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ORIGINAL ARTICLE

Correlation of plasma and salivary oxytocin in healthy young men – Experimental evidence

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Abstract

OBJECTIVES: The neuroactive hormone oxytocin (OT) has significant influence on human behavior, and it has been measured peripherally in venous blood and in saliva in many behavioral neuroscience studies. Assessment of salivary hormone levels is popular due to non-invasiveness, but there is a controversy as to whether OT can be reliably measured in saliva and how possible time lags between plasma and salivary OT levels influence correlation.
DESIGN AND METHODS: In order to shed light on the question whether salivary and plasma OT levels correlate, we designed an experiment where healthy young men had to look at a presentation of trustworthy faces on a computer screen (faces were taken from an established database in trust research). During three points in time, plasma and saliva samples were collected and analyzed using ELISA.
RESULTS: Plasma and salivary OT levels did not correlate even when considering a time lag of 15 or 30 minutes.
CONCLUSIONS: Our results suggest that plasma and salivary OT levels do not correlate in healthy young men, and hence comparison of results across plasma and salivary studies is neither informative nor warranted. However, we recommend replicating this study based on mixed-gender samples.

INTRODUCTION

Oxytocin (OT) is a neuropeptide that is synthesized in magnocellular neurons of the paraventricular and supraoptic nuclei of the hypothalamus and then secreted by the pituitary gland into the blood stream. Additionally, it is distributed throughout the central nervous system (for a review, see Riedl & Javor 2012). OT has been shown to influence human behavior, predominantly by enhancing pro-social behaviors, such as trust and approach (Heinrichs *et al.* 2009). Trust behavior has been in the focus of medical and neuroscientific research for several years (e.g. Javor *et al.* 2013). Hence, there are many studies published that measured OT, mostly in plasma, in various experimental designs (Modahl *et al.* 1998; Zak *et al.* 2005). Assessment of hormone levels in plasma

involves venous puncture, whereas saliva samples are non-invasive, a fact that explains the popularity of salivary hormone measurements, especially in research designs involving multiple specimen collections, e.g. daily hormone profiles (Susoliakova *et al.* 2014). While reliable salivary measurement of several hormones is undisputed (e.g., cortisol), there is a controversy as to whether OT can be reliably assessed in saliva, primarily because OT levels are often below the detection limit of most assays (Horvat-Gordon *et al.* 2005; Carter *et al.* 2007). It has been proposed to solve these shortcomings by drying (in the cold), re-suspending, and concentrating samples to produce levels within the detection range (White-Traut *et al.* 2009). McCullough *et al.* (2013) further argue that salivary assays only weakly correlate with measurements of OT in unextracted plasma (Grewen *et al.* 2010; Feldman *et al.* 2011). This raises the questions whether salivary OT levels are a reliable marker at all (if plasma OT is used as benchmark), and hence whether a comparison across studies based on different assessment techniques is informative and warranted. In order to shed light on the question whether plasma and salivary OT levels correlate, we designed the following protocol that was approved by the local ethics committee.

MATERIALS AND METHODS

A total of 30 healthy, right-handed, male volunteers between 18 and 40 years were recruited. All of them were students or young employees of the local university. Written informed consent was obtained from all participants prior to the study. Standard exclusion criteria for salivary hormone assessment studies, such as smoking, alcohol abuse, taking medicine, suffering from acute or chronic hormonal dysregulations, and psychosomatic or psychiatric diseases, were applied (Takahashi 2005). Participants were instructed not to drink beverages containing alcohol or caffeine on the day before their participation and not to eat and drink anything except water within two hours prior to and during the experiment. At the beginning subjects were required to rinse their mouths thoroughly with water, and then a venous catheter was introduced at the arm. Participants were seated alone in a quiet room for the entire protocol and had to look at a presentation of trustworthy faces on a computer screen; faces were taken from an established database (Lundqvist *et al.* 1998). Perception of a trustworthy face has been frequently shown to positively correlate with OT (see a recent review by Riedl & Javor 2012), and it has been argued that automatic positive reactions to trustworthy human faces constitute evolved capacities, shaped by natural selection and human evolution (Riedl *et al.* 2014). Therefore, we used trustworthy faces as stimuli in order to elicit an OT response in our study. Analysis of our data revealed that perception of the faces indeed increased OT levels monotonically (n.s.). All blood samples were collected through the catheter into pre-chilled ethylenediaminetetraacetic acid (EDTA) tubes (1 mg/mL blood) and kept cold during every step of processing. Saliva samples were collected using the Greiner Saliva Collection System (Greiner Bio One GmbH, Kremsmünster, Austria) in a standardized time frame of 2 minutes and then frozen at –80°C until assay. In total, blood and saliva samples were taken at 3 points in time: at the beginning of the experiment (t1), 15 minutes later (t2) and 30 minutes later (t3), for an illustration of the experimental protocol see Figure 1. Blood samples were immediately cold-centrifuged at 1600 × g for 15 min at 4 °C. Plasma was then transferred into chilled tubes and frozen at −80 °C until assay. The specimen preparation was processed based on the extraction protocol for sample concentration according to "LUCIO-Medical ELISA Oxytocin" (Nal von Minden GmbH, Regensburg, Germany), which includes elution over C18 columns, cold evaporation, and resuspension. Plasma and saliva samples were extracted using the same procedure. In a subsequent step, enzyme-linked immunosorbent assay (ELISA) was identically performed on resuspended plasma and saliva samples with inter- and intra-assay coefficients of variability (%CV) of 11.8 to 20.9 and 10.2 to 13.3 (according to LUCIO-Medical ELISA Oxytocin. Ref. ELI-4982; Version 1.3, 2013-11-06, p. 5).

RESULTS

Statistical analyses were performed using SPSS®, Version 20. First, we corrected all measured values, which were under the detection limit of <13.5 pg/ml according to Helsel (1990). Second, we plotted the data and drew the line of equality on which all points should lie if the two measurements gave exactly the same values at each time point (see Figure 2). The illustrations show the degree of agreement between salivary (horizontal axis) and plasma (vertical axis) OT measured at t1, t2 and t3.

Third, we calculated the correlation coefficient (r) between the two measurements; t1: r=0.252 (p=0.180), t2: r=–0.005 (p=0.980), and t3: r=0.043 (p=0.821). These results indicate that there is a very weak insignificant correlation at t1, and no correla-

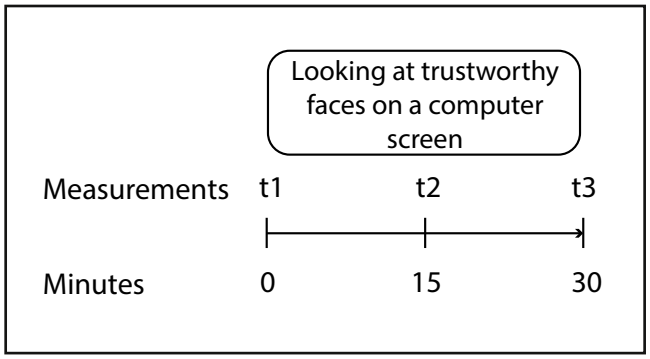

Fig. 1. Experimental protocol

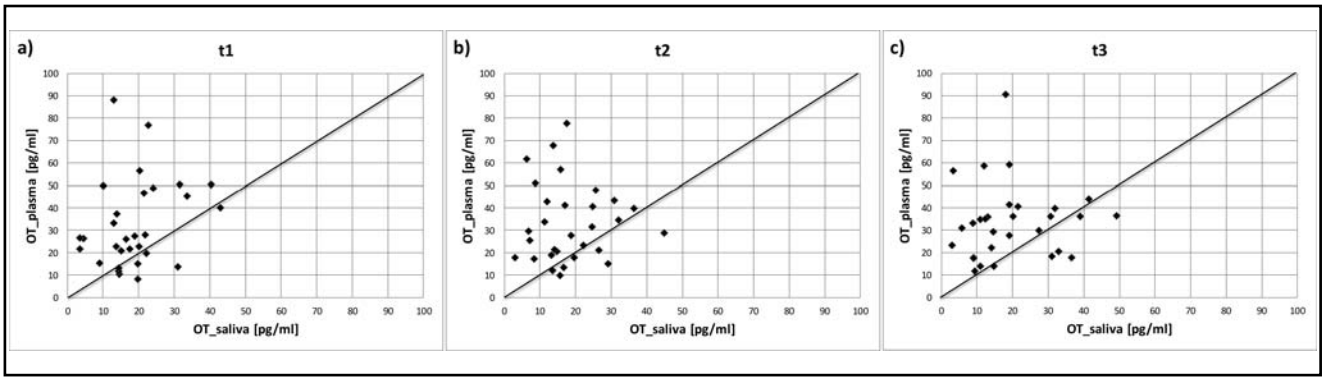

Fig. 2. Scatter plots of salivary and plasma oxytocin measurements at the three time points

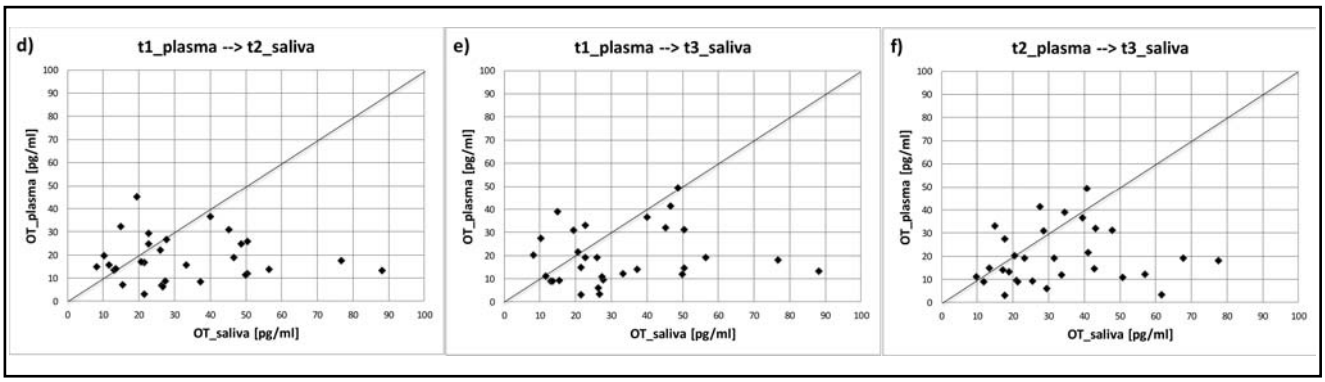

Fig. 3. Scatter plots of salivary and plasma oxytocin measurements taking into account a time lag of 15 and 30 minutes between plasma and saliva

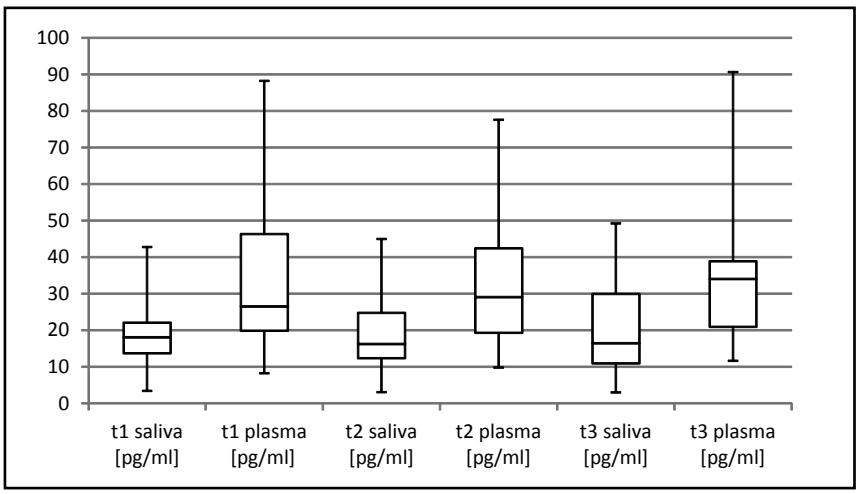

Fig. 4. Box-plots showing median, minimum and maximum concentrations of oxytocin in saliva and plasma at different points in time

tion between the OT values measured in saliva and plasma at t2 and at t3. It follows that these two OT measures indicate entirely different OT levels.

Fourth, we run regression analyses to determine whether plasma OT at t1 predicts saliva OT in t2 or in t3, because of a possible time lag caused by the transition of OT from plasma to saliva. We also calculated the regression between plasma OT at t2 and saliva OT at t3 (see Figure 3). Fifth, regression analyses on the saliva OT at t2 as dependent variable and plasma OT at t1 as independent variable confirmed that there is no effect (β=–0.021; p=0.911). Similar results were obtained for saliva OT at t3 and plasma OT at t1 (β=0.152; p=0.422), as well as saliva OT at t3 and plasma OT at t2 (β=0.088; p=0.644).

A box-plot analysis comparing plasma and salivary measurements revealed higher spreading and medians of plasma values at all three time points (see Figure 4; values in pg/ml: t1_saliva median 18.10; minimum 3.39; maximum 42.73; t1_plasma median 26.51; minimum

8.24; maximum 88.20; t2_saliva median 16.19; minimum 3.01; maximum 44.95; t2_plasma median 29.07; minimum 9.81; maximum 77.58; t3_saliva median 16.42; minimum 2.98, maximum 49.22; t3_plasma median 34.02; minimum 11.60; maximum 90.63).

DISCUSSION

The goal of this study was to find out whether plasma and salivary OT levels correlate during a perception task well-known to elicit an OT response (namely looking at trustworthy faces). An answer to this question is important because it reveals whether results of studies using these two different ways of assessing peripheral OT are comparable. Our results show that plasma and salivary OT levels do not correlate, suggesting that comparison of results across plasma and salivary studies is neither informative nor warranted. Even though other papers reported low or non-correlation of salivary and plasma OT (e.g. Grewen *et al.* 2010; Feldman *et al.* 2011), the authors of these investigations emphasize that it was not the goal of their studies to identify a correlation. Thus, the designs of these studies cannot rule out that different time lags of changes in plasma and salivary OT levels are the source of the non-correlation. This is the first study explicitly designed to compare salivary and plasma OT levels. There exists, to the best of our knowledge, no empirical study which considered a time lag between plasma and salivary OT levels. Evidence from intranasal OT administration showed an increase of salivary OT within 15 min (Weisman *et al.* 2012). In our data even a suspected time lag of 15 or 30 min does not lead to a significant correlation of plasma and salivary OT levels. We hypothesized that higher spreading in plasma OT concentrations might be the result of different levels of cross-reacting antigens in saliva and plasma. Lower levels in saliva compared to plasma, as found in the present study, are a known phenomenon reported in other hormone studies (e.g., cortisol), likely due to enzymatic degradation in the salivary epithelial cells during transcellular diffusion (Hellhammer *et al.* 2009).

Against the background of the results reported in this paper, we argue that scientists should be careful in comparing results from studies using plasma and salivary OT measurements. Moreover, we suggest replication studies, based on a larger sample size and more measurement points. Another important avenue for future research is the investigation of whether correlation of plasma and salivary OT is related to sex. Specifically, replication of our study with a female sample, or a mixed-gender sample, is recommended.

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Conflicts of interest statement: *The authors disclose no conflicts.*

REFERENCES

1 Carter CS, Pournajafi-Nazarloo H, Kramer KM, Ziegler TE, White-Traut R, Bello D, et al (2007). Oxytocin: Behavioral associations and potential as a salivary biomarker. Ann New York Acad Sci. **1098:** 312–322.
2 Feldman R, Gordon I, Zagoory-Sharon O (2011). Maternal and paternal plasma, salivary, and urinary oxytocin and parent–infant synchrony: considering stress and affiliation components of human bonding. Dev Sci. **14:** 752–761.
3 Grewen KM, Davenport RE, Light KC (2010). An investigation of plasma and salivary oxytocin responses in breast- and formula-feeding mothers of infants. Psychophysiology. **47:** 625–32.
4 Heinrichs M, von Dawans B, Domes G (2009). Oxytocin, vasopressin, and human social behavior. Front Neuroendocrinol. **30:** 548–557.
5 Hellhammer DH, Wüst S, Kudielka BM (2009). Salivary cortisol as a biomarker in stress research. Psychoneuroendocrinology. **34:** 163–171.
6 Helsel DR (1990). Less than obvious-statistical treatment of data below the detection limit. Environ Sci Technol. **24:** 1766–1774.
7 Horvat-Gordon M, Granger DA, Schwartz EB, Nelson VJ, Kivlighan KT (2005). Oxytocin is not a valid biomarker when measured in saliva by immunoassay. Physiol Behav. **84:** 445–448.
8 Javor A, Koller M, Lee N, Chamberlain L, Ransmayr G (2013). Neuromarketing and consumer neuroscience: contributions to neurology. BMC Neurol. **13:** 13.
9 Lundqvist D, Flykt A, Ohman A (1998). Karolinska Directed Emotional Faces [Database of standardized facial images]. Psychology Section, Department of Clinical Neuroscience, Karolinska Hospital, S-171 76 Stockholm, Sweden.
10 McCullough ME, Churchland PS, Mendez AJ (2013). Problems with measuring peripheral oxytocin: can the data on oxytocin and human behavior be trusted. Neurosci Biobehav Rev. **37:** 1485–1492.
11 Modahl C, Green LA, Fein D, Morris M, Waterhouse L, Feinstein C, et al (1998). Plasma oxytocin levels in autistic children. Biol Psychiatr. **43:** 270–277.
12 Riedl R, Javor A (2012). The biology of trust: Integrating evidence from genetics, endocrinology and functional brain imaging. J Neurosci Psychol Econ. **5:** 63–91.
13 Riedl R, Mohr P, Kenning P, Davis F, Heekeren H (2014). Trusting humans and avatars: A brain imaging study based on evolution theory. J Manage Inform Syst. **30:** 83–113.
14 Susoliakova O, Smejkalova J, Bicikova M, Potuznikova D, Hodacova L, Grimby-Ekman A, et al (2014). Salivary cortisol in two professions: Daily cortisol profiles in school teachers and firefighters. Neuroendocrinol. Lett. **35:** 314–321.
15 Takahashi T (2005). Social memory, social stress, and economic behaviors. Brain Res Bull. **67:** 398–402.
16 Weisman O, Zagoory-Sharon O, Feldman R (2012). Intranasal oxytocin administration is reflected in human saliva. Psychoneuroendocrinology. **37:** 1582–1586.
17 White-Traut R, Watanabe K, Pournajafi-Nazarloo H, Schwertz D, Bell A, Carter CS (2009). Detection of salivary oxytocin levels in lactating women. Dev Psychobiol. **51:** 367–373.
18 Zak PJ, Kurzban R, Matzner WT (2005). Oxytocin is associated with human trustworthiness. Horm Behav. **48:** 522–527.