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Animal behaviour

Salivary oxytocin increases concurrently with testosterone and time away from home among returning Tsimane' hunters

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Oxytocin, testosterone and cortisol can have opposing effects on social behaviour, yet few studies have examined their interactions. We measured changes in salivary oxytocin, testosterone and cortisol among Tsimane' men returning home after hunting, an ancient context of male status competition, parental investment and cooperation. Contra normal diurnal rhythm, oxytocin increased relative to baseline and this increase was positively associated with duration of the hunt and change in testosterone, but not cortisol, social context, hunting outcome or physical activity. The concurrent increase in endogenous peripheral oxytocin and testosterone is unexpected given their opposing independent effects on social cognition and behaviour, and has not been observed before. We discuss the potential significance of these effects for the biology of pair-bonding, parenting and social foraging in humans and other species.

1. Introduction

The neuropeptide oxytocin (OT) and the steroids testosterone (T) and cortisol affect social behaviour in different ways across species. By reducing anxiety and enhancing social cognition, OT can foster affiliation and help build enduring partner preferences that facilitate cooperation [1–4]. Conversely, T has opposite effects on social cognition and stimulates status competition [1,2,5]. Parenting and pair-bonding in humans are therefore typically associated with high baseline OT and low T [1,2]. In non-reproductive contexts, OT administration increased trust and generosity [6–9], whereas T decreased them [10,11], and meat sharing among chimpanzees was associated with high urinary OT [12] but low T [13]. Cortisol, indicating immediate energetic demands, decreased cooperation among cleaner fish [14]. As cooperation requires investment in social partners despite diverging individual interests (status, energy), interactions between OT, T and cortisol may be crucial, yet few studies have examined them [7,15].

Leveraging a previous study showing effects of hunting success on T and cortisol among Tsimane' men [16], we measured salivary OT in the same sample. Hunting and meat sharing are ancient contexts of male status competition, parental investment and reciprocal cooperation [17], and therefore well suited to reveal evolved endocrine mechanisms underlying male strategies [1,12,13,16]. To this end, we tested whether OT increased when hunters returned home compared with baseline, and associated changes in OT with changes in T and cortisol as well as hunting outcomes, duration, physical activity and social context (table 1 and see electronic supplementary material, table S1 for full data).

2. Material and methods

(a) Study population

The Tsimane' are an indigenous population of approximately 16 000 living in the Bolivian Amazon and subsisting primarily on hunting, fishing and horticulture [16].

Table 1. Predictors of percentage changes in OT when returning home compared with baseline.

variable	description	association with OT change
% T change	percentage change in T home − baseline (mean = 5.4%, range = −50 to 74%)	$r = 0.36$, $n = 25$, $p = 0.07$*
% cortisol change	percentage change in cortisol home − baseline (mean = −7.9%, range = −78 to 154%)	$r = 0.15$, $n = 25$, $p = 0.48$
cooperative hunting	was another hunter present? (19% yes)	$t = -0.29$, $n = 25$, $p = 0.78$
hunt duration[a]	hours between leaving and returning home (mean = 8.6 h, range = 2.8–13.1 h)	$r = 0.35$, $n = 25$, $p = 0.08$*
accelerometry count[a]	average accelerometry count during hunt (vector magnitude units) (mean = 360, range = 253–579)	$r = 0.18$, $n = 12$, $p = 0.60$
heart rate[a]	average heart rate during hunt (mean = 89, range = 80–100)	$r = 0.37$, $n = 10$, $p = 0.29$
hunting success	did the hunter make a kill? (59% yes)	$t = 1.24$, $n = 25$, $p = 0.23$
meat sharing	did the hunter bring meat home? (78% yes)	$t = 0.41$, $n = 25$, $p = 0.69$
audience	did the returning hunter encounter people from other families? (74% yes)	$t = -1.62$, $n = 25$, $p = 0.14$
family size	number of live children (median = 5, range = 1–12, all men were married)	$r = 0.20$, $n = 25$, $p = 0.34$
age	mean = 37.8, range = 18–82	$r = 0.06$, $n = 23$, $p = 0.78$
age^2	mean = 1627, range = 324–6724	$r = 0.01$, $n = 23$, $p = 0.96$
BMI	body mass index (mean = 23.8, range = 19.6–29)	$r = 0.28$, $n = 23$, $p = 0.20$

[a]Duration was highly correlated with total distance travelled (mean = 11 km, range = 3.7–17; $r = 0.83$, $p < 0.001$), which we therefore abandoned for direct measures of exercise (accelerometer count, heart rate) uncorrelated with duration.
*$p < 0.1$.

Hunts are typically solitary and last several hours (table 1). Meat may be shared both within and between families. In this study, meat was virtually always given to the women of the household for processing immediately upon return, but no sharing outside the household occurred prior to sample collection.

(b) Sample collection and analysis

Thirty-one married Tsimane′ men provided saliva samples during 31 independent hunting follows [16]. After collection, specimens were stored in liquid nitrogen before transfer on dry ice to −80°C freezers in the USA. Methods and data for measuring T (PAb R156/7) and cortisol (PAb R4866) are published elsewhere and are publicly available [16]. We measured OT in a random selection of samples with sufficient remaining volume at baseline, i.e. leaving home ($n = 8$) or 3 h into the hunt ($n = 19$), and 10 min after hunters returned home ($n = 25$). Electronic supplementary material, table S2 details this sampling timeline and how it relates to clearance times of the different hormones.

OT was measured in duplicate at the UCSB Biodemography Laboratory after two freeze–thaw cycles using Enzo Life Sciences enzyme immunoassay kit ADI-901-153A. Specimens were thawed and centrifuged at 1500*g*, and 250 µl of the aqueous portion of specimens was extracted in 6 ml ethanol and desiccated under a Mini-vap evaporator before being reconstituted in 250 µl of assay buffer. The within and between plate CVs were 1.2% and 7.7%, for the high control (539 pg ml^{-1}), and 1.3% and 1.4% for the low control (31 pg ml^{-1}) for $n = 2$ plates.

(c) Validity of oxytocin measures

Extraction should improve the specificity and accuracy of the assay [18,19], and all specimens were within its detection limits. Salivary OT should correlate moderately with plasma OT [20,21]. However, peripheral OT does not always reflect central OT [9,18,19,22–24] and may have different functions [23], such that interpretation of the results should be limited to effects described for peripheral OT [1,4].

(d) Statistical analysis

There was no significant difference in OT levels between the pre-hunt or 3 h baseline (Wilcoxon test: $V = 7$, $p = 0.56$); hence they were combined. Percentage changes in OT, T and cortisol were calculated using the same baseline sample for each individual. To test for an association between percentage changes in OT and other variables (table 1), we used bivariate analyses (Pearson correlations, *t*-tests), before including variables with strong associations ($p <$ 0.1, two-tailed) in multiple regression to assess their partial effects. Standard diagnostic plots were used to identify potential outliers. All analyses and graphs were done in R v. 3.0.2 [25].

3. Results

Salivary OT levels were over 50% higher when hunters returned home (mean = 49.8 pg ml^{-1}) compared with baseline (mean = 30.2 pg ml^{-1}, Wilcoxon paired test: $V = 20$, $p < 0.001$). Men with higher baseline OT experienced lower increases in OT upon returning home (Pearson's $r = -0.59$, $n = 25$, $p < 0.01$). Percentage change in OT was strongly associated with percentage change in T and hunt duration but no other variables (table 1). In multiple regression, both T change ($\beta = 0.47$, $p < 0.05$) and hunt duration ($\beta = 0.46$, $p < 0.05$) were significantly positively associated with OT change (table 2 and figure 1). Diagnostic plots revealed three outliers (electronic supplementary material, figure S1), removal of which resulted in a weaker association with T ($\beta = 0.35$, $p = 0.08$) and stronger association with duration ($\beta = 0.62$, $p < 0.01$, electronic supplementary material, table S3). There was no correlation between baseline OT and baseline T ($p = 0.90$) or baseline cortisol ($p = 0.88$).

4. Discussion

Contra diurnal rhythm [26], salivary OT levels among Tsimane′ hunters were significantly higher when returning

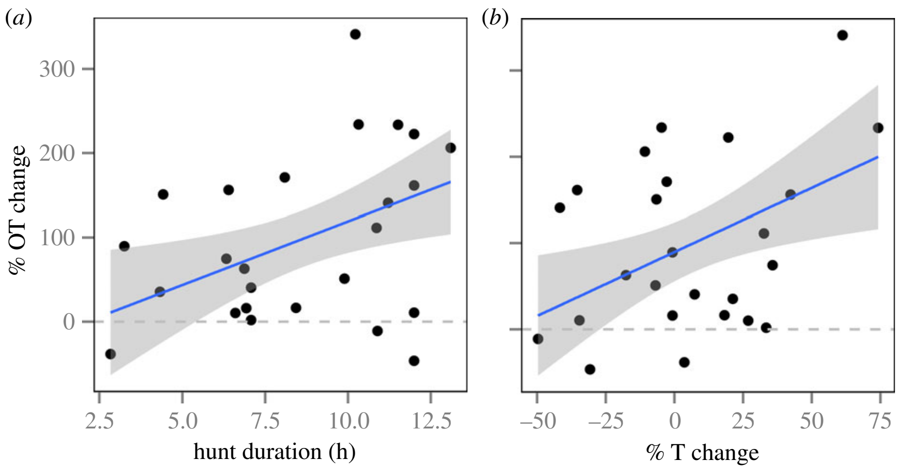

Figure 1. Percentage change in oxytocin when hunters returned home compared with baseline, as a function of (*a*) hunt duration, holding T change at average, and (*b*) percentage T change, holding duration at average. The horizontal dashed lines indicate no change in OT. Fitted lines and 95% CIs (shaded areas) are based on the model in table 2. (Online version in colour.)

Table 2. Multiple regression model predicting percentage change in oxytocin upon returning home compared with baseline. Shown are parameter estimates *b* (with standard errors s.e.), *t*- and *p*-values, and standardized coefficients β. $R^2 = 0.27$.

	b (s.e.)	*t*	*p*	β
(intercept)	−39.74 (54.1)	−0.73	0.47	
hunt duration (h)	15.06 (5.9)	2.56	0.018	0.46
percentage change in testosterone	1.48 (0.6)	2.61	0.016	0.47

home compared with baseline. Percentage changes in OT were not predicted by variables previously shown to affect peripheral OT, including meat sharing in chimpanzees [12] and (extreme but not moderate) exercise [27], as well as various social variables that could impact male strategies, including cooperative hunting, family size and audience [1,16,17] (table 1). However, some effects were in the expected direction but statistical power was low, and we further cannot exclude the possibility that these variables did have independent effects on central OT. Cortisol, which could make cooperation condition-dependent [14] and can be suppressed by OT [28], was not associated with OT here, possibly because energy demand was not enough to elevate cortisol in this sample [16] (compared with extreme exercise [27]), or because salivary OT in men is not always associated with stress [21].

Men who were hunting for longer durations had higher increases in OT upon returning home (figure 1*a*). Given that peripheral OT levels may track partner value [4], we speculate that this duration effect could facilitate familial social contact and help reinforce pair-bonding and parenting behaviour after male absence [1,2], which was probably common in our recent evolutionary history owing to the sexual division of labour [17]. Although correlated with distance travelled, the duration effect is unlikely owing to physical activity as neither duration nor OT change were associated with accelerometry counts or heart rates (table 1; although the correlation between OT change and heart rate was moderate but statistical power was low), and only extreme exercise may increase peripheral OT [27].

Finally, changes in T positively predicted changes in OT (figure 1*b*), independent of baseline levels and hunt duration (table 1). This concurrent change in peripheral T and OT is unexpected given their opposing independent effects on social cognition and cooperation [1–13], and highlights the need to consider interactions between multiple hormones [2,7,15]. We cannot be certain if changes in salivary T and OT levels were prompted by the same events given their differing half-lives and the long time periods between sample collection (electronic supplementary material, table S2). Nonetheless, positive associations between T and OT might occur during sexual intimacy [2], and exogenous OT increased salivary T levels in fathers, thus enhancing their enjoyment in parenting [15]. Similarly, short-term T increases in successful hunters could reinforce this subsistence strategy [16,29], whereas concurrent OT increases could enhance its social salience [17]. As such, interactions between T and OT might underlie aspects of pair-bonding, parenting and social foraging in humans, and perhaps other species [1,2]. Additionally, both T and OT could be involved in muscle regeneration in some species [16,30]. While T can increase OT receptor-binding in rodent brains [31], and OT may increase T production through OT receptors in the testes [32,33], future research is needed to determine the physiological mechanism for the observed positive interaction between peripheral T and OT, its prevalence across species, and significance for cooperative behaviours.

Ethics statement. Participants provided informed consent and procedures were approved by the University of Washington, University of New Mexico, and University of California Santa Barbara Internal Review Boards.

Data accessibility. All data supporting this article are available in the electronic supplementary material, table S1.

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Authors' contributions. A.J. conceived the oxytocin study, helped carry out laboratory work, conducted the statistical analyses and wrote the manuscript; B.T. helped design the study, collected samples and field data, carried out the laboratory work, and helped write the manuscript; H.K. and M.G. facilitated the research, provided laboratory space and funding, and edited the manuscript. All authors gave final approval for publication.

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