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Testosterone and estrogen impact social evaluations and vicarious emotions: A double-blind placebo-controlled study

Running title: Sex hormonal effects on social evaluation and vicarious emotion

Andreas Olsson[1]*, Eleni Kopsida[1], Kimmo Sorjonen[1], & Ivanka Savic[2]

[1]Department of Clinical Neuroscience, Division of Psychology, and [2]Department of Women's and Children's Health, Karolinska Institutet, Sweden

* to whom correspondence should be addressed:

Karolinska Institutet, Department of Clinical Neuroscience,

Nobels väg 9, 171 77, Stockholm, Sweden

Tel:+46-(0)8-524-824-59

e-mail: andreas.olsson@ki.se

Summary

The abilities to 'read' other peoples' intentions and emotions, and to learn from their experiences, are critical to survival. Previous studies have highlighted the role of sex hormones, notably testosterone and estrogen, in these processes. Yet, it is unclear how these hormones affect social cognition and emotion using acute hormonal administration. In the present double-blind, placebo-controlled study, we administered an acute exogenous dose of testosterone or estrogen to healthy female and male volunteers, respectively, with the aim to investigate the effects of these steroids on social cognitive and emotional processes. Following hormonal and placebo treatment, participants made (1) facial dominance judgments, (2) mental state inferences (Reading the Mind in the Eyes Test, RMET), and (3) learned aversive associations through watching others' emotional responses (observational fear learning, OL). Our results showed that testosterone administration to females enhanced ratings of facial dominance, but diminished their accuracy in inferring mental states. In men, estrogen administration resulted in an increase in emotional (vicarious) reactivity when watching a distressed other during the OL task. Taken together, these results suggest that sex hormones affect social cognitive and emotional functions at several levels linking our results to neuropsychiatric disorders where these functions are impaired.

Key-words: affect, hormone, social cognition, observational learning, empathy, mentalizing

Introduction

The abilities to decode the intentions and emotions of others, and to learn from them, are critical to survival. Indeed, misreading the behavioral intentions of a potentially threatening conspecific, or missing the opportunity to learn about his or her dangerousness by observing others' fearful expressions, can be fatal. These social abilities require the integrity of a flexible tool-kit of social-cognitive and emotional skills, enabling the individual to interpret social signals, such as facial cues, and learn from them (Frith & Frith, 2007; Lieberman, 2007; Mitchell, Macrae, & Banaji, 2006; Ochsner et al., 2004; Olsson & Ochsner, 2008; Saxe, Carey, & Kanwisher, 2004).

An increasing number of studies report that specific social cognitive abilities differ between sexes. Specifically, females have been shown to outperform males on certain 'mind-reading' and empathic tasks (Baron-Cohen, 2005; Derntl et al., 2010; Singer et al., 2006), whereas males tend to be more sensitive to facial cues related to dominance and threat (Aleman & Swart, 2008; Mazur & Booth, 1998). In terms of learning fear vicariously by observing emotional expressions of others, findings of sex differences are scarce. One earlier study suggested an advantage in avoidance learning through observation in female toddlers (Gerull & Rapee, 2002). Another study reported that females showed stronger fear learning in terms of rated distress, but not autonomic conditioning (Kelly & Forsyth, 2007). Interestingly, other research has shown that females are more sensitive to both self- (Bartley & Fillingim, 2013) and vicariously experienced pain (Decety & Svetlova, 2012) . Although self-experienced pain has been directly linked to differences in levels of sex hormones (Bartley & Fillingim, 2013), vicarious pain might be especially sensitive to conformation with stereotypic gender roles

(Martel, Thibault, & Sullivan, 2011). Supporting this suggestion is the fact that sex differences in empathic responses to others' suffering are larger for self-reported than autonomic and brain based measures (Decety & Svetlova, 2012). Studies of classical fear conditioning, which shares important features with observational fear learning (Olsson & Phelps, 2007), have reported both a male (Milad et al., 2010) and a female (Guimaraes, Hellewell, Hensman, Wang, & Deakin, 1991) advantage, and have suggested that observed sex differences in fear conditioning may critically depend on an interaction between sex and stress hormones (Stark et al., 2006; Zorawski, Cook, Kuhn, & LaBar, 2005).

The reported sex differences in the social cognitive and emotional domains are likely to depend on both social and biological (sex-linked genes and sex hormones) factors, and the interaction between them (Kret & De Gelder, 2012). Among these factors, increasing attention has recently been paid to sex hormones that are known to affect the neuronal networks involved in social cognitive and emotional processes (Bos, Panksepp, Bluthé, & van Honk, 2012). In addition to having an impact on cerebral development, these steroids can directly influence social cognitive and emotional responses (McCall & Singer, 2012).

Of particular interest are studies suggesting that testosterone impairs 'mind-reading' while facilitating threat related judgments. For example, exogenous administration of testosterone has been shown to impair women's ability to recognize mental states of people during the Reading the Mind in the Eyes test (RMET, Van Honk et al., 2011), decrease women's empathic mimicry of emotional facial expressions (Hermans, Putman, & Van Honk, 2006), attenuate women's ability to recognize angry

faces, and facilitate processing of facial cues that signal competition and dominance in both men and women (Eisenegger et al., 2011; Van Honk and Schutter, 2007; Wirth and Schultheiss, 2007). However, it remains unknown if judgments of facial dominance change as a function of treatment with testosterone. In addition, although testosterone has been shown to have fear reducing effects in females (Hermans et al., 2006), no research has to date addressed the role of testosterone in observational learning.

In comparison to the role of testosterone, even less is presently known about how estrogen affects social cognitive and emotional behavior. Most of the existing studies have focused on fluctuating levels of estrogen across the menstrual cycle in females. However, using natural variation of sex steroids limits the opportunity to disentangling potential effects of estrogen and progesterone, because their levels co-fluctuate and could interact with each other (van Wingen, Ossewaarde, Bäckström, Hermans, & Fernández, 2011). This may explain that high estrogen levels across the menstrual cycle have been shown to both enhance (Pearson & Lewis, 2005) and attenuate (Derntl, Kryspin-Exner, Fernbach, Moser, & Habel, 2008) female ability to recognize facial emotional expressions. During the cycle phase when both estrogen and progesterone levels are high, progesterone might dampen, or even reverse, the facilitating effect of estrogen on emotion recognition. Indeed, progesterone has been observed to impair recognition of facial emotions (Derntl, Hack, Kryspin-Exner, & Hable, 2012). Thus, it remains unclear what role estrogen might play in human social cognitive and emotional responses.

The present study addressed the aforementioned issues by investigating the impact of testosterone and estrogen in females and males, respectively, on the sensitivity to facial dominance, the ability to recognize mental states, and the learning to fear a

previously neutral stimulus through watching others' emotional expressions. Our design enabled both with-in subjects comparisons of hormonal levels (hormone vs placebo), and between-subject comparisons between males and females in the placebo conditions. A single dose of testosterone was administered to female participants and a single dose of estrogen to male participants. The reason for using this 'opposite sex design' was to avoid potential ceiling effects driven by the already high doses of sex hormones (estrogen in women and testosterone in men) in a young, healthy population.

We included a series of well-established behavioral tasks that tapped into different modes of social cognitive and emotional processes: (1) reflexive social cognitions were measured through fast and intuitive ratings of level of dominance in facial images that parametrically varied in normative level of dominance (from low to high, Oosterhof & Todorov, 2008), (2) reflective social cognitions were measured through inferences of mental states from images of the eye region in the RMET task, and (3) social emotional learning was assessed through an observational fear learning task. To the best of our knowledge, this was the first study administering both testosterone and estrogen in the same design to examine their respective impact on various components of social cognitive and emotional performance. Our main hypotheses were the following: For females, we first predicted that testosterone would enhance dominance ratings as compared to the placebo condition. Second, we expected to find that exogenous administration of testosterone would hamper female performance on 'mind-reading' on the RMET. Third, testosterone should either impair or have no effect on observational fear learning.

For males, due to the scarcity of previous research on the effects of estrogen on social cognitive and emotional processing, our approach was more explorative. First, because of the lack of previously reported results, we did not expect any effects on the dominance ratings. Although some studies have suggested a facilitating effect of estrogen on the recognition of emotional faces (Pearson & Lewis, 2005), no research has examined its influence on mentalizing abilities. Therefore, we expected that estrogen would either have no effect, or slightly improve performance, on the RMET task. Third, and related to observational fear learning, the demonstration of a facilitating effect of estrogen on facial emotion recognition (Pearson & Lewis, 2005), argued for an enhancing effect on vicarious fear. This would also be consistent with the findings that females are more sensitive to both self (Bartley & Fillingim, 2013) and vicariously experienced pain (Decety & Svetlova, 2012). Although hormonal effects in other animals should be interpreted with care, research on rodents has demonstrated that estrogen enhances learning trough the transmission of social signals (Choleris, Clipperton-Allen, Phan, Valsecchi, & Kavaliers, 2012). Taken together, these findings suggested that estrogen would enhance the reactivity to the emotional expressions of the learning model in the observational fear learning task, and as a result of this, the ensuing learning. However, the inconsistent findings of hormone-specific effects on direct fear learning (Pavlovian conditioning) left open the possibility of no hormonal effects on the learning aspect of the observational learning task.

Materials and methods

Participants

Sixty-six healthy participants (33 men, 33 women; mean age 26.9, SD=5, age range 20-45 years) were paid for their participation. All subjects were right-handed, heterosexual (according to Kinsey scale), and able to read and speak Swedish fluently. History of chronic and/or psychiatric disease, use of recreational drugs, CNS medication, or analgesics served as exclusion criteria. All women in the study used estrogen containing single-phase contraceptives, and were not pregnant or breastfeeding. The rationale behind restricting female participants to those using contraceptives was to avoid major influences of sex hormone fluctuations over the menstrual cycle. All experiments were carried out on days with estrogen intake. Individual variation in the second-to-fourth digit ratio, which has been shown to be related to cognitive empthy in women (van Honk et al., 2011), was assessed. The study was approved by the Karolinska Institutet local ethic board.

Placebo, hormone gel and saliva samples

Ten hours before the tests were carried out, participants were asked to smear gel (placebo or hormone) on the lower part of the belly. Female participants received testosterone (Testogel 50mg) and male participants received estrogen (Divigel 2mg). Although it was recently shown that testosterone and estrogen levels peak already 2-4 h after application of gel (Eisenegger, von Eckardstein, Fehr, & von Eckardstein, 2013) we choose the 10 h interval because testing was performed in the morning and we wanted to avoid asking participants to apply the gel in the early morning. The order of the placebo and hormone administration was double blinded and randomized. In order to measure hormonal levels (estrogen and testosterone in both women and men), samples of saliva were collected

between 9 and 10.30 in the morning on both occasions. The samples were frozen within 4 hours and stored at –20°C until shipping to the U. K. for analysis (Genova Diagnostics Europe, U.K). Estradiol and testosterone levels were determined using CE marked ELISA kits. Hormonal analysis was conducted according to the manufacturer's instructions (Salimetrics, U.S.A).

Behavioral paradigms

A battery of three tests was administered twice (hormone and placebo treatment) with 1 to 2 ½ weeks gap for any hormonal effects to wash out. There was no systematic difference between groups with respect to time gap. Each participant took part in both the hormone and placebo treatments.

Dominance rating of faces

Processing of facial cues of dominance was assessed employing a set of artificial faces generated by Oosterhof and Todorov (2008) using a 3D statistical model (FaceGen 3.1 software). The faces were emotionally neutral male faces that parametrically differed in the degrees of normative dominance. In the present study, seven different versions of a face that varied in dominance (SD: -4.5 [least dominant], -3.0, -1.5, 0, 1.5, 3.0, 4.5 [most dominant] on this dimension) were presented in a randomized fashion on a computer screen in front of the participants. The same faces were presented on both days. Participants were asked to make fast and 'intuitive' judgments about the degree of dominance, using a 7-items Likert scale (from 0 = not at all dominant to 6 = very dominant).

Reading the Mind in the Eyes test (RMET)

RMET was used to assess the ability to recognize mental states (intentions and emotions). The test comprises a total of 37 pictures of the eye region, including one practice picture. For the present study, a validated Swedish adaptation of the test (Hallerbäck, Lugnegård, Hjärthag, & Gillberg, 2009) was administered, using the pictures of the original RMET version (Autism Research Center, University of Cambridge, UK). For each picture, participants were asked to choose between four alternative adjectives (e.g. 'reflective', 'aghast', 'irritated', 'impatient') the one that best described what the person in the image felt or thought. There was no time limit, but participants were asked to answer as quickly and accurately as they could. Because previous studies have reported no practice effect (Hallerbäck et al., 2009; Handford, Lemon, Grimm, Vollmer-Conna, 2013), the same pictures were shown during both testing days (day 1 and 2).

Observational Fear Learning (OL)

As a measure of social-emotional reactivity and learning, we introduced a previously used OL task, in which the participant (the observer) learns to fear a conditioned stimulus (CS) by mere observation of somebody else's (the 'learning model's') expressions of distress towards the CS (Crişan et al., 2009; Olsson, Nearing, & Phelps, 2007; Olsson & Phelps, 2004). In addition to assessing the learning of a basic association between the CS and the learning model's emotional expression, OL enables the measure of an immediate emotional, vicarious, response to the learning model's distress (serving as a natural aversive 'social' unconditioned stimulus, US). Observational

fear learning is thought to depend on both basic mechanisms of associative learning and the observer's evaluation of the learning model's emotional expression (Olsson & Phelps, 2007).

The OL task consisted of two parts in the following order: an observational and a test phase. The stimuli and design used in this experiment were similar to those reported previously (Olsson et al., 2007). Prior to the observational phase, participants were told that they were going to watch a movie of another individual participating in an experiment similar to the one that they themselves later were going to take part in. During the observational phase, participants watched a movie (3 min, 54 sec) displaying the learning model undergoing a fear conditioning procedure. Two colored squares (red and green on day 1, blue and yellow on day 2) served as the CS and were presented on a computer screen in front of the learning model. To control for potential observer X model interactions, female participants watched a female learning model, and male participants watched a male learning model. Each CS presentation lasted 10 seconds, with an inter-stimulus-interval (ITI) varying between 10 and 14 seconds (during which the word "rest" appeared on the screen). Each colored square (CS) was presented five times in a pseudorandomized order. Three of the five presented stimuli (CS+) co-terminated with the administration of an unpleasant shock to the learning model's right wrist. The model reacted to the administration of the shock by twisting his/her arm and displaying facial signs of discomfort, such as frowning, towards the CS. The other color served as a control stimulus (CS-) and was never paired with a shock. The participants never received any shocks during the observational phase to ensure that learning occurred indirectly, solely through social means, by watching the learning model's responses.

After the completion of the observational phase, participants were presented with the same CS (in a different order) as the learning model had been exposed to in the movie. Again, no shock was administered during the test phase to ensure that emotional learning of the CS-US contingencies remained social in nature. At the end of the experiment, participants were debriefed and asked whether they had believed the instructions.

Observational Fear Learning data analysis

During the observational and test phase, participants' skin conductance response (SCR) was recorded as measure of aversive learning. The SCR was measured through Ag-AgCl electrodes, attached to the distal phalanges of the second and third digits of the left hand, and grounded through a RF filter panel. A BIOPAC Systems (Santa Barbara, California) skin conductance module was used to record and amplify the signal. Analysis Analogue SCR waveforms were analyzed off-line with Acknowledge software (BIOPAC Systems Inc., Goleta, California). The peak-to-peak amplitude (microSiemens, μS) was calculated from the largest responses beginning in the 0.5-4.5 second latency window following stimulus onset. The SCR data were square-root transformed to normalize the distributions. For each subject, three average scores (CS+, CS-, US [onset of shock to the learning model]) were produced for the observational phase and two (CS+, CS-) for the test phase. Data from the two phases were analyzed separately.

Statistical analysis

Statistical analysis (using IBM SPSS 22) was conducted using Mixed Linear Model (Condition [hormone, placebo] was the default fixed factor) in order to control more

accurately for within subjects variation. Level of dominance (0= not at all dominant; 6= very dominant) and Stimulus (CS+, CS-) were used as additional factors for analysis of the Dominance rating of faces test and OL, respectively. Following Van Honk and colleagues (2011), data from RMET were analyzed using paired t-tests in order to allow for a direct comparison between the results.

Results

Elevated hormonal levels after exogenous administration of testosterone and estrogen were verified for women and men separately. Data on testosterone and estrogen levels (pg/mL) were not available for six participants (3 women, 3 men). Wilcoxon signed ranks test was performed to account for deviation from normal distribution. As expected, testosterone levels, in women, were significantly higher after hormonal manipulation than after receiving placebo ($T = 0$, $p < .001$). Similarly, estrogen administration to men resulted in higher estrogen levels than after placebo administration ($T = 0$, $p < .001$). Because the hormone levels of estrogen and testosterone exceeded the range for the assay kits in the intended direction, the numeric values are not reported. Individual variation in the second-to-fourth digit ratio, which has been shown to be related to cognitive empthy in women (van Honk et al., 2011), did not yield any effects in the present study, and are therefore not discussed further. The relationships between the main dependent variables in males and females in the placebo and hormone conditions are presented in table 1 and 2, respectively.

Dominance rating of faces

In the placebo condition, gender had no significant main effect on dominance ratings, $F(1, 64) = 0.795$, $p = .376$, Cohen's $d = .128$. The effect of occasion (first or second), irrespective of condition, on dominance ratings was significant among women, $F(1, 428) = 6.002$, $p = .015$, Cohen's $d = .214$, but not among men, $F(1, 428) = 1.089$, $p = .297$, Cohen's $d = .091$. Women rated the faces as more dominant on the first ($M = 3.65$, $SD = 1.72$) as compared to the second ($M = 3.28$, $SD = 1.70$) occasion. This effect of occasion among women was not qualified by an occasion × condition interaction effect, $F(1, 31) < 0.001$, $p = .993$, indicating that the effect of testosterone treatment was not significantly different on the first compared with the second occasion. Neither did we find an occasion x condition among men, F(1, 31) = 0.147, p = .704. Similarly, the occasion × condition × dominance level interaction effect was not significant among women, $F(1, 423) = 0.099$, $p = .753$, or among men, $F(1, 423) = 0.632$, $p = .427$. Separate analyses revealed no general effect of condition on dominance ratings among women, $F(1, 428) = 0.434$, $p = .510$, Cohen's $d = .058$ or men, $F(1, 428) = 0.567$, $p = .452$, Cohen's $d = .065$. However, for both women ($p = .072$) and men ($p = .051$), condition interacted marginally with level of dominance. As seen in figure 2, as compared to the placebo group, females treated with testosterone displayed a flattened increase in rated dominance across increasing levels of the dominance dimension. In other words, testosterone treatment weakened the association between level of dominance and rated dominance of faces among females (the effect of an increase in level of dominance by one, on the scale from 0 to 6, decreased from $\beta = .376$ to $\beta = .325$, corresponding to 13%). For males, estrogen treatment strengthened the link between rated dominance and increasing levels on the dominance dimension (the effect increased from $\beta = .303$ to $\beta = .360$, corresponding to

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19%). Visual inspection of figure 2 indicates that among female participants, the weakened link between rated, and dimensional level of, dominance in the testosterone condition was primarily due to a relative increase in rated dominance at the lowest levels of dominance in the faces. If restricting the analysis to the two lowest levels of dominance (level 0 and 1), female participants tended to rate the faces as more dominant in the testosterone as compared to the placebo condition at a marginally significant level, $F(1, 98) = 3.450$, $p = .066$, Cohen's $d = .185$.

Reading the Mind in the Eyes Test

There was no significant difference between female and male performance in the placebo condition, $t_{63} = 0.891$, $p = .371$, Cohen's $d = .221$. The effect of occasion was not significant among women, $t_{31} = -1.494$, $p = .145$, Cohen's $d = .296$, or among men, $t_{32} = -0.938$, $p = .355$, Cohen's $d = .138$. Neither was the occasion $\times$ condition interaction effect significant among women, $F(1, 30) = 2.769$, $p = .107$, or among men, $F(1, 31) = 0.001$, $p = .973$, indicating that the effect of hormone treatment was not significantly different on the first compared with the second occasion. Our aim was to test whether hormonal treatment would affect women and men separately. This was motivated by previous findings that testosterone treatment impaired female performance on the RMET (Van Honk, 2011). Replicating this finding, we found that women were less accurate in inferring the emotional state depicted in the RMET pictures during testosterone treatment as compared to when they had received placebo ($t_{32} = 1.711$, $p = 0.048$, one-tailed, Cohen's $d = .322$, see Fig. 3). In contrast, male performance was not affected by estrogen treatment ($t_{32} = 0.133$, $p = 0.895$, Cohen's $d = .009$).

Observational Fear Learning

Observational phase

Observing CS presentations to the learning model. When observing the model being exposed to a CS, participants displayed a greater SCR to the CS+ than to the CS-, $F(1, 941) = 5.742$, $p = .017$, Cohen's $d = .100$, (Figure 4). This effect of stimulus was not qualified by a stimulus × occasion, $F(1, 939) = 0.246$, $p = .620$, or a stimulus × occasion × condition, $F(1, 936) < 0.001$, $p = .986$, interaction effect, indicating that a possible stimulus × condition interaction effect did not look significantly different on the first and second occasion. However, this stimulus × condition interaction effect was not significant among women, $F(1, 489) = 0.502$, $p = .479$, or among men, $F(1, 436) = 0.991$, $p = .320$.

Observing shock presentations to the learning model. In the placebo condition, there was no significant difference between female and male participants SCR when observing the model receiving a shock (US; measuring the SCR at the onset of the shock to the learning model, $F(1, 48) = 0.024$, $p = .878$, Cohen's $d = .031$). The effect of occasion on SCR was significant both among women, $F(1, 134) = 4.356$, $p = .039$, Cohen's $d = .240$, and among men, $F(1, 127) = 7.361$, $p = .008$, Cohen's $d = .323$. However, while the SCR increased among women (from $M = .409$, $SD = .232$ at the first to $M = .466$, $SD = .261$ at the second occasion), it decreased among men (from $M = .577$, $SD = .204$ to $M = .469$, $SD = .201$. The occasion × condition interaction effect did not reach significance among women, $F(1, 27) = 0.729$, $p = .401$ or among men, $F(1, 25) = 0.020$, $p = .889$, indicating that the effect of hormone treatment was not significantly different on the first compared

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with the second occasion. Separate follow-up analyzes revealed a significant effect of condition for men, $F(1, 131) = 10.886$, $p = .001$, Cohen's $d = .384$.), but not for women, $F(1, 136) = 0.057$, $p = .812$, Cohen's $d = .028$, indicating that males treated with estrogen as opposed to placebo responded stronger when watching the learning model receiving a shock (Figure 5).

Test phase

As predicted, participants displayed greater SCR to the CS+ compared to the CS-, verifying that learning had taken place, $F(1, 1243) = 167.931$, $p < .001$, Cohen's $d = .679$ (Figure 4). Separate analyses for women and men indicated that this effect of stimulus was not qualified by occasion or condition.

Discussion

With the aim to investigate the effects of sex hormones on social cognitive and emotional skills, we administered a single dose of testosterone and estrogen to female and male participants, respectively. Both testosterone and estrogen levels, as assessed immediately before the experiment, showed a significantly increased level of the respective hormone. In sum, our results showed that females treated with testosterone, as compared to placebo, displayed an enhanced tendency to rate low dominant faces as dominant, and hampered the ability to accurately attribute mental states to others. In contrast, estrogen administered to males did not affect social cognitive performance, but affected vicarious emotional reactivity.

The effect of testosterone on dominance ratings should be interpreted with caution, because it was seen mainly at lower levels of facial dominance. It is possible that the strength of dominance features at higher levels caused the ratings to reach a ceiling, thus masking any potential differential impact of the testosterone manipulation. This possibility remains to be investigated in future research. Notwithstanding, our results are supportive of the notion that women with high levels of testosterone were more vigilant of facial cues signaling dominance. According to the model proposed by Oosterhof and Todorov (2008), faces high in dominance might also be perceived as low in submission and trustworthiness and subsequently of high threat value. Testosterone administration has been shown to decrease trustworthiness towards strangers (Bos, Terburg, & Van Honk, 2010), a finding that the authors relate to increased social vigilance. Our findings are also supportive of previous research showing that testosterone can increase cardiac response to angry faces in females (Van Honk et al., 2001), and increase attention towards fearful and angry faces in both females and males (Van Honk et al, 1999), suggesting that high testosterone levels can prepare the organism for approach and 'fight' behavior in the face of an encountered threat.

With regards to the more reflective mode of social cognition, attributing mental states to another person (mentalizing), our study replicated previous findings reported by Van Honk and colleagues (2011), showing that exogenous testosterone administration impaired female performance on the RMET. The RMET, which was initially developed for testing social cognitive abilities of individuals with autistic spectrum disorders, has been widely used to assess the ability to accurately attribute mental states to others (Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001). Previous research has related

fetal testosterone, another index of androgen levels, to impaired performance on the RMET and other social cognitive tasks, as well as to the development of autistic spectrum disorders (Baron-Cohen et al., 2011). The effects of testosterone administration in the present study taken together with earlier findings suggest an intriguing role of the testosterone in social interactions. According to this, individuals with high levels of testosterone might be more prone to engage in competitive and approach behavior, aided by the suppressed capacity to infer the competitors' mental states (Eisenegger et al., 2011). Unlike the study by van Honk et al. (2011), we did not find any relationship between the second-to-fourth digit ratio and RMTE scores. It is unclear whether this failure to replicate was due to procedural differences, sample characteristics, chance alone or a combination of factors. It should be noted that the current study contained a larger sample (n=33) than the van Honk study (n=16).

Social cognitive abilities do, however, only represent one aspect of the social tool-kit enabling us to successfully interact with, and learn from, each-other. Social *emotional* capacities, such as the ability to respond to others' emotional responses and learn from them, are of equal importance. In the present study, we found that the administration of estrogen in males resulted in an increased autonomic reactivity to another individual's expression of distress. This effect was unlikely to be an effect of a generalized arousal, because the SCR to the presentation of the CS did not interact with hormonal manipulation or gender during the two (observational and test) phases. Rather, this increased reactivity is likely to result from an enhanced sensitivity to others' emotional expressions, an ability linked to the affective aspects of empathy (Singer et al., 2006). Previous studies on the impact of estrogen on responsiveness to emotional expressions

have revealed conflicting results. For example, high estrogen levels across the menstrual cycle have been shown to both enhance (Pearson & Lewis, 2005) and attenuate (Derntl et al., 2008) female ability to recognize static facial emotional expressions. However, conflicting results in previous studies might have been due to the interaction between estrogen and progesterone in free cycling females. In our study, we administered estrogen to males. Another important difference between previous studies and our experimental paradigm is our use of moving images of another individual's anticipation and exposure to aversive stimulation, containing a higher degree of naturalism. Related to this, it is also possible that the exaggerated emotional reaction of estrogen treated male participants in our study was a function of the participants' anticipation of being in the same situation themselves at a later time. An alternative, non-exclusive, explanation is that estrogen facilitated emotional learning. Indeed, earlier research in human and non-human primates suggests that the learning model's distress functions as a 'social' US (Mineka & Cook, 1993; Olsson & Phelps, 2007), and studies in rodents have shown an enhancing role of estrogen on social emotional learning in female mice (there were no comparable data from male rodents) (Choleris et al., 2012). This latter alternative is, however, less likely to explain the present findings considering that estrogen did not affect the expression of learned fear during the subsequent test phase. The conflicting results in the past research on estrogen in social and emotional tasks, underscore the importance of replicating the present findings.

An important question arising from the present results relates to the mechanisms through which testosterone and estrogen exert their effects on social cognitive and emotional capacities. A substantial body of brain imaging studies examining the impact

of testosterone has demonstrated that this hormone modulates both subcortical and cortical brain regions (van Wingen et al., 2011). Both acute exogenous administration and measurement of endogenous levels of testosterone have been associated with increased amygdala activity during detection of salient stimuli (Bos, Panksepp, Bluthé, & van Honk, 2012), such as angry and fearful faces (Derntl et al., 2009; Hermans, Ramsey, & van Honk, 2008), as well as a functional decoupling between the amygdala and the orbitofrontal cortex (OFC) (van Wingen, Mattern, Verkes, Buitelaar, & Fernández, 2010). The amygdala is a core structure in the brain's emotional circuitry and crucial for the detection and processing of salient negative stimuli, and damage to this brain area is related to decrements in social cognitive abilities (Adolphs, 2003), including performance on the RMET (Shaw et al., 2005). The OFC has been associated with capacities underlying social cognition, such as decision making, processing of emotional information and updating of the effective value of potential outcomes (Amodio and Frith, 2006). It is thus plausible that high testosterone levels alter expressions of emotionally and impulsively driven behaviors by decreasing the regulatory control that OFC exerts over the amygdala.

Similarly, estrogen could exert its effect on social emotional behavior by mediating the activity of networks in the brain including the amygdala, OFC, and cingulate that have been implicated in previous studies of natural fluctuation during the menstrual cycle (Goldstein et al., 2005). However, to date, research on acute administration of estrogen in relation to social emotional behavior has been lacking.

In sum, the present study revealed an effect of testosterone treatment in females on social-cognitive processing, as indexed by ratings of facial dominance and the RMET.

In addition we showed an effect of estrogen on emotional reactivity to another individual's distress in an observational fear learning paradigm. These social-cognitive and emotional functions of the social tool-kit are likely to be highly interconnected despite their unique features. Indeed, processing of social information almost always have an emotional flavor to it (Olsson & Ochsner, 2008). The conceptual dissociation between the effects of testosterone and estrogen in our study suggests that these hormones could at least partially tap different functional systems. This suggestion is, however, based on the assumption that men and women respond similarly to estrogen and testosterone, which was not tested in the present study. Another methodological consideration is the fact that the hormonal gel was applied ten hours before testing, an interval that, according to recent suggestions (Eisenegger et al., 2013), might cause the levels of hormone to peak before the time of the tests. Although the time of experimental testing was possibly beyond the peak, the levels of testosterone and estrogen significantly increased in our participants as measured by the pre versus post hormonal levels, indicating that the observed effects were hormonally influenced. This is in agreement with previous research showing that application of hormonal gel leads to high hormonal levels, which stay constant for more than 10 hours (Slater et al., 2001).

Our findings are consistent with the observation that testosterone in males has been associated with autistic spectrum disorders (Baron-Cohen, 2005), critically involving dysfunctional social cognition, and higher levels of aggression and risk taking behavior (Holden, 2005). Women are more prone to develop affective disorders with aberrant social emotional reactivity (i.e. depression, generalized anxiety disorder, and phobias, McLean & Anderson, 2009). Sex hormones constitute, however, only one of

many factors predisposing individuals into developing social cognitive and emotional dysfunctions. More studies are needed to further specify the roles of testosterone and estrogen on social cognitive and emotional behavior. To this aim, it will be instrumental to use neuroimaging techniques in combination with the administration of both testosterone and estrogen to men and women.

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Figures

Figure 1. Procedure of the Observational Fear Learning (OL) task. During the observational phase (A), the participant watched a short movie depicting the learning model being submitted to a fear conditioning procedure. In three out of five trials, the presentation of the conditioned stimulus (CS+) co-terminated with the administration of an unpleasant shock (US) to the wrist of the learning model. No shocks were delivered to the participant. During the subsequent test phase (B), the participant was presented with the same CSs, but in a different order, as the learning model in the movie. No shocks were delivered to the participant.
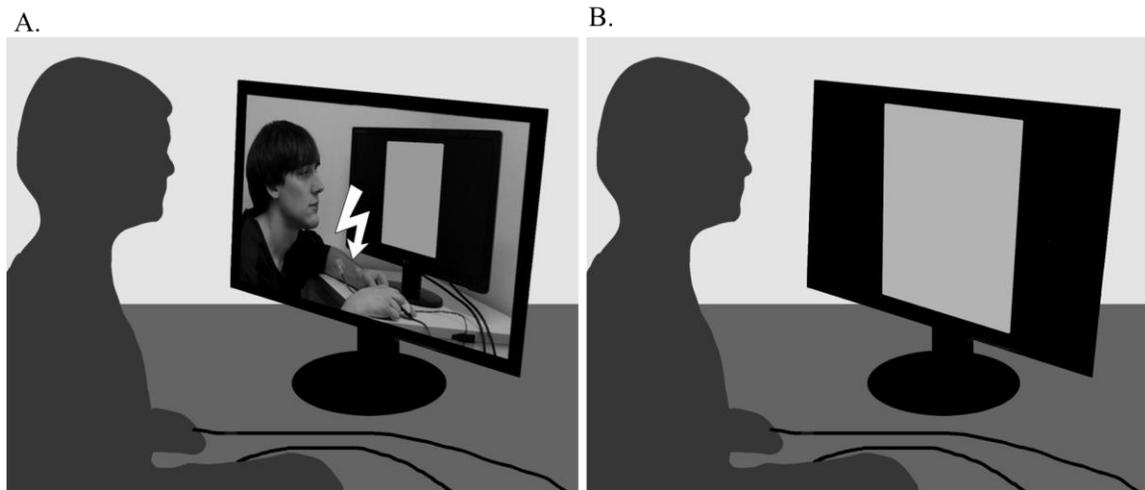

A.

B.

Figure 2. Women receiving a single administration of testosterone rated facial stimuli as more dominant than women receiving placebo. This effect was mostly pronounced at the two lowest levels (0 and 1) of facial dominance.
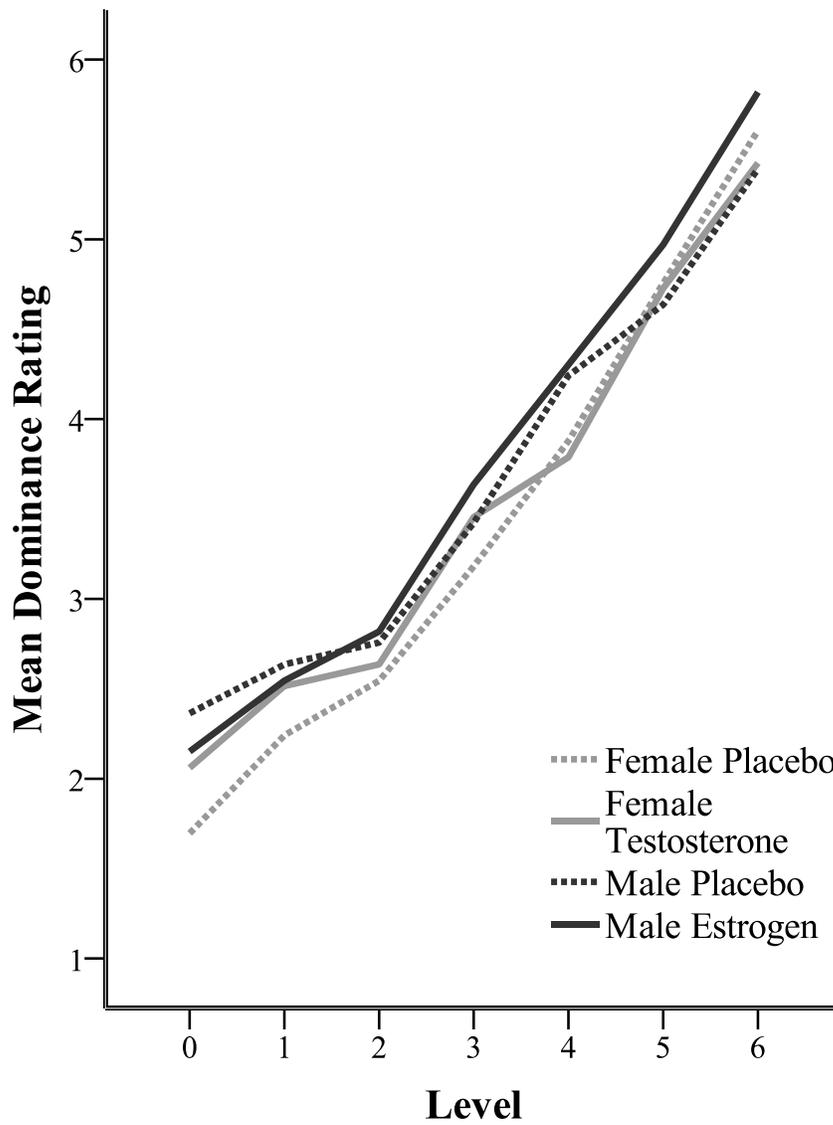
Figure 3. Female participants made more errors in the RMET after receiving testosterone versus placebo (p<0.05). Hormonal treatment did not have any effect on performance in males (p= 0.895). * p<0.05
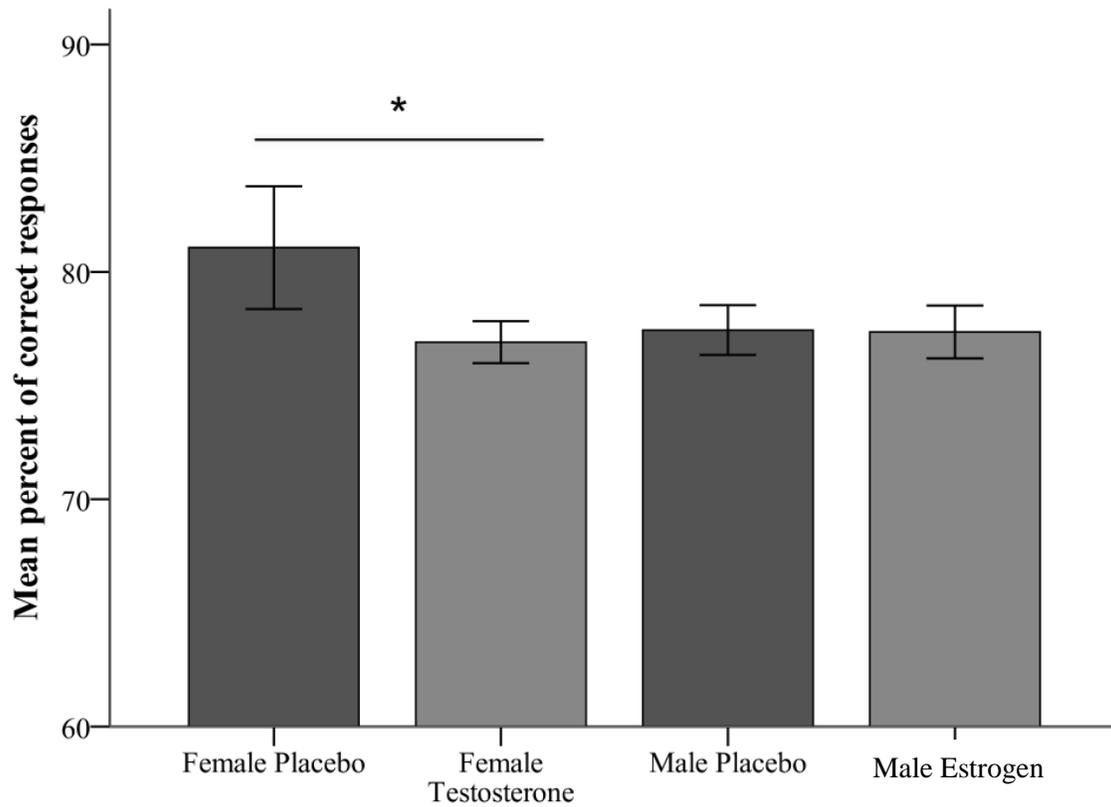
Figure 4. The mean SCR (microSiemens, μS) across gender was larger to the CS+ versus the CS-, indicating successful learning. * p<0.05
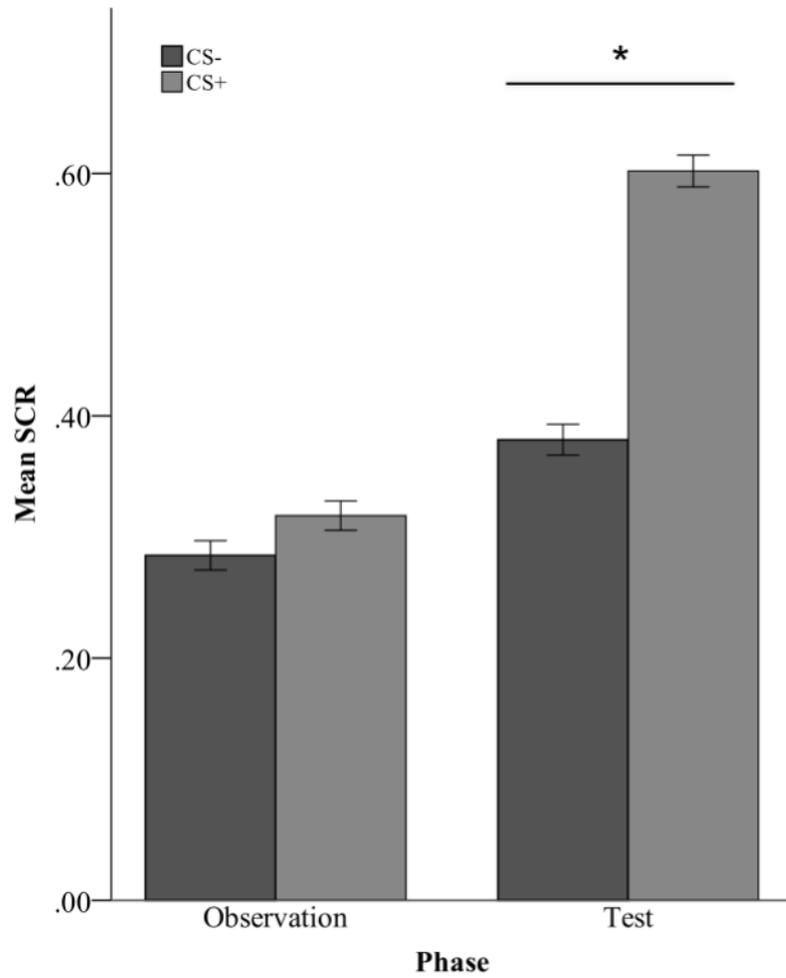
Figure 5. Males receiving estrogen as compared to placebo displayed a greater SCR (microSiemens, µS) when watching the learning model's distressed response to receiving a shock. Testosterone administration in women did not have any significant effects.
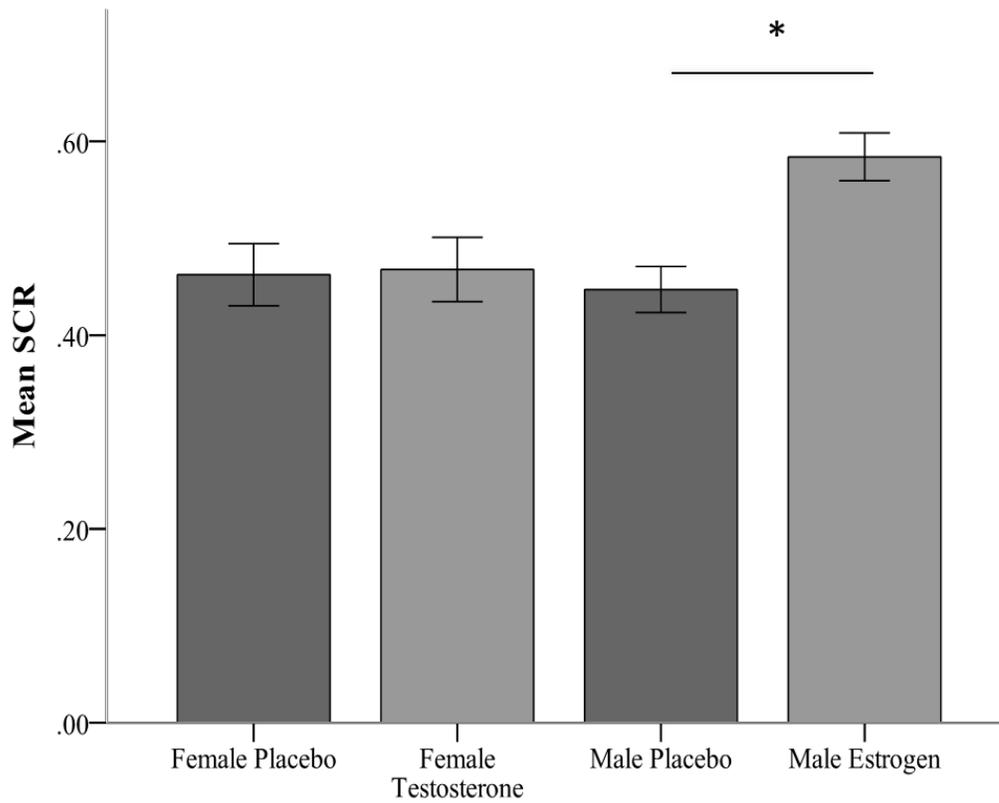
Table 1. Pearson correlations between the main dependent variables in the placebo condition. Correlations for female participants are given above the diagonal and correlations for male participants below the diagonal.

	1	2	3	4	5	6	7
1.Domi	-	.018	-.246	-.015	-.095	-.130	-.047
2.RMET	.044	-	-.400*	-.204	-.253	.028	-.099
3.Shock	.067	.092	-	.575**	.713**	.543**	.415*
4.Obs-	.187	.230	.514*	-	.892**	.697**	.298
5.Obs+	.047	.064	.784**	.604**	-	.665**	.422*
6.Test-	.082	.089	.619**	.500*	.614**	-	.536**
7.Test+	.012	.215	.357	.125	.327	.436*	-

*$p < .05$; ** $p < .01$

Table 2. Pearson correlations between the main dependent variables in the hormone treatment condition. Correlations for female participants are given above the diagonal and correlations for male participants below the diagonal.

	1	2	3	4	5	6	7
1.Domi	-	-.087	.067	-.154	.149	.028	.355*
2.RMET	-.166	-	.354	.080	.144	-.021	.223
3.Shock	.273	-.031	-	.612**	.815**	.130	.044
4.Obs-	.150	-.443*	.235	-	.800**	.533**	-.327
5.Obs+	.266	-.430*	.663**	.676**	-	.414*	.014
6.Test-	.312	-.129	.076	.720**	.420*	-	-.005
7.Test+	.284	.188	.301	-.158	.149	.015	-

*$p < .05$; ** $p < .01$