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CortiLove: A pilot study on hair steroids in the context of being in love and separation

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ABSTRACT

While romantic infatuation and separation influence psychological and physiological functioning, the hypothalamic-pituitary-adrenal axis with its biomarkers cortisol, dehydroepiandrosterone (DHEA), and progesterone central for coping and distress has been scarcely researched in this context. In particular, endocrine hair analyses assumed to be more valid than saliva or blood assessments for studying long-term processes have not yet been conducted in the context of romantic love. Thus, 101 female subjects in phases of infatuation ($n = 16$), separation ($n = 14$), long-term relationships ($n = 40$), and singlehood ($n = 31$) reported psychological distress and provided 1 cm hair samples for the assessment of long-term integrated cortisol, DHEA, and progesterone over the last month. Separated, infatuated, and single women exhibited higher cortisol levels than those in a long-term relationship (all $ps \leq .031$), while self-reported distress was only evident in separated individuals. Further, no group differences for progesterone ($p = .602$), but higher DHEA levels in the separation ($p = .009$) and single group ($p = .016$) compared to the long-term relationship group were detected. This is the first study showing that compared to women in long-term relationships, infatuation, separation, and single groups exhibit higher levels of physiological, but not necessarily self-reported indicators of distress. These findings, albeit on a very small and preliminary sample, are discussed in the context of the stress-buffering effect of relationships, and provide important starting points for bigger, more balanced studies combining multimodal self-report and biological markers in psychological research of romantic love.

1. Introduction

Intense romantic experiences such as falling in love and breaking up are biographically highly relevant phases requiring self-regulatory and adaptational processes (e.g., Ref. [1]). Divorce or separation represent massive transitions related to increased psychological distress and decreased life satisfaction [2]. In particular for younger populations, divorce or separation may be associated with clinical issues such as major depression (e.g., Ref. [3]). However, also the start of a new relationship has been identified as crucial stressor (see modified Holmes and Rahe Stress Scale for younger populations, [4]. While infatuation is generally considered to be associated with increased energy, serenity, and focused attention (e.g. Ref. [5]), it is also believed to require intense transition efforts. Due to the novelty, the socio-evaluative context [6], and the accompanying reactions such as euphoria, insomnia, heart palpitations, or accelerated breathing (e.g. Ref. [5]), infatuation phases can be considered stressful. In the long run, however, people in stable, fulfilling relationships experience fewer mental health problems, higher life satisfaction and expectation, and better cardiovascular health than singles (e.g., Ref. [7]).

While there is a plethora of research on self-reported stress during phases of separation or infatuation, the knowledge of underlying bio-psychological processes is sparse. Of relevance in this context might be the hypothalamic-pituitary-adrenal (HPA) axis with its central effector hormone cortisol as the crucial component of the endocrine stress response (e.g., Ref. [8]). While endocrine levels may be studied via blood, saliva or urine samples, these methods are relatively sensitive to situational influences, and only allow insights into short periods of minutes to hours of HPA axis activation. Consequently, for research on long-term processes relevant for chronic conditions, the recent

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development of endocrine hair analyses allowing researchers to examine several weeks to months of hormonal activity, represents a major advance in the field [9].

Next to the well-known association of the HPA axis and, in particular, the so-called “stress hormone” cortisol with interpersonal and socio-evaluative stress (reviewed, e.g., in Ref. [6]), data suggest that in the aftermath of bereavement or severe divorce/separation, morning blood or saliva cortisol levels are elevated compared to control subjects (e.g. Refs. [10,11]), and may remain so for several months (reviewed in Ref. [12]). Moreover, even short absences of the partner, such as during day trips, seem to result in higher salivary cortisol observing the transition from separation to reunion in high-anxious partners staying at home [13]. With respect to infatuation phases, there are two studies suggesting increased salivary and blood cortisol levels during infatuation [14,15], while another study reports contradictory results showing lower daytime salivary cortisol production and a truncated cortisol awakening response in infatuated individuals (CAR; [16]). Other studies report no group differences for blood serum or plasma cortisol levels between infatuated and non-infatuated individuals [17] or heterosexual couples in very fresh relationships compared to singles [1]. Yet, there is evidence that individuals in long-term relationships compared to singles exhibit lower salivary cortisol levels (observed for Caucasian, Hispanic, and Asian, but not African-American individuals [18], while this could not be replicated in a more recent study also using salivary cortisol but only examining women [19].

Besides cortisol, other, less well-researched steroid hormones of the HPA axis might play a role in the context of romantic love. Several authors postulate a role of progesterone for commitment motivation potentially relevant for establishing a stable relationship (e.g., Refs. [20–22]). However, no differences in acutely infatuated compared to non-infatuated individuals were observed [15]. Further, dehydroepiandrosterone (DHEA), frequently discussed in the context of chronic as well as traumatic stress [23,24] might be of relevance for romantic love particularly due to its postulated antagonistic interplay with cortisol. However, available studies so far did not reveal differences between infatuated and long-term relationship groups, albeit having only focused on the metabolic form DHEA-S [1,15]. In general, available longitudinal data on endocrine [15], but also neurotrophine and monoamine levels in blood samples [25,26] revealed that endocrine differences between acutely infatuated and control groups were usually no longer detectable at follow-up measurements after 1–2 years of relationship.

In sum, while important first insights into psychoendocrine associations with different stages of romantic love exist, as of yet, no integrative comparison of long-term hormonal secretion during infatuation and separation phases, such as possible with hair analyses, has been conducted. Further, the chosen control groups greatly vary with individuals in long-term relationships [25], singles [1,16,25], mixed groups [15], or unspecified "healthy controls" [26].

Thus, the aim of the current pilot study was to, for the first time, contrast women currently in phases of infatuation ($n = 16$) and separation ($n = 14$) with women in long-term relationships ($n = 40$) and singles ($n = 31$) with respect to self-report, but also the long-term integrated cortisol, progesterone, and DHEA levels. The study focused on young adult cis women in order to minimize sex-specific influences on endocrine levels [27] and to take into account the expected broad diversity in the relationship life of young adults. Based on the available literature, we expected elevated levels of hair cortisol in both the infatuation and the separation compared to the long-term relationship and single group, while for progesterone, we hypothesized elevations to only be present in the infatuation group. Further, we exploratively assessed the role of DHEA in the context of romantic love, as well as associations of endocrine markers with self-reported characteristics romantic love, distress, and self-regulation factors.

2. Methods

2.1. Participants and procedures

Young female adults aged between 18 and 29 years were recruited via flyers, social media announcements, and invitations during university courses. Prerequisites for the study were an identity as a cis woman (i.e., identifying oneself as a woman and assigned female at birth) and a hair length of at least 1 cm. Exclusion criteria were current severe chronic physical (e.g., diabetes, asthma) or psychological diseases (e.g., psychosis, severe depressive disorder with psychotic symptoms, or bipolar disorder), endocrine (e.g., glucocorticoid-containing or thyroid) medication within 6 weeks prior to participation, smoking more than 15 cigarettes per day, pregnancy or breastfeeding, or being the romantic partner of another study participant. Allocation to the study groups was based on a preliminary online screening using LimeSurvey (BPS Bildungsportal Sachsen GmbH, Chemnitz, Germany). Study groups were defined as follows: infatuation (acutely in love and in a stable relationship for at least one and maximum three months), separation (acutely separated since at least one and maximum three months), long-term relationship (in a stable relationship for at least twelve months), single (no relationship for at least twelve months). In case of study inclusion, participants were invited to the laboratory where they completed self-report inventories via LimeSurvey and had their hair samples taken. In return for participation, course credits and the chance to win gift vouchers (1 x 50 €, 10 x 5 €) were offered. All participants had provided written informed consent before the start of the study. The study protocol was approved by the ethics committee of the Technische Universität Dresden (EK 364092018) and conducted in accordance with the Declaration of Helsinki.

2.2. Measures

Sociodemographic (age, education status, stressful life events, self-identified sexual orientation, relationship history, and subjective degree of infatuation on a scale from 0 to 100) and health-related variables (BMI, regular medication intake, alcohol and drug use, and smoking) were assessed via self-developed questionnaires. For an assessment of self-reported infatuation in the infatuation and long-term relationship group, the Passionate Love Scale (PLS; [28]) was implemented, while relationship satisfaction was measured via the Relationship Assessment Scale (RAS; [29]).

With respect to distress measures, potential adjustment disorder symptomatology in the separation group was assessed via the Adjustment Disorder - New Module 20 (ADNM-20; [30]). For all four study groups, the Perceived Stress Scale (PSS; [31]) measured the extent to which life situations in the last month were perceived as stressful or uncontrollable, while the Patient Health Questionnaire 9 (PHQ-9; [32]) examined core diagnostic criteria for depressive disorders. The trait part of the State-Trait Anxiety Depression Inventory (STADI; [33]) determined the extent of anxiety and depression as an enduring disposition. Regarding self-regulatory capacities, the Difficulties in Emotion Regulation Scale (DERS; [34]) and the Resilience Scale (RS-11; [35]) were applied. On a last note, the Sense of Coherence Scale 13 (SOC-13; [36]) recorded individual manifestations of Antonovsky’s Sense of Coherence concept [37].

2.3. Hair sample collection and preparation

Hair samples were cut as closely as possible to the scalp at the posterior vertex position [9], the region with the proposedly most uniform growth [38]. Data collectors sampled three strands of hair with an overall diameter of approximately 3 mm. Hair samples were wrapped in aluminum foil and stored in a dry, dark place. The 1 cm hair segment closest to the scalp reflecting one month of hormone secretion based on a growth rate of 1 cm per month [39] was analyzed with respect to cortisol, progesterone, and DHEA in the biopsychological laboratory of the Technische Universität Dresden following the published liquid chromatography-tandem mass spectrometry (LC-MS/MS) protocol [40]. This method has been shown to achieve excellent sensitivity, specificity, and reliability with intra- and inter-assay coefficients of variance ranging between 3.7% and 8.8% for cortisol, between 4.3% and 8.3% for progesterone, and between 4.5% and 9.1% for DHEA, respectively [40].

2.4. Statistical analyses and data exclusion

Analyses were performed using SPSS 25 (IBM, Armonk, NY), and R [41]. As the endocrine levels proved to not be normally distributed, for hypothesis testing, log-transformed values were used, while for descriptive purposes, data were reported in original units (pg/mg). For endocrine analyses, eight subjects were excluded due to the intake of endocrine medication. Further, in six subjects, the progesterone concentration was below the detection limit, which was dealt with by conservative ad-hoc single imputation such as recommended for <15% of non-detectable values (replacing the respective non-detectable values with detection limit/2; (e.g. Ref. [42]), resulting in a sample size of n = 14 (infatuation group), n = 12 (separation group), n = 38 (long-term relationship group) and n = 29 (single group) for endocrine analyses. Based on the frequently used criterion of 3 SD above or below the group mean, no individuals had to be excluded as outliers for cortisol and progesterone. However, with respect to DHEA, one individual from the long-term relationship and one from the single group emerged as outlier, who thus were excluded from DHEA analyses.

For group comparisons regarding demographic and health-related data as well as psychological and endocrine characteristics, univariate analyses of variance (ANOVAs; for continuous variables) with Bonferroni-corrected post-hoc tests and χ^2 contingency tables (for dichotomous variables) were used. In cases of ordinal data or too small case numbers, non-parametric tests (i.e., the Kruskal-Wallis or Fisher's exact test, respectively) were used. Age, contraception status, and storage time of hair samples, as well as, in case of group differences, demographic and health-related variables were one by one included as covariates into hypothesis testing. Associations between biomarkers and self-report were studied via Pearson correlations.

3. Results

3.1. Demographic data and self-report facets of romantic love

With respect to demographic data, the four groups differed in age ($F_{3, 97}$ = 4.43, p = .006, η^2_p = .120, see Table 1), with Bonferroni-corrected post-hoc tests showing the separation group to be on average older than the long-term relationship group (p = .003) and no other differences (all $ps \geq .116$). Further, the groups differed in terms of educational status

Table 1
Comparison of demographic and health-related characteristics of the infatuation, separation, long-term relationship, and single group.

	Infatuation (n = 16)	Separation (n = 14)	Long-term relationship (n = 40)	Single (n = 31)	Test statistic	p
Demographics						
Age (years) (*M, SD*)	21.44 (2.28)	23.07 (3.25)	20.38 (2.08)	21.23 (2.45)	$F_{3, 97}$ = 4.43	.006[i]
BMI (*M, SD*)	21.51 (1.93)	21.52 (2.25)	20.97 (2.75)	22.08 (3.05)	$F_{3, 97}$ = 1.00	.395
Highest educational status					Fisher's exact	
Academic degree (%)	2 (12.5)	7 (50.0)	7 (17.5)	29 (93.5)		.008
A level (%)	14 (87.5)	7 (50.0)	33 (82.5)	2 (6.5)		
Stressful life events (last three months) (%)[a]	4 (25.0)	2 (14.3)	2 (5.0)	3 (9.7)	Fisher's exact	.147
Medication intake (last three months) (%)[b]					Fisher's exact	
yes, rarely/seldom	4 (25.0)	3 (21.4)	11 (27.5)	5 (16.1)		.632
yes, regularly	4 (25.0)	2 (14.3)	11 (27.5)	5 (16.1)		
No	8 (50.0)	9 (64.3)	18 (45.0)	21 (67.7)		
Hormonal contraceptives (%)	11 (68.8)	6 (42.9)	26 (65.0)	5 (16.1)	χ^2_3 = 20.16	<.001
Alcohol consumption (%)					H_3 = 1.63	
Never	–	1 (7.1)	3 (7.5)	1 (3.2)		.654
on singular occasions	3 (18.8)	1 (7.1)	8 (20.0)	6 (19.4)		
once or twice a month	6 (37.5)	4 (28.6)	12 (30.0)	9 (29.0)		
once or twice a week	7 (43.8)	7 (50.0)	17 (42.5)	14 (45.2)		
daily or almost daily	–	1 (7.1)	–	1 (3.2)		
Drug use (%)[c]					H_3 = 9.72	
Never	8 (50.0)	11 (78.6)	34 (85.0)	27 (87.1)		.021
on singular occasions	6 (37.5)	2 (14.3)	4 (10.0)	3 (9.7)		
once or twice a month	2 (12.5)	–	1 (2.5)	1 (3.2)		
once or twice a week	–	1 (7.1)	1 (2.5)	–		
Self-Identified Sexual Orientation (%)[d]					Fisher's exact	
Heterosexual	13 (81.3)	12 (85.7)	38 (95.0)	24 (77.4)		.113
Bisexual	3 (18.8)	2 (14.3)	1 (2.5)	6 (19.4)		
Pansexual	–	–	1 (2.5)	–		
Asexual	–	–	–	1 (3.2)		
Duration of current relationship status (months) (*M, SD*)	2.46 (1.33)[e]	2.29 (1.20)	35.27 (19.06)[f]	27.07 (13.11)[g]		
Storage Time of hair samples in days (*M, SD*)	61.01 (38.95)[h]	60.14 (38.56)[h]	92.87 (12.26)[g]	69.33 (35.43)[g]	$F_{3, 89}$ = 7.05	<.001[j]

[a] Included family conflicts (n = 3) and illness (n = 3) or death (n = 5) of a close person.
[b] Included non-steroidal anti-inflammatory drugs (e.g., ibuprofen; n = 17), oral contraceptives (n = 11), glucocorticoid-containing or thyroid medication (n = 8, excluded for later endocrine analyses), antibiotics (n = 4), psychotropic (n = 3), and other medication (n = 4).
[c] Included cannabis (n = 16), stimulants (n = 6), and other substances (n = 1).
[d] Participants were asked to describe their sexual orientation (self-identification label that best describes them either exclusively or predominantly).
[e] Based on n = 13, which only corresponds to infatuated women who have reported.
[f] Four (10%) individuals from the long-term relationship, but none from the single group reported a current open relationship.
[g] Based on n = 14 corresponding to only those individuals from the single group who had reported to have had any previous relationships.
[h] Based on infatuation (n = 14), separation (n = 12), long-term relationship (n = 38) and single group (n = 29).
[i] Separation > long-term relationship group (p = .003) with the infatuation and the single group in between (all $ps \geq .116$).
[j] Infatuation = separation = single < long-term relationship group ($ps \leq .01$).

(Fisher's exact test, $p = .008$), albeit with all individuals having attained at least A level status. With regard to health-related characteristics, the groups differed in terms of hormonal contraceptives ($\chi^2_3 = 20.16$, $p < .001$) and drug use ($H_3 = 9.72$, $p = .021$). Numerical inspection indicated the first to be mainly driven by women in relationships (i.e., the infatuation and long-term relationship group) reporting higher use of hormonal contraceptives. Five subjects mentioned a lifetime diagnosis of psychological disease, with, however, only two reporting a recent diagnosis (depressive episode, borderline personality disorder).

As expected, the groups differed with respect to their self-reported degree of infatuation ($F_{3,\ 97} = 78.76$, $p < .001$, $\eta^2_p = .709$; see Table 2), with the separation and the single group reporting lower levels than both the infatuation and the long-term relationship group according to Bonferroni-corrected post-hoc tests (all $ps < .001$), and the latter also not differing regarding experienced passionate love (PLS) and relationship satisfaction (RAS; all $ps \geq .202$). With respect to the ADNM-20, five separated women (35.7%) had a high risk of adjustment disorder according to the diagnosis algorithm (cf. [43]). Seven individuals (50%) indicated that the separation had been initiated by their partner, three (21.4%) had initiated the separation by themselves, and four (28.6%) reported a mutual decision.

While the four groups did not differ in trait anxiety, depression, or the global score of the STADI (all $ps \geq .216$), for the PSS ($F_{3,\ 97} = 3.06$, $p = .032$, $\eta^2_p = .086$), at trend level, the separated group reported higher subjective stress than the infatuation and the long-term relationship group ($p = .097$ and $p = .081$, respectively), with the single group in between (all $ps \geq .466$). According to PHQ-9 ($F_{3,\ 97} = 3.05$, $p = .032$, $\eta^2_p = .086$), separated women further had higher levels of depression than the long-term relationship group ($p = .038$), with the infatuation and the single group in between (all $ps \geq .261$). No group differences emerged for emotion regulation, sense of coherence and resilience (all $ps \geq .133$).

3.2. Endocrine data from hair samples

With respect to endocrine hair data, a group difference for cortisol emerged ($F_{3,\ 89} = 7.78$, $p < .001$, $\eta^2_p = .208$). Bonferroni-corrected post-hoc tests indicated that the long-term relationship group had lower cortisol levels than the infatuation ($p = .001$), the separation ($p = .031$) and the single one ($p = .003$), and no other group differences (all $ps = 1.000$, see Fig. 1). While for progesterone, no group differences emerged ($F_{3,\ 89} = 0.62$, $p = .602$, $\eta^2_p = .021$), for DHEA ($F_{3,\ 87} = 5.24$, $p = .002$, $\eta^2_p = .153$), Bonferroni-corrected post-hoc tests showed higher DHEA concentrations in the single ($p = .016$) and the separation ($p = .009$) compared to the long-term relationship group, with the infatuated women in between (all $ps \geq .256$). Including the covariates age, medication intake and storage time in the GLMs did not affect the main findings.

Correlational analyses revealed positive associations of hair DHEA both with cortisol ($r = .29$; $p = .006$) and progesterone ($r = .21$; $p = .043$), but not between the latter ($r = -.01$; $p = .958$). Neither hair cortisol, nor progesterone were associated with self-reported love, self-regulation, or distress facets (all $ps \geq .192$). DHEA, however, emerged to be positively associated with depressiveness (PHQ-9; $r = .18$; $p = .086$), as well as inversely with sense of coherence (SOC; $r = -.18$; $p = .096$), but both only as a non-significant trend.

4. Discussion

The aim of the current pilot study was to, for the first time, contrast individuals in acute infatuation and separation phases with others in long-term relationships and singlehood regarding self-reported and psychoendocrine indicators of distress and self-regulation. Here, for the first time, endocrine hair analyses better suited for studying long-term processes (e.g. Ref. [9]), were conducted in the context of romantic love. Although only some elevations of self-reported distress concerning the separation group were observable, and the groups did not differ with respect to hair progesterone, for cortisol and DHEA, contrasting patterns could be shown. While, compared to individuals in a long-term relationship, higher hair cortisol concentrations emerged in the infatuation, the separation and the single group, for DHEA, only the separated and the single group exhibited elevated levels. Although the interpretation of these findings remains preliminary due to the pilot character of the study, it allows the derivation of starting points for further research on bigger, more balanced samples.

While the higher hair cortisol levels of the infatuation and the separation group compared to the long-term relationship group are in line with our hypothesis, contrary to our expectations, this also emerged for the single group. On a first note, the finding for the separation group supports and extends the literature from blood and saliva sampling suggesting increased HPA axis activation in the context of separation and bereavement (reviewed, e.g., in Refs. [10–13]). While the available data on infatuation, at the first glance, seem heterogeneous, they stem

Table 2
Comparison of self-report facets of romantic love, distress and self-regulation in the infatuation, separation, long-term relationship, and single group.

	Infatuation ($n = 16$)	Separation ($n = 14$)	Long-term relationship ($n = 40$)	Single ($n = 31$)	Test statistic	P	Effect size
Romantic love							
Subjective degree of infatuation (0 – 100)	82.94 (15.73)	25.57 (25.99)	79.05 (16.62)	14.42 (23.82)	$F_{3,\ 97} = 78.76$	<.001[I]	$\eta^2_p = .709$
PLS	200.94 (39.21)	–	214.73 (22.42)	–	$t_{19.05} = -1.32$	.202	$d = .432$
RAS	4.05 (0.62)	–	4.23 (0.53)	–	$t_{54} = -1.07$	.291	$d = .304$
Distress and self-regulation							
ADNM-20 (symptom severity)	–	42.64 (14.60)	–	–			
PSS	15.50 (8.73)	21.64 (7.77)	16.58 (5.85)	19.16 (5.92)	$F_{3,\ 97} = 3.06$	.032[II]	$\eta^2_p = .086$
PHQ-9	6.69 (5.38)	9.14 (5.02)	5.33 (4.05)	7.48 (4.02)	$F_{3,\ 97} = 3.05$	.032[III]	$\eta^2_p = .086$
STADI Global value	39.25 (11.01)	40.43 (8.97)	42.93 (9.05)	43.97 (10.79)	$F_{3,\ 97} = 1.02$	.390	$\eta^2_p = .030$
Trait-Anxiety	21.44 (5.61)	22.07 (5.36)	23.13 (5.65)	23.10 (6.20)	$F_{3,\ 97} = 0.43$	.732	$\eta^2_p = .013$
Trait-Depression	17.81 (6.41)	18.36 (4.73)	19.80 (4.73)	20.87 (5.42)	$F_{3,\ 97} = 1.51$	.216	$\eta^2_p = .045$
RS-11	63.25 (8.25)	60.93 (6.87)	61.35 (6.09)	58.48 (7.20)	$F_{3,\ 97} = 1.91$	.133	$\eta^2_p = .056$
DERS	90.06 (21.73)	85.50 (23.42)	86.35 (17.23)	86.32 (19.21)	$F_{3,\ 97} = 0.18$	.907	$\eta^2_p = .006$
SOC-13	61.63 (13.50)	58.14 (13.72)	60.30 (9.75)	57.77 (9.68)	$F_{3,\ 97} = 0.60$	.619	$\eta^2_p = .018$

Note. Data are presented as *(M, SD)*.
Abbreviations: PLS = Passionate Love Scale, RAS = Relationship Assessment Scale, ADNM-20 = Adjustment Disorder - New Module 20, PSS = Perceived Stress Scale, PHQ-9 = Patient Health Questionnaire 9, STADI = State-Trait Anxiety Depression Inventory, RS-11 = Resilience Scale, DERS = Difficulties in Emotion Regulation Scale, SOC-13 = Sense of Coherence Scale 13.
[I] Infatuation = long-term relationship > separation = single group ($ps < .001$); [II] Infatuation = long-term relationship < separation group at trend level (all $ps \leq .097$), with the single group in between (all $ps \geq .466$); [III] Long-term relationship < separation ($p = .038$), with the infatuation and the single group in between (all $ps \geq .261$).

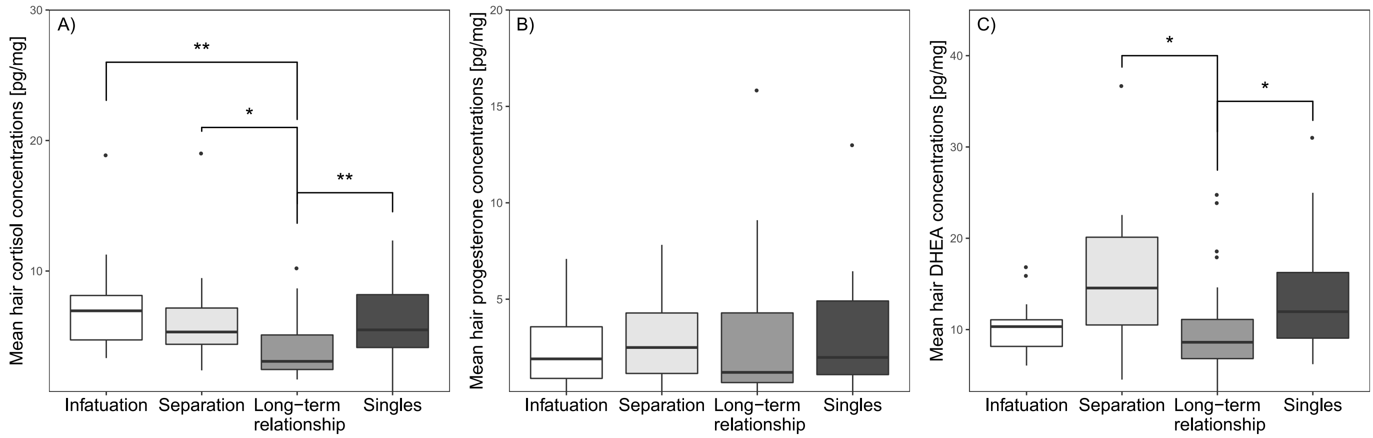

Fig. 1. Boxplots of A) cortisol, B) progesterone, and C) DHEA from hair samples of the infatuation, separation, long-term relationship, and single group (***p < .001., **p < .01, *p < .05). For raw data see supplementary material.

from vastly different group contrasts. Importantly, elevated cortisol levels in infatuation were only reported when contrasted with a mixed group of long-term relationship and single individuals [15], but not with exclusively single groups [1,16,18]; with, in the latter case, the levels of the single group even exceeding the ones for the infatuated group). Thus, our data very well integrate those findings, and elaborate on similar suggestions from the study of [18] on saliva samples.

In sum, the results for cortisol can be explained within the framework of the stress-buffering effect of stable relationships, which is related to social support (e.g. Refs. [44–46]), or less loneliness (e.g., Ref. [7]). Here, oxytocin might play a major role, as there are experimental data suggesting that it enhances the stress-buffering effect of social support on the HPA axis (e.g., Refs. [45,47]) and reduces cortisol levels during couple conflict [48]. Furthermore, as suggested by the polyvagal theory (cf. [49]) as well as the model of neurovisceral integration (cf. [50], these effects might result from the promotion of self-regulatory communication between the brain and the autonomic nervous systems. Thus, an extension of following research to include also markers of the functional status of the proposed brain body connection (such as heart rate variability) would allow a better understanding of the processes of stress and romantic love. While it remains challenging to assess, in particular, long-term oxytocin output (e.g., Ref. [51]), it would also be highly interesting to study whether the lower cortisol secretion of (female) individuals in a long-term relationship suggested here might be related to oxytocin secretion. Further, in order to allow a more holistic picture of other potentially influencing factors, such as lifestyle factors/daily routines for which the groups studied here might differ, it would be intriguing to use ecological momentary assessment (EMA; e.g., Ref. [52]) for real-life, day-by-day analyses.

Earlier attempts of integrating the seemingly conflicting findings further suggested a two-track process of HPA activity in infatuation. While general cortisol secretion might be buffered in romantic love in order to enable approach behavior, acute stressors related to the attachment bond (such as the requirement to think intensely about the new partner; [14] might trigger stress responses [16]. This could cause inconsistent results particularly with respect to singular measurements sensitive to situational influences. Because our study, using endocrine levels from hair samples constituting an integrated estimate of secretion over a longer time frame, is not susceptible to short-term cortisol secretion changes, it provides important long-term support for the notion that not any relationship, but only stable, long-term ones may be associated with smaller cortisol output.

It must be noted, however, that due to its cross-sectional design, the current study precludes causal interpretations. It might also be possible to explain our findings by the notion that generally more stress-resilient individuals with lower cortisol output might be more successful in leading stable relationships. However, this does not seem too probable as our self-report data show no group differences in terms of resilience and coping. Of note, our results also suggest that subjective infatuation is of little significance for cortisol levels, as infatuated women and women in long-term relationships did not differ in terms of self-reported degree of infatuation, subjectively experienced passionate love (PLS), and relationship satisfaction (RAS).

With respect to hair DHEA, a different picture emerged, with increased levels observed in the separated and the single, but not the infatuated compared to the long-term relationship group. This corresponds with previous studies, albeit on DHEA-S, which also reported no increased levels in infatuated individuals compared to single or mixed single and long-term relationship groups [1,15]. While it is also in line with the proposed association of DHEA and stress, and might be explained by the suggested anti-glucocorticoid properties of the hormone (for reviews, see Refs. [23,24]), the deviating picture for the infatuation group compared to the separation group, with only cortisol, but not DHEA elevated, is notable. Importantly, in contrast to cortisol and DHEA, and at variance with our hypothesis, no group differences with respect to progesterone emerged. While this concurs with a previous examination [15], it remains striking due to progesterone's properties as a sex hormone (e.g. Ref. [53]), as well as the postulated associations with commitment motivation (e.g., Refs. [20–22]). In general, it must be noted that both for progesterone and DHEA the literature with respect to romantic love, but also stress research in general is still very sparse, which, at this point, precludes a more in-depth interpretation of the respective findings.

It must be noted that while we initially also had intended to report on testosterone (such as had been done in the study of [15], showing reductions in male, but increases in female infatuated participants in contrast to individuals in long-term relationships, this had proven not feasible due to the fact that in our exclusively female, and relatively young sample, testosterone levels of 89 participants (88.1%) were below the limit of quantification. While high levels of non-detectable values for LC-MS/MS-based testosterone analyses are not uncommon in female samples (e.g. Ref. [54]), this highlights the need for more sensitive analysis methods, as well as better procedures regarding non-detectable values.

The main strength of the study is that it, for the first time, utilizes endocrine hair analyses ideally suited for long-term processes in the context of romantic love. Additionally, for the first time, individuals from infatuation, separation, long-term relationship, and single groups were contrasted within one study, allowing an integration of previous findings. Further, the present study again underlines the necessity to study physiological, in addition to self-report data in psychological research, as, notably, self-report group differences with respect to stress and depressiveness only emerged for the separation in contrast to the infatuation and the long-term relationship, and only the long-term relationship group, respectively, while for anxiety and self-regulation, they were completely missing. This can be considered another observation of the so-called lack of psychoendocrine covariance (i.e., the frequently reported lower-than-assumed associations between endocrine and self-report data), which is considered to be particularly prevalent when studying everyday, non-clinical manifestations of stress [27]. In line with this observation, only weak associations between endocrine and self-report indicators emerged. However, for DHEA, due to the observed polarity of findings for distress and self-regulation facets the directionality of associations, tentatively supports the group-level results.

Major limitations of the study are the unequal and small group sizes, mainly attributable to the fact that subjective romantic experiences are notoriously difficult to operationalize for psychoendocrine studies. This precludes more in-depth statistical analyses with respect to the influences of relationship satisfaction or duration of the relationship status potentially affecting hormonal secretion. Further, as this is the first study in this context focusing on hair analyses, and the previous studies vastly differed with respect to their methodology (e.g., singular versus repeated blood or saliva sampling at fixed or random time intervals), no prior power analysis was feasible. Thus, the results can only be seen as preliminary in the context of a pilot study and urgently need replication in bigger and more balanced samples. Particularly the necessity of setting strict time criteria for the inclusion of especially infatuated and separated individuals encumbered recruiting. In this context, it must be noted that we had applied rather strict criteria for these groups (less than three months since having falling in love/separating, compared to other studies applying criteria of up to six months, (e.g. Refs. [15,16,18]), in order to achieve high discriminatory power between the infatuation and the long-term relationship, as well as the separation and the single group, respectively. While a certain heterogeneity with respect to the individual and potentially fluent emotional and romantic situation, as well as group differences with respect to medication and hormonal contraception intake cannot be precluded, the subjective infatuation from the self-report data, as well as the fact that respective covariance analyses did not show substantial changes of the reported results support the validity of our findings. Further studies could, however, benefit from even more rigorous inclusion criteria, as well as the application of standardized clinical interviews, objective health data and/or medical examinations. Due to its cross-sectional nature, the study is furthermore limited with respect to its potential in illuminating causal processes. Thus, further studies equipped to identify causes and consequences of endocrine alterations in the context of romantic love are of high relevance.

On a last note, in the current study, female subjects were included regardless of their sexual orientation, resulting in singular non-heterosexual participants, which, however, were too few in number to allow specific analyses. As a recent study showed differences between hetero- and homo-/bisexual women with regard to reactive endocrine secretion (i.e., with respect to cortisol [55], further, bigger studies on those groups could bear important implications for the understanding of endocrine processes underlying romantic love. In addition, due to suggested differences with respect to sex/gender in previous studies in this context (e.g. Refs. [1,15]), the study does not allow a generalization of the results to cis men (or, notably, individuals not identifying as cis). Thus, further studies including or focusing on men are warranted. Further, in particular the consideration of dyads or the evaluation of variability as a function of sexual orientation remain exciting questions.

In sum, our results from a pilot study on a preliminary and small sample suggest that different agents of the HPA axis (cortisol, DHEA, and progesterone) may be associated differentially with different relationship situations. This is particularly notable in view of the fact that for self-reported distress, only the separation group showed increased levels. While the results for elevated hair cortisol in the infatuated, the separated, and the single compared to the long-term relationship group might be explained by the stress-buffering effects of stable partnerships, the fact that with respect to hair DHEA, only the separated and the single group showed higher levels than the long-term one suggests further associated mechanisms. This advocates the validity of an integrated examination of endocrine markers in order to physiologically differentiate between different stages of romantic love, which is in line with the general trend towards combined and multimodal panel markers for explaining mechanisms behind psychological states (e.g., Refs. [24,56]). Moreover, however, it also shows the necessity of bigger studies with more heterogeneous participant groups, as well as multimodal long- and short-term biomarker and self-report panels in order to allow a better understanding of the complex mechanisms underlying different stages of romantic love.

Declaration of competing interest

All authors have no conflicts to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cpnec.2021.100061.

Author note

J.R.: conceptualization, data curation, formal analysis, investigation, project administration, writing – original draft, writing – review & editing. M.S.: formal analysis, writing – review & editing. A.W.: conceptualization, writing – review & editing. L.S.: conceptualization, formal analysis, visualization, supervision, writing – original draft, writing – review & editing. J.R. was supported by the Friedrich-Ebert-Stiftung. L.S. was supported by the German Academic Scholarship Foundation. The authors would like to thank Clemens Kirschbaum and the members of the biopsychological laboratory of the Technische Universität Dresden for making the endocrine hair analyses possible and providing helpful advice throughout the study process. Further, we are grateful to Franca Ohde, Anne Peschel, and Kelly Schaunsland for their great help in conducting this research, and to Magdalena Wekenborg for her helpful comments during the revision process. All authors have no conflicts to disclose. Neither of the experiments reported in this article was formally preregistered. Neither the data nor the materials have been made available on a permanent third-party archive; however, requests for the data or materials can be sent via email to the corresponding author. This data has been previously presented in part at the 2019 annual conference "Psychologie und Gehirn", Dresden/Germany, and in totality at the 2020 virtual annual conference of the International Society of Psychoneuroendocrinology (ISPNE), Chicago/IL, and has been published as an abstract in "Psychoneuroendocrinology" [57].

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