compared between males and females using independent-samples *t*-tests to rule out their possible effects on the results associated with sex differences.

Before performing statistical analyses, Shapiro–Wilk tests were conducted to evaluate the normality of the data (i.e., pain sensitivity variables and negative emotions). For skewed distributed variables, non-parametric methods (i.e., Mann-Whitney *U*-tests and Spearman rank correlation analyses) were used to perform the statistical analysis. For normally distributed variables, parametric methods (i.e., independent-samples *t*-tests and Pearson correlation analyses) were used. Results showed that the values of pain threshold and pain tolerance were skewed distributed (all *p* < 0.001), and the values of negative emotion variables (i.e., FPQ, PASS, STAI-T, and BDI scores) were normally distributed (all *p* > 0.05). Therefore, nonparametric Mann-Whitney *U*-tests were performed to test whether there were sex differences in pain threshold and pain tolerance. Parametric independent-samples *t*-tests were used to compare sex differences in negative emotions as evaluated by FPQ, PASS, STAI-T, and BDI scores. Moreover, Spearman rank correlation analyses were performed to explore the relationship between pain threshold/tolerance and negative emotions with sex differences. Pearson correlation analyses were performed to reveal the relationship between different negative emotions with sex differences. To account for multiple comparisons in the analyses, a false discovery rate (FDR) procedure (Benjamini and Hochberg, 1995) was adopted to adjust the *p*

values. All tests were 2-tailed, and the level of significance was $p = 0.05$. Please note that participants who withdrew their left hand until 300 s (13 males and 11 females) were also included in statistical analyses, since excluding these participants may misrepresent the range of participants' experiences and bias the results (Birnie et al., 2012). To exclude the influence of including participants with a fixed pain tolerance value (i.e., 300 s) on the results, we also performed the statistical analyses after excluding these participants ($n = 24$). Notably, almost the same statistical results were obtained after excluding these participants.

A general linear model was used at each voxel in the whole brain to identify brain regions where males showed significant cortical thickness differences relative to females while controlling for age (based on the FreeSurfer's statistical tool Qdec). Moreover, univariate analyses of covariance (ANCOVA) were conducted to test sex differences in subcortical volumes (i.e., the nucleus accumbens, caudate nucleus, putamen, pallidum, thalamus, amygdala, and hippocampus), while controlling for intracranial volume (ICV) and age. The same ANCOVA procedures were also performed on amygdala subnuclei volumes to explore whether amygdala volumes exist sex differences at the subnuclei level. In addition, Spearman rank correlation analyses were performed to test the relationship between brain variables (i.e., cortical thickness, subcortical volumes, and amygdala subnuclei volumes) with sex differences and pain threshold/tolerance after controlling for ICV (if necessary) and age. Pearson correlation analyses were performed to test the relationship between brain variables (i.e., cortical thickness, subcortical volumes, and amygdala subnuclei volumes) with sex differences and the negative emotions with sex differences after controlling for ICV (if necessary) and age. To correct for multiple comparisons in the above analyses, the FDR procedure was used to adjust the p values. The above statistical analyses were conducted using SPSS 22.0 (SPSS Inc., Chicago, IL, USA).

Independent-samples t -tests were also performed to assess whether there were sex differences in fALFF values at the voxel level, with the age and head motion parameter (indexed by the mean frame-wise displacement [FD]) as covariate variables in DPABI. To explore the possible relationship between fALFF values and behavioral characteristics with sex difference (i.e., pain threshold, pain tolerance, and negative emotions), voxel-wise whole-brain multiple regression analyses were performed with age and FD as covariate variables. To further confirm the results obtained at the voxel level, the same analyses were also performed at the regional level (i.e., the average fALFF values from 90 regions of interest based on the Anatomical Automatic Labeling atlas were extracted) (Tzourio-Mazoyer et al., 2002). The level of significance was set as $p = 0.05$ after FDR correction.

To explore the influence factors of the relationship between sex and pain sensitivity, we hypothesized a series of mediation models based on the significant results of the above statistical analyses. The adequacy of the hypothetic mediation models was tested using path analyses or structural equation modeling with maximum likelihood estimation. Specifically, the hypothetic mediation models included the following four categories. First, to assess the possible effect of negative emotions on the relationship between sex and pain sensitivity, sex was used as the independent variable, pain sensitivity indices (pain threshold and tolerance, respectively) were used as dependent variables, and negative emotion variables (i.e., FPQ score, PASS score, and both) were used as mediator variables. Second, to assess the possible effect of amygdala volumes on the relationship between sex and pain sensitivity, sex was used as the independent variable, pain sensitivity indices (pain threshold and tolerance, respectively) were used as dependent variables, and amygdala subnuclei volumes were used as mediator variables. Third, considering the close association between amygdala volumes and negative emotions (Baur et al., 2012; Hartley et al., 2011; Hayano et al., 2009; Rogers et al., 2009), sex was used as the independent variable, negative emotion variables (i.e., FPQ score, PASS score, and both) were used as dependent variables, and amygdala volumes (at whole or subnuclei level) were used as mediator variables. Finally, based on the above modeling re-

sults, we proposed a compositive model to characterize the relationship among all these variables.

The model fit was assessed using the following criteria: the significance of chi-square (χ^2) statistic (p value) > 0.05 , the ratio of chi-square to degrees of freedom (χ^2/df) < 2 (Kline, 2015), the root mean square error of approximation (RMSEA) ≤ 0.06 (Hu and Bentler, 1999), both the goodness-of-fit index (GFI) and the adjusted goodness-of-fit index (AGFI) ≥ 0.90 , both the comparative fit index (CFI) and the normed fit index (NFI) ≥ 0.95 (Hooper et al., 2008). Besides, to assess the significance of the indirect and direct effects in the mediation model, bias-corrected 95% confidence intervals (CIs) were calculated using the bootstrapping procedure (Preacher and Hayes, 2008). The estimate was considered statistically significant when the 95% CI (based on 5000 bootstrap samples) excluded zero. Standardized estimate (b), standard error (SE), 95% CI, and p value were reported for both indirect and direct effects. There are three patterns of mediating effect: (a) a complementary mediation exhibits both significant indirect and direct effects, which point to the same (positive or negative) direction; (b) a competitive mediation exhibits both significant indirect and direct effects, which point to opposite directions; and (c) an indirect-only mediation exhibits a significant indirect effect but insignificant direct effect (Carrión et al., 2017). These statistical analyses were performed using Amos 24.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Sex differences in pain sensitivity and negative emotions

Descriptive statistics of pain sensitivity and negative emotions were summarized in Table 1. There were no significant sex differences in age ($t(429) = 1.48$, $p = 0.139$) or education level ($t(429) = 0.78$, $p = 0.438$). Significant sex differences were found in pain threshold and tolerance, FPQ score, and PASS score (Table 1 and Fig. 1). In details, males exhibited significantly higher pain threshold (mean rank = 240.16 and 202.84 for males and females, respectively) and pain tolerance (mean rank = 240.20 and 202.81 for males and females, respectively) than females (pain threshold: $U = 17,531.00$, $z = -2.97$, $p = 0.003/0.006$; pain tolerance: $U = 17,525.00$, $z = -2.98$, $p = 0.003/0.006$; p = value uncorrected/FDR corrected, the same hereinafter). In addition, males reported significantly lower FPQ ($t(429) = -4.26$, $p < 0.001/0.001$) and PASS ($t(429) = -2.85$, $p = 0.005/0.007$) scores than females. In contrast, there were no significant sex differences in STAI-T ($t(429) = -0.70$, $p = 0.485/0.485$) and BDI ($t(429) = 1.13$, $p = 0.261/0.313$) scores (Table 1 and Fig. 1).

Since STAI-T and BDI scores showed no significant sex differences, both variables were not considered in subsequent statistical analyses. Spearman rank correlation analyses showed that pain threshold was strongly correlated with pain tolerance ($r(431) = 0.65$, $p < 0.001/0.001$), FPQ score was negatively correlated with pain threshold ($r(431) = -0.15$, $p = 0.002/0.009$) and tolerance ($r(431) = -0.20$, $p < 0.001/0.001$), and PASS score was negatively correlated with pain threshold ($r(431) = -0.17$, $p < 0.001/0.001$) and tolerance ($r(431) = -0.19$, $p < 0.001/0.001$). In addition, Pearson correlation analysis showed that FPQ score was positively correlated with PASS score ($r(431) = 0.46$, $p < 0.001/0.001$) (Fig. 2).

3.2. Sex differences in structural MRI data

For cortical thickness, no significant differences were observed between males and females after controlling for age ($p > 0.05$). For subcortical volumes, males showed significantly larger volumes in the left putamen ($F(1, 427) = 12.14$, $p < 0.001/0.003$, $\eta_p^2 = 0.03$, Fig. 3A), right putamen ($F(1, 427) = 6.63$, $p = 0.010/0.029$, $\eta_p^2 = 0.02$, Fig. 3B), left amygdala ($F(1, 427) = 26.01$, $p < 0.001/0.001$, $\eta_p^2 = 0.06$, Fig. 3C), right amygdala ($F(1, 427) = 14.83$, $p < 0.001/0.001$, $\eta_p^2 = 0.03$, Fig. 3D), and left pallidum ($F(1, 427) = 11.46$, $p < 0.001/0.003$, $\eta_p^2 = 0.03$, Fig. 3E)

Table 1

Descriptive statistics (*Mean ± SE*) of demographic information, pain sensitivity indices, and negative emotion variables, as well as their statistical comparisons between males and females.

	Males (<i>n</i> = 152)	Females (<i>n</i> = 279)	Statistics		
			<i>t/z</i>	Uncorrected <i>p</i> value	FDR-corrected <i>p</i> value
Age (year)	20.96 ± 0.17	20.66 ± 0.12	1.48	0.139	–
Education levels (year)	14.72 ± 0.15	14.57 ± 0.12	0.78	0.438	–
Pain threshold (s)	11.30 ± 0.88	8.74 ± 0.52	–2.97	0.003	0.006**
Pain tolerance (s)	58.86 ± 6.80	41.52 ± 3.81	–2.98	0.003	0.006**
FPQ score	86.55 ± 1.39	93.53 ± 0.94	–4.26	< 0.001	< 0.001***
PASS score	41.15 ± 1.22	45.20 ± 0.81	–2.85	0.005	0.007**
STAI-T score	41.37 ± 0.74	41.99 ± 0.52	–0.70	0.485	0.485
BDI score	10.53 ± 0.60	9.73 ± 0.41	1.13	0.261	0.313

Since sex differences in pain threshold and tolerance were compared using Mann-Whitney *U*-tests, their statistics were expressed in *z* values. For other variables that were compared using independent-samples *t*-tests, their statistics were expressed in *t* values. FPQ, Fear of Pain Questionnaire; PASS, Pain Anxiety Symptoms Scale; STAI-T, Trait version of State-Trait Anxiety Inventory; BDI, Beck Depression Inventory. ***p* < 0.01, ****p* < 0.001.

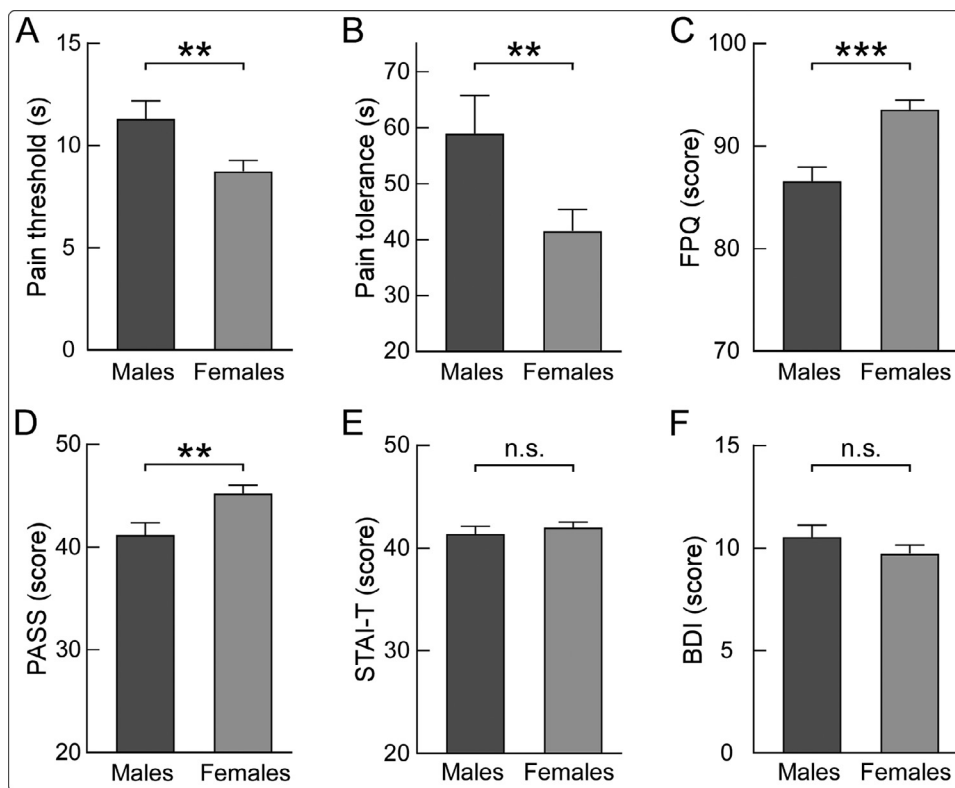


Fig. 1. Sex differences in pain sensitivity and negative emotions. (A) Pain threshold and (B) tolerance were significantly higher in males than females, while (C) FPQ and (D) PASS scores were significantly lower in males than females. No significant sex differences were observed in (E) STAI-T and (F) BDI scores. Bar plots denote mean, error bars denote *SE*. FPQ, Fear of Pain Questionnaire; PASS, Pain Anxiety Symptoms Scale; STAI-T, Trait version of State-Trait Anxiety Inventory; BDI, Beck Depression Inventory. n.s. not significant, ***p* < 0.01, ****p* < 0.001.

than females after controlling for ICV and age. No significant sex differences were observed in other subcortical volumes (i.e., the left and right nucleus accumbens, left and right caudate nucleus, left and right thalamus, left and right hippocampus, and right pallidum, all *p* > 0.05). For amygdala subnuclei volumes, males also showed significantly larger volumes in eight subnuclei (i.e., the lateral nucleus, basal nucleus, accessory basal nucleus, anterior amygdaloid area, central nucleus, cortical nucleus, cortico-amygdaloid transition area, and paralaminar nucleus) in the left hemisphere and five subnuclei (the lateral nucleus, basal nucleus, accessory basal nucleus, cortical nucleus, and cortico-amygdaloid transition area) in the right hemisphere than females (all *p* < 0.05, FDR corrected. Supplementary Table 1).

Spearman rank/Pearson correlation analyses showed that the right amygdala volume was positively correlated with pain tolerance ($r(431) = 0.13$, *p* = 0.008/0.031, Fig. 3F), and the left amygdala volume was negatively correlated with FPQ score ($r(431) = -0.12$, *p* = 0.012/0.039, Fig. 3G), after controlling for ICV and age. No other significant correlations were observed between subcortical volumes and

pain sensitivity indices or negative emotion variables (all *p* > 0.05). Furthermore, when considering amygdala subnuclei volumes, significant correlations were observed between FPQ score and the volumes of the lateral nucleus ($r(431) = -0.13$, *p* = 0.007/0.0497, Fig. 4B), basal nucleus ($r(431) = -0.11$, *p* = 0.023/0.0497, Fig. 4C), accessory basal nucleus ($r(431) = -0.11$, *p* = 0.018/0.0497, Fig. 4D), and cortico-amygdaloid transition area ($r(431) = -0.11$, *p* = 0.025/0.0497, Fig. 4F) in the left hemisphere, after controlling for ICV and age. No other significant correlations were observed between amygdala subnuclei volumes and pain sensitivity indices or negative emotion variables (all *p* > 0.05).

3.3. Sex differences in fMRI data

In resting-state fMRI data, males showed significantly higher fALFF values in the right putamen, right insula, left anterior insula, bilateral precuneus, left middle cingulate gyrus, right inferior frontal gyrus, right middle frontal gyrus, bilateral precentral gyrus, bilateral postcentral gyrus, right superior parietal gyrus, left inferior parietal gyrus, right

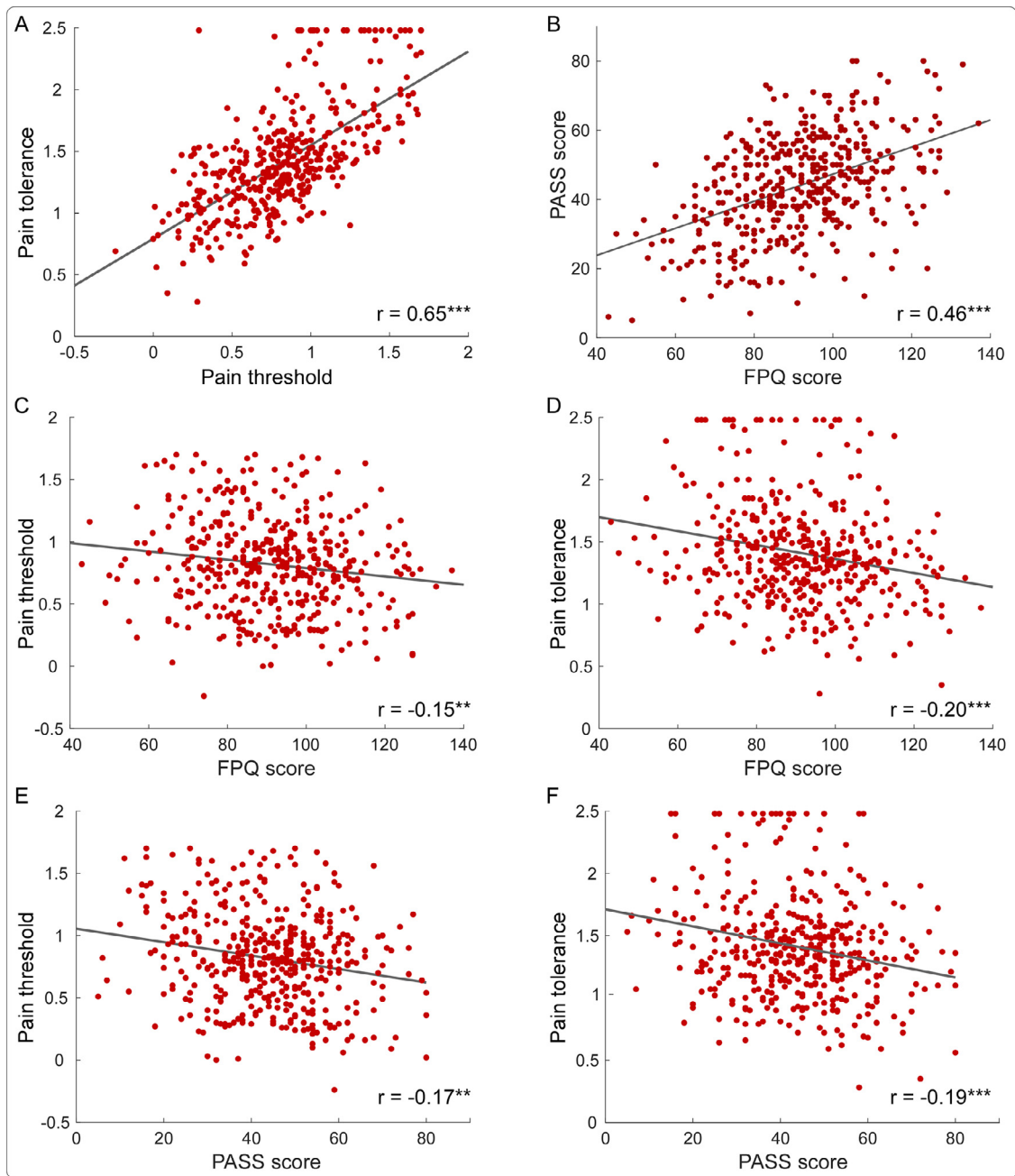


Fig. 2. Correlations between pain sensitivity indices and negative emotion variables. (A) Pain threshold was positively correlated with pain tolerance. (B) FPQ score was positively correlated with PASS score. (C-D) FPQ score was negatively correlated with pain threshold and tolerance. (E-F) PASS score was negatively correlated with pain threshold and tolerance. For the aesthetics of the scatter plot, the values of pain threshold and tolerance were log-transformed. ** $p < 0.01$, *** $p < 0.001$.

middle and inferior occipital gyrus, right fusiform gyrus, right lingual gyrus, bilateral middle and superior temporal gyrus, right inferior temporal gyrus, and right cerebellum_crus1 than females ($p < 0.05$, FDR corrected. Fig. 5 and Supplementary Table 2).

In contrast, males showed significantly lower fALFF values in the bilateral caudate, left putamen, right thalamus, right hippocampus, bilateral midbrain, bilateral parahippocampal gyrus, right cingulate gyrus, bilateral superior and middle frontal gyrus, left orbital and medial frontal gyrus, right superior parietal gyrus, bilateral inferior parietal gyrus, left supramarginal gyrus, right angular gyrus, bilateral inferior and middle temporal gyrus, bilateral cerebellum_8, and cerebellum_crus2 than females ($p < 0.05$, FDR corrected. Fig. 5 and Supplementary Table 2). However, within all brain regions with sex differences of fALFF values, no significant correlations were observed between fALFF values and

pain threshold, pain tolerance, FPQ score, or PASS score (all $p > 0.05$, FDR corrected).

The results obtained at the regional level also showed that no significant correlations were observed between fALFF values in any region of interest and pain threshold, pain tolerance, FPQ score, or PASS score (all $p > 0.05$, FDR corrected).

3.4. Mediation effects of the relationship between sex and pain sensitivity

When assessing the possible effect of negative emotions on the relationship between sex and pain sensitivity, we observed that sex showed both a direct effect (FPQ: $b = -0.10$, $SE = 0.05$, $CI = [-0.19, -0.01]$, $p = 0.034$; PASS: $b = -0.10$, $SE = 0.05$, $CI = [-0.19, -0.01]$, $p = 0.027$) and an indirect effect (FPQ: $b = -0.03$, $SE = 0.01$, $CI = [-0.06, -0.01]$, p

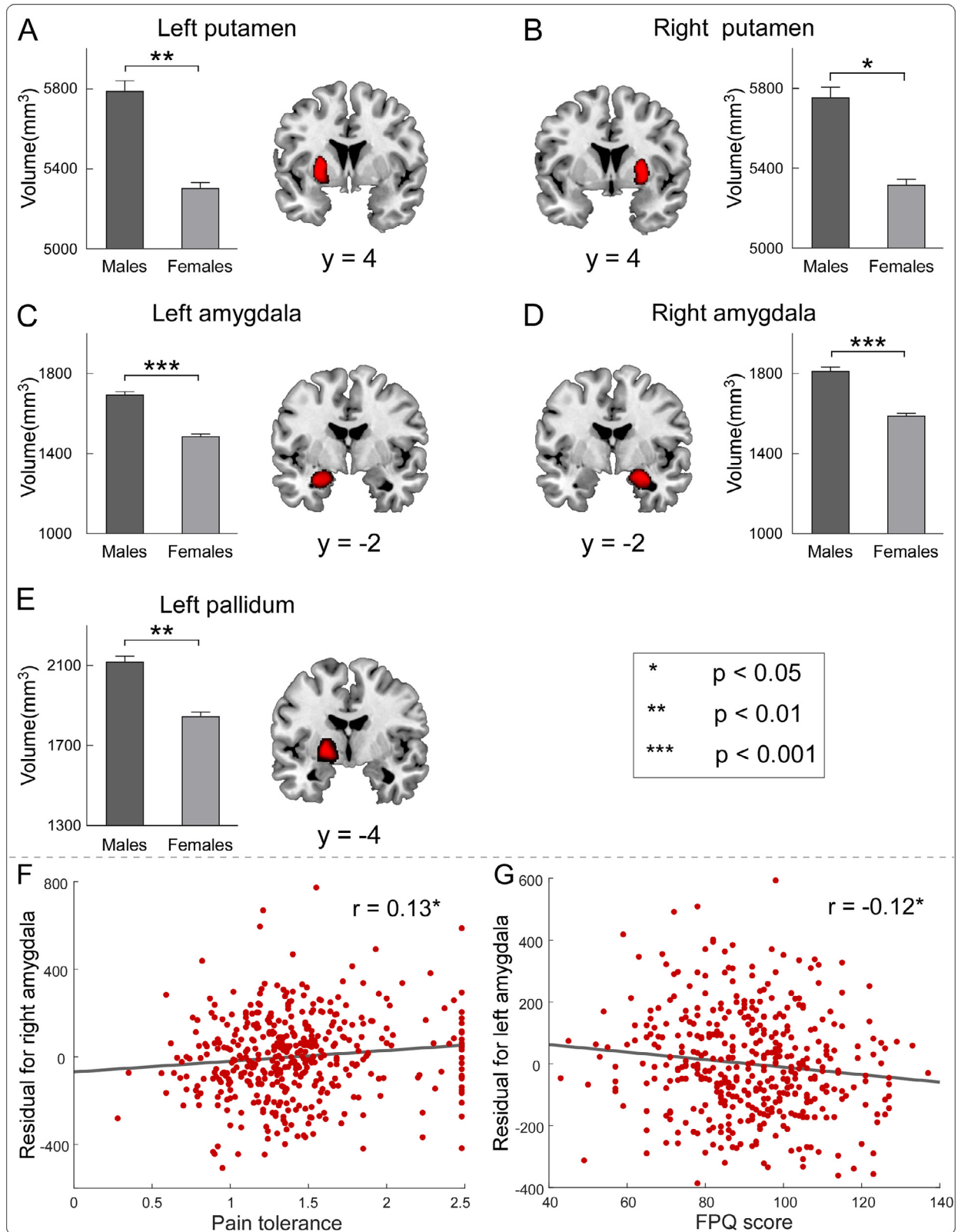


Fig. 3. Sex differences in subcortical volumes and correlations between amygdala volumes and pain tolerance/FPQ score. The volumes of the (A) left putamen, (B) right putamen, (C) left amygdala, (D) right amygdala, and (E) left pallidum were significantly larger in males than females after controlling for ICV and age. Bar plots denote mean, error bars denote SE. The diagrammatic sketches of these subcortical regions are displayed in their respective panels. Correlation results showed that (F) the volume of the right amygdala was positively correlated with pain tolerance, and (G) the volume of the left amygdala was negatively correlated with FPQ score, after controlling for ICV and age. For the aesthetics of the scatter plot, the values of pain tolerance were log-transformed. The ordinate values in the scatter plots were the residuals of the amygdala volumes controlling for ICV and age. FPQ, Fear of Pain Questionnaire.

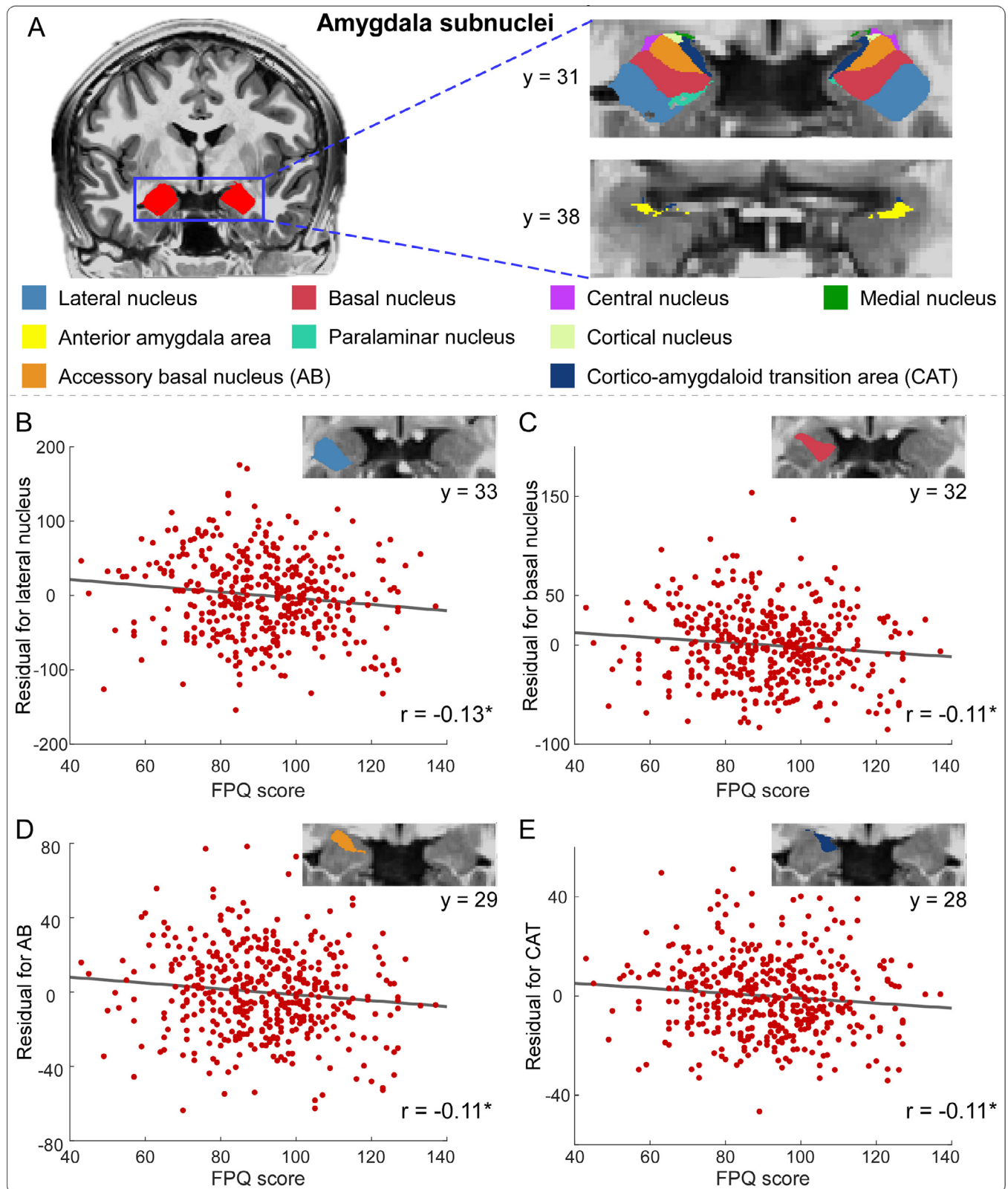


Fig. 4. Correlations between FPQ score and amygdala subnuclei volumes in the left hemisphere. (A) Coronal view of the amygdala subnuclei segmentation in one participant derived from Freesurfer software package. FPQ score was negatively correlated with the volumes of (B) the lateral nucleus, (C) basal nucleus, (D) accessory basal nucleus, and (E) cortico-amygdaloid transition area in the left hemisphere. The ordinate values in the scatter plots were the residuals of the amygdala subnuclei volumes controlling for ICV and age. FPQ, Fear of Pain Questionnaire. $*p < 0.05$.

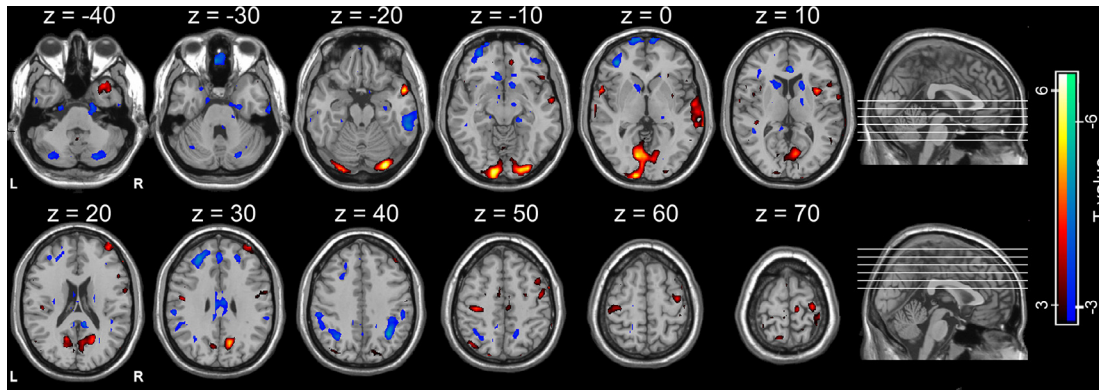


Fig. 5. Brain regions exhibiting sex differences in resting-state fMRI data (indexed using the fALFF value). Warm color represents regions with significantly higher fALFF values in males than females, and cold color represents regions with significantly lower fALFF values in males than females. The significance threshold was set as $p < 0.05$ with FDR correction. fALFF, the fractional amplitude of low frequency fluctuations.

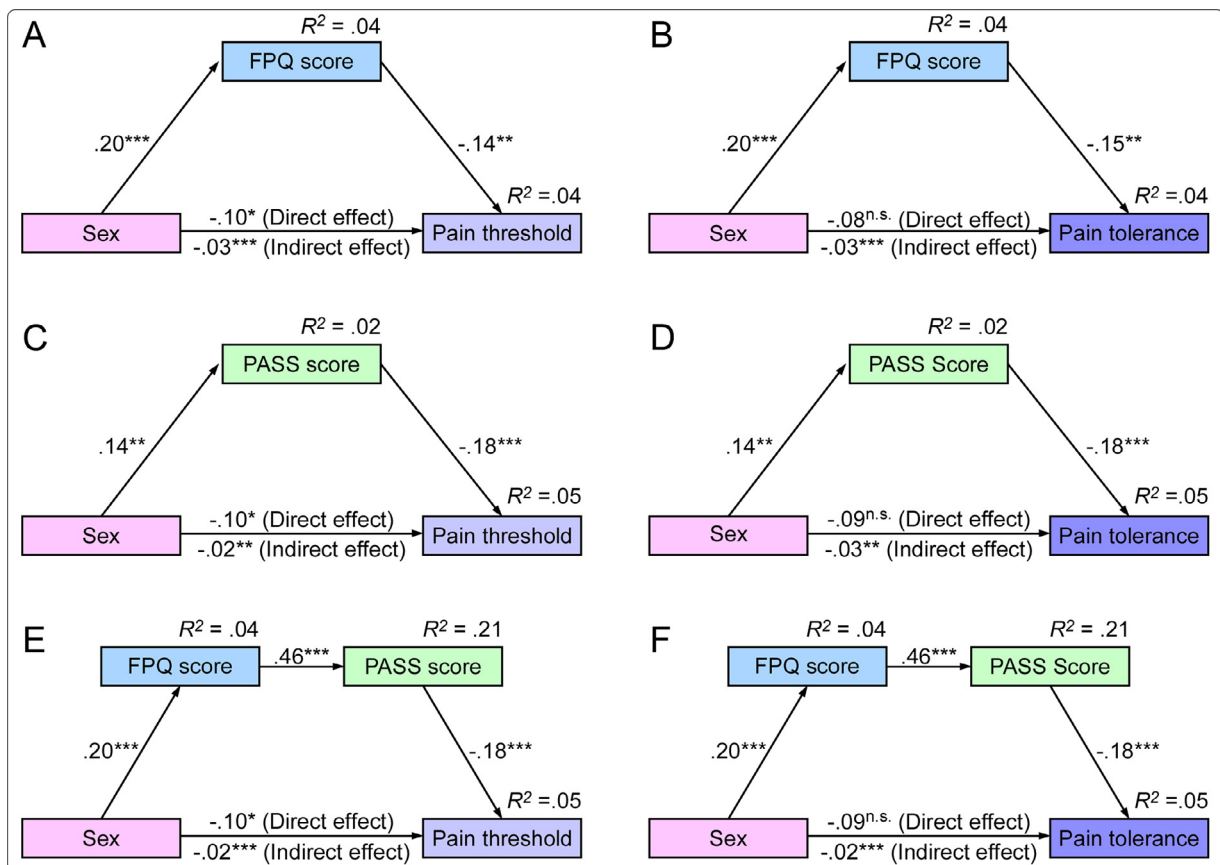


Fig. 6. Pain-related negative emotions mediate the relationship between sex and pain sensitivity. The relationship between sex and pain threshold was complementarily mediated by (A) FPQ score, (C) PASS score, and (E) both. However, the relationship between sex and pain tolerance was indirect-only mediated by (B) FPQ score, (D) PASS score, and (F) both. Standardized regression weights and squared multiple correlation coefficients were shown for each model. For the sex variable, females were coded with a higher value than males. FPQ, Fear of Pain Questionnaire; PASS, Pain Anxiety Symptoms Scale. n.s. not significant, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

< 0.001 ; PASS: $b = -0.02$, $SE = 0.01$, $CI = [-0.05, -0.01]$, $p = 0.004$) on pain threshold through FPQ score (Fig. 6A) or PASS score (Fig. 6C). In contrast, sex only showed an indirect effect (FPQ: $b = -0.03$, $SE = 0.01$, $CI = [-0.06, -0.01]$, $p < 0.001$; PASS: $b = -0.03$, $SE = 0.01$, $CI = [-0.05, -0.01]$, $p = 0.004$) on pain tolerance through FPQ score (Fig. 6B) or PASS score (Fig. 6D), but not a direct effect (FPQ: $b = -0.08$, $SE = 0.05$, $CI = [-0.18, 0.01]$, $p = 0.077$; PASS: $b = -0.09$, $SE = 0.05$, $CI = [-0.19, 0.01]$, $p = 0.068$). These results revealed that FPQ and PASS scores were

the complementary mediators of the relationship between sex and pain threshold, while they were the indirect-only mediators of the relationship between sex and pain tolerance.

In light of the significant correlation between pain-related fear and anxiety, we expected that both negative emotions (FPQ and PASS scores) could jointly mediate the relationship between sex and pain sensitivity. When assessing whether FPQ and PASS scores serially mediated the effect of sex on pain threshold, we observed a direct effect ($b = -0.10$,

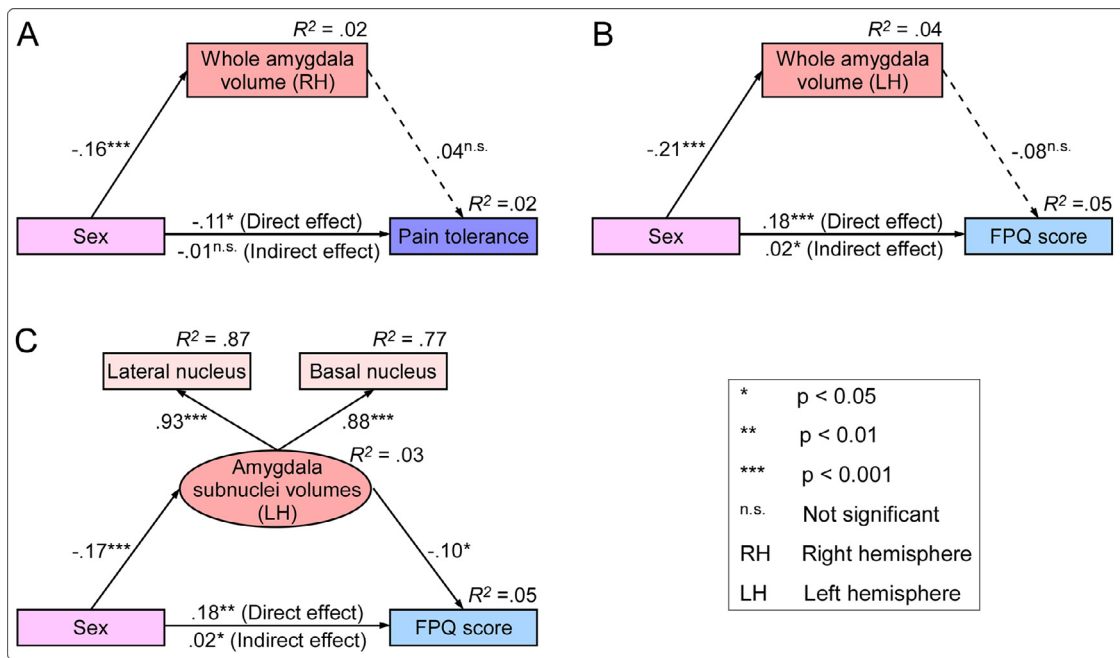


Fig. 7. The mediation effects of amygdala volumes on pain sensitivity and negative emotions. (A) The relationship between sex and pain tolerance was not mediated by the right amygdala volume. (B) The relationship between sex and FPQ score was complementarily mediated by the left amygdala volume, while the left amygdala volume was not directly related to FPQ score. However, (C) the relationship between sex and FPQ score was complementarily mediated by the left amygdala subnuclei volumes, which were loaded by the lateral and basal nuclei. Standardized regression weights and squared multiple correlation coefficients were shown for each model. For the sex variable, females were coded with a higher value than males. FPQ, Fear of Pain Questionnaire.

$SE = 0.05$, $CI = [-0.19, -0.01]$, $p = 0.027$) and an indirect effect ($b = -0.02$, $SE = 0.01$, $CI = [-0.03, -0.01]$, $p < 0.001$). The model fit the data well and exhibited adequate fit indices: $\chi^2(2) = 3.24$, $p = 0.198$, $\chi^2/df = 1.62$, $RMSEA = 0.04$, $GFI = 0.996$, $AGFI = 0.98$, $CFI = 0.99$, $NFI = 0.98$ (Fig. 6E). When assessing whether FPQ and PASS scores serially mediated the effect of sex on pain tolerance, we only observed an indirect effect ($b = -0.02$, $SE = 0.01$, $CI = [-0.03, -0.01]$, $p < 0.001$), but not a direct effect ($b = -0.09$, $SE = 0.05$, $CI = [-0.19, 0.01]$, $p = 0.068$). The model also fit the data well and exhibited adequate fit indices: $\chi^2(2) = 3.87$, $p = 0.145$, $\chi^2/df = 1.93$, $RMSEA = 0.05$, $GFI = 0.996$, $AGFI = 0.98$, $CFI = 0.99$, $NFI = 0.97$ (Fig. 6F). In contrast, when assessing whether PASS and FPQ scores serially mediated the effect of sex on pain threshold or tolerance, the models exhibited poor fit indices. These results suggested that FPQ and PASS scores were the serial and complementary mediators of the relationship between sex and pain threshold, while they were the serial and indirect-only mediators of the relationship between sex and pain tolerance.

In light of the significant correlation between the right amygdala volume and pain tolerance, we tested whether the right amygdala volume could mediate the relationship between sex and pain tolerance. We observed that sex had a direct effect ($b = -0.11$, $SE = 0.05$, $CI = [-0.21, -0.01]$, $p = 0.034$), but not an indirect effect ($b = -0.01$, $SE = 0.01$, $CI = [-0.03, 0.01]$, $p = 0.287$) on pain tolerance through the right amygdala volume (Fig. 7A). This result suggested that the right amygdala volume had no mediation effect on the relationship between sex and pain tolerance. In light of the significant correlation between the left amygdala volume and FPQ score, we tested whether the left amygdala volume could mediate the relationship between sex and pain-related fear. We observed that sex had both a direct effect ($b = 0.18$, $SE = 0.05$, $CI = [0.01, 0.28]$, $p < 0.001$) and an indirect effect ($b = 0.02$, $SE = 0.01$, $CI = [0.001, 0.04]$, $p = 0.044$) on FPQ score through the left amygdala volume (Fig. 7B). This result suggested that the left amygdala volume was a complementary mediator of the relationship between sex and FPQ score. However, the path from the left amygdala volume to FPQ score was not significant ($p = 0.057$), suggesting that the left amygdala volume as a whole did not relate directly to FPQ score in this model.

dala volume as a whole did not relate directly to FPQ score in this model.

Furthermore, we assessed whether the amygdala volumes at the subnuclei level could reliably mediate the relationship between sex and pain-related fear. Specifically, the amygdala subnuclei volumes were the mediators and loaded by the lateral and basal nuclei in the left hemisphere. The final model fit the data well and exhibited adequate fit indices: $\chi^2(1) = 0.38$, $p = 0.539$, $\chi^2/df = 0.38$, $RMSEA < 0.001$, $GFI = 1.00$, $AGFI = 0.996$, $CFI = 1.00$, $NFI = 0.999$ (Fig. 7C). Sex demonstrated both a direct effect ($b = 0.18$, $SE = 0.05$, $CI = [0.08, 0.28]$, $p = 0.001$) and an indirect effect ($b = 0.02$, $SE = 0.01$, $CI = [0.001, 0.05]$, $p = 0.033$) on FPQ score through the volumes of amygdala subnuclei. These results suggested that the volumes of amygdala subnuclei (i.e., the lateral and basal nuclei in the left hemisphere) were the complementary mediators of the relationship between sex and pain-related fear. Please note that although significant correlations were also observed between the volumes of the left accessory basal nucleus, cortico-amygdaloid transition area, and FPQ score, these nuclei were not considered in the analysis due to the insignificant model path.

Based on the well-fitted models as described above, we proposed a composite model to characterize the relationship among these variables by modifying the model paths and removing non-significant paths (Fig. 8). The final model fit the data well and exhibited adequate fit indices: $\chi^2(11) = 8.10$, $p = 0.704$, $\chi^2/df = 0.74$, $RMSEA < 0.001$, $GFI = 0.995$, $AGFI = 0.99$, $CFI = 1.00$, $NFI = 0.99$. When considering the relationship between sex and pain threshold, the final model suggested a direct path and two indirect paths (path 1: serially mediated by FPQ and PASS scores; path 2: serially mediated by the amygdala subnuclei volumes loaded by the lateral and basal nuclei in the left hemisphere, FPQ score, and PASS score). When considering the relationship between sex and pain tolerance, the final model suggested four indirect paths (path 1: mediated by pain threshold; path 2: mediated by FPQ score; path 3: serially mediated by FPQ score, PASS score, and pain threshold; path 4: serially mediated by the amygdala subnuclei volumes, FPQ score, PASS score, and pain threshold).

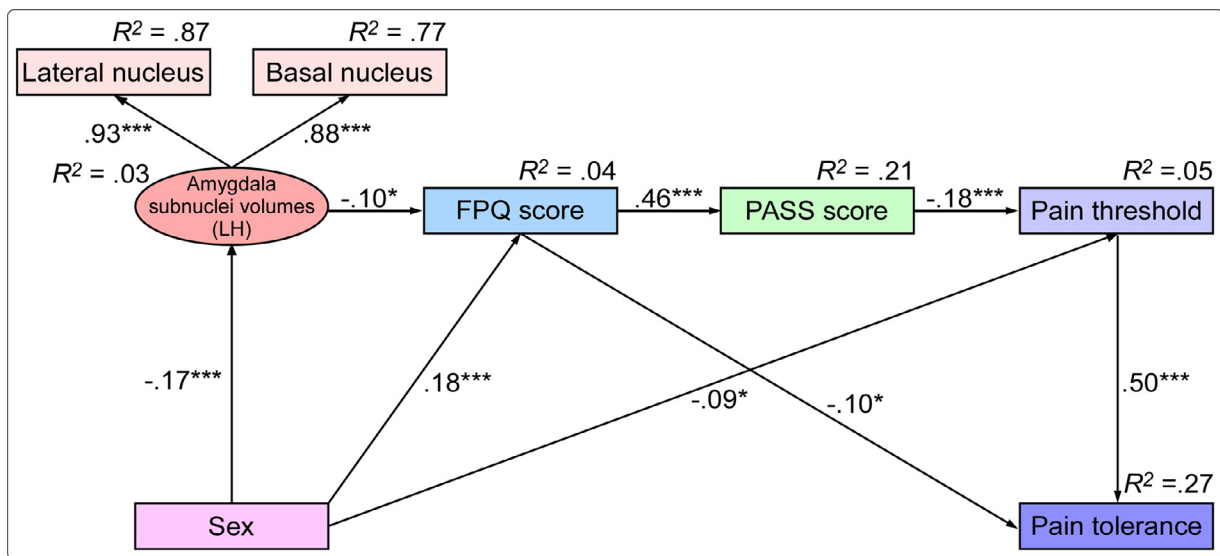


Fig. 8. The composite model characterizing the detailed relationship between sex and pain sensitivity. When considering the relationship between sex and pain threshold, the final model suggested a direct path and two indirect paths (path 1: serially mediated by FPQ and PASS scores; path 2: serially mediated by the amygdala subnuclei volumes loaded by the lateral and basal nuclei in the left hemisphere, FPQ score, and PASS score). When considering the relationship between sex and pain tolerance, the final model suggested four indirect paths (path 1: mediated by pain threshold; path 2: mediated by FPQ score; path 3: serially mediated by FPQ score, PASS score, and pain threshold; path 4: serially mediated by the amygdala subnuclei volumes, FPQ score, PASS score, and pain threshold). Standardized regression weights and squared multiple correlation coefficients were shown for each model. For the sex variable, females were coded with a higher value than males. FPQ, Fear of Pain Questionnaire; PASS, Pain Anxiety Symptoms Scale. * $p < 0.05$, *** $p < 0.001$.

4. Discussion

The present study aimed to investigate the neuropsychological mechanism underlying sex differences in pain sensitivity, i.e., the extent to which negative emotions and brain structural and functional features accounted for these differences in 450 healthy subjects. We obtained four main findings. First, as compared to males, females showed lower pain threshold and pain tolerance in response to cold pain elicited by CPT (Fig. 1). Second, sex differences in pain sensitivity were mediated by pain-related negative emotions. Specifically, pain-related fear and anxiety were the complementary mediators of the relationship between sex and pain threshold, and they were the indirect-only mediators of the relationship between sex and pain tolerance (Fig. 6). Third, some brain structural features subserved the neural underpinnings of sex differences in pain sensitivity through the modulation of pain-related fear. Specifically, the amygdala subnuclei (i.e., the lateral and basal nuclei in the left hemisphere) volumes were the complementary mediators of the relationship between sex and pain-related fear (Fig. 7). At last, the composite model quantified the mediation relationship among sex, negative emotions, structural brain features, and pain sensitivity, and provided an insight into the role of amygdala subnuclei volumes and pain-related negative emotions in sex differences in pain sensitivity (Fig. 8).

4.1. The role of negative emotions in sex differences in pain sensitivity

In line with previous findings (Hunter et al., 2019; Pud et al., 2009), we also observed lower pain threshold and tolerance in females than males (Fig. 1). Interestingly, sex difference in pain threshold was complementarily mediated by pain-related fear, pain-related anxiety, and both. Nevertheless, sex difference in pain tolerance was indirectly-only mediated by pain-related fear, pain-related anxiety, and both. These findings are highly consistent with the notion that pain threshold, which emphasizes the discrimination of nociceptive quality, and pain tolerance, which indicates an unwillingness to receive more intense stimuli, differ considerably in their loading on physiological and psychological components (Harris and Rollman, 1983). Specifically, pain threshold could be influenced not only by physiological components (i.e., sex-

related biological factors including the sex hormones and the sensitivity of nociceptors) (Maeng and Milad, 2015; Zhang et al., 2021) but also by psychological components (i.e., negative emotions including pain-related fear and anxiety) (Horn et al., 2014; Thibodeau et al., 2013). In contrast, pain tolerance, the moment that a person can no longer endure the pain, could be easily influenced by psychological components and seems to be more amenable to experimental manipulation through psychological variables than pain threshold (Gelfand, 1964).

Fear and anxiety are common responses to pain events in daily life (Wiech and Tracey, 2009). Evidence demonstrated that pain-related fear and anxiety are closely tied to the experience of pain (Hirsh et al., 2008; Thibodeau et al., 2013). In the current study, we observed that females reported higher levels of pain-related fear and anxiety, and these pain-related negative emotions were highly correlated with pain sensitivity across all participants (Fig. 2). However, no significant sex differences in trait anxiety and depression and no significant correlations between them and pain sensitivity were observed in the present study. These results suggested that the lower-order pain-related psychological constructs (e.g., pain-related anxiety) may be more predictive of sex differences in pain sensitivity than higher-order personality traits (e.g., trait anxiety) (Lee et al., 2010). Moreover, pain-related fear and anxiety are considered to be strong psychological predictors in the transition from acute pain events to chronic pain conditions (Hasenbring et al., 2014). Individuals with elevated pain-related fear or anxiety had a higher risk of developing chronic pain and more severe disability in chronic pain conditions (Zale and Ditte, 2015; Zvolensky et al., 2001). Therefore, the higher level of pain-related fear and anxiety in females might be a contributing factor for the greater prevalence of chronic pain conditions in females than males (Holley et al., 2017; Meints et al., 2018; Vetvik and MacGregor, 2017).

4.2. The role of the amygdala in sex differences in pain-related fear

Studies across species showed that the amygdala is a critical neural substrate in fear acquisition, learning, extinction, and potentiation (Cho et al., 2013; LaBar et al., 1998; Phelps et al., 2004; Trouche et al., 2013). Recent human and animal literature provided solid evidence for

the important link between amygdala structure and fear-related behaviors, i.e., the smaller amygdala volume was related to the increased fear acquisition magnitude (Hartley et al., 2011). Our findings supported this observation in the context of pain. Females showed significantly smaller volumes in most subnuclei in the left and right amygdala (Supplementary Table 1). In addition, individuals with smaller amygdala volume (especially the lateral nucleus, basal nucleus, accessory basal nucleus, and cortico-amygdaloid transition area in the left hemisphere) have higher levels of pain-related fear (Fig. 4). Moreover, we observed that the left amygdala subnuclei volumes (i.e., the lateral and basal nuclei) were the complementary mediators of the relationship between sex and pain-related fear (Fig. 7). Notably, the amygdala is a complex structure that consists of several functionally distinct nuclei with different patterns of connectivity (Bzdok et al., 2013; Roy et al., 2009). Of all amygdala nuclei, the basolateral complex of the amygdala (BLA, consists of the lateral, basal, and accessory basal nuclei) receives sensory and threat-related information and is thought to process emotions via the communication with regions, such as visual association cortices, medial prefrontal cortex, and the cingulate gyrus (Krabbe et al., 2018; Pessoa and Adolphs, 2010; Sergerie et al., 2008). Evidence showed that connections between the anterior cingulate gyrus, medial prefrontal cortex, and amygdala are critical neural substrates in fear acquisition, learning, extinction, and potentiation (Cho et al., 2013; Hakamata et al., 2020; Sharma et al., 2020). Animal models demonstrated that sensory inputs concerning threats are relayed to the lateral amygdala and typically travel through the basal nucleus to reach the central nucleus ultimately, then predominantly project to the hypothalamus and brainstem for the generation of fear-related physiological (e.g., autonomic nervous system), emotional, and behavioral responses (e.g., freezing, escape, fight, and avoidance) (Neugebauer, 2015; Simons et al., 2014). In short, our results support these viewpoints in the context of pain and provide evidence into the neuroanatomical basis (i.e., the lateral and basal nuclei in the left hemisphere) of sex differences in pain-related fear.

It should be noted that although with evident sex differences (Fig. 5), functional brain features (i.e., fALFF values) were not significantly correlated with pain sensitivity indices and negative emotion variables. These negative results could be associated with the relatively poorer spatial resolution of fMRI data than structural MRI data. Moreover, we focused on exploiting regional brain activities, but did not assess the possible brain circuits or networks related to sex differences in pain sensitivity in the present study. Indeed, this possibility needs further investigation in the future.

4.3. The mediation relationship among sex, negative emotions, amygdala volume, and pain sensitivity

Based on the aforementioned pieces of evidence, we built a compositional model to characterize the relationship among sex, negative emotions, structural brain features, and pain sensitivity. The compositional model provided an insight into the neuropsychological mechanism underlying sex differences in pain sensitivity. The final model emphasized the mediation effects of the structural underpinning of amygdala subnuclei volumes (i.e., the lateral and basal nuclei in the left hemisphere) on sex differences in pain-related fear and the important contributions of negative emotions (pain-related fear and anxiety) on sex differences in pain sensitivity. Specifically, the compositional model demonstrated that although the amygdala subnuclei volumes were not directly related to sex differences in pain sensitivity, they may influence sex differences in pain sensitivity through pain-related fear. For example, females with smaller amygdala volumes accompanied with a higher level of pain-related fear may be more likely to tag the incoming nociceptive signal as aversive before the further processing in the brain compared to males (Méndez-Bértolo et al., 2016). Finally, in line with the result that pain threshold and pain tolerance are highly correlated, the compositional model provided novel evidence that the pain threshold could mediate

sex differences in pain tolerance, which highlighted the indirect influence of physiological components on pain tolerance.

4.4. Limitations

There are several limitations of the present study that merit consideration in the future. First, in the present study, mediation analyses were performed based on the sex difference results, correlation results, and the assumption of the roles of negative emotions or brain structures in sex differences in pain sensitivity. Although our analytical strategy met the conditions and assumptions of mediation analyses, it should be noted that the interpretation of the results may be very complicated and it could be difficult to rule out other explanations (MacKinnon et al., 2002). It will be more insightful if negative emotions or brain structures can be manipulated directly to investigate their relationship with sex differences in pain sensitivity. Second, to account for multiple comparisons, FDR correction was used to adjust the *p* values in this study. By definition, FDR correction is a method of estimating the proportion of wrongly rejected null hypotheses and is the mathematical quantity given by false positive/(true positive + false positive). FDR correction tries to achieve a balance between false positives and false negatives and can control the ratio of false/true positives to a certain range. However, FDR correction is relatively liberal and might result in a higher false-positive rate (Bennett et al., 2009). Third, some correlations were significant but relatively weak in the present study (e.g., correlations in Figs. 3 & 4). Considering that participants recruited in the present study were healthy subjects, whether the results obtained could be replicated in patients with painful conditions and whether these findings have apparent clinical significance remain unclear. Further investigations are needed to address these issues in clinical samples in the future. Lastly, other factors, such as sex hormones, particularly estrogen, likely contribute to sex differences in pain responses (Fillingim, 2017). Estrogen can regulate fear and stress responses in women and female rodents (Maeng and Milad, 2015). However, the sex hormone levels were not collected in this study. For this reason, we cannot exclude the influence of this potentially confounding variable on our results, and future studies are suggested to consider its potential impacts.

5. Conclusions

Altogether, our study provided solid evidence for the crucial role of pain-related anxiety and fear on sex differences in pain sensitivity elicited by CPT and highlighted the indirect influence of the amygdala subnuclei volumes on sex differences in pain sensitivity via its modulation on pain-related fear. Further investigations are needed to explore these associations in clinical samples, which may help tailor a personalized approach to treat pain based on sex and the level of pain-related negative emotions for patients with painful conditions. For instance, more attention should be paid to females with painful conditions during treatment considering their higher pain sensitivity and higher levels of pain-related fear and anxiety than males. Besides, pain patients with high levels of pain-related negative emotions may require more clinical interventions to manage their pain.

Credit authorship contribution statement

Huijuan Zhang: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Visualization, Writing – original draft, Writing – review & editing. **Yanzhi Bi:** Conceptualization, Methodology, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Xin Hou:** Investigation, Data curation. **Xuejing Lu:** Writing – review & editing. **Yiheng Tu:** Formal analysis, Writing – review & editing. **Li Hu:** Conceptualization, Methodology, Formal analysis, Data curation, Visualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

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Data and code availability statements

The data in the present study are available upon reasonable request to the corresponding author. The codes for analyzing MRI data can be found in the FreeSurfer software (version 7.0.0, <http://surfer.nmr.mgh.harvard.edu>) and Data Processing & Analysis for Brain Imaging toolbox (DPABI, version 6.0, <http://rfmri.org/dpabi>). No customized codes were used in this study.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.neuroimage.2021.118685](https://doi.org/10.1016/j.neuroimage.2021.118685).

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