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Statements

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Impact Statement

This study provides the first evidence of circadian variation in salivary oxytocin levels, observed in both healthy controls and individuals with borderline personality disorder (BPD). These findings challenge the assumption of constant oxytocin secretion and highlight the importance of considering time-of-day effects when studying oxytocin's role in psychological processes.

Abstract

This brief report presents circadian variation in salivary oxytocin levels in a sample of 58 female participants, including 35 healthy controls and 23 patients with borderline personality disorder (BPD). A significant increase in salivary oxytocin levels was observed between awakening and early afternoon (1–2 PM) ($F = 20.54$, $p < 0.001$, partial eta² = 0.27, 99% achieved power). There were no significant group differences and no Group × Time interaction. These findings have implication for research held in the field and suggest the need to control for time of assessment, as done in studies assessing cortisol.

Keywords

Circadian variation, oxytocin, salivary biomarkers, borderline personality disorder, hormonal cycle

Introduction

Oxytocin, a neuropeptide primarily associated with parturition and lactation, has gained increasing attention for its role in social bonding, emotion regulation, stress response and cognitive flexibility [1-5]. Despite growing research on oxytocin in both clinical and non-clinical populations, the question of whether endogenous oxytocin exhibits circadian variation remains unresolved [6]. As evidenced by well-established research on the circadian assessment of cortisol and melatonin, the study of hormonal rhythms requires repeated sampling throughout the day [7, 8]. Thus, the lack of reliable methods to quantify oxytocin may have contributed to the limited exploration of its diurnal fluctuations. While early research primarily focused on quantifying oxytocin in blood and cerebrospinal fluid, salivary assessment has emerged as a non-invasive alternative, despite ongoing debates regarding its methodological reliability [9]. Recent studies suggest that repeated measures using radioimmunoassay (RIA) can increase the reliability of salivary oxytocin assessment [10-12].

Existing evidence on oxytocin's circadian rhythm remains scarce and inconclusive. Van Dam and colleagues [6] investigated salivary oxytocin levels in a sample of 12 adolescents (8 males, 4 females, aged between15–17 years-old) using enzyme-linked immunosorbent assay (ELISA) across three consecutive days in a controlled sleep laboratory setting. Their findings revealed a sharp reduction in oxytocin immediately following awakening, followed by stable levels at later time points (8 and 12 hours post-awakening). However, their small sample size

and limited age range restrict the generalizability of their results. Kagerbauer [13] assessed oxytocin levels in a sample of 20 neurosurgical patients recovering from intracranial haemorrhage or tumours (8 males, 6 premenopausal females, 6 postmenopausal females, median age 54.5 years old) using radioimmunoassay (RIA). They assessed oxytocin concentrations in cerebrospinal fluid blood, and saliva at four time points (06:00, 12:00, 18:00, and 24:00). Their results indicated no significant diurnal fluctuations in any of these biological fluids, except for a minor increase in CSF oxytocin at noon in postmenopausal women. Although these studies were well designed, limitations can be highlighted, such as sample heterogeneity, particularly the sex ratio, and a lack of control for menstrual cycle phase. These factors are essential when studying oxytocin, as menstrual cycle phase and hormonal contraceptive use influence oxytocin levels [14]. Graugaard-Jensen and colleagues [15] controlled for these aspects by comparing diurnal plasma oxytocin levels between 15 naturally cycling women in the follicular phase and 11 women using oral contraceptives. They did not find significant changes in circadian levels. However, besides the small sample size, morning samples were not taken immediately after individual awakening (as done in cortisol circadian assessments).

This current report refers to partial data from a larger registered trial (NCT05357521) and focuses on investigating potential circadian variation in salivary oxytocin levels in a female sample, including both healthy controls and clinical participants diagnosed with borderline personality disorder (BPD) *(refer to our oxytocin pilot study for more details on the this psychiatric condition [16]*). By employing rigorous methodological controls, including a homogenous sample regarding sex and age range, standardized saliva collection times, menstrual cycle phase (assessment in the luteal phase), and accounting for oral contraceptive use and antidepressant intake in the clinical sample, this study contributes to the growing understanding of oxytocin's circadian trend. Given the growing interest in oxytocin's role in psychological and physiological functions, understanding its temporal dynamics is crucial for improving research methodologies, reduce result variability and accurately interpreting findings.

Methods:

Participants

Reported findings refer to 58 consecutive female participants (35 healthy controls and 23 patients with borderline personality disorder) who completed a larger protocol NCT05357521. Healthy controls were recruited by advertisement and patients with BPD within patients treated at the Emotion Regulation Unit of the Division of Psychiatry, Geneva University Hospitals as previously described [17]. All participants provided informed consent, and the study was approved by the local ethics committee.

Participants were eligible if they were between 18 and 35 years of age (M = 24.3, SD = 5.9), free from neurohormonal or neurological disorders, not using systemic corticosteroids, and not pregnant or breastfeeding within the last 6 months. Participants with a history of alcohol or drug addiction or a body mass index under 17 were excluded. Those who had experienced major stressful life events (such as the loss of a close relative, loss of employment, or significant changes in marital status or medical condition) within the two months prior to enrolment were either excluded or contacted after a few months for a re-assessment of eligibility. Alcohol, tobacco, and occasional cannabis use were permitted, but use on sampling days was excluded. Substance use was controlled through interviews, and sampling was rescheduled if necessary.

For specific subgroups, patients with BPD were required to meet DSM-5 criteria, confirmed by a structured clinical interview. They were excluded if they had a formal diagnosis of psychosis, were daily users of neuroleptics, or were using antidepressants outside of the SSRI or SNRI classes. For healthy participants, those who met three or more of the BPD diagnostic criteria were excluded (with five being the threshold for a formal diagnosis).

Salivary Oxytocin Sampling

Saliva was collected using Salivettes with synthetic swab (Sarstedt,Germany) chewed for at least 1min. Swabs were temporarily stored at -20°C, followed by a longer-term storage at -80 ^{o}C until analysis. Temperature was assured with dry-ice during shipping. Oxytocin concentrations were quantified by radioimmunoassay (RIAgnosis, Sinzing, Germany) following the procedures described in previous studies, including ours [16, 18, 19].

Saliva samples were collected at two time points:

1. Awakening (T1): Participants were instructed to collect their saliva sample immediately upon waking, following standardized cortisol awakening protocols to minimize confounds from movement or activity.
2. Early afternoon- between 1 and 2 PM (T2): The second saliva sample was collected the same at the laboratory, under controlled conditions.

For participants who were not using hormonal contraception, sampling was scheduled one week before the expected onset of menstruation, within the luteal phase, to control for menstrual cycle effects. Participants tracked their cycles and reported their average cycle length. The mid-cycle was then estimated individually for each participant. Ovulation was estimated with a 3-day safety margin added. Then, 6 days were subtracted from the expected end of the cycle to estimate the ideal luteal phase timeframe. If a participant had already started their period before the scheduled session, data collection was rescheduled to ensure cycle consistency.

Statistical Analysis

Statistical analyses were conducted using SPSS 28.0. We performed a mixed 2 X 2 repeated-measures ANOVA with Group (BPD vs. healthy participants) as a between-subject factor and Time (T1 – morning, T2 – early afternoon) as a within-subject factor was performed to assess circadian variation in salivary oxytocin levels. Effect sizes were reported using partial eta squared (η^2p)**.** Box's test of equality of covariance showed that assumption of homogeneity was met ($p>0.05$).

To explore the potential influence of hormonal contraception, a repeated-measures ANCOVA was conducted, including contraceptive use as a covariate. Potential interactions were followed by exploratory analyses of variance and t-test to examine potential effects. Given that antidepressant use was exclusive to the BPD group, a separate repeated-measures ANCOVA was conducted within this group to test whether antidepressant use influenced circadian changes in salivary oxytocin. All statistical tests were two-tailed, with a significance threshold set at $p < 0.05$

Results

Main ANOVA without covariates

The repeated-measures ANOVA revealed a significant main effect of Time, indicating that salivary oxytocin levels significantly differed between T1 and T2 ($F(1,56) = 20.54$, $p < 0.001$, $\eta^2p = 0.27$), with a 99.4% achieved power. There was no significant main effect of Group ($F(1,56) = 1.81$, $p = 0.18$), nor significant Group × Time interaction ($F(1,56) = 0.82$, $p = 0.37$).

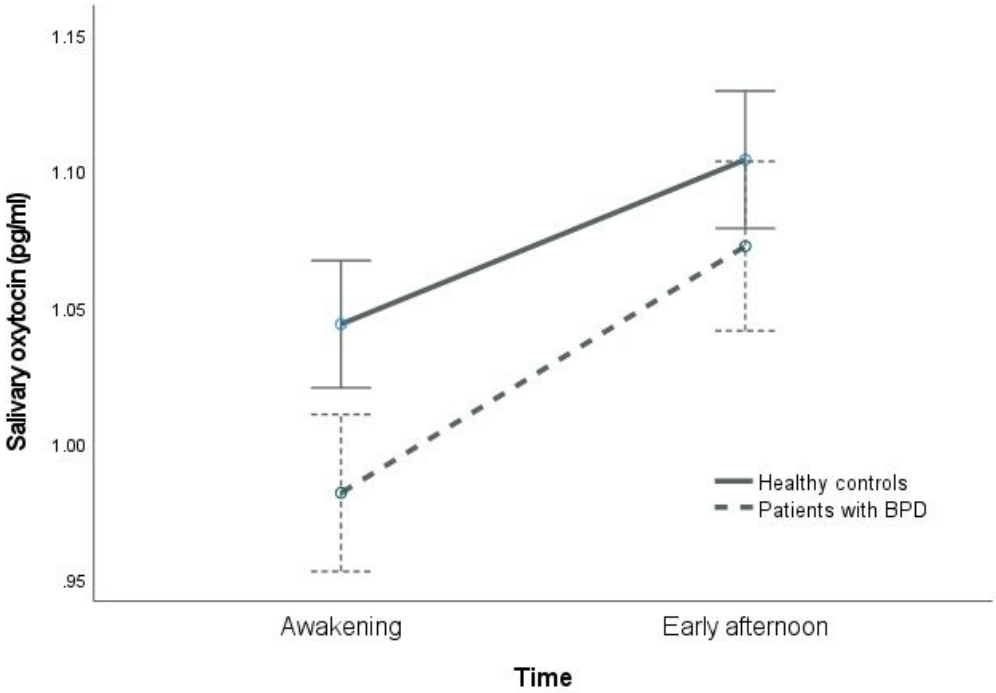

Figure 1. Salivary oxytocin levels of healthy controls and patients with borderline personality disorder (BPD) at awakening and early afternoon

(Error bars represent Standard Error of the mean)

Effect of Hormonal Contraception

A total of 16 participants in the entire sample reported the use of hormonal contraception. When hormonal contraception was included as a covariate, the main effect of Time remained significant ($F(1,55) = 9.18$, $p = 0.004$), with no significant main effect of group or Group X Time interaction. The interaction Time × Contraception was significant ($F(1,55) = 4.09$, $p = 0.048$). The interaction was mainly driven by a greater increase between T1 and T2 in the

group with hormonal contraception (graphical analysis), but a separate ANOVA with Contraception as group factor, did not yield significant effect of group ($F(1,56)= 2.12$, $p=0.15$), with a trend to an interaction between Contraception and time ($F(1,56)= 3.25$, $p=0.08$) and a maintenance of the significant effect of time ($F(1,56) =23.63$, $p<0.001$). An exploratory paired t-test run in each subgroup (without and with hormonal contraception), showed significantly higher levels of salivary oxytocin in the early afternoon compared to awakening in both groups: (without hormonal contraception $t(41) = -2.79$, $p=0.008$; with hormonal contraception $t(15) = -4.44$, $p< 0.001$).

Effect of Antidepressants in the Clinical Group

In the Clinical group, a repeated-measures ANCOVA with antidepressant use as a covariate still showed a significant main effect of Time ($F(1, 21) = 6.40$, $p = 0.02$), indicating a circadian variation in oxytocin levels but no significant Time X Antidepressant interaction ($F(1,21) = 0.54$, $p = 0.47$), suggesting that antidepressant use did not influence oxytocin variation across time points.

Discussion

This study provides clear evidence of circadian variation in salivary oxytocin levels, with a significant increase from awakening to early afternoon. These findings challenge the prevailing assumption that oxytocin secretion remains stable throughout the day and points to the importance of considering time-of-day effects in future research on oxytocin's role in psychological and physiological processes. Given the large effect size and high statistical power, our results are robust and unlikely to be due to chance.

While the few existing studies did not report significant diurnal variation in oxytocin levels [13] or reported a different pattern of variation (as Van Dam's study reporting a sharp drop at awakening with stable levels throughout the day [6]), our study suggests that salivary oxytocin may exhibit time-dependent fluctuations. The divergence between our findings and those of prior studies may be attributed to methodological differences, including our noticeably larger sample size, sample homogeneity (only young female participants) and control of menstrual cycle phase as well as hormonal contraception and medication. Further, we framed different time-points (awakening and early afternoon) and unlike previous studies that used an in-patient protocol, these data involved a non-hospitalised sample, within

participant's natural environment, minimizing potential confounds related to stress and medical interventions.

Beyond the circadian effect, we observed that hormonal contraceptive users exhibited a trend to higher oxytocin increase compared to naturally cycling women, consistent with previous research, suggesting that hormonal contraception affect oxytocin release [20]. However, due to the lower proportion of hormonal contraceptive users, this should be confirmed in our larger sample or future studies. Further, antidepressant use (SSRI, SNRI) did not significantly affect oxytocin secretion patterns, suggesting that circadian variation is preserved despite these specific molecules. In future studies, it could be of interest to target other antidepressant classes. Of note, it is important to point out, that in a previous pilot study of our group [16], the circadian pattern of oxytocin increase was observed in our healthy participants (n=9) but not in our patient's sample (n=10). However, as it was a pilot protocol, we did not yet control for type of antidepression intake, current co-morbidities, menstrual cycle phase or hormonal contraception.

In the current sample, no significant group differences or interactions between time and clinical status were observed. This suggests a consistent circadian pattern across both healthy controls and individuals with BPD, at least in a natural environment without stress conditions. Indeed, our ongoing larger trial will explore differences under naturalistic and experimental stress-induced conditions, and we also aim to explore other relevant parameters such as depressive and anxiety symptoms, adverse childhood experience, symptoms of post-traumatic stress-disorder or attachment patterns.

Despite the robust effect size and high statistical power observed in our primary analysis, some limitations must be acknowledged. As the sample was restricted to premenopausal females in the luteal phase, our findings cannot be generalized to males or to women who are premenarchal or postmenopausal. Additionally, while the current sample size was sufficient to detect within-subject circadian variation, future protocols aimed at assessing circadian oxytocin variation could include additional time points throughout the day, with later assessments (as done for cortisol). Moreover, it would be of particular interest to examine the relationship between oxytocin and cortisol circadian variations. Further analyses of the interplay between oxytocin and cortisol rhythms will be presented in subsequent reports as part of the larger study, which investigates their dynamics in both natural and experimental settings.

Finally, our findings have important methodological and theoretical implications. The presence of circadian variation in salivary oxytocin suggests that future studies should carefully control for time-of-day effects when measuring oxytocin levels. Such adaptations would reduce variability in results, thereby enhancing the reproducibility and interpretability of findings. Failure to do so may contribute to inconsistencies across studies and limit the reproducibility of findings.

Conclusion

This study highlights a clear diurnal variation in salivary oxytocin in pre-menopausal women, emphasizing the need to account for time-of-day effects in oxytocin research.

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Impact statement

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