

Effects of Intranasal Oxytocin on Steroid Hormones in Men and Women

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Key Words

Intranasal oxytocin · Humans · Men · Women · Salivary hormones · Cortisol · Progesterone · Testosterone · Hypothalamic-pituitary-adrenal axis · Hypothalamic-pituitary-gonadal axis

Abstract

Background: Recent interest in the social and cognitive effects of intranasal oxytocin prompts a need for understanding its physiological effects in humans. Few studies have examined the effects of intranasal oxytocin on steroid hormones. Filling this gap is especially important given the evidence that steroid hormones participate in some of the same behavioral functions as oxytocin, e.g. in stress, processing of emotional stimuli, aggression, trust, empathy, and parental care. **Methods:** In randomized, double-blind experiments, we administered oxytocin (24 IU) or saline placebo to 97 healthy participants. Saliva samples were collected before and at several time points after the oxytocin/placebo administration to assess the levels of cortisol, progesterone, and testosterone. **Results:** Oxytocin had no effects on testosterone, progesterone, or cortisol in women or men. **Conclusion:** Acute intranasal oxytocin does not affect the levels of cortisol, testosterone or progesterone in humans, at least in the absence of a stressful context. These data suggest that acute oxytocin does not have a direct impact on the human hypothalamic-pituitary-adrenal or hypothalamic-pituitary-gonadal axes under nonstressful circumstances. This knowl-

edge helps rule out potential mechanisms for some of the effects of oxytocin in humans and adds to the generally limited body of knowledge on the basic physiological or psychological effects of intranasal oxytocin in human beings.

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Introduction

The peptide hormone oxytocin plays important roles in mammalian social behavior, including maternal care, pair bonding, and social memory [1–3]. Recently, there has been interest in the effects of oxytocin on emotional processing, social cognition, and social behavior in humans. This research is made possible by the use of a nasal spray which allows a noninvasive experimental manipulation of oxytocin. Increases in cerebrospinal fluid are observed after the nasal administration of oxytocin and related peptides [5–7], and oxytocin accumulation in brain sites relevant to emotion and memory was observed after nasal administration to laboratory animals [8]. There are several possible routes by which this peptide could directly or indirectly affect the human brain after nasal administration [9, 7]. Whether direct or indirect, acute intranasal oxytocin does exert effects on the brain: many effects of oxytocin have been described on behavior, cognition, and physiology, including changes in neural activation in functional magnetic resonance imaging studies [10–13; for reviews see 14–16].

One topic which has not been adequately explored is the effect of oxytocin manipulation on steroid hormones. There are several reasons to elucidate any such effects. Steroid hormones participate in many of the emotional and social behaviors of interest to psychologists studying oxytocin, including stress response [17–19], processing of emotional stimuli [20–25], dominance and aggression [26–29], and pro-social behaviors such as trust and empathy [30–32]. For example, several studies have investigated the effects of acute intranasal oxytocin on attention to, memory for, and the ability to recognize emotional facial expressions [33–37]. Interestingly, the acute administration of testosterone or cortisol has also been shown to impact the processing of emotional face stimuli [24, 38, 39]. In two studies, testosterone administration affected empathy-related responses to emotional facial expressions, which is relevant to the theorized effects of oxytocin [31, 40]. Testosterone has also been implicated in trust [30], relationship or marital status [41], and in human parental behavior and closeness [42–44] – behaviors that also are associated with oxytocin [45–48]. In fact, researchers have emphasized the importance of a joint consideration of neuropeptides like oxytocin and steroids in understanding biological contributors to human social behavior [49].

Oxytocin might have direct effects on brain regions responsible for social behavior and/or the cognitive processing of social stimuli, but it is also possible that oxytocin exerts some of its effects in part by impacting other hormonal systems. This could be achieved through effects on brain systems that control hormone production (e.g. hypothalamus) and/or effects on glands in the periphery: intranasal oxytocin seems to cause dramatic increases of oxytocin in the bloodstream (i.e. periphery) as well as in the brain [7], making it theoretically possible that oxytocin could act on peripheral hormone-producing glands. Either way, if oxytocin impacts the steroid hormone levels, this could help explain the observed effects of oxytocin. This makes it important to investigate the effects of oxytocin on steroid hormones.

There is reason to suspect that oxytocin could impact the levels of the steroid hormone cortisol. Oxytocin seems to have anxiety- or stress-reducing properties in animals [50–52] and may also have such effects in humans, as shown by a reduction in cortisol responses to stressful situations [53–55], although others have found that this effect depends on individual differences [56, 57]. Importantly, oxytocin may affect cortisol in humans in the absence of subjective mood or anxiety changes: intranasal oxytocin does not seem to cause any changes in self-re-

ported mood or affect compared with placebo; in fact, participants guess at chance levels whether they received oxytocin or placebo [58]. Also, it is unknown whether oxytocin reduces cortisol in these studies by directly affecting the hypothalamic-pituitary-adrenal (HPA) axis, which is responsible for cortisol synthesis, or by cognitive pathways such as an affecting construal of the stressful situation. Few studies have reported on the effects of oxytocin on cortisol at rest – i.e. in a nonstressful situation – which would help elucidate whether oxytocin decreases the HPA axis activity across the board. Thus, one goal of the present study was to investigate the effect of acute oxytocin administration on cortisol levels in the absence of overt stress.

Progesterone is another steroid hormone that has received recent attention for potential roles in human social behavior, in particular social affiliation and rejection [59–63]. Given the role of oxytocin in mammalian social behavior, some have speculated that oxytocin may actually exert prosocial effects in part by increasing the progesterone levels [59–61]; this idea is based in part on *in vitro* evidence that oxytocin application causes an increased release of progesterone from (bovine) ovarian tissue [64]. However, whether this effect also occurs *in vivo* in human beings is not known. Investigating the effect of oxytocin on progesterone was therefore a second goal of the present study.

Few studies have investigated the effects of oxytocin manipulation on the levels of progesterone or testosterone in humans [11, 65, 66]. Although Gossen et al. [65] found suggestive evidence of an increase in testosterone and no change in progesterone, the study was underpowered with only 8 men included. Weisman et al. [66] found that oxytocin caused an increase in the testosterone levels in fathers of infants, in the context of a structured father-infant interaction, but this hormonal response might be specific to fathers of infants. Domes et al. [11] found no change in progesterone after oxytocin administration in women, but only 2 samples were collected (1 before and 1 after oxytocin), and hormonal measurement was not the main goal of the study. With the present study, we aimed to investigate the effect of oxytocin on testosterone levels in both men and women, apart from a parent-child interaction.

In summary, information is needed on whether oxytocin impacts cortisol, progesterone, and testosterone in humans of both sexes. We addressed this need by measuring steroid hormones in saliva samples collected repeatedly before and after the acute administration of intranasal oxytocin or placebo. We chose a dose of oxytocin that

is most commonly used in the literature and has been associated with changes in trust [47], stress responses [54, 56], emotional processing [34, 35], and other behavioral and physiological measures in humans. Discovering the effects of oxytocin on steroid hormones in humans will not only address the gaps in the literature discussed above and elucidate the effects of oxytocin on peripheral physiology, but may also be useful for understanding many of the interesting and complicated effects of oxytocin on human social behavior.

Method

Study 1: Men

Our study used a parallel-group, double-blind, placebo-controlled design. Forty-six college-aged men were recruited from the University of Notre Dame community between July and December 2012. The exclusion criteria included current psychological disorders, major health problems, or use of psychiatric medications. All study procedures had prior approval by the Notre Dame Institutional Review Board. All participants provided their informed consent and were compensated 10 USD/h for their participation. Two participants were excluded for incomplete cortisol data, 3 for incomplete progesterone data, and 4 for incomplete testosterone data, leaving sample sizes of 44, 43, and 42 participants, respectively.

All study sessions began between 15:00 and 16:00 to control for potential circadian/diurnal effects in the impact of oxytocin. The participants were asked to avoid eating, vigorous exercise, caffeine, or brushing their teeth for at least 2 h prior to their study time. Due to uncertainty about possible pharmacological interactions with oxytocin, the participants were also asked to refrain from drinking alcohol and taking recreational or over-the-counter drugs from 48 h before the study session until 2 days later.

Following their consent, the participants provided a baseline saliva sample (sample No. 1, ~25 min before drug administration) and completed questionnaires to assess baseline mood/affect using the Positive and Negative Affect Scale questionnaire (PANAS [67]) and a symptom checklist to assess the potential side effects of intranasal treatment (e.g. headache, runny nose, dry mouth, elation, anxiety [58]). Scale reliabilities for the PANAS have been reported as Cronbach's $\alpha = 0.89$ for positive affect (PA) and 0.85 for negative affect (NA) [68]. The participants were then fitted with sensors for psychophysiology recording to test different hypotheses. Next, they were escorted to a private room with a sealed opaque box containing the treatment bottle and detailed instructions, a stopwatch, and a box of tissues. The participants self-administered 3 puffs/nostril (24 IU) of synthetic oxytocin (Syntocinon, Novartis, Basel, Switzerland) or a placebo saline solution (Salinex, Muro Pharmaceutical Inc., Tewksbury, Mass., USA).¹ Any labeling identifying the solution inside the bottle had been removed prior to the experiment. Experimenters never viewed the contents of the box to help ensure that the administration was double-blind. Following the oxytocin/placebo administration, the participants rested while initial physiology data was collected and then completed a second PANAS as well as providing 2 saliva samples (45 and 70 min after

the administration, samples No. 2 and 3) for later steroid hormone measurement. Next, they completed 2 cognitive tasks for purposes not related to the present report: a picture-memory task and a working memory (n-back) task in randomized order. They provided 2 additional saliva samples (95 and 120 min after the administration, samples No. 4 and 5), between/after the tasks and completed PANAS questionnaires between and after the tasks, completing the symptom checklist again as part of two posttreatment questionnaire packets.

To evaluate the efficacy of the double-blinding, the study experimenters were asked to report which condition they thought participants were in, and why, at the end of each study session. Along with other questionnaires, the participants also reported which condition they believed they were in. The experimenters and participants could respond 'oxytocin', 'control', or 'not sure'.

Study 2: Women

Sixty-two college-aged women were recruited from the University of Notre Dame community.² The exclusion criteria included use of hormonal contraceptives, currently pregnant or nursing, a history of being pregnant, current psychological disorders, major health problems, or the use of psychiatric medications. All study procedures had prior approval by the Notre Dame Institutional Review Board. All participants provided their informed consent and were compensated 10 USD/h for their participation. Six participants were excluded for incomplete cortisol data, 7 for progesterone, and 4 for testosterone, leaving samples of 56, 55, and 58 participants, respectively.

All study sessions began at 16:00 to control for potential circadian/diurnal effects in the impact of oxytocin. The participants were scheduled to complete the study in the early follicular phase (i.e. days 1–6) of the menstrual cycle during both study sessions. As in Study 1, the participants were asked to avoid eating, vigorous exercise, caffeine, or brushing their teeth for at least 2 h prior to their study time. The participants were also asked to refrain from drinking alcohol and taking recreational or over-the-counter drugs from 48 h before the study session until 2 days later.

Following their consent, the participants were directed to the restroom to complete a pregnancy test. After showing a negative result on the test, they provided a baseline saliva sample (~5 min before drug administration; sample No. 1) and completed the PANAS and other questionnaires as well as a symptom checklist. Next, they were given detailed verbal and written instructions to self-administer a single dose (3 puffs/nostril; 24 IU total) of intranasal oxytocin or saline placebo (see Study 1 for instruction details). As in Study 1, the drug assignment was double-blind. Fol-

¹ The participants were first instructed to prime the pump and to blow their nose to clear it. For each spray, they were instructed to keep their head upright, hold 1 nostril closed and insert the nozzle straight up in the other nostril, administer 1 spray, inhale through the open nostril and exhale through the mouth. The participants were instructed to give 1 spray to each nostril, wait 30 s, then give a second set of sprays, wait 30 s, and then give a third set of sprays, for 6 sprays total.

² An initial 42 women were recruited and completed the study from November 2011 to March 2012. In the original sample, there appeared to be a marginal effect of oxytocin to decrease testosterone at time point No. 4; we therefore collected data from an additional 20 women from June to December 2013 in order to increase the statistical power.

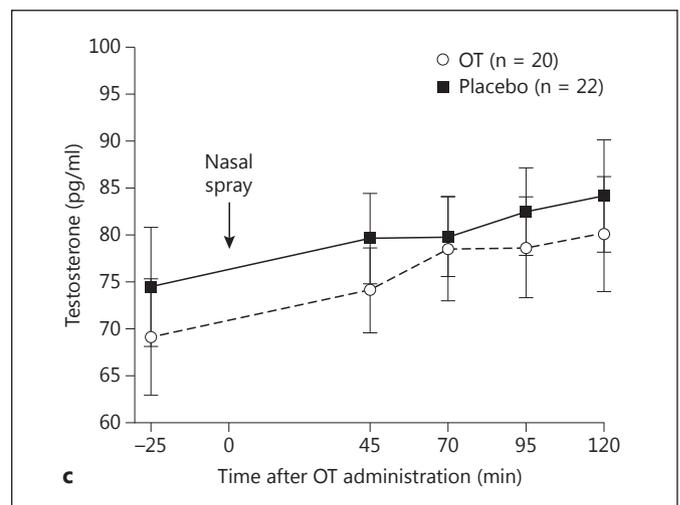
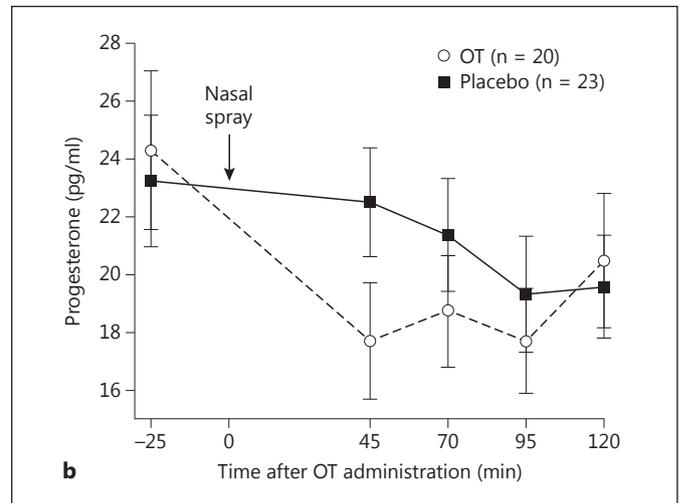
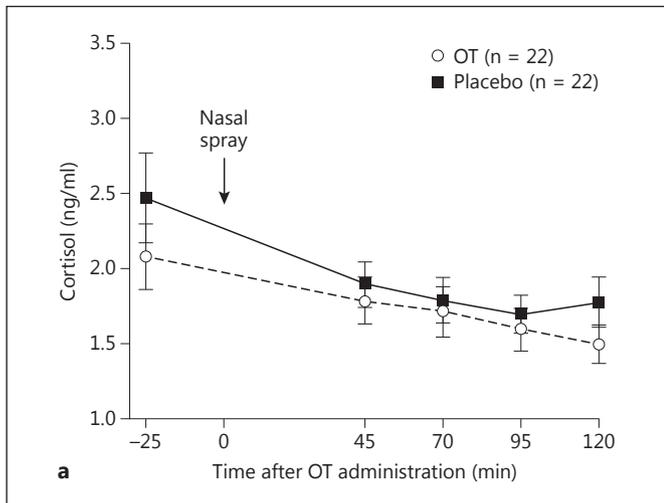


Fig. 1. Men's salivary cortisol (a), progesterone (b), and testosterone (c) by treatment condition. Oxytocin (OT) = circles/broken lines; placebo = squares/solid lines. The error bars show standard errors of the mean. In post hoc tests, the two groups did not significantly differ on any time point for any hormone.

Following the oxytocin or placebo administration, the participants completed additional PANAS questionnaires and provided 2 saliva samples (40 and 65 min after the administration, samples No. 2 and 3). They then completed the 2 cognitive tasks used in Study 1, provided 2 additional saliva samples (90 and 115 min after the administration, samples No. 4 and 5), completed PANAS questionnaires between and after the tasks, and completed the symptom checklist again.

As in Study 1, the experimenters were asked to report which condition they thought participants were in, and why, at the end of each study session. The participants also reported which condition they believed they were in. Possible responses included 'oxytocin', 'control', or 'not sure'.

Saliva Collection and Hormone Analysis

All saliva samples were collected without stimulants (i.e. passive drool) in sterile 15-ml polypropylene tubes. The tubes were immediately capped and frozen at -20°C , and within 2 weeks the samples underwent 2 freeze-thaw cycles to break up mucopolysaccharides, followed by centrifugation and transfer of the super-

natant to fresh tubes for freezer storage (again at -20°C) prior to analysis. The samples were then assayed using DPC Coat-A-Count radioimmunoassay kits (Siemens Healthcare Diagnostics, Athens, Ga., USA) with I^{125} and incubation overnight at room temperature. The kit standards were water diluted to match the ranges of hormones typical in saliva and were measured in triplicate. For cortisol, the standards (in addition to a zero standard) ranged from 0.5 to 50 ng/ml; for progesterone, from 1.5 to 400 pg/ml; and for testosterone, from 1 to 500 pg/ml. The samples were assayed in duplicate along with pools and external controls. Across the 6 assays/hormone, lower limits of detection ($B_0 - 3 \times \text{SD}$ method) averaged 0.12 ng/ml for cortisol, 3.96 pg/ml (i.e. 0.00396 ng/ml) for progesterone, and 3.03 pg/ml (0.00303 ng/ml) for testosterone. Samples with coefficients of variation $>60\%$ were removed from the analysis. The intra-assay coefficients of variation calculated on the samples averaged 10.1% for cortisol, 15.5% for progesterone, and 11.4% for testosterone. Note that higher coefficients of variation are typical for progesterone and testosterone versus cortisol, as the concentrations of these hormones are much lower.

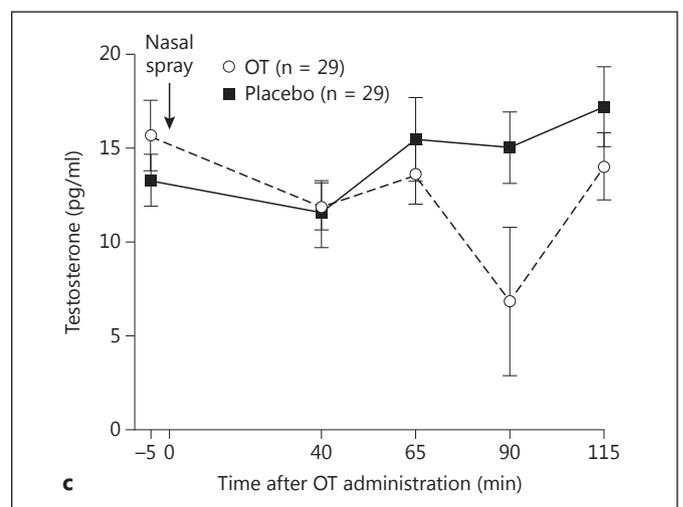
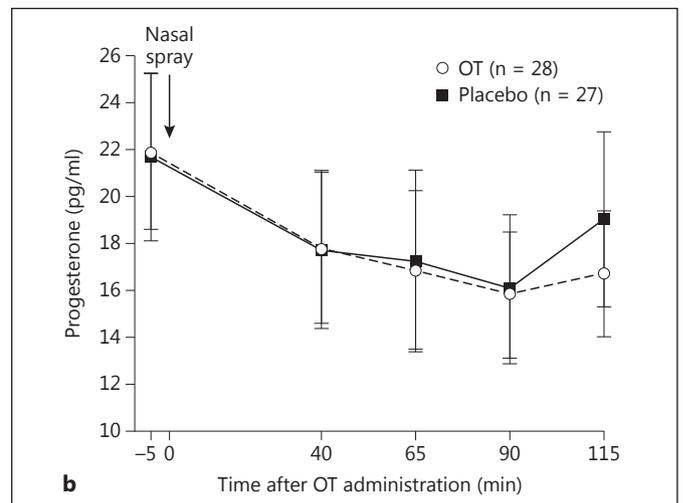
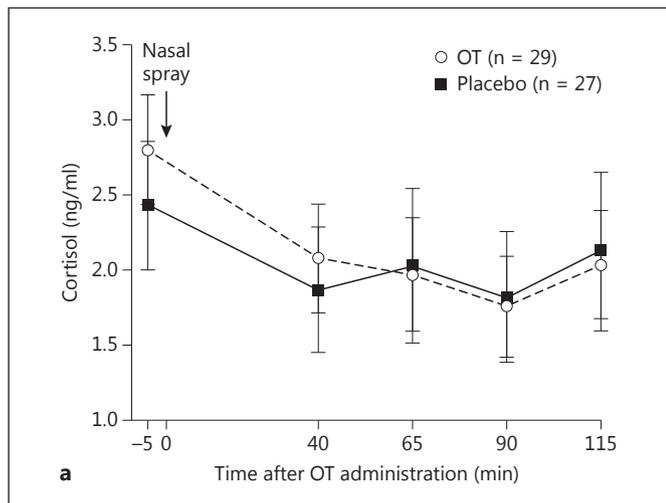


Fig. 2. Women's salivary cortisol (a), progesterone (b), and testosterone (c) by treatment condition. Oxytocin (OT) = circles/broken lines; placebo = squares/solid lines. The error bars show standard errors of the mean. In post hoc tests, the two groups did not significantly differ on any time point for any hormone.

Results

Symptoms, Mood, and Blinding

In both studies, crosstabulation analysis indicated that there were no differences between the number of people who endorsed any of the symptoms on the symptoms checklist in the oxytocin versus the placebo groups (i.e. effects of condition), nor in the oxytocin group from the preoxytocin to the postoxytocin checklist (effects of Time; all $p > 0.05$).

In mixed ANOVAs on the PANAS PA and NA scores, with the factors Time (pre- and several posttreatment measurements) and Condition (oxytocin vs. placebo), there were no effects of oxytocin treatment, as evidenced by no significant Condition \times Time interactions and no main effect of Condition for men [PA, Condition: $F(1, 43) = 3.51$,

$p = 0.07$, partial $\eta^2 = 0.08$; Time \times Condition: $F(5, 39) = 0.21$, $p = 0.96$, partial $\eta^2 = 0.03$; NA, Condition: $F(1, 43) = 1.657$, $p = 0.04$, partial $\eta^2 = 0.00$; Time \times Condition: $F(5, 39) = 0.93$, $p = 0.47$, partial $\eta^2 = 0.11$] nor for women [PA, Condition: $F(1, 55) = 0.01$, $p = 0.94$, partial $\eta^2 = 0.00$; Time \times Condition: $F(5, 51) = 1.01$, $p = 0.41$, partial $\eta^2 = 0.02$; NA, Condition: $F(1, 55) = 10.29$, $p = 0.68$, partial $\eta^2 = 0.00$; Time \times Condition: $F(5, 51) = 2.33$, $p = 0.06$, partial $\eta^2 = 0.19$]. Thus, oxytocin had no effects on PA or NA in either study. There were main effects of Time in both PA and NA for men [PA, $F(5, 39) = 6.87$, $p = 0.00$, partial $\eta^2 = 0.47$; NA, $F(5, 39) = 2.92$, $p = 0.03$, partial $\eta^2 = 0.27$] and for women [PA, $F(5, 51) = 16.99$, $p = 0.00$, partial $\eta^2 = 0.63$; NA, $F(5, 51) = 16.00$, $p = 0.00$, partial $\eta^2 = 0.30$], indicating that both PA and NA decreased over the course of the study session equivalently for oxytocin and placebo participants.

Crosstabulation analyses indicated that, in each study, there was no relationship between the participants' actual and perceived condition; i.e. the participants were unable to guess which treatment they had received [men: $\chi^2(2, 45) = 2.298, p = 0.32, \phi = 0.23$; women: $\chi^2(2, 56) = 1.28, p = 0.53, \phi = 0.15$]. The same held for the experimenters' guesses [men: $\chi^2(1, 45) = 1.84, p = 0.18, \phi = 0.20$; women: $\chi^2(1, 60) = 0.00, p = 1.00, \phi = 0.00$], demonstrating that the double-blinding was effective in both studies.

Steroid Hormones

To address the main goals of the studies, 6 separate mixed ANOVAs – 1 for cortisol, progesterone, and testosterone for each study – were conducted on steroid hormone concentrations in the 5 samples across the study session, with between-subjects factor Condition (oxytocin or placebo) and within-subjects factor Time (across the 5 samples). There were no main effects of Condition, nor were there Condition \times Time interactions, in any of the 3 ANOVAs for men [cortisol, Time \times Condition: $F(4, 39) = 0.54, p = 0.71, \text{partial } \eta^2 = 0.05$; Condition: $F(1, 42) = 1.89, p = 0.18, \text{partial } \eta^2 = 0.04$; progesterone, Time \times Condition: $F(4, 38) = 1.68, p = 0.18, \text{partial } \eta^2 = 0.15$; Condition: $F(1, 41) = 0.35, p = 0.56, \text{partial } \eta^2 = 0.01$; testosterone, Time \times Condition: $F(4, 37) = 0.35, p = 0.85, \text{partial } \eta^2 = 0.04$; Condition: $F(1, 40) = 0.33, p = 0.57, \text{partial } \eta^2 = 0.01$; fig. 1], nor were there any such effects for women [cortisol, Time \times Condition: $F(4, 51) = 0.63, p = 0.65, \text{partial } \eta^2 = 0.05$; Condition: $F(1, 54) = 0.19, p = 0.89, \text{partial } \eta^2 = 0.00$; progesterone, Time \times Condition: $F(4, 50) = 0.53, p = 0.72, \text{partial } \eta^2 = 0.04$; Condition: $F(1, 53) = 0.01, p = 0.91, \text{partial } \eta^2 = 0.00$; testosterone, Time \times Condition: $F(4, 52) = 0.84, p = 0.51, \text{partial } \eta^2 = 0.06$; Condition: $F(1, 55) = 1.29, p = 0.26, \text{partial } \eta^2 = 0.02$; fig. 2]. Thus, the oxytocin treatment did not affect salivary cortisol, progesterone, or testosterone in women and men.

In Study 1 (men), there were significant main effects of Time for cortisol [$F(4, 39) = 2.71, p = 0.04, \text{partial } \eta^2 = 0.22$] and progesterone [$F(4, 38) = 3.70, p = 0.01, \text{partial } \eta^2 = 0.28$], reflecting the normal circadian decline seen in these hormones over the course of the study sessions. Interestingly, there was also a marginal main effect of Time for testosterone, which appeared to increase over the course of the study session [$F(4, 37) = 2.28, p = 0.08, \text{partial } \eta^2 = 0.20$].

Similarly, in Study 2 (women), there were significant main effects of Time for cortisol [$F(4, 50) = 8.85, p < 0.001, \text{partial } \eta^2 = 0.41$] and progesterone [$F(4, 50) = 7.11, p < 0.0005, \text{partial } \eta^2 = 0.36$], and a marginal main effect of Time for testosterone [$F(4, 52) = 2.35, p = 0.07, \text{partial } \eta^2 = 0.15$].

Discussion

The present data address an important gap in current knowledge about the effects of oxytocin. Our data provide evidence that, at least in the absence of stress or an overt social situation, acute oxytocin manipulation exerts no effects on cortisol, progesterone, or testosterone levels in men or women.

There is currently disagreement over the mechanisms by which oxytocin manipulation causes its behavioral effects. For example, in a review, Campbell [16] identified 3 different possible pathways: (1) oxytocin directly boosts trust in others, (2) it enhances social cognition, or (3) it reduces fear and anxiety – which removes impediments against trusting others and other social approach behaviors. Although these 3 pathways are not mutually exclusive, many researchers adhere to one or another. For example, Churchland and Winkielman [4] argue that the effects of oxytocin are likely to be explained entirely by simpler mechanisms, such as anxiety reduction. On the other hand, Guastella and MacLeod [37] argue for a much more elaborate theory, i.e. that oxytocin enhances the detection and appraisal of emotion-related information from social cues, particularly so for information related to positive affect/emotion. Also of note, Bartz et al. [15] reviewed evidence that social context and individual differences dramatically change the effects of oxytocin, so that opposite (e.g. antisocial) effects are produced in some individuals or in some contexts (see also [57, 80]).

To help resolve these differing perspectives, research on whether oxytocin affects basic physiological and cognitive processes is essential. Our data have many implications for the field. First, these data help eliminate some potential pathways for oxytocin's social/behavioral effects. Our data suggest that acute, intranasal oxytocin does not have direct, across-the-board impacts on the HPA or hypothalamic-pituitary-gonadal axes in humans. This helps us begin to rule out some possible mechanisms. For example, if oxytocin caused testosterone reductions, this could be a potential mechanism by which oxytocin is associated with empathy and trust, two behaviors that are impacted by testosterone [30–32]. Also, if oxytocin had dramatic effects on the HPA axis, this would have to be reckoned with as a potential mediator of some of its social effects. Of course, ruling out changes in steroid hormone levels does not preclude the possibility that oxytocin exerts its effects through some other basic cognitive or physiological pathway.

Importantly, our data only speak to the acute administration of oxytocin; chronic or repeated administration

could produce different effects on steroid hormones. Our data also only indicate a lack of direct, physiological impact on the HPA and hypothalamic-pituitary-gonadal axes and/or a lack of impact of oxytocin on hormone levels in people at rest, without overt stress or a social situation. Decreasing NA across both studies suggests that neither the cognitive tasks nor any other parts of the experiment were stressful or negative for the participants. In contrast, Heinrichs et al. [54] found that oxytocin administration did cause a modest reduction in cortisol response to a potent laboratory stressor, the Trier Social Stress Test. Cardoso et al. [69] found even more modest, marginal effects of oxytocin on the cortisol response to exercise stress.³ Another study [57] found suggestive evidence that oxytocin modestly reduced resting cortisol, specifically in men without early parental separation, but the total sample size was only 19; the authors acknowledged that replication is needed.

Potentially, intranasal oxytocin could dampen the effects of *stress-induced* HPA axis function [54, 69], without affecting *resting* HPA axis function. This would suggest a top-down effect, e.g. an effect on emotion regulation, rather than a direct effect on hypothalamic CRH-producing neurons, pituitary ACTH production, and/or the adrenal glands. Future work assessing oxytocin receptor binding, etc. in humans will be needed to elucidate this. A similar pattern could also be true for other hormones; investigating the effects of oxytocin on stress-induced progesterone or testosterone, or on testosterone stimulated by competition [27], will be important future directions for this research. Similarly, as Bartz et al. [15] point out, social context seems to moderate the effects of oxytocin on social behavior. In our studies, the participants did interact with the experimenter throughout the study session, but this person was a stranger; the interaction was professional and presumably somewhat distant. It will be important to characterize whether the effects of oxytocin on steroid hormones are different in social contexts related to closeness or bonding.

Our finding of a lack of effect of oxytocin on progesterone levels is notable especially since there is growing evidence linking progesterone to affiliative behavior and/or social rejection in humans [for a review, see 63]. This has led some authors to point to the relationship between oxytocin and progesterone to help explain the prosocial effects of oxytocin and/or progesterone [60, 61]. This argument was based in part on evidence that oxytocin application causes a release of progesterone from bovine ovary tissue *in vitro* [64]. The present data suggest that this effect may not generalize to all mammals and/or does

not apply *in vivo*. In any case, our data help rule out the possibility that any of oxytocin's effects in humans occur via causing an increase in circulating progesterone levels.⁴ Our data also echo those of Domes et al. [11], who found no effect of 24 IU of intranasal oxytocin on plasma progesterone levels in 16 healthy adult women (progesterone measured 45 min following oxytocin administration).

Our finding of a lack of effect on testosterone levels contrasts with Gossen et al. [65] and Weisman et al. [66], who report evidence that oxytocin causes testosterone increases in men.⁵ Notably, however, the study by Gossen et al. [65], in only 8 men, was likely underpowered and stands the risk of Type I error. Weisman et al. [66], in contrast, collected data from a sample similar to ours in size. We can offer several possible explanations for the discrepancy between our findings and those of Weisman et al. [66]. First, Weisman et al. specifically studied new fathers interacting with their infants, whereas our study population was made up of younger men without children. It is possible that oxytocin-induced testosterone increases only occur in new fathers, or only occur in certain affiliative social contexts. Second, Weisman et al. [66] used Salivettes to collect saliva, and they acknowledge that limitations have been shown in accurate testosterone measurement using these devices versus passive drool [66, 73]. Finally, at pharmacological levels, oxytocin may activate the receptors of the structurally similar hormone arginine vasopressin [74, 75]. Receptors for oxytocin and vasopressin are highly homologous [76, 77]; the two peptides' receptors are not highly selective and may bind each other's ligands. Vasopressin levels are higher in male mammals and, along with testosterone, play important roles in mating, territoriality, and aggression in nonhuman animal species. It is currently unknown whether intranasal oxytocin at doses commonly used in human studies results in brain concentrations of central oxytocin

³ Although older findings suggested that oxytocin does dampen activity in the HPA axis at rest [70, 71], these studies were conducted with intravenous oxytocin, which does not cross the blood-brain barrier, complicating the interpretation of the data. Furthermore, other research from the same time period failed to replicate this effect [72].

⁴ This also raises the important issue that circulating (blood) oxytocin does not necessarily reflect the brain levels or activity in oxytocin neurons. It is currently an open question whether the cognitive and behavioral effects of intranasal oxytocin result from actions of oxytocin in the brain, the periphery, or both.

⁵ For comparison to the present findings, Gossen et al. [65] collected samples 5 min prior to and 30, 90, 150, and 210 min following oxytocin administration. Weisman et al. [66] collected samples immediately prior to and 40, 65, and 85 min following oxytocin administration.

high enough to cause vasopressin receptor binding. However, if this were the case, it is possible that intranasal oxytocin can exert effects on testosterone release via actions at the vasopressin receptors. Furthermore, this might occur in some studies and not others, since researchers are unable to control or even measure the amount of oxytocin that reaches the brain after nasal spray application (see below).

A limitation of our study, as in all studies utilizing intranasal oxytocin, is that we cannot be sure how much oxytocin reached the brain. There are several potential routes to the brain from the nasal mucosa [9]; very little research to date quantifies cerebrospinal fluid levels after intranasal oxytocin [7], and there could be individual differences in how much enters the brain and at what rate. Perhaps even more importantly, we currently lack tools to assess oxytocin receptor binding in the living human brain. Development of such tools, such as oxytocin radioligands for use in positron emission tomography imaging, will be crucial to understand the impact of oxytocin manipulation using nasal spray on the human brain and might help us understand the mixed pattern of findings on behavioral, cognitive, and physiological outcomes.

A second limitation of our study is that we risk making Type II error when arguing that oxytocin does not affect levels of cortisol, progesterone, or testosterone – as is always true when a case is made for null findings. This concern can be mitigated by considering that our study had a sufficient number of participants to detect a medium effect size (as found in the study by Weisman et al. [66]) with 95% power using the G*Power program [78]. However, if the true effect sizes in the population are in fact small [79], it would take as many as 186 participants to detect such effects with 95% power.

Another potential limitation of the study is that the interval between oxytocin administration and the first sample after oxytocin administration was 40–45 min.

Since there is evidence that plasma oxytocin increases within 15 min of intranasal administration [7], it is possible that there were very rapid effects of oxytocin on steroid hormones that we could not detect. Future studies should attempt to replicate our findings with more frequent sampling. However, our first sample after oxytocin administration does correspond with the time interval in most human behavioral studies of oxytocin nasal spray, so our findings are relevant to those behavioral effects.

In conclusion, our findings provide evidence that the acute administration of intranasal oxytocin, at the dose most commonly used to examine behavioral effects in humans, has no effect on the levels of the hormones cortisol, progesterone, or testosterone in women or men in the absence of stress or an overtly social context. To our knowledge, our study is the largest and most comprehensive to date (in terms of sampling frequency, number of hormones, and by including both sexes) to examine the effects of oxytocin on steroid hormones in humans. Future research should continue to investigate under what conditions, if any, oxytocin might exert effects on these hormones. Our data add an important piece of evidence as to the basic physiological effects of oxytocin and help rule out some potential mechanisms for observed effects of oxytocin in humans.

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