Testosterone modulates preattentive sensory processing and involuntary attention switches to emotional voices

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1Institute of Neuroscience, National Yang-Ming University, Taipei, Taiwan; 2Department of Surgery, National Yang-Ming University Hospital, Yilan, Taiwan; 3Department of Education and Research, Taipei City Hospital, Taipei, Taiwan; 4Institute of Microbiology and Immunology, National Yang-Ming University, Taipei, Taiwan; and 5Department of Rehabilitation, National Yang-Ming University Hospital, Yilan, Taiwan

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Chen C, Chen CY, Yang CY, Lin CH, Cheng Y. Testosterone modulates preattentive sensory processing and involuntary attention switches to emotional voices. J Neurophysiol 113: 1842–1849, 2015. First published December 24, 2014; doi:10.1152/jn.00587.2014.—Testosterone is capable of altering facial threat processing. Voices, similar to faces, convey social information. We hypothesized that administering a single dose of testosterone would change voice perception in humans. In a placebo-controlled, randomly assigned, double-blind crossover design, we administered a single dose of testosterone or placebo to 18 healthy female volunteers and used a passive auditory oddball paradigm. The mismatch negativity (MMN) and P3a in responses to fearful, happily, and neutrally spoken syllables dada and acoustically matched nonvocal sounds were analyzed, indicating preattentive sensory processing and involuntary attention switches. Results showed that testosterone administration had a trend to shorten the peak latencies of happy MMN and significantly enhanced the amplitudes of happy and fearful P3a, whereas the happy- and fearful-derived nonvocal MMN and P3a remained unaffected. These findings demonstrated acute effect of testosterone on the neural dynamics of voice perception. Administering a single dose of testosterone modulates preattentive sensory processing and involuntary attention switches in response to emotional voices.

testosterone; voice; mismatch negativity; MMN; P3a

THE FEAR-REDUCING PROPERTIES of testosterone have been established and verified using an excessive range of behavioral assessment paradigms in animals (e.g., Aikey et al. 2002; Boissy and Bouissou 1994; Bouissou and Vandenheede 1996; Frye and Seliga 2001) and in humans (e.g., Burris et al. 1992; Pope et al. 2003; van Honk et al. 2005; Wang et al. 1996). These findings, in relation to humans, were primarily based on people’s responses to threatening (angry, fearful) faces. Human voices, similar to faces, can convey a wealth of social information (Belin et al. 2004). However, little is known regarding the effect of acute testosterone administration on emotional voice processing.

Data from animal research indicate that testosterone predominantly affects emotion and motivation by binding to steroid receptive neurons that occupy the amygdaloid-centered nuclei in the limbic system (Schulkin 2003; Wood 1996). Research on humans suggests the crucial role of amygdala in the effects of testosterone on emotional processing (van Wingen et al. 2009). In healthy volunteers, a single administration of testosterone reduced the recognition of angry and fearful facial expressions (van Honk et al. 2005; van Honk et al. 2001). Vigilant attention responses to angry faces were also positively related to testosterone levels (van Honk et al. 1999).

Mismatch negativity (MMN) and P3a, which are event-related potentials (ERPs), can be elicited by a passive auditory oddball paradigm in which participants engage in a task and ignore the stimuli that are presented in a random series, with one stimulus (standard) occurring more frequently than the other stimulus (deviant). MMN, which is elicited irrespective of the person’s direction of attention (Näätänen et al. 1978), is presumed to reflect the preattentive sensory processing and the function of the N-methyl-D-aspartate (NMDA) receptor (Näätänen et al. 2007). The modeling of generator sources suggests that predominantly frontocentral scalp distribution of MMN is primarily explained by the activity in the bilateral supratemporal cortex (Rinne et al. 1999). P3a, which is the ERP component that occurs after MMN is induced in response to deviants in the unattended stream (Näätänen et al. 2011), is associated with involuntary attention switches caused by sound changes (Escera et al. 2000). Given an increasing visuospatial attentional load, the P3a response becomes smaller in relation to decreased involuntary attention switching (Fan et al. 2013; Zhang et al. 2006). MMN and P3a in response to emotional syllables have recently indicated the emotional salience of voice perception at the preattentive level (Chen et al. 2014; Cheng et al. 2012; Fan et al. 2013). Similarly, youths with autism spectrum conditions and conduct disorder symptoms exhibited altered MMN and P3a in response to emotional syllables (Fan and Cheng 2014; Hung et al. 2013). In a previous study, female participants displayed stronger fearful MMN than males did (Hung and Cheng 2014), inferring the sex hormone-mediated processing of emotional voices. However, these findings are only correlational and do not clarify the causal relation between testosterone and emotional voices.

To explore the possible causal role of testosterone in the perception of emotional voice and, in particular, to test whether testosterone modulates the neural correlates of fearful voice processing, we investigated the effect of a single administration of testosterone on the presentation of the emotionally spoken syllables dada and acoustically matched nonvocal sounds, respectively, in a passive oddball paradigm. Based on a randomized, double-blind, crossover design, we sublingually administered either 0.5 mg of testosterone or a placebo to female volunteers on 2 separate days. Only women participated, because the quantity and time course for inducing...
neuropsychological effects in women after a single sublingual administration of 0.5 mg of testosterone have been established but are unknown for men (Tuiten et al. 2000). We hypothesized that if testosterone were involved in the preattentive sensory processing of voices, then MMN in response to emotional syllables, but not acoustically matched nonvocal sounds, would be altered after testosterone administration. If involuntary attention switches to voices were related to testosterone, then P3a in relation to emotional syllables, rather than acoustically matched nonvocal sounds, would be changed accordingly. Furthermore, this design might enable us to clarify whether the fear-reducing effect of testosterone would be associated with the decreased neural response to fearful voices or the increased neural response to both happy and fearful voices (Archer 2006; Bos et al. 2012, 2013; Wingfield et al. 1990). In addition, considering that the right-hand second digit-to-fourth digit (2D: 4D) ratio as a proxy of fetal testosterone is substantiated by evidence derived from animals and humans (Hönekopp et al. 2007; van Honk et al. 2011), we conducted analyses on the correlation of the 2D:4D ratio with MMN and P3a, respectively. Given that intertribal variability is characteristic of the ERP signal (Spencer 2005), we measured both the amplitude and peak latency of MMN and P3a.

MATERIALS AND METHODS

Participants. Twenty-one healthy right-handed, aged between 19 and 27 years (23 ± 1.8 yr), participated in this study after providing written informed consent. As a result of poor electroencephalogram (EEG) qualities and less than a twofold increase in testosterone levels after administration, a total of 18 participants were finally included in the data analysis. They had normal menstruation cycles and were nonsmokers. All the participants had normal hearing; did not have neurological, endocrinial, and psychiatric disorders; and were not taking any medication at the time of testing. This study was approved by the Ethics Committee at Taipei City Hospital and conducted in accordance with the Declaration of Helsinki. The participants received monetary compensation for their participation.

Testosterone administration. In the double-blind, crossover, within-in-subjects design used in this study, the participants received a single dose of 0.5 mg of testosterone on one day and a single dose of placebo 48 h later. The drug samples consisted of 0.5 mg of testosterone undecanoate (Andriol; Organon). Testosterone was omitted from the placebo sample, and both testosterone and placebo were administered sublingually. The dosage of 0.5 mg should be sufficient to result in a 10-fold blood testosterone level within 90 min (Tuiten et al. 2000). The interval between two administrations, 48 h, was used to enable exogenous testosterone to metabolize (Slater et al. 2001).

Auditory stimuli. The stimulus materials consisted of two categories: emotional syllables and acoustically matched nonvocal sounds. Fearfully, happily, and neutrally spoken syllables dada were selected as the stimuli (please refer to Cheng et al. 2012; Fan and Cheng 2014; Fan et al. 2013; Hung et al. 2013; Hung and Cheng 2014 for validation). To create a set of control stimuli that retain acoustical correspondence, i.e., the temporal and spectral features of emotional syllables, we synthesized nonvocal sounds with Praat (Boersma 2001) and MATLAB (MathWorks). The fundamental frequency (f0) of emotional (fearful, happy, neutral) syllables was extracted to produce the nonvocal sounds using a sine waveform and then multiplied by the syllable envelope. The duration (550 ms) and loudness (minimum: 57 dB; maximum: 62 dB; mean: 59 dB) of the stimuli were controlled for the emotional syllables and nonvocal sounds.

Digit ratio measurement. The digit ratio was measured using the scanned images of the right hand of each participant, which is a valid method for measuring finger lengths (van Honk et al. 2011). When scanning the images, we ensured that details of major creases could be clearly seen. We used Adobe Photoshop to measure the lengths of the second and fourth digits from the ventral proximal crease of the digit to the fingertip. If a band of creases existed at the base of the digit, the measurement was obtained from the most proximal crease.

Salivary testosterone measurement. The level of salivary testosterone one indicates the free, unbound, or biologically available portion of testosterone in circulation. We used the method that has been reliably applied to measure free testosterone levels in humans (Arregger et al. 2007). Saliva was collected within 15 min before and after the administration of testosterone or placebo. The participants were required to clean their mouth and abstain from drinking and eating for at least 1 h before saliva sampling. After they chewed a cotton wool swab (Salivette; Sarstedt) for ~60 s, the samples were collected in a Salivette tube. The tubes were subsequently stored at −20°C for further analysis.

The saliva samples were recovered by centrifuging the Salivette (combined with the centrifuge vessel) at 1,000 g for 2 min. The clear fluid saliva supernatant was obtained in the conical base of the centrifuge vessel. Based on the manufacturer instructions for an ELISA kit (IBL, Hamburg, Germany), we measured optical density by using a photometer at 450 nm (reference wavelength: 600–650 nm) within 15 min after pipetting the Stop Solution for concentration measurements.

Procedures. Figure 1 shows the placebo-controlled, randomly assigned, double-masked, and crossover experimental design. We sublingually administered either 0.5 mg of testosterone or a placebo to female participants on 2 separate days, both of which were conducted 10 days after the menstruation period of each participant had ended. To control the diurnal effect of hormones, we performed all tests in the morning (09:30 AM to 12:30 PM).

During the passive oddball paradigm for EEG recordings, all participants were required to watch a silent movie with subtitles while task-irrelevant emotional syllables or nonvocal sounds in oddball sequences were presented. The oddball paradigm for emotional syllables employed happily and fearfully spoken syllables dada as deviants and neutrally spoken syllables dada as the standard. The corresponding nonvocal sounds were applied with the same oddball paradigm but were presented in separated blocks. Each stimulus categories comprised two blocks. Each block consisted of 500 trials, of which 80% were neutral syllables or sounds, 10% were fearful syllables or sounds, and the other 10% were happy syllables or

Fig. 1. Experimental procedures. The design is placebo-controlled, randomly assigned, double-masked, and crossover. We sublingually administered either testosterone (T) or placebo (P) to participants on 2 separate days. Saliva was collected before and after the administration. Participant needed to fill in the Profile of Mood States (POMS). During EEG recordings, participants were required to watch a silent movie with subtitles while task-irrelevant emotional syllables or nonvocal sounds in oddball sequences were presented. The digit ratio was measured using the scanned images of the right hand. Finally, participants had to indicate (guessing) in which sessions they believe they received testosterone or placebo.
sounds. The sequences of blocks and stimuli were quasirandomized to avoid successive blocks from identical stimulus categories and successive deviant stimuli. The stimulus-onset-asynchrony was 1,200 ms (550 stimulus, 650 idle).

To control for the potential secondary mood-generated effects of testosterone on voice perception, we used the shortened version of the Profile of Mood States (POMS) (Shacham 1983) for analyzing the participants who received the placebo and testosterone.

Considering that assumptions regarding the effects of the testosterone hormone can influence the performance of human participants under experimental conditions in which participants believe they have been administered the hormone (Eisenegger et al. 2010), we asked the participants to indicate (by forced choice) in which sessions they believe they received testosterone or placebo after the two administrations.

**EEG apparatus and recording.** The EEG was continuously recorded from 32 scalp sites using electrodes mounted in an elastic cap and positioned according to the modified International 10–20 system, with the addition of two mastoid electrodes. The electrode at right mastoid (A2) was used as online reference. Eye blinks and vertical eye movements were monitored with electrodes located above and below left eye. The horizontal electro-oculogram (EOG) was recorded from electrodes placed 1.5 cm lateral to the left and right external canthi. A ground electrode was placed on the forehead. Electrode/skin impedance was kept <5 kΩ. Channels were re-referenced offline to the average of left and right mastoid recordings [(A1 + A2)/2]. Signals were sampled at 500 Hz, band-pass filtered (0.1–100 Hz), and epoched over an analysis time of 600 ms, including prestimulus of 100 ms used for baseline correction. An automatic artifact rejection system excluded from the average all trials containing transients exceeding ±70 µV at recording electrodes and exceeding ±100 µV at the horizontal EOG channel. Furthermore, the quality of ERP traces was ensured by careful visual inspection in every subject and trial and by applying appropriate digital, zero-phase shift band-pass filtered (0.1–50 Hz, 24 dB/octave). The first 10 epochs of each sequence were omitted from the averaging to exclude unexpected large responses elicited by the initiation of the sequences.

The paradigm was edited by MATLAB (MathWorks). Each event in the paradigm was associated with a digital code that was sent to the continuous EEG, thereby enabling the offline segmentation and the average of selected EEG periods to be obtained for analysis. The ERPs were processed and analyzed using Neuroscan 4.3 (Compumedics, Australia).

**Statistical analysis.** The amplitudes of MMN and P3a were analyzed as an average within a 50-ms time window surrounding the peak latency at the electrode sites F3, Fz, F4, C3, Cz, and C4. The MMN peak was defined as the largest negativity in the subtraction between the deviant and standard ERPs during a period of 150–250 ms after sound onset. Only the standards presented before the deviants were included in the analysis. The P3a peak was defined as the highest positivity during a period of 300–500 ms.

Statistical analyses on MMN and P3a were conducted using four-way ANOVA comprising the factors of the administration (testosterone or placebo), category (emotional syllables or nonvocal sounds), affect (fearful or happy), and electrode (F3, Fz, F4, C3, Cz, or C4). The dependent variables were the mean amplitudes and peak latencies of the MMN and P3a components at the selected electrode sites. Degrees of freedom were corrected using the Greenhouse-Geisser method. The post hoc comparisons were only conducted when preceded by significant main effects.

**RESULTS**

**Mood measurement.** The shortened version of POMS was used to indicate the possible effects of testosterone on tension, anxiety, depression, anger, fatigue, and vigor. The Wilcoxon signed-rank test revealed no significant differences in mood between those who received the placebo and testosterone ($P > 0.11$). Given that testosterone did not affect mood, the observed effects of testosterone on emotional voice processing cannot be attributed to secondary mood-generated response biases.

**Control of belief effects and subjective biases.** Performance was at the chance level ($n = 21$, binomial $P = 0.78$), which confirmed that the participants were unaware of what they had received.

**Salivary testosterone.** The salivary testosterone level increased to 1,061% after testosterone administration, but 105% after placebo [$t(17) = 6.52, P < .001$]. The testosterone and placebo concentrations were 1,908.64 ± 481.80 and 265.62 ± 133.08 pg/ml, respectively.

**MMN elicited by emotional syllables and nonvocal sounds.** The preattentive discrimination of emotional voices was studied using MMN and was determined by subtracting the neutral ERP from fearful and happy ERPs (Fig. 2). The repeated-
measures ANOVA model was used to analyze MMN amplitudes and revealed that the main effects of category [emotional syllables vs. nonvocal sounds: $F(1,17) = 11.35, P = 0.004, \eta^2 = 0.40$], affect [fearful vs. happy: $F(1,17) = 62.44, P < 0.001, \eta^2 = 0.79$], and electrode [$F(5,85) = 7.69, P = 0.001, \eta^2 = 0.31$] exerted no effects. Fearful MMN had longer latencies than did happy MMN. In addition, an interaction occurred between administration and category [$F(1,17) = 5.85, P = 0.027, \eta^2 = 0.26$], but no interaction occurred between administration and affect [$F(1,17) = 0.22, P = 0.64, \eta^2 = 0.01$]. Emotional MMN relative to nonvocal MMN had longer latencies after placebo ($P = 0.007$), whereas this effect was diminished after testosterone administration ($P = 0.90$). Particularly, the interaction among administration, category, and affect had a marginal significance with medium to large effect size [$F(1,17) = 4.11, P = 0.059, \eta^2 = 0.20$]. Post hoc analysis indicated that happy MMN exerted an interaction between administration and category [$F(1,17) = 5.36, P = 0.033, \eta^2 = 0.24$] but fearful MMN did not [$F(1,17) = 0.21, P = 0.65, \eta^2 = 0.01$]. The administration effect of happy MMN showed a trend that approached significance [$t(17) = 1.828, P = 0.085, d = 0.43$], indicating that testosterone relative to placebo administration tended to shorten happy MMN latencies. Instead, fearful MMN failed to achieve a customary level of statistical significance [$t(17) = -1.451, P = 0.165, d = 0.34$; Fig. 4].

The repeated measures ANOVA conducted on MMN peak latencies revealed that the affect (fearful vs. happy) produced a main effect [$F(1,17) = 12.34, P = 0.003, \eta^2 = 0.42$], but the administration [$F(1,17) = 0.24, P = .63, \eta^2 = 0.01$], category [emotional syllables vs. nonvocal sounds: $F(1,17) = 4.11, P = 0.059, \eta^2 = 0.20$], and electrode [$F(5,85) = 1.85, P = 0.16, \eta^2 = 0.10$] exerted no effects. Fearful MMN had longer latencies than did happy MMN. In addition, an interaction occurred between administration and category [$F(1,17) = 5.85, P = 0.027, \eta^2 = 0.26$], but no interaction occurred between administration and affect [$F(1,17) = 0.22, P = 0.64, \eta^2 = 0.01$]. Emotional MMN relative to nonvocal MMN had longer latencies after placebo ($P = 0.007$), whereas this effect was diminished after testosterone administration ($P = 0.90$). Particularly, the interaction among administration, category, and affect had a marginal significance with medium to large effect size [$F(1,17) = 4.11, P = 0.059, \eta^2 = 0.20$]. Post hoc analysis indicated that happy MMN exerted an interaction between administration and category [$F(1,17) = 5.36, P = 0.033, \eta^2 = 0.24$] but fearful MMN did not [$F(1,17) = 0.21, P = 0.65, \eta^2 = 0.01$]. The administration effect of happy MMN showed a trend that approached significance [$t(17) = 1.828, P = 0.085, d = 0.43$], indicating that testosterone relative to placebo administration tended to shorten happy MMN latencies. Instead, fearful MMN failed to achieve a customary level of statistical significance [$t(17) = -1.451, P = 0.165, d = 0.34$; Fig. 4].

P3a elicited by emotional syllables and nonvocal sounds.

The repeated measures ANOVAs of P3a amplitudes revealed main effects produced by the administration [testosterone vs.
Moreover, a significant interaction occurred between administration, category, and affect with medium to large effect size \((P = 0.001)\). Follow-up analysis indicated that testosterone relative to placebo administration tended to shorten happy MMN latencies \((+P < 0.05\), one-tailed\), but not to fearful MMN \((P = 0.16)\). As for P3a, neither the administration nor related interactions reached any statistical significance.

Fig. 4. Peak latencies of MMN and P3a to emotional syllables and nonvocal sounds after administration of testosterone and placebo. MMN had a marginal interaction among administration, category, and affect \([\text{emotional syllables vs. nonvocal sounds: } F(1,17) = 6.61, P = 0.02, \eta^2 = 0.28], \text{category [emotional syllables vs. nonvocal sounds: } F(1,17) = 4.79, P = 0.043, \eta^2 = 0.22], \text{affect [fearful vs. happy: } F(1,17) = 76.29, P < 0.001, \eta^2 = 0.82], \text{and electrode } [F(5,85) = 27.85, P < 0.001, \eta^2 = 0.62; \text{Fig. 3}]. P3a significantly differed between the administration of testosterone and placebo. Emotional syllables elicited stronger P3a than did nonvocal sounds. Fearful P3a exhibited stronger amplitudes than did happy P3a. The midline electrodes had larger P3a than did the lateral electrodes \((Fz \text{ vs. F3: Bonferroni-corrected } P < 0.001; Fz \text{ vs. F4: } P < 0.001; Cz \text{ vs. C3: } P < 0.001; Cz \text{ vs. C4: } P < 0.001)\). Moreover, a significant interaction occurred between administration and category \([F(1,17) = 11.10, P = 0.004, \eta^2 = 0.40]\), but none occurred between administration and affect \([F(1,17) = 0.87, P = 0.36, \eta^2 = 0.05]\) nor among administration, category, and affect \([F(1,17) = 0.14, P = 0.72, \eta^2 = 0.01]\). Follow-up analysis indicated that testosterone relative to placebo administration achieved significance only for emotional P3a \((P = 0.002)\) rather than nonvocal P3a \((P = 0.32)\). Testosterone administration increased P3a in response to happy and fearful syllables, but not to acoustically matched nonvocal sounds.

The ANOVA of P3a peak latencies did not reveal any effect caused by the administration \([F(1,17) = 0.47, P = 0.50, \eta^2 = 0.03]\), category \([F(1,17) = 2.27, P = 0.15, \eta^2 = 0.12]\), affect \([F(1,17) = 0.29, P = 0.60, \eta^2 = 0.02]\), or electrode \([F(5,85) = 0.75, P = 0.52, \eta^2 = 0.04; \text{Fig. 4}]. None of the administration-related interactions reached significance \([\text{administration } \times \text{category: } F(1,17) = 0.82, P = 0.38, \eta^2 = 0.05; \text{administration } \times \text{affect: } F(1,17) = 0.34, P = 0.57, \eta^2 = 0.02; \text{administration } \times \text{category } \times \text{affect: } F(1,17) = 0.09, P = .77, \eta^2 = 0.01]\).

**Correlations between 2D:4D ratio and MMN amplitudes.** Table 1 lists that neither emotional nor nonvocal MMN and P3a were correlated with the 2D:4D ratio, irrespective of whether testosterone or placebo was administered \((all P > 0.05)\).

**DISCUSSION**

We investigated the causal role of testosterone in the neural correlates of emotional voices. Healthy young women received a single dose of testosterone in a randomized, placebo-controlled, crossover manner, and MMN and P3a elicited by emotional (happy, fearful) syllables and acoustically matched nonvocal sounds were recorded in a passive auditory oddball paradigm. The results indicated that a single dose of testosterone administration tended to accelerate the peak latencies of happy MMN and significantly enhanced the amplitudes of
Testosterone acts through NMDA receptors to affect the motor synaptic currents (Brann et al. 1993; White et al. 1999). This method. These two techniques are used to examine extremely diverse markers of brain activity: steroid receptors and androgen receptors in the insula of humans is currently available (Bancroft et al. 2014) are not likely attributed to acute testosterone effect. Theoretically, three possible reasons can explain this phenomenon. First, the cortical generator of MMN likely originates from the primary auditory cortex or its vicinity (Alho 1995). The insula, located near the primary auditory cortex, is capable of integrating multisensory modalities (Rodgers et al. 2008) and is involved in processing emotional voices (Belin et al. 2004; Chen et al. 2014; Morris et al. 1999; Sander and Scheich 2001). However, no evidence of androgen receptors in the insula of humans is currently available (Bancroft 2005). If the insula underpins emotional voice processing at the preattentive level, acute testosterone administration would not modulate fearful MMN. Second, physiological levels of testosterone may be necessary for obtaining a generalized pattern of interactive brain activity, much of which is independent of the direct testosterone effect. Currently, only a limited overlap exists between localized testosterone effects in the brain based on a comparison androgen receptors and neuroimaging studies (Peper et al. 2011). This may partially reflect the methodological limitations of ERPs; certain relevant areas of the brain, particularly the amygdala, are too deep to be recorded using this method. These two techniques are used to examine extremely diverse markers of brain activity: steroid receptors and postsynaptic potentials. Third, the testosterone effect on MMN does not appear without long-term exposure. NMDA receptor antagonists and agonists affect MMN generation (e.g., Javitt et al. 1996; Leung et al. 2008; Umbricht et al. 2000). Testosterone modulates NMDA receptor-mediated mRNA levels and postsynaptic currents (Brann et al. 1993; White et al. 1999). Testosterone acts through NMDA receptors to affect the motor neuron response to glutamate and blockage of NMDA receptors decreases testosterone levels (Hsu et al. 2000). Accordingly, acute testosterone effect on MMN will not be evident without following these cascade steps.

A single dose of testosterone administration enhances P3a responses to emotional (happy, fearful) syllables in a passive auditory oddball paradigm. Testosterone plays a crucial role in affective behavior, which is mediated by various brain regions within the emotional circuitry, including the prefrontal cortex and amygdala (van Wingen et al. 2010; Volman et al. 2011). Endogenous testosterone levels were related to attentional orienting to angry faces (van Honk et al. 1999; Wirth and Schultheiss 2007) and smiling faces (Cashdan 1995; Dabbs 1997). Exogenous testosterone moderated attentional responses to threatening stimuli (Lacreuse et al. 2010; van Honk et al. 2005). Testosterone might particularly increase attentional biases to negative stimuli during early exposure to the stimuli accompanied by acute treatment, or when the stimuli are highly aroused by chronic treatment (King et al. 2012). P3a reflects involuntary attentional switches caused by novel stimuli (Harmony et al. 2000). Lesion studies have indicated that the prefrontal cortex might contribute to P3a generation (Knight 1984). This study demonstrated that exogenous testosterone enhanced involuntary attention switches to emotional voices, as indicated by heightened responses to both happy and fearful P3a. This finding concurs with the hypothesis that the function of testosterone could facilitate any form of adaptive goal-directed behavior, such as the sensitivity to socially relevant signals (Archer 2006; Bos et al. 2012; Wingfield et al. 1990). Interestingly, one recent fMRI study showed that testosterone reduced fear by increasing the amygdala activity to fearful and happy faces (Bos et al. 2013). We thus ascribed the fear-reducing properties of testosterone to strengthening, rather than attenuating, the response to emotional faces and voices. These findings might lend support acute testosterone effect to be associated with more elaborate processing of social signals. Incorporating with previous findings that anterior insular cortex activity responded to MMN elicited by hearing disgust (Chen et al. 2014), we supposed that augmented P3a to fearful and happy voices in the present study could be in parallel with enhanced amygdala activity to fearful and happy faces (Bos et al. 2013). The source localization of emotional MMN and P3a should be dissociated.

Particularly, the salivary testosterone level increased to 1061% after testosterone administration with large between-subject variability (385–2,789%). It could be attributed to the weak serum-saliva correlation for testosterone in our female subjects. Only 1–10% of testosterone is in its unbound or biologically active form in blood. The remaining testosterone is bound to serum proteins whereas the majority of testosterone in saliva is not protein bound. The salivary testosterone levels are modestly correlated with serum levels for males, but not necessarily for females (Shirtcliffe et al. 2002). Here, we administered sublingual testosterone in females, whose psychological and physiological effects had been testified by an amount of studies (e.g., Slater et al. 2001; Tuiten et al. 2000; van Honk et al. 2001). Taken together, these data first provide the direct evidence for examining acute testosterone effect on the neural dynamics of emotional voice processing in humans. Based on a passive auditory oddball paradigm, testosterone administration tended to advance the peak latencies of MMN in response to happy

Table 1. Correlations between the 2D:4D ratio and MMN/P3a amplitudes at Fz

<table>
<thead>
<tr>
<th>Administration</th>
<th>2D:4D Ratio</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emotional Happy MMN/P3a</td>
<td>−0.18/−0.13</td>
<td>0.48/0.62</td>
<td></td>
</tr>
<tr>
<td>Fearful MMN/P3a</td>
<td>−0.13/0.02</td>
<td>0.62/0.94</td>
<td></td>
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<tr>
<td>Nonvocal Happy-derived MMN/P3a</td>
<td>−0.15/0.23</td>
<td>0.55/0.35</td>
<td></td>
</tr>
<tr>
<td>Fearful-derived MMN/P3a</td>
<td>−0.11/0.13</td>
<td>0.66/0.60</td>
<td></td>
</tr>
<tr>
<td>Placebo Emotional Happy MMN/P3a</td>
<td>−0.22/−0.18</td>
<td>0.38/0.46</td>
<td></td>
</tr>
<tr>
<td>Fearful MMN/P3a</td>
<td>−0.16/0.34</td>
<td>0.53/0.16</td>
<td></td>
</tr>
<tr>
<td>Nonvocal Happy-derived MMN/P3a</td>
<td>0.09/0.16</td>
<td>0.72/0.52</td>
<td></td>
</tr>
<tr>
<td>Fearful-derived MMN/P3a</td>
<td>−0.02/0.18</td>
<td>0.95/0.48</td>
<td></td>
</tr>
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2D:4D, second digit-to-fourth digit; MMN, mismatch negativity; Fz, electrode site; n = 18.
syllables, as well as significantly increased the amplitudes of P3a to happy and fearful syllables. Testosterone-modulated preattentive sensory discrimination and involuntary attention switches to emotional voice processing. The findings suggest that acute testosterone effect is unlikely to cause the emergence of sex differences in fearful MMN during adulthood (Cheng et al. 2012; Hung and Cheng 2014). Considering the role of testosterone in attention control (e.g., Burris et al. 1992; Pope et al. 2003; van Honk et al. 2005; Wang et al. 1996), we argued that testosterone might reduce fear by facilitating preattentive sensory processing of positive social cues as well as elaborate attentional processing of both positive and negative social signals.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

Author contributions: C.C. and C.-Y.Y. performed experiments; C.C., C.-Y.C., and C.-Y.Y. analyzed data; C.C., C.-Y.C., C.-Y.Y., and Y.C. interpreted results of experiments; C.C. prepared figures; C.C., C.-H.L., and Y.C. drafted manuscript; C.C., C.-Y.C., C.-H.L., and Y.C. edited and revised manuscript; C.C., C.-Y.C., C.-Y.Y., and Y.C. approved final version of manuscript; C.-H.L. and Y.C. conception and design of research.

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