Psychoneuroendocrinology (2012) 37, 1582—1586

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SHORT COMMUNICATION

# Intranasal oxytocin administration is reflected in human saliva

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Received 18 January 2012; received in revised form 27 February 2012; accepted 27 February 2012

**KEYWORDS**
Intranasal oxytocin;
Salivary oxytocin;
ELISA;
Neuropeptide;
Administration paradigms

**Summary** Following the discovery that intranasal administration of neuropeptides can reach the central nervous system, a growing number of studies applied intranasal oxytocin (OT) paradigms to demonstrate the positive effects of OT on social and emotional processes. The three-step paradigm typically included: OT administration, a 45-min waiting period, and approximately 1-h period of active drug effects when experimental manipulations are applied. Yet, this schedule has not been put to systematic validation. Utilizing a double-blind placebo-control within-subject design, ten individuals were administered OT or placebo and salivary OT was measured ten times, at baseline and nine times over four consecutive hours. OT administration induced substantial increases in salivary OT across the entire period. OT rose dramatically 15 min after administration (from 6.9 pg/ml at baseline to 1265.4 pg/ml), reached plateau at 45—120 min (range = 131.6 and 105.3 pg/ml), and did not return to baseline by 4 h. Results contribute to discussion on brain-periphery coordination of OT and highlight the need for further research on the temporal dynamics and durations of OT administration effects.


## 1. Introduction

Since intranasal administration of neuropeptides has shown to cross the brain barrier and reach the central nervous system (Born et al., 2002), a growing number of studies utilized this experimental paradigm to explore the involvement of oxytocin (OT) in human behaviors, cognitions, and brain activation patterns (Heinrichs and Domes, 2008). In fact, a search for 'intranasal oxytocin' in PubMed reveals nearly 70 published studies dated from the last five years demonstrating the positive effects of OT on social and emotional processes in both healthy subjects and individuals diagnosed with a variety of psychiatric disorders. Generally, intranasal OT was found to enhance social functioning, including trust, empathy, and "theory-of-mind" (Macdonald and Macdonald, 2010), and its beneficial effects in cases of autism or schizophrenia suggest that OT may serve as a therapeutic agent in conditions associated with severe social dysfunction (Meyer-Lindenberg et al., 2011).

Despite the blooming research and promising results, very little is known about the effects of intranasal OT on the peripheral expression of OT or the timing of the effects. Typically (Meyer-Lindenberg et al., 2011), 24 IU of OT are administered intranasally, subjects wait approximately 40—45 min for the initiation of drug effects, and effects are estimated to last about 1 h, a period when experimental manipulations are conducted. Yet, while these schedules

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doi:10.1016/j.psyneuen.2012.02.014

have been readily adapted, further validation is clearly needed and little research examined the effects of OT administration on peripheral OT levels. Burri et al. (2008) found significant increases in plasma OT approximately 40 min after administration and these high levels were maintained for over an hour, findings which support preliminary data based on three male subjects (Landgraf, 1985). Systematic research assessing whether OT administration is expressed in peripheral OT levels, how soon peripheral effects are noted, and how long they last is therefore much in need.

The links between the expression of OT in brain and the periphery is an issue of continuous debate, although several researchers have suggested that the two are coordinated (Carter et al., 2007; Ross and Young, 2009). Because human research is unable to assess OT at the brain neurochemical level, research has generally been limited to peripheral measures which are considered proxies for brain OT. While no study has conclusively demonstrated links between central and peripheral OT activity in humans, some findings may point in this direction, albeit tentatively. First, studies in humans and non-human primates found correlations between peripheral OT and maternal behavior, particularly maternal touch (Maestripieri et al., 2009; Feldman et al., 2010). Such findings are consistent with those reported in rodents, which show associations between maternal touch and brain OT (Meaney, 2001). Taken together, these studies raise the possibility that central and peripheral activity of the OT system is connected through mechanisms that are not yet fully understood. In addition, risk alleles on the *OXTR* and *CD38* genes, an ectoenzime that mediates the release of OT from hypothalamic neurons to the bloodstream through the mobilization of calcium, were found to be associated with lower levels of plasma OT (Feldman et al., in press). Risk alleles on the *OXTR* are thought to index less optimal production of OT at the brain level and *CD38* knockout mice were found to show lower levels of plasma OT (Feldman et al., in press), and thus, these findings accord with the hypothesis that central and peripheral OT levels are inter-related. Alternations in peripheral OT in response to OT administration may thus be consistent with the hypothesis that manipulations of brain OT are inter-related with measurable changes in peripheral activity of the OT system.

As such, the current study had two goals. First, we examined whether OT administration would be reflected in peripheral assessments and salivary OT would increase following administration. Second, we assessed the dynamics of these effects over several hours. Utilizing a double-blind placebo-controlled design, salivary OT was measured ten times — at baseline and periodically over four consecutive hours. We expected salivary OT levels to markedly increase following administration and explored the trajectory of this increase over the 4-hour periodline.

## 2. Methods

### 2.1. Participants

Ten individuals (5 men, 5 women) participated in a randomized double-blind, placebo-control within-subject design. Participants' age averaged 28.25 years (SD = 4, range = 20.5—33.0) and all reported being healthy with no history of chronic mental or physical illness, medication intake, or smoking. Participants were instructed to abstain from food, caffeine, or beverage other than water 2 h prior to experiment. Since no behavioral measures or other factors were assessed in this study apart from salivary OT, we did not control for women's menstrual cycle or hormonal contraception. However, pregnant women or those trying to get pregnant were excluded. The study was approved by the Institutional Review Board and conducted according to ethical standards, and all participants signed an informed consent. Participants received gift vouchers for their participation.

### 2.2. Procedure

Following arrival, participants signed informed consent and provided the first (baseline) saliva sample. Immediately after, participants self-administered either drug — a single dose of intranasal OT including 24 IU, 3 puffs per nostril, each puff containing 4 IU (Syntocinon-Spray, Novartis, Basel, Switzerland)-or placebo. Each participant visited the lab twice, a week apart. Participants were encouraged to drink water between samples to produce sufficient saliva, and to eat a small meal just after the eighth sample (2 h) to prevent fatigue. It should be noted, however, that there are special warnings for prolonged use of excessive doses together with large volumes of fluid as this may lead to water intoxication with hyponatraemia. The rare adverse reaction is thought to be related to the close similarity in chemical structure between OT and the hormone vasopressin (for a review of safety issues related to human OT administration research, see MacDonald et al., 2011).

### 2.3. Salivary oxytocin collection and analysis

Saliva samples were collected using a Salivette (Sarstedt, Rommelsdorft, Germany). Ten samples at each session were collected: at baseline, and 15, 30, 45, 60, 80, 100, 120, 180, and 240 min following administration. Sessions were held between 1230 h and 1730 h, to accommodate diurnal variations in OT.

Salivettes were immediately stored at −20 °C to be centrifuged twice at 4 °C at 1500 × *g* for 15 min within two weeks. In the placebo condition, saliva were lyophilized over night to concentrate the samples by 3 or 4 times and kept at −20 °C until assayed. In the OT condition, samples were kept at −20 °C until assayed. The first sample in the OT condition (baseline; prior to administration) was treated as the other samples in the placebo condition.

Determination of salivary OT was performed using a 96-plate commercial OT-ELISA kit (ENZO, NY, USA), consistent with previous research (Gordon et al., 2010). Although the trustworthiness of OT measurement in saliva has been questioned in the past (Horvat-Gordon et al., 2005), more recent studies have shown that OT values are reliable when measured in saliva using enzyme immunoassay (e.g., White-Traut et al., 2009; Feldman et al., 2010). Measurements were performed in duplicate. Samples were diluted 1:5 in the assay buffer and treated according to kit's instructions. At the final step, the optical density of the samples and

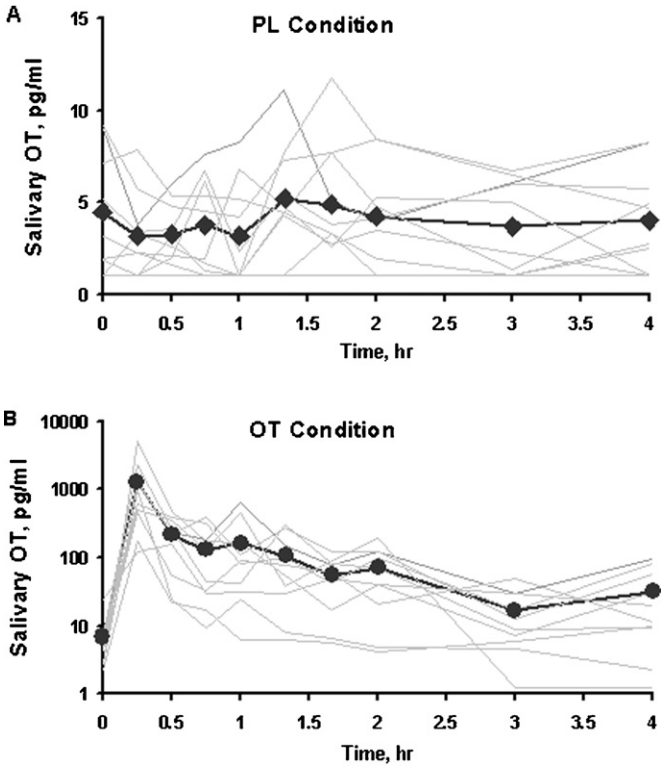


**Figure 1** Salivary oxytocin concentrations before and 4 h following administration in the (A) PL condition and (B) OT condition. The bold line is the average oxytocin level of the ten participants (gray lines).

standards was measured with a wave length of 405 and of 590 for additional correction. Sample concentrations were calculated by MatLab-7 according to relevant standard curve. The assay's reported intra-assay and inter-assay coefficients of variability are 12—19.1% and 5.2—14.5%, respectively. The intra-assay and inter-assay coefficient we received were $<$ 13.2% and 16.1%, respectively.

### 2.4. Statistical analysis

Repeated-measures ANOVA was conducted with drug (OT, PL) and time (10 samples) as within-subjects factor and measures. The Repeated polynomial was used to determine changes from one time-point to the next and parameters of the curve. Following, paired *t*-tests were computed to compare levels at each time-point between conditions.

## 3. Results

A significant main effect for condition was found, $F(1,9) = 14.30$, $p = .004$, $Eta^2 = .61$. Salivary OT levels were significantly higher (Mean = 207.17, SE = 53.77) in the OT condition compared to placebo (Mean = 4.01, SE = .66). Significant main effect was found for time, $F(9,81) = 6.42$, $p = .000$, $Eta^2 = .41$, which was mainly related to condition-by-time interaction, $F(9,81) = 6.45$, $p = .000$, $Eta^2 = .42$ (Fig. 1). Paired *t*-tests revealed no differences at baseline but significant differences at each of the follwoing assessments (Table 1).

Salivary OT increases in the OT condition were fit by linear, $F(1,9) = 7.63$, $p < .05$, $Eta^2 = .46$, and cubic, $F(1,9) = 10.57$, $p = .01$, $Eta^2 = .54$ trends, indicating that OT changed in non-linear ways. Significant differences emerged between baseline and second assessment, $F(1,9) = 7.24$, $p < .05$, $Eta^2 = .44$, second and third assessments, $F(1,9) = 5.71$, $p < .05$, $Eta^2 = .44$, and eighth and ninth assessments, $F(1,9) = 6.85$, $p < .05$, $Eta^2 = .43$. No linear, quadratic, or

**Table 1** Salivary OT levels at baseline and in multiple assessments following the administration of OT vs placebo.

| Time, min | Mean salivary oxytocin, pg/ml (SE) | | *T* |
|---|---|---|---|
| | OT condition | PL condition | |
| 0 (baseline) | 6.95 (1.99) | 4.11 (1.30) | NS |
| 15 min | 1265.46 (466.99) | 3.20 (.69) | 2.70* |
| 30 min | 221.09 (50.77) | 3.25 (.52) | 4.30** |
| 45 min | 131.59 (41.50) | 3.76 (.81) | 3.08** |
| 60 min (1 h) | 165.63 (67.12) | 3.24 (.86) | 2.43* |
| 80 min | 105.33 (34.01) | 5.18 (.95) | 2.99** |
| 100 min | 55.28 (12.17) | 4.92 (1.01) | 4.34** |
| 120 min (2 h) | 71.91 (19.62) | 4.27 (.82) | 3.52** |
| 180 min (3 h) | 16.55 (4.80) | 3.74 (.79) | 2.53* |
| 240 min (4 h) | 31.93 (10.75) | 4.06 (.88) | 2.74* |

* $p \leq .05$.
** $p \leq .01$.

cubic trends were found in the placebo condition, indicating no change over time. No gender effects were found and changes in OT were consistent across genders.

## 4. Discussion

The current findings are the first to demonstrate that intranasal administration of OT is reflected in human saliva. The repeated-measure design of ten samples over four consecutive hours enabled us to model the dynamics of OT expression in a human peripheral endocrine system following manipulation which is considered to affect brain OT. Results indicate that OT peaks already 15 min after administration, declines in the next 30 min, reaches plateau between 45 and 120 min, and further declines to levels higher than baseline. Four hours after administration salivary OT levels were still higher than baseline, suggesting that the system may still be actively responding to the drug. Although the mechanisms underlying this marked and lengthy increase are still unclear, it has been suggested that the OT system employs feed-forward mechanisms, as seen, for instance, in human lactation. Overall, the findings may be consistent with perspectives contending that OT in brain and periphery are coordinated (Ross and Young, 2009), by showing that inhaling OT resulted in marked increases in peripheral levels. However, since OT in CSF was not assessed and we did not measure brain OT, this hypothesis should be treated with caution and much further research is required to detail the mechanisms through which changes in brain OT are expressed in various peripheral systems. It is important to note that since the effects of OT administration on the human brain have not been studied in depth, it is unclear how administration impacts OT receptors. From a kinetic viewpoint, it may take only a few seconds for the increase in brain OT to be released into the periphery, as is the case during labor, and the latency to the OT effects on various behaviors and cognitions may differ. Much further research is thus needed to more fully evaluate how alterations in brain OT are expressed in the periphery and the current findings may provide a first step.

From an experimental viewpoint, our findings call to further examine the ''golden standard'' of intranasal OT research, which suggest to begin experimental manipulations 40—45 min after administration when OT is thought to reach the central nervous system and plateau (Born et al., 2002). OT indeed reached plateau 45 min after administration but unlike Burri et al. (2008), our data show that OT levels climax much earlier, at 15 min after administration. These findings may suggest that OT effects may be expressed much earlier than previously thought. However, an alternative interpretation for the sharp increase in saliva OT at 15 min post-administration may relate to a possible dripping back into the mouth of parts of the sniffs of OT and, thus, the findings may be an artifact of the administration paradigm. Following this reasoning, such dripping back effect is only temporary and, as seen, disappears after about a half an hour.

Overall, the data suggest the need for much further research on the dynamic expression of OT administration in peripheral OT across a lengthy period. Future research is needed to examine OT effects on brain and behavior at multiple time-points starting immediately after administration and across several hours to assess when the effects are strongest and whether the timing of the effects change for various cognitive, social, and physiological processes. It is important to carefully assess whether different psychiatric conditions are associated with alterations in the timing of OT effects and whether timing effects are mediated by OT-related biological and social factors, such as social support, attachment history, genetic variability, or the provision of touch.

## Contributors

Authors RF, OZS and OW designed the study. OW ran the experiment. OZS conducted hormonal analyses. RF and OW conducted statistical analyses and wrote the manuscript. All authors contributed to and have approved the final manuscript.

## Role of funding source

Funds received from the funding sources support general activity in Prof. Feldman's lab

## Conflict of interest statement

Drs. Weisman, Zagoory-Sharon, and Feldman have no conflict of interest to disclose.

## Acknowledgments

Research at Dr. Feldman's laboratory during the study period was supported by the Israel Science Foundation (1318/08), the US-Israel Bi-National Science Foundation (2005-273), the NARSAD foundation (independent investigator award, 2006, 2008), the Katz Family Foundation, the Kor Family Foundation, and the Irving B. Harris Foundation.

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