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Single-dose testosterone administration modulates instant empathic responses to others' pain: an EEG study

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Abstract

Whether or not testosterone can impair empathy remains unclear in the literature. Given that empathic responses to others' emotional experiences depend strongly upon top–down controlled mechanisms of attention, here we investigated whether the effects of testosterone administration on pain empathy could be modulated by manipulating attention. We used a double-blind, placebo-controlled within-participant design, in which either testosterone or placebo was administrated in separate sessions. Images depicting painful or nonpainful scenes were presented to induce instant empathic responses. Experiment 1 adopted the pain-judgment and hands-counting tasks to direct attention toward painful or nonpainful aspect of the images, respectively. Experiment 2 employed the pain-rating task to estimate affective and cognitive aspects of pain empathy. When discriminating nonpainful aspects of the images in the hands-counting task, accuracies were lower and empathic late positive potential responses were greater in testosterone sessions than in placebo sessions. This suggested that testosterone enhanced empathic responses to task-irrelevant pain-related features, which interfered with task performance. When providing empathic ratings to the images in the pain-rating task, empathic event-related potentials in the early stage were only observed in the testosterone session. This suggested that testosterone facilitated automatic affective reactivity to others' pain when elaborately processing empathic stimuli. Nevertheless, when discriminating painful aspects of the images in the pain-judgment task, we did not observe any significant differences between the two sessions. These results demonstrated that testosterone effects on enhancing brain reactivity to empathic stimuli were dependent upon task demands deploying attention allocation. The enhancement likely arose from the altered brain state (e.g., increased vigilance and arousal levels) after testosterone administration, as evidenced by the reduced amplitude of spontaneous α-oscillation recorded before the onset of the images. It expands our understanding of the neurobiological mechanisms that affect empathy, and highlights the role of testosterone.

Keywords: testosterone, pain, empathy, attention, event-related potentials, pre-stimulus α-oscillations

1. Introduction

Empathy refers to sharing and understanding the mental and emotional states of others, and is thought to play a crucial role in social cognition, cooperation, and prosocial behavior (Batson, 2008; Decety, 2010). The ability to empathize with others' emotions or feelings allows us to share the affective states of people around us and to predict and respond appropriately to their behaviors. Neurocognitive models have proposed that empathy is a complex and multifaceted construct composed of both affective and cognitive components (Decety and Jackson, 2004; Shamay-Tsoory, 2011; Singer, 2006). Affective empathy refers to our ability to emotionally "resonate" with other people's emotions and sensations, and involves automatic, bottom-up processes that occur in response to empathic stimuli, such as mimicry and emotional contagion. Cognitive empathy constitutes our capacity to infer from observed emotions, beliefs, and goals of other people, and involves mental operations such as cognitive appraisal, self-other distinction, and perspective-taking. Therefore, affective empathy allows us to vicariously experience others' emotional states, while cognitive empathy helps us to understand their experiences, intentions, and needs.

The *extreme male brain theory* proposes that the impaired empathy observed among people with autism might arise from elevated prenatal exposure to the sex hormone testosterone (Baron-Cohen et al., 2005). This theory is supported by evidence showing a direct association between testosterone and empathy (Knickmeyer et al., 2006; Nitschke and Bartz, 2020; Olsson et al., 2016). For instance, endogenous testosterone levels were reported to be negatively correlated with self-reported empathic behaviors (Chen et al., 2018) and empathic accuracy (Nitschke and Bartz, 2020). The causal effects of testosterone on empathy have also been assessed by manipulation studies. In one study, a single dose of testosterone attenuated empathic mimicry of emotional facial expressions (Hermans et al., 2006), which might indicate testosterone effects on impairing affective empathy. Other studies have shown that testosterone impaired cognitive empathy as indicated by reduced performance in the mindreading paradigm, whereby one infers the emotional state of another from limited information (Olsson et al., 2016; Van Honk et al., 2011). Nevertheless, a recent study reported two large-scale experiments that employed different testosterone-administration protocols, but neither showed any significant effects of pharmacological testosterone manipulations on cognitive empathy by using mindreading paradigms (Nadler et al., 2019). This piece of evidence has thus cast doubt on the effects that testosterone has on empathy.

Pain empathy is a specific subtype of empathy that involves sharing and understanding another person's pain. The perception of images or sounds that portray other people in pain triggers a unique pain-empathy response. Palmieri et al. (2020) investigated pain-empathy neural responses among patients with spino-bulbar muscular atrophy (SBMA), a disease in which abnormal androgen receptors leads to poor cellular uptake of testosterone. Compared with healthy controls, patients with SBMA exhibited enhanced early empathic neural responses to painful faces that were preceded by painful contexts, whereas pain-empathy behaviors did not differ between groups. This finding supports the notion that the amount of testosterone in the body is inversely related to the size of pain-empathy neural responses. Nevertheless, Heany et al. (2020) reported that when observing pain in others, neural

responses in the bilateral insula and anterior cingulate cortex, which comprise a core pain-empathy network, were unaffected by application of testosterone. In their study, participants were simply told to pay attention to the stimuli, without any further instruction. Importantly, empathic responses to others' pain are known to be sensitive to top–down control of attention (Fan and Han, 2008; Gu and Han, 2007; Meng et al., 2019). Previous studies (Fan and Han, 2008; Gu and Han, 2007) manipulated top–down attention to others' pain by instructing participants either to estimate the pain intensity felt by a person in the image (attention directed toward the painful aspects of the stimuli) or to count the number of hands in the image (attention directed toward the nonpainful aspects of the stimuli). The attentional modulation on empathic pain processing was confirmed by the temporal dynamics of event-related potential (ERP) responses while participants performed the task (Han et al., 2008; Li et al., 2020; Meng et al., 2019). Late ERP components related to cognitive appraisals of others' pain (e.g., P3 and late positive potential), but not earlier ERP components related to automatic reactions to others' pain (e.g., N1 and N2 components), largely disappeared when participants withdrew their attention away from the pain cues in the images (Han et al., 2008). Given that neural correlates of empathic pain processing are sensitive to top–down modulation of attention, the same is likely true for the effects of testosterone on pain empathy.

Therefore, the present study aimed to clarify the effects of testosterone administration on pain empathy, specifically, whether these effects can be altered via top–down manipulation of attention. We adopted a double-blind, placebo-controlled within-participant experimental design. Each participant received a single dose of testosterone in one session and placebo in the other session. Pain empathy paradigms were used to assess instant empathic responses to the observation of others' pain. Images depicting other people in either painful or nonpainful situations were presented, and participants directed their attention toward or away from the pain cues in the images. Behavioral responses and ERP temporal dynamics were characterized and compared between testosterone and placebo sessions.

2. Materials and Methods

2.1 Participants

Thirty-two single male college students were recruited for this study. None participant reported history of acute or chronic pain, history of psychiatric, neurological, or endocrine diseases, and current use of any medication. Written-informed consent was obtained from each participant before the experiments in accordance with the Declaration of Helsinki. The experimental procedures were approved by the local ethics committee.

2.2 General procedure and gel administration

We adopted a double-blind, placebo-controlled within-participant experimental design. Each participant completed two sessions in which 150 mg of either testosterone (Androgel®) or placebo was topically applied to their shoulders and upper arms. The placebo gel lacked testosterone but was otherwise identical. A single application of testosterone at this dosage to the shoulder and upper arm can significantly increase serum testosterone concentration as well as testosterone levels in saliva samples (Eisenegger et al., 2013; Wu et al., 2018; Wu et

al., 2019). Each session started at 13:00 to minimize the confounding effects of circadian rhythm as much as possible (Diver et al., 2003). A male research assistant who was blind to the treatment condition applied the Androgel® or placebo gel between 13:10 and 13:20, as in previous studies (Fang et al., 2021; Wu et al., 2018; Wu et al., 2019). The experimental and control sessions were identical except for the type of gel that was applied. The session order was counterbalanced across participants, and the sessions were spaced at least one week apart (mean: 9.14 ± 2.98 days; range: 7–17 days). The overall experiment comprised two sub-experiments. Experiment 1 used two-choice discrimination tasks to assess top–down attentional effects on the discrimination of others' pain. Experiment 2 used a pain-rating task to assess empathic behavioral and ERP responses to others' pain. The experiment began 3 hours after gel administration, as previous pharmacokinetic data has shown that salivary testosterone concentration peaks 3 hours after Androgel® application (Eisenegger et al., 2013; Wu et al., 2018; Wu et al., 2019). During the 3-hour waiting period, participants rested in the laboratory and were provided with magazines that were unrelated to the experiment.

2.3 Stimuli and pain-empathy tasks

The stimuli used in the experiment were 120 color digital images of people's hands or forearms in painful or nonpainful situations (termed painful and nonpainful images; 60 each, Meng et al., 2013). All situations depicted in the images were ordinary daily life events and the events shown in the nonpainful images corresponded to those in the painful images, but without the nociceptive component. The luminance, contrast, and color were matched between painful and nonpainful images. All images were presented on a black background in the center of a computer monitor (visual angle of 12.8° × 7.7°, viewing distance of 100 cm). The same images were used in Experiments 1 and 2. Stimulus presentation was controlled using E-prime 3.0 software (Psychology Software Tools, Inc; Pittsburgh, USA).

2.3.1 Experiment 1: pain-judgment and hands-counting tasks

In this experiment, participants were instructed to either discriminate the painful (pain-judgment task) or nonpainful (hands-counting task) aspects of the same images. The order to these two discrimination tasks was counterbalanced across participants. As illustrated in Figure 1A, each trial began with a 1000-ms fixation cross presented in the center of blank screen. After a blank 1000-2000 ms interval, either a painful or nonpainful image was presented. In the pain-judgment task, participants were required to determine whether the situation depicted in the image was painful or not. In the hands-counting task, they were instructed to determine whether the image contained one hand or two hands. For both tasks, participants made their choices by pressing one of two keys on the keyboard ('F' or 'J') as quickly and as accurately as possible. Key assignment for both tasks was counterbalanced across participants. The inter-trial interval varied randomly between 2000 ms and 4000 ms. Each task consisted of two blocks, with 60 trials per block (30 painful and 30 nonpainful). Reaction times (RTs) and accuracies (ACCs) were recorded throughout both discrimination tasks.

2.3.2 Experiment 2: pain-rating task

As shown in Figure 1B, painful and nonpainful images were presented for 2000 ms, and

participants were prompted by instructions to estimate the pain intensity felt by the person in the image and their own self-experienced feelings of unpleasantness on 11-point numerical rating scales (NRS; 0 = no pain/unpleasantness, 10 = unbearable pain/unpleasantness). We emphasized that the pain-intensity ratings referred to the experiences of the people in the images, while the unpleasantness ratings referred to the experiences of the participants themselves in response to what they saw in the images.

Insert Figure 1 approximately here

2.4 EEG data collection

Continuous EEG data were recorded throughout the two experiments. Participants sat on a comfortable chair in a sound-treated and temperature-controlled room. They were instructed to focus on the stimuli, keep their eyes open, and gaze at a fixation point on the screen. EEG data were recorded using 64 Ag-AgCl scalp electrodes placed according to the International 10–20 system (Brain Products GmbH; bandpass filter: 0.01–100 Hz; sampling rate: 1000 Hz). The electrode-to-skin impedances were kept below 10 kΩ for all electrodes. The TP10 electrode (right mastoid) was used as the online-recording reference. Electro-oculographic (EOG) signals were simultaneously recorded using surface electrodes to monitor ocular movements and eye blinks.

2.5 EEG data analysis

EEG data were preprocessed in EEGLAB (Delorme and Makeig, 2004), an open source toolbox for the MATLAB environment (The MathWorks, Inc., Natick, Massachusetts, USA). Continuous EEG data were band-pass filtered between 0.1 and 40 Hz. EEG epochs were extracted using a window-analysis time of 2000 ms (−1000 ms pre-stimulus to 1000 ms post-stimulus), and baseline corrected using the pre-stimulus interval. For each participant, EEG epochs were visually inspected and epochs contaminated by gross movements were removed. Trials contaminated by eye-blinks and movements were corrected using an Independent Component Analysis (ICA) algorithm (Jung et al., 2001). In all datasets, independent components of ocular artifacts featured a large EOG channel contribution and a frontal scalp distribution. After ICA and an additional baseline correction, EEG data were re-referenced to the bilateral mastoid electrodes so that the results would be comparable with other empathy-related studies (Cui et al., 2016; Li et al., 2020).

Time-domain analysis was performed to assess the effects of testosterone administration on ERP responses to the observation of others' pain. The analysis focused on a 1200-ms time-window (−200 pre-stimulus ms to 1000 ms post-stimulus). For each participant, single-trial ERP waveforms elicited by painful and nonpainful images were averaged for each experimental condition (12 conditions: 2 sessions × 3 experimental tasks × 2 image types). This procedure yielded 12 single-participant average waveforms that were time-locked to the onset of the images (painful and nonpainful) for each session (testosterone and placebo) and each experimental task (pain-judgment, hands-counting, and pain-rating). The single-participant average ERP waveforms were subsequently averaged across participants to obtain group-level ERP waveforms for each experimental condition. The scalp

topographies were computed by spline interpolation.

Dominant ERP components were identified according to the grand average waveforms and scalp topographies. According to previous studies (Cui et al., 2016; Jiao et al., 2017; Meng et al., 2013; Wang et al., 2014), N1 and N2 were defined as the most negative components within 100–200 ms and 200–300 ms after stimulus onset, respectively, with maximum distribution over the fronto-central area. P3 and the late positive potential (LPP) were defined as long-lasing positive components within 300–500 ms and 500–1000 ms after stimulus onset, respectively, over the centro-parietal region. Based on the grand averaged ERP activity and previous ERP studies of pain empathy (Decety et al., 2010; Fan and Han, 2008; Meng et al., 2013; Peng et al., 2019b), ERP-component amplitudes were measured at different sets of electrodes and latency intervals. Specifically, N1 and N2 amplitudes were measured at fronto-central electrodes (F1, Fz, F2, FC1, FCz, FC2), during the 115–155 ms and 240–280 ms latency intervals, respectively. P3 amplitudes were measured at parietal electrodes (P1, Pz, P2, PO3, POz, PO4) during the 340–400 ms latency interval. LPP amplitudes were measured at centro-parietal electrodes (CP1, CPz, CP2, P1, Pz, P2) at the 500–900 ms latency interval. For each participant and experimental condition, the amplitudes of these ERP components were derived from single-participant ERP waveforms by averaging the amplitudes at the corresponding electrode sets and latency intervals.

2.6 Pre-stimulus EEG spectral analysis

To assess the effect of testosterone on spontaneous neuronal oscillations before the onset of the images, pre-stimulus EEG signals were extracted using a 1000-ms time window (from −1000 ms to 0 ms before image onset). For each participant and each of the two sessions, pre-stimulus EEG signals were transformed to the frequency domain using a Fast Fourier Transform. This yielded two amplitude spectra (in μV) ranging from 1 to 30 Hz for each participant. Single-participant EEG spectra was subsequently averaged across participants to obtain a group-level pre-stimulus EEG spectrum for each session. Previous studies have demonstrated that spontaneous EEG oscillations in the alpha frequency band (α-oscillations, 8–12 Hz) can reflect states of sustained attention (Clayton et al., 2015; Macdonald et al., 2011) and can influence subsequent processing of affective or cognitive events (Peng et al., 2019a; Wu et al., 2020). We compared spontaneous α-oscillation amplitudes before image onset between testosterone and placebo sessions. Scalp topographies of pre-stimulus α-oscillation amplitudes were computed by spline interpolation. Single-participant α-oscillation amplitudes at fronto-central (Fz, FC1, FCz, FC2, Cz) and parietal electrodes (P1, P2, P3, P4, Pz) were averaged within the 8–12 Hz frequency interval for each session.

2.7 Statistical analysis

All statistical analyses were carried out using the IBM SPSS statistical analysis package (version 22; IBM Corp., Armonk, NY). To assess whether testosterone administration affects pain empathy, and whether any effects depend on where attention is focused, we compared pain-empathy related responses in Experiment 1 between testosterone and placebo sessions. Behavioral responses (RTs and ACCs) and neural responses (amplitudes of dominant ERP components) to painful and nonpainful images during pain-judgment and hands-counting

tasks were obtained for each participant. These responses were entered into a three-way repeated-measures analysis of variance (ANOVA), with three within-participant factors of Image (painful vs. nonpainful), Task (pain-judgment vs. hands-counting task), and Session (testosterone vs. placebo). If the three-way interaction was significant, a post hoc two-way ANOVA with factors of Image and Session was performed separately for the pain-judgment and hands-counting tasks. To assess whether testosterone administration affects explicit empathic responses to the observation of others' pain, we compared pain-empathy related responses in Experiment 2 between testosterone and placebo sessions. Subjective ratings (estimation of others' pain intensity and self-experienced unpleasantness) and neural responses (amplitudes of dominant ERP components) were obtained for each participant. These responses were entered into a two-way repeated-measures ANOVA with two within-participant factors of Image and Session. When a significant main effect or interaction was found, post hoc pairwise comparisons were performed.

To assess whether testosterone administration affects brain states that can be characterized by neuronal oscillations in the α-band, α-oscillation amplitudes at fronto-central electrodes and parietal electrodes were separately compared between placebo and testosterone sessions using paired-sample t-tests.

3. Results

No participant reported any side effects after testosterone application and the experimental protocol was well tolerated. Data from five participants were excluded from analysis because of either equipment failure during data collection or noise contamination in the EEG data, leading to a final sample of 27 participants (age: 21.30 ± 0.36 years; height: 172.52 ± 1.17 cm; weight: 66.57 ± 2.65 kg; mean ± standard error of the mean [SEM]).

3.1 Experiment 1: pain-judgment and hands-counting tasks

Grand average RTs and ACCs on the pain-judgment and hands-counting tasks, as well as their statistics, are summarized in the right panel of Figure 1A and Table 1. Analysis of RTs showed significant main effects of Task ($F_{(1,26)} = 20.98$, $p < 0.001$, $\eta_p^2 = 0.45$) and Image ($F_{(1,26)} = 6.06$, $p = 0.021$, $\eta_p^2 = 0.19$). Responses were slower for painful images than for nonpainful images and for the pain-judgment task than for the hands-counting task. The interaction between Task and Image was also significant ($F_{(1,26)} = 25.54$, $p < 0.001$, $\eta_p^2 = 0.50$). Post hoc comparisons showed that RTs for painful images were slower than those for nonpainful images during the hands-counting task ($p < 0.001$), while RTs to painful and nonpainful images were comparable during the pain-judgment task ($p = 0.358$). We observed no other significant main effects or interactions.

Analysis of ACCs showed significant main effects of Task ($F_{(1,26)} = 7.72$, $p = 0.010$, $\eta_p^2 = 0.23$) and Image ($F_{(1,26)} = 54.15$, $p < 0.001$, $\eta_p^2 = 0.68$), which manifested as lower ACCs for painful images than for nonpainful images, and for the pain-judgment task than for the hands-counting task. The interaction between Session and Task was significant ($F_{(1,26)} = 8.49$, $p = 0.007$, $\eta_p^2 = 0.25$). Post hoc comparisons showed that ACCs during the hands-counting task were lower in testosterone sessions than in placebo sessions (90.4% ± 0.1% vs. 93.0%

± 0.1%, p = 0.017). In contrast, they were comparable across sessions during the pain-judgment task (p = 0.284). These results indicate that testosterone administration increased the degree to which pain interfered with the ability to process nonpainful information in the images.

Insert Table 1 approximately here

Grand average ERP waveforms elicited by painful and nonpainful images during the pain-judgment and hands-counting tasks are shown in Figure 2 (measured at centro-parietal electrodes) and Figure S1 (measured at fronto-central electrodes). According to visual inspection of the ERP waveforms, painful and nonpainful images elicited N1 and N2 waves maximally over the fronto-central region, P3 and LPP waves over the centro-parietal region. Statistics for these ERP amplitudes are summarized in Table 1.

Analysis of fronto-central N1 and N2 amplitudes did not show any significant main effect or interaction (p > 0.05 for all comparisons). The main effect of Task was significant for parietal P3 amplitudes ($F_{(1,26)}$ = 18.30, p < 0.001, η_p^2 = 0.41) and centro-parietal LPP amplitudes ($F_{(1,26)}$ = 20.04, p < 0.001, η_p^2 = 0.44). In both cases, greater amplitudes were elicited during the pain-judgment task than during the hands-counting task. Analysis of LPP amplitudes also showed a significant main effect of Image ($F_{(1,26)}$ = 29.12, p < 0.001, η_p^2 = 0.53) such that greater amplitudes were elicited by painful images than by nonpainful images. The interaction between Task and Stimulation was also significant ($F_{(1,26)}$ = 8.02, p = 0.01, η_p^2 = 0.24) such that the difference in amplitude between painful and nonpainful image trials was greater during the pain-judgment task than during the hands-counting task. Importantly, the three-way interaction among Session, Task, and Image was significant ($F_{(1,26)}$ = 7.49, p = 0.01, η_p^2 = 0.22). Post hoc two-way ANOVA on the LPP amplitudes during the hands-counting task revealed a significant main effect of Image ($F_{(1,26)}$ = 6.12, p = 0.020, η_p^2 = 0.19) and a significant interaction between Session and Image ($F_{(1,26)}$ = 9.00, p = 0.006, η_p^2 = 0.26). In the testosterone session, LPP amplitudes were greater in response to painful images than to nonpainful images (p < 0.001), but this difference was not significant in the placebo session (p = 0.979). In contrast, post-hoc two-way ANOVA on the LPP amplitudes during the pain-judgment task only revealed a significant main effect of Image ($F_{(1,26)}$ = 25.88, p < 0.001, η_p^2 = 0.50) such that painful images elicited greater LPP amplitudes than did nonpainful images. These results indicate that testosterone only enhanced the effect of pain empathy on LPP responses when attention was directed away from pain cues, but not when it was directed toward the pain cues.

Insert Figure 2 approximately here

3.2 Experiment 2: pain-rating task

Grand average pain-empathy ratings (pain intensity and unpleasantness), as well as their statistics, are summarized in the right panel of Figure 1B. Analysis showed a significant main effect of Image on both pain-intensity ($F_{(1,26)}$ = 231.42, p < 0.001, η_p^2 = 0.90) and unpleasantness ($F_{(1,26)}$ = 271.29, p = 0.007, η_p^2 = 0.91) ratings. Ratings for both were higher

for the painful images than for the nonpainful images. Neither the main effect of Session nor the Image × Session interaction was significant. This suggests that participants had experienced significant empathy when viewing painful images, and that the amount of empathy was not significantly affected by testosterone administration.

Grand average ERP waveforms elicited by the painful and nonpainful images during the pain-rating task are shown in Figure 3. According to visual inspection of the ERP waveforms, painful images evoked larger LPP waveforms than nonpainful images did. A two-way ANOVA revealed a significant interaction between Session and Image on both N1 ($F_{(1,26)} = 5.47$, $p = 0.027$, $\eta_p^2 = 0.17$) and N2 ($F_{(1,26)} = 5.65$ $p = 0.025$, $\eta_p^2 = 0.18$) amplitudes. Post hoc comparisons showed both ERP amplitudes differed significantly between painful and nonpainful image conditions in the testosterone sessions (N1: $p = 0.018$; N2: $p = 0.044$), but not in the placebo sessions (N1: $p = 0.899$; N2: $p = 0.215$). Analysis also indicated a significant main effect of Image on centro-parietal LPP amplitudes ($F_{(1,26)} = 49.89$, $p < 0.001$, $\eta_p^2 = 0.66$), with amplitudes being greater for painful images than for nonpainful images. We did not observe any significant effects on parietal P3 amplitudes. These results indicate that testosterone selectively increased the effects of pain empathy on fronto-central N1 and N2 responses, which might reflect early automatic reactions to others' pain.

Insert Figure 3 approximately here

3.3 Pre-stimulus EEG spectra results

Grand average spectra of pre-stimulus EEG oscillation measured at fronto-central electrodes are shown in Figure 4. Pre-stimulus α-oscillation amplitude in the 8–12 Hz range showed a maximal distribution over fronto-central and parietal regions in both testosterone and placebo sessions. Paired-sample t-tests showed that the amplitudes of fronto-central α-oscillation were significantly lower in the testosterone session than in the placebo session ($t_{(26)} = -2.13$, $p = 0.043$, Cohen's d = −0.14). In contrast, parietal α-oscillation amplitudes did not differ significantly between sessions ($t_{(26)} = 0.68$, $p = 0.51$, Cohen's d = 0.04). These results indicate that testosterone induced a state of reduced spontaneous fronto-central brain activity (α-oscillation) recorded prior to the onset of empathy-eliciting stimuli.

Insert Figure 4 approximately here

4 Discussion

The present study investigated how testosterone administration affects pain empathy and whether the effects can be modulated by manipulating attention. Experiment 1 included two choice-discrimination tasks (pain-judgment and hands-counting). While the neural and behavioral responses in the pain-judgment task were comparable between testosterone and placebo sessions, discrimination accuracies in the hands-counting task were lower during the testosterone session, and was accompanied by larger LPP amplitudes in response to empathic stimuli. This indicated that even when attention was directed away from the painful aspects of the images, in the testosterone session, the painful images evoked late empathic neural responses that interfered with stimulus discrimination. Experiment 2 comprised a

pain-rating task in which participants evaluated emotional and cognitive empathy as they viewed the painful and nonpainful images. Although testosterone did not overwhelming affect self-reported empathic ratings, it did evoke greater pain empathic effects on the amplitudes of N1 and N2 components. This suggests that testosterone facilitated early automatic reactions to others' pain when people deliberately processed empathic stimuli. Importantly, testosterone generally decreased the amplitudes of frontal-central α-oscillation recorded before the onset of the images. Thus, the effects of testosterone on pain empathy could be arisen from the altered brain state after testosterone administration. These results provide comprehensive evidence for the causal effects of testosterone on pain empathy and highlight the fact that the effects partially depend upon where attention is directed.

4.1 Testosterone affected pain empathy during pain-judgment and hands-counting tasks

Two-choice stimulus-discrimination tasks (pain-judgment and hands-counting tasks) were employed in Experiment 1 to assess top–down attentional modulation of pain empathy. Given that the images used in these two tasks were identical, behavioral and neural differences should reflect the contribution of attention manipulation and be unrelated to stimulus properties (Gu and Han, 2007; Han et al., 2008). During these two tasks, RTs and ACCs were measured as indirect reflections of empathy-related processes. Longer RTs and lower ACCs were observed in the pain-judgment task than in the hands-counting task, indicating that judging pain was more difficult than counting hands. In addition, ACCs were lower for painful images than for nonpainful images, indicating that painful content interfered with the ability to discriminate image properties. Importantly, this interference during the hands-counting task was greater in the testosterone session than in the placebo session. Therefore, when participants were instructed to direct attention away from the painful content depicted in the images, testosterone-induced increases in individual sensitivity to the painful content led to greater task interference.

Previous ERP studies of pain empathy have identified the temporal dynamics of neural responses to witnessing others' pain (Decety et al., 2010; Fan and Han, 2008; Meng et al., 2013). Early ERP components (e.g., N1 and N2) have been associated with stimulus-driven, automatic neural reactions to empathic stimuli, whereas late ERP components (e.g., P3 and LPP) are considered as indexing later controlled process that reflects cognitive appraisal and emotion regulation (Dennis and Hajcak, 2009; Fan and Han, 2008). These empathy-related ERP components have been shown to be modulated differently by attention, with only the later empathy-related components being sensitive to task requirements that involve attention deployment (Fan and Han, 2008). Consistent with this notion, greater empathy-related LPP responses were found in the pain-judgment task than in the hands-counting task. Importantly, for the testosterone session of the hands-counting task, painful images elicited greater LPP responses than nonpainful images, indicating significant empathy-related LPP responses when attention was directed away from pain cues. In contrast, this empathy-related LPP response was not observed in the placebo session, which was expected based on previous studies (Li et al., 2020; Meng et al., 2019). Indeed, the LPP component can be considered as a reliable electrophysiological index of pain empathy (Coll, 2018). The amplitude of LPP

component varied with trait empathy of the observer (Choi et al., 2014) and increased when processing others' pain in depth (Li and Han, 2010). The greater empathy-related LPP responses in the testosterone session indicated that more cognitive resources were allocated to the painful stimuli for further appraisal and evaluation when attention was directed away from painful aspects of the images. It was likely that testosterone enhanced empathy-related cognitive appraisal of pain-related aspects of the images. However, because those aspects were irreverent to the task, it interfered with the task performance (count the hands).

4.2 Testosterone affected pain empathy during the pain-rating task

To provide a more direct measurement of pain empathy, in Experiment 2 we instructed participants to estimate the intensity of pain experienced by the person in the picture (cognitive empathy) and to report self-experienced feelings of unpleasantness in response to the images (affective empathy). Although the cognitive and affective empathy ratings were comparable between testosterone and placebo sessions, we observed significant empathy-related neural responses in the N1 and N2 components, but only in the testosterone session. The painful images were likely more salient and more arousing than the nonpainful images, thus preferentially attracting attention early in the information processing stream. Therefore, when participants evaluated the affective content of the images, testosterone facilitated automatic affective reactivity to painful images. However, because testosterone did not alter the self-reported empathy ratings, we presume that its effects on pain empathy might be more easily captured by measurements of automatic and unconscious responses (Hermans et al., 2006; Terburg et al., 2012). Note that the effects of testosterone on empathy-related neural responses during the pain-rating task do not match the findings obtained during the pain-judgment task (comparable neural responses between the two sessions). Although both tasks required participants to pay attention to the painful aspects of the images, providing empathic ratings was a more elaborate and deliberate process than simple yes/no decisions, and thus required more attentional resources to be deployed.

The enhanced early empathy-related ERP components (N1 and N2) that we observed in the pain-rating task after testosterone administration are seemingly inconsistent with findings in Palmieri et al. (2020). In that study, early empathic neural responses to painful faces preceded by painful contexts were enhanced in patients with SBMA, whose body tissue cannot uptake testosterone very well. This inconsistency might be related to the different stimulation materials that were used to elicit pain empathy. In the current study, neural empathic responses were induced by viewing images depicting physically painful situations, while in Palmieri et al. (2020), they were induced by the superimposed presentation of painful face expressions and painful contexts. More importantly, despite low testosterone uptake, patients with SBMA have paradoxically high levels of testosterone, which is caused by abnormal androgen receptors (Rhodes et al., 2009). Moreover, while the biological effect of testosterone mediated by androgen receptors is impaired in patients with SBMA, testosterone can increase estradiol levels through aromatization (Steinmetz et al., 2022), thus further influencing neural processing of pain empathy. Thus, it is difficult to draw any conclusion about the causal relationship between testosterone levels and neural pain-empathy responses from the SMBA data. The opposing results between our study and

Palmieri et al. (2020) likely results from the difference in pain-empathy tasks and the complexity of SBMA.

4.3 Testosterone affected brain states and pain empathy

Our data in Experiments 1 and 2 support the idea that overall, testosterone enhances neural processing of pain empathy, and that this effect is sensitive to where attention is focused on. Because pain signals a potential threat or danger in the environment and urges individuals to escape or avoid the source (Yamada and Decety, 2009), observing others in pain also triggers the threat detection system and intensively provokes distress (Goubert et al., 2005). The heightened neural sensitivity to pain empathy could reflect testosterone-induced facilitation of attention toward observed pain, which would allow more efficient detection of and reaction to salient and threatening events in the environment. Indeed, many studies have shown an association between testosterone and attention allocation (Fang et al., 2021; van Honk et al., 1999). For example, testosterone facilitated involuntary attentional orientation toward novel and salient stimuli (Fang et al., 2021) and enhanced amygdala reactivity to threat (Goetz et al., 2014). Individuals with higher endogenous testosterone levels exhibited stronger selected attention toward threat information (van Honk et al., 1999).

Rather than being an invariant process, perception of external stimuli depends crucially on the current brain state (Weisz et al., 2014). The heightened neural sensitivity to pain empathy that we observed after testosterone application might have arisen from an altered brain state. Fluctuations of brain states are characterized by ongoing oscillations of neuronal activity, which occur at different frequency bands and continuously modulate how neurons process forthcoming sensory events (Hanslmayr et al., 2007). Here, we have shown that testosterone generally reduced frontal-central α-oscillation amplitudes that were recorded before image onset. Functionally, spontaneous α-oscillation amplitudes are considered an important neural index of sustained attention and the ability to maintain vigilance over protracted periods of time (Clayton et al., 2015; Klimesch, 2012). Reduced amplitudes of spontaneous α-oscillations has primarily been interpreted as strictly “local” enhancement of neuronal ensemble excitability (Jensen and Mazaheri, 2010; Klimesch et al., 2007). Thus, testosterone administration likely increased vigilance and arousal levels throughout the session, but especially just before image onset, which led to enhanced neural processing of the empathic stimuli, presumably for facilitating threat detection and reaction.

Combining our results from the different pain-empathy tasks, as well as the spontaneous EEG activity, we propose a theoretical model to explain how testosterone affects pain empathy that is elicited by images depicting painful events (Figure 5). Testosterone facilitates pain empathic neural responses by altering brain states to allow for better detection and reaction to threatening information in the environment. The effects of testosterone on pain-empathy neural responses are dependent upon top-down attention. Specifically, when a task requires a large amount of attention be directed toward pain and involves elaborate and deliberate processes (e.g., people need to provide empathy ratings of the observed pain), testosterone heightens the early automatic neural response to the salient painful information. When a task simply requires attention be directed towards pain for discrimination (i.e., pain is

task-relevant), testosterone does not exert an overwhelming influence on the neural responses to the observed pain. However, when the task requires attention be directed away from pain (i.e., pain is task-irrelevant), testosterone heightens late controlled neural responses to the observed pain, thus leading to task interference.

Insert Figure 5 approximately here

Our finding that testosterone enhanced neural pain-empathy responses contrasts with previous studies showing that testosterone impairs empathy (Hermans et al., 2006; Olsson et al., 2016; Van Honk et al., 2011). These inconsistent findings might have arisen from the different paradigms used to measure empathy. In the current study, images depicting others in painful situations were used to elicit pain empathy. This paradigm has been shown to activate affective-motivational and sensory-discriminative aspects of the pain matrix (Jackson et al., 2006; Jackson et al., 2005). Enhanced neural responses to pain empathy after testosterone administration might largely reflect how testosterone facilitates the affective aspect of empathy (e.g., emotional contagion and personal distress in response to the perception of others' pain). In contrast, empathy in other studies was quantified by the skill at inferring other's mental abilities (Bos et al., 2016; Carré et al., 2015; Grainger et al., 2021; Olsson et al., 2016; Van Honk et al., 2011), e.g., using the 'Reading the Mind in the Eyes' Test. The findings that testosterone impaired empathic accuracy (Nitschke and Bartz, 2020) and the theory of mind (Khorashad et al., 2018) could largely reflect how testosterone suppresses the cognitive aspect of empathy (e.g., our capacity to infer from the observed emotions, beliefs, and goals of other people). Therefore, testosterone likely impacts the two components of empathy differently: it facilitates affective empathy but inhibits cognitive empathy. Testosterone-induced facilitation of vigilance and emotional reactivity to others' distress may have an adaptive function for survival (e.g., avoiding or escaping from danger). This is consistent with evidence showing that testosterone increases facial emotional responses (Bos et al., 2021) and insula activations (a key brain region related to affective empathy, Bos et al., 2010) when facing people who are distressed (e.g., a crying infant). On the other hand, testosterone-induced suppression of the ability to infer others' mental states might be beneficial in the sense that it helps avoid boosting concern for others, which allows aggression and dominance to be maintained as a way to seek higher status (Eisenegger et al., 2011). Indeed, this assumption is supported by evidence showing impaired social understanding, but heightened empathic arousal, among individuals with autism, whose etiology has been associated with excessive fetal exposure to testosterone (Baron-Cohen et al., 2005; Fan et al., 2014).

5 Conclusion

The current study provided evidence for the causal effects of testosterone on empathic responses when observing others in pain. While both early automatic and late controlled empathic responses can be enhanced by testosterone, these effects depended upon the task demands, specifically, where attention was allocated. Testosterone likely increases neural reactivity to empathic stimuli by altering brain states to allow increased vigilance and arousal levels, as evidenced by the reduced amplitudes of spontaneous α-oscillation prior to the

onset of empathic stimuli. These results provide a more complete story regarding the relationship between empathy and testosterone, and suggest a role of testosterone in the atypical empathy observed in some psychiatric conditions such as autism and schizophrenia.

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Declarations of interest

None.

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Figure legends

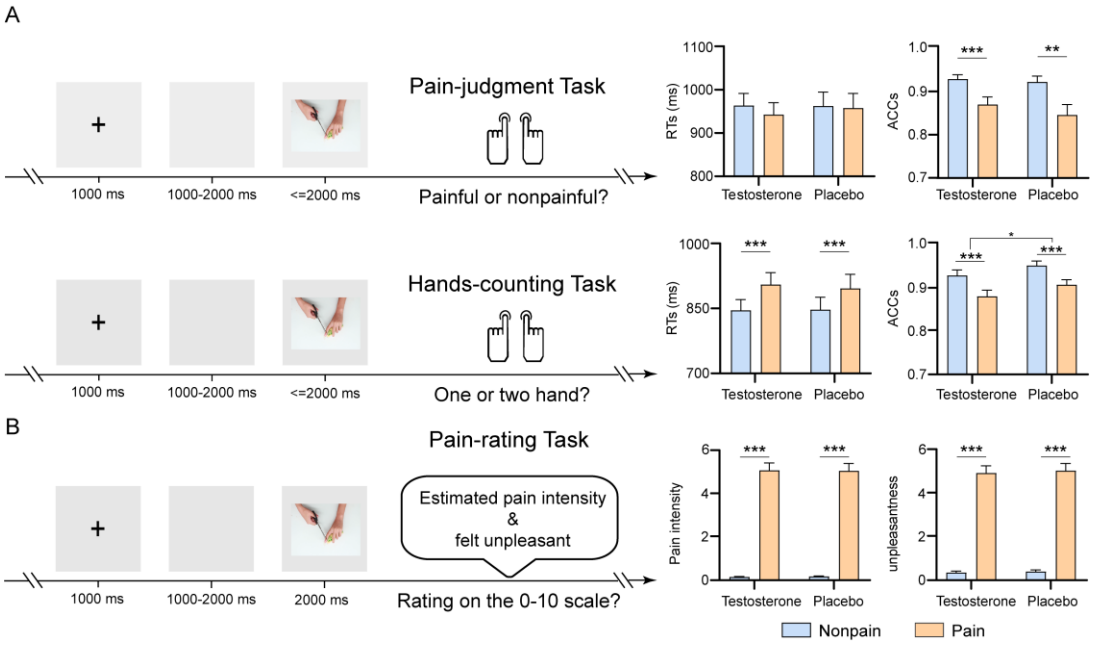

Figure 1. Pain empathy paradigms and behavioral results

Pain empathy paradigms were used to estimate instant empathic responses to others' pain. Experiment 1 comprised pain-judgment and hands-counting tasks, both of which were two-choice discrimination tasks (A). Participants were instructed to determine whether the images depicted painful or nonpainful scenes (pain-judgment task) or to determine whether they contained one or two hands (hands-counting task). The task order was counterbalanced across participants. RTs and ACCs for the nonpainful (blue bars) and painful (orange bars) images during the two tasks were compared between testosterone and placebo sessions. Experiment 2 included a pain-rating task in which participants explicitly reported the intensity of the depicted pain and their own feelings of unpleasantness on a 0–10 numerical rating scale (B). Subjective ratings of estimated pain intensity and self-experienced unpleasantness for nonpainful (blue bars) and painful (orange bars) images were compared between testosterone and placebo sessions. Data in the bar plots are expressed as Mean ± SEM; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; paired-sample t-test.

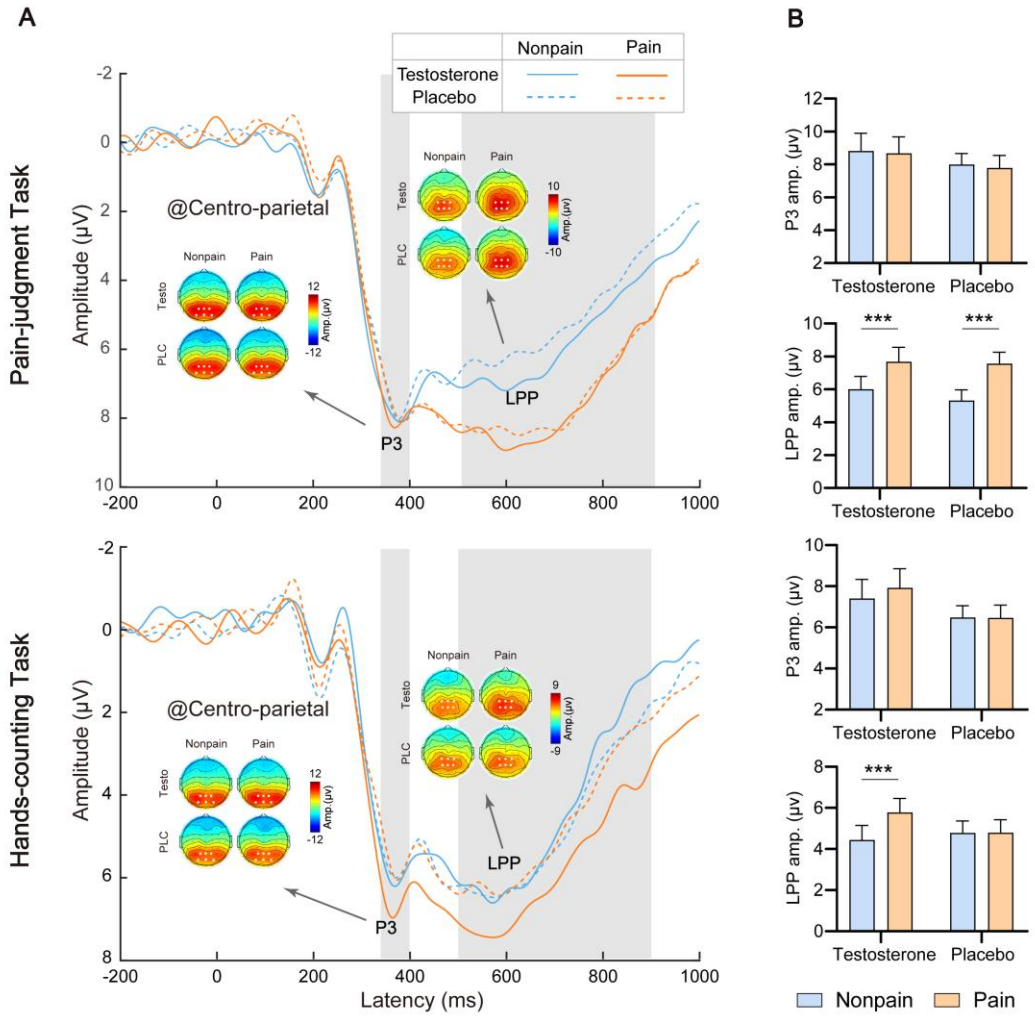

Figure 2. ERP responses during the pain-judgment and hands-counting tasks
Grand average ERP waveforms and scalp topographies for testosterone (solid lines) and placebo (dashed lines) sessions were elicited by nonpainful (blue lines) or painful (orange lines) images during the pain-judgment and hands-counting tasks (A). Displayed waveforms were measured at centro-parietal electrodes (CP1, CPz, CP2, P1, Pz, P2). Amplitudes of P3 and LPP components elicited by nonpainful (blue bars) and painful images (orange bars) were compared between testosterone and placebo sessions (B). Electrodes used to measure ERP amplitudes were marked using enlarged white dots on the corresponding scalp topographies. Data in the bar plots are expressed as Mean ± SEM. ***: $p < 0.001$; paired-sample t-test.

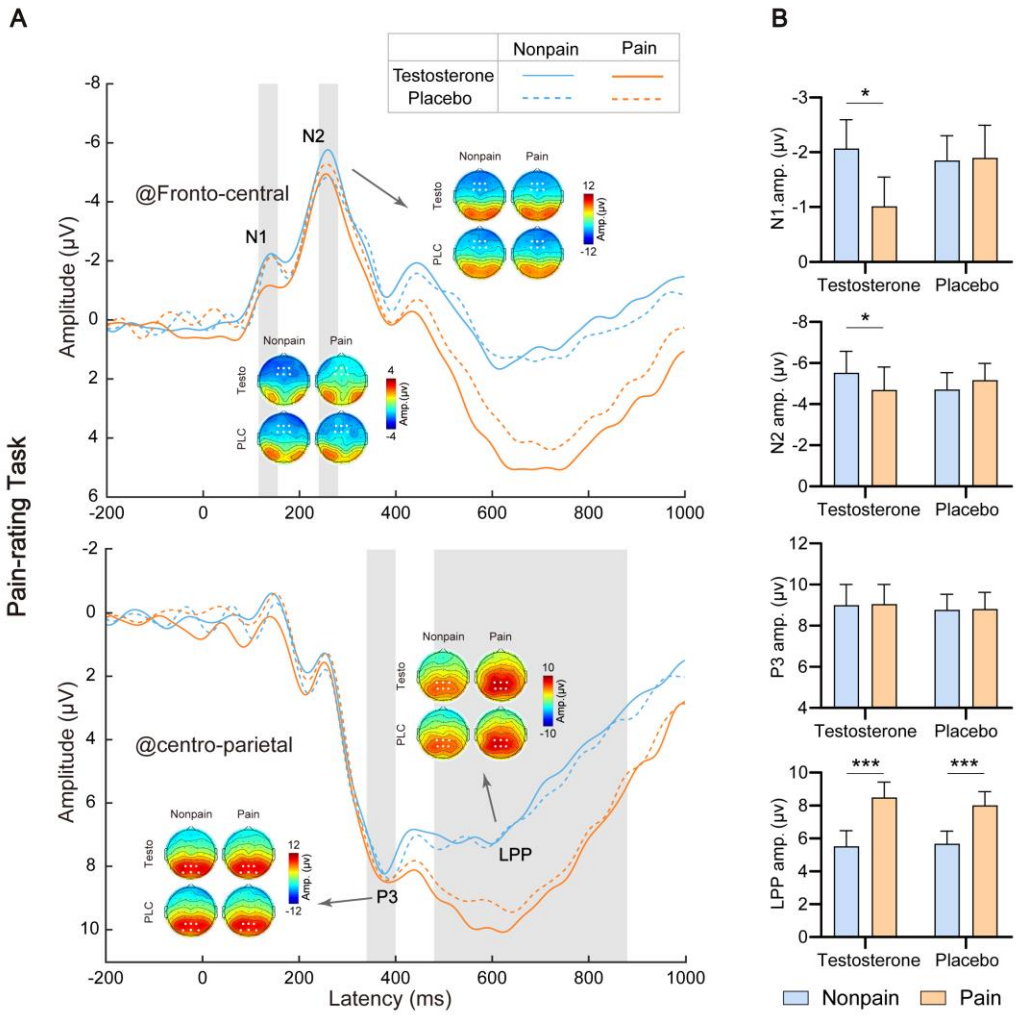

Figure 3. ERP responses during the pain-rating task

Grand average ERP waveforms and scalp topographies for testosterone (solid lines) and placebo (dashed lines) sessions were elicited by nonpainful (blue lines) and painful (orange lines) images during the pain-rating task (A). Displayed waveforms were measured at fronto-central (F1, Fz, F2, FC1, FCz, FC2) and centro-parietal (CP1, CPz, CP2, P1, Pz, P2) electrodes. Amplitudes of dominant ERP components (N1, N2, P3, and LPP) elicited by nonpainful (blue bars) and painful images (orange bars) were compared between testosterone and placebo sessions (B). Electrodes used to measure ERP amplitudes were marked using enlarged white dots on the corresponding scalp topographies. Data in the bar plots are expressed as Mean ± SEM. *: $p < 0.05$; ***: $p < 0.001$; paired-sample t-test.

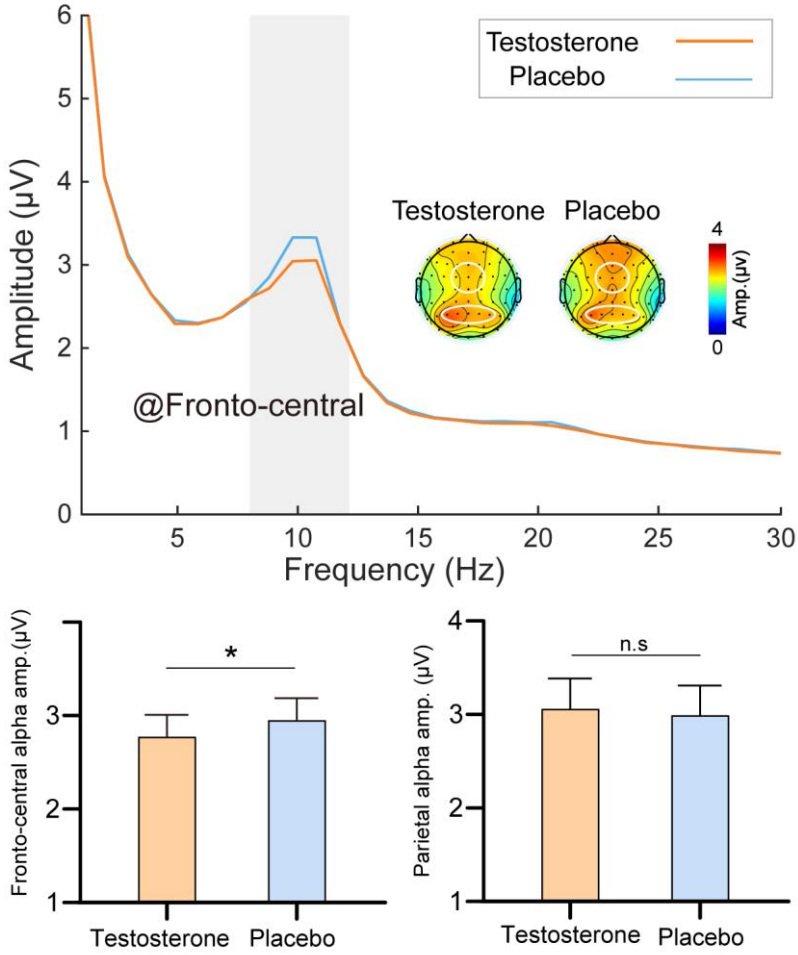

Figure 4. Pre-stimulus EEG oscillations during the testosterone and placebo sessions Pre-stimulus EEG spectra just before onset of empathy-eliciting stimuli (−1000 to 0 ms) were measured from fronto-central electrodes (Fz, FC1, FCz, FC2, Cz) in testosterone (orange line) and placebo (blue line) sessions (A). Scalp topographies of pre-stimulus α-oscillations (8–12 Hz in frequency, marked in grey rectangles) showed maximal distribution over both frontal-central and parietal electrodes (outlined in white on the topographies) regardless of the session. Amplitudes of pre-stimulus α-oscillations were compared between testosterone and placebo sessions. Pre-stimulus α-oscillation amplitudes in the testosterone session were significantly lower than those in the placebo session at fronto-central electrodes (Fz, FC1, FCz, FC2, Cz), but not at parietal electrodes (P1, Pz, P2, P3, P4). Data in the bar plots are expressed as Mean ± SEM; *: $p < 0.05$; n.s.: $p > 0.05$; paired-sample t-test

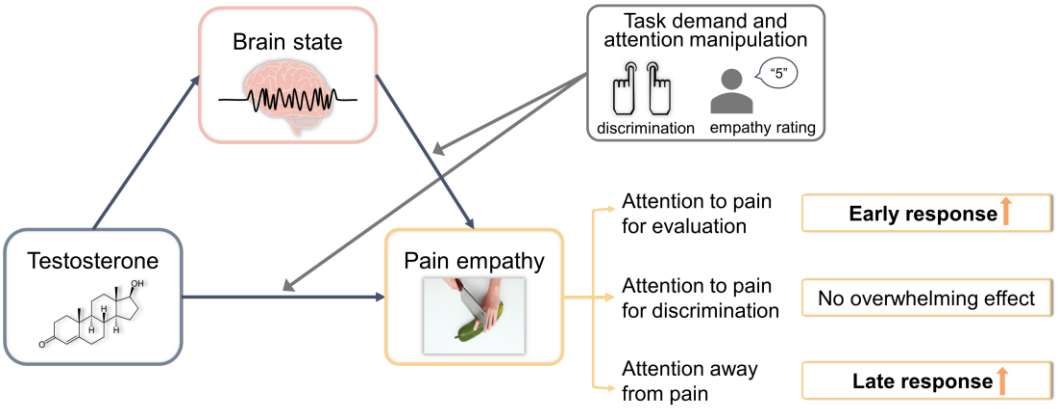

Figure 5. Theoretical model illustrating how testosterone affects pain empathy
Testosterone facilitates pain-empathy neural responses by altering brain states, as indexed by ongoing oscillations of neuronal activity. The effect of testosterone on the neural responses depends on how attention is allocated during the task. When the task requires a large amount of attention be directed toward pain and participants must provide empathy ratings, testosterone enhances the early empathic neural response. When the task requires attention be directed toward pain for discrimination, testosterone exerts no overwhelming effect. However, when the task requires attention be directed away from pain, testosterone enhances the late empathic neural response.

Supplementary materials

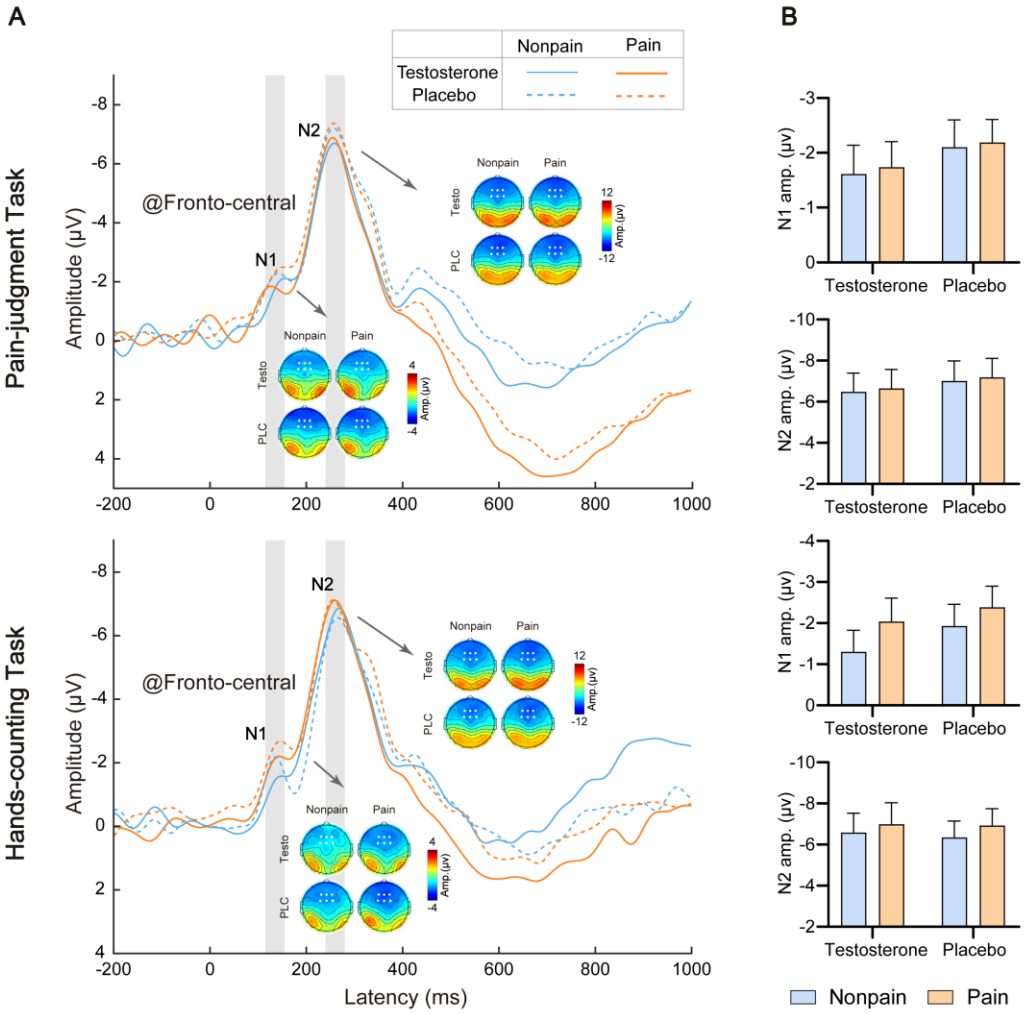

Figure S1. Neural responses measured at fronto-central electrodes during the pain-judgment and hands-counting tasks

Grand average ERP waveforms and scalp topographies for testosterone (solid lines) and placebo (dashed lines) sessions were elicited by nonpainful (blue lines) and painful (orange lines) images during the pain-judgment and hands-counting tasks (A). Displayed waveforms were measured at fronto-central electrodes (F1, Fz, F2, FC1, FCz, FC2). Amplitudes of the N1 and N2 ERPs elicited by nonpainful (blue bars) and painful images (orange bars) were compared between testosterone and placebo sessions (B). Electrodes used to measure ERP amplitudes were marked using enlarged white dots on the corresponding scalp topographies. Data in the bar plots are expressed as Mean ± SEM.