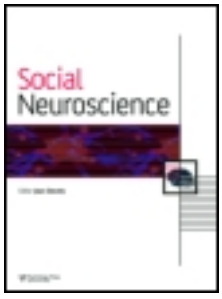

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Mutual influences between partners' hormones shape conflict dialog and relationship duration at the initiation of romantic love

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Early-stage romantic love involves reorganization of neurohormonal systems and behavioral patterns marked by mutual influences between the partners' physiology and behavior. Guided by the *biobehavioral synchrony* conceptual frame, we tested bidirectional influences between the partners' hormones and conflict behavior at the initiation of romantic love. Participants included 120 new lovers (60 couples) and 40 singles. Plasma levels of five affiliation and stress-related hormones were assessed: oxytocin (OT), prolactin (PRL), testosterone (T), cortisol (CT), and dehydroepiandrosterone sulfate (DHEAS). Couples were observed in conflict interaction coded for empathy and hostility. CT and DHEAS showed direct actor effects: higher CT and DHEAS predicted greater hostility. OT showed direct partner effects: individuals whose partners had higher OT showed greater empathy. T and CT showed combined actor–partner effects. High T predicted greater hostility only when partner also had high T, but lower hostility when partner had low T. Similarly, CT predicted low empathy only in the context of high partner's CT. Mediational analysis indicated that combined high CT in both partners was associated with relationship breakup as mediated by decrease in empathy. Findings demonstrate the mutual influences between hormones and behavior within an attachment bond and underscore the dynamic, co-regulated, and systemic nature of pair-bond formation in humans.

Keywords: Romantic attachment; Oxytocin; Testosterone; Prolactin; Cortisol; DHEAS; Couple Conflict; Empathy

Social bonds provide the foundation for the individual's well-being, growth, and adaptation throughout life (Carter, 2014; Insel & Young, 2001). Due to their critical importance, periods of bond formation involve major reorganization of brain, hormonal, and behavioral systems, and successful bonding is underpinned by coordination between the physiological and behavioral responses of attachment partners (Hofer, Robinson, Stehouwer, Mandell, & Selz, 1995; Meaney, 2001). In humans, a central feature of affiliative bonds is that of dyadic synchrony. According to our *biobehavioral synchrony* conceptual model (Feldman, 2007, 2012a, 2012b, 2013), periods of bond formation—such as becoming a parent or falling in love—are characterized by brain, hormonal, and behavioral attunement between attachment partners

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as well by mutual influences between the physiology of one and the behavior of the other and vice versa. In support, we found associations between peripheral levels of oxytocin (OT) and reciprocal social behavior within the three forms of social bonds in humans—parental, romantic, and friendship attachment (Feldman, Gordon, Influs, Gutbir, & Ebstein, 2013; Feldman, Gordon, & Zagoory-Sharon, 2011; Schneiderman, Zagoory-Sharon, Leckman, & Feldman, 2012). Similarly, allelic variations on the oxytocin receptor gene (*OXTR*) were linked with peripheral OT and behavioral synchrony in parental and romantic attachment (Feldman et al., 2012, 2013; Schneiderman, Kanat-Maymon, Ebstein, & Feldman, 2013). Biological synchrony between maternal and infant heart rhythms was observed during the moments of interactive synchrony (Feldman, Magori-Cohen, Galili, Singer, & Louzoun, 2011), and brain-to-brain coupling was found between maternal and paternal brain response to their infant cues in areas implicated in mentalization and empathy, including the insula, dorsomedial prefrontal cortex, and inferior frontal gyrus (Atzil, Hendler, Zagoory-Sharon, Winetraub, & Feldman, 2012). These studies lend support to the conclusion that periods of bond formation are accompanied by complex neurohormonal changes that involve mutual adaptation between the physiological responses of attachment partners as mediated by social behavior. Yet, to date, no study has tested bidirectional associations between the physiological and behavioral responses of romantic partners during the period of falling in love.

In contrast to the extant body of research on the neurobiology of maternal–infant bonding, much less is known about pair-bond formation in humans, and the neurobiological basis of romantic attachment requires much further research (de Boer, van Buel, & Ter Horst, 2012). Early-stage romantic love is thought to constitute a distinct phase, associated with unique brain alterations and hormonal changes (Emanuele et al., 2006), some of which resemble those observed in parental–infant attachment. Studies point to alterations in testosterone (T), increase in cortisol (CT), and dramatic rise in OT levels at the initiation of romantic love (Emanuele et al., 2006; Marazziti & Canale, 2004; Marazziti, Akiskal, Rossi, & Cassano, 1999; Schneiderman et al., 2012), findings that echo those observed for OT, CT, and T in parental–infant bonding (Fleming, Ruble, Krieger, & Wong, 1997; Gettler, McDade, Feranil, & Kuzawa, 2011; Gordon, Zagoory-Sharon, Leckman, & Feldman, 2010a). Studies have underscored the central role of OT in pair-bond formation in mammals (Ross & Young, 2009). Human imaging studies showed that brain areas activated in romantic partners to cues of their loved ones are those rich in OT receptors (Acevedo, Aron, Fisher, & Brown, 2012); OT administration increased positive communication between couples (Ditzen et al., 2009); and low risk on the *OXTR* was associated with greater empathy between new lovers (Schneiderman et al., 2013). Animal studies similarly pointed to the role of OT in the regulation of pair-bond formation in monogamous mammals (Ross & Young, 2009) and blockade of OT receptors impaired the formation of pair bonds (Insel & Hulihan, 1995).

Notwithstanding the central role of OT, research has also described the involvement of other hormones and pointed to the interchange between OT and other neurohormonal systems during the periods of bonding. Human imaging studies showed that romantic attachment is associated with increased activations in dopamine-rich reward areas, including the caudate, nucleus accumbens (NAcc), and ventral tegmental area (Acevedo et al., 2012; Aron, 2005; Bartels & Zeki, 2000; Kim et al., 2009). Associations between the OT and dopamine systems have been observed in both human romantic attachment and animal pair bonding. Following OT administration, romantic partners showed greater activations in dopamine-rich brain areas (Scheele et al., 2013) and expression of the OT receptor in NAcc-enhanced partner preference in female prairie voles (Ross & Young, 2009). OT has also been linked with changes in stress hormones and sex steroids in the context of parental and romantic attachment. OT administration to parent altered the production of CT and T in fathers as mediated by parenting behavior (Weisman et al., 2013; Weisman, Zagoory-Sharon, & Feldman, 2014). In parallel, OT administration to couples reduced CT levels and improved negative communications during conflict discussion (Ditzen et al., 2009). Associations were also found between OT and prolactin (PRL) in human and rodent parents, reciprocally related to parenting behavior (Gordon, Zagoory-Sharon, Leckman, & Feldman, 2010b; Neumann, 2009; Wynne-Edwards, 2001). Although the relations between central and peripheral activity of the OT system are not fully understood, research showing correlations between allelic variations on the *OXTR* and plasma OT (Feldman et al., 2012) and studies indicating marked increases in peripheral OT levels following intranasal OT administration (van Ijzendoorn, Bhandari, van der Veen, Grewen, & Bakermans-Kranenburg, 2012; Weisman, Zagoory-Sharon, & Feldman, 2012) suggest coordination between central and peripheral activity and point to the utility of assessing peripheral hormonal levels in the study of bonding-related processes. Overall, the aforementioned studies suggest that multiple hormonal systems,

possibly triggered by OT changes, interact dynamically to form the affiliative bond and that hormonal levels are bidirectionally impacted by social behavior between attachment partners.

As such, the current study focused on the initial stages of romantic love and tested OT in combination with four other hormones implicated in mammalian bonding: T, PRL, CT, and dehydroepiandrosterone sulfate (DHEAS). To test bidirectional influences between hormones and behavior, we employed a multilevel dyadic analysis, which tests how each partner's hormones affect both his/her own behavior and the partner's behavior and how these mutual influences of hormones and behavior lead to relationship consolidation or breakup. We observed couples during conflict interactions and coded the behavioral dimensions of hostility and empathy. Hostility and empathy in couple conflict discussion provide reliable indicators of relationship quality and predict relationship deterioration and even divorce in longitudinal research (Cramer, 2000; Gottman & Krokoff, 1989; Levenson & Gottman, 1985; Mosek-Eilon, Hirschberger, Kanat-Maymon, & Feldman, 2013).

T is an androgenic steroid produced by the hypothalamic–pituitary–gonadal (HPG) axis that modulates reproductive behavior and plays an important role in human social behavior. Across species, T has been implicated in the search for and maintenance of social status, sometimes in combination with hostile and aggressive behavior (Eisenegger, Haushofer, & Fehr, 2011; Mazur & Booth, 1998; Wingfield, Hegner, Dufty, Jr., & Ball, 1990). In humans, T levels correlate with high dominance (Grant & France, 2001; Rowe, Maughan, Worthman, Costello, & Angold, 2004) and rise in anticipation of competition and following winning (Booth, Shelley, Mazur, Tharp, & Kittok, 1989; Mazur & Lamb, 1980; Oliveira, Gouveia, & Oliveira, 2009). Since competitive behavior often undermines empathy and affiliative interest, T has been shown to decrease interpersonal trust and impair cognitive empathy (Bos, Terburg, & Van Honk, 2010; van Honk et al., 2011). As such, T appears to modulate social behavior by shifting social focus from affiliation to competition (Eisenegger et al., 2011). In the context of romantic attachment, T has been linked with the likelihood of entering relationships (Edelstein, Chopik, & Kean, 2011; Van Anders & Watson, 2007). High T is found in single men and low in men and women within committed relationships or in new fathers (Booth & Dabbs, 1993; Burnham et al., 2003; Gettler et al., 2011; Mazur & Michalek, 1998; Van Anders & Goldey, 2010). Consistent with the hypothesis that early-stage romantic love constitutes a unique neuroendocrine phase, Marazziti and Canale (2004) found low T in men and high T in women during the first 6 months of romantic attachment as compared to a control group including both singles and individuals in long-term relationships.

PRL is a peptide hormone originating mainly in the anterior pituitary lactotroph cells (Freeman, Kanyicska, Lerant, & Nagy, 2000) that has multiple effects on reproduction and lactation and, similar to OT, is thought to mediate the formation of affiliative bonds in humans and other mammals (Neumann, 2009). PRL is released within the hypothalamus and other limbic areas during mother–infant contact in rodents (Luz Torner et al., 2004) and its administration stimulates maternal care in rats (Bridges, DiBiase, Loundes, & Doherty, 1985). PRL reduces hypothalamic–pituitary–adrenal (HPA) activity and was found to have antistress effects in rats (Donner, Bredewold, Maloumby, & Neumann, 2007; Schlein, Zarrow, & Denenberg, 1974; Torner, Toschi, Pohlinger, Landgraf, & Neumann, 2001). In addition, PRL appears to be involved in human fatherhood (Gettler, McDade, Feranil, & Kuzawa, 2012; Gordon et al., 2010b), and lower levels of PRL were found in fathers compared to nonfathers, which further decline after father–infant interaction (Gettler et al., 2011). Yet, while two studies showed correlations between PRL and depression following spouse loss (Jacobs et al., 1986; Lane et al., 1987), no study examined PRL in romantic attachment in humans.

CT is a steroid hormone, secreted by the HPA axis in conditions of stress, and much research addressed the relations between CT and psychological, physiological, and physical health. Extant research documents the effects of social threat on CT in humans (Dickerson & Kemeny, 2004), and CT is involved in the regulation of parenting behaviors in women and men (Fleming et al., 1997; Wynne-Edwards, 2001; Ziegler, 2000). Furthermore, evidence points to the involvement of CT in human pair-bonding. The HPA axis is active during the early stages of romantic attachment (Laurent & Powers, 2007; Marazziti & Canale, 2004), and women engaged in romantic relationships were found to show an increase in circulating CT after merely thinking about their partners (Loving, Crockett, & Paxson, 2009). However, the relationships between romantic partners' CT levels and their joint impact on social behavior have not been explored.

Like CT, dehydroepiandrosterone (DHEA) and its more stable sulfated metabolite DHEAS are secreted from the adrenal cortex in response to adrenocorticotropin-releasing hormone (ACTH). It is suggested that DHEA and DHEAS, which convert into one another by enzymes, protect from the neurotoxic effects of CT during stress (Morgan III et al., 2004). During acute psychological stress elevations of plasma DHEA and DHEAS levels are observed (Lennartsson, Kushnir,

Bergquist, & Jonsdottir, 2012). Furthermore, evidence suggests that both DHEA and DHEAS have antioxidant, anti-inflammatory, neuroprotective, and antiglucocorticoid effects (Maninger, Wolkowitz, Reus, Epel, & Mellon, 2009). Human studies found negative associations between DHEA or DHEAS and depression, panic disorder, and schizophrenia (Barrett-Connor, Von Mühlen, Laughlin, & Kripke, 1999; Fava et al., 1989; Michael, Jenaway, Paykel, & Herbert, 2000; Oades & Schepker, 1994). However, only one study examined DHEAS in the context of romantic bonding and found no difference between romantically attached individuals and controls (Marazziti & Canale, 2004).

The quality of intimate relationships and particularly the dialog of conflict between partners is a central aspect of the relationship that mediates the effects of close couple attachment on health and well-being (Appelberg, Romanov, Heikkilä, Honkasalo, & Koskenvuo, 1996; Friedman et al., 2005; Levenson, Carstensen, & Gottman, 1994; Singer & Ryff, 1999). Warmth and support within close relationships carry beneficial effects on stress-related systems (Holt-Lunstad, Birmingham, & Light, 2008), reducing blood pressure and stress hormones (Grewen, 2005; Holt-Lunstad et al., 2008; Matsunaga et al., 2008). On the other hand, hostile partner conflict increases blood pressure and alters endocrine and immune functioning (Broadwell & Light, 1999; Ewart, Taylor, Kraemer, & Agras, 1991; Kiecolt-Glaser et al., 1993, 1996). The empathic and hostile conflict dialog styles represent distinct and stable interactive styles within couples (Mosek-Eilon et al., 2013) and were found to predict relationship satisfaction and relationship dissolution in longitudinal research (Cramer, 2000; Gottman & Krokoff, 1989; Levenson & Gottman, 1985).

In light of the above, the present study examined the involvement of five bonding-related hormones at the initiation of romantic love; OT, T, PRL, CT, and DHEAS. We first compared levels of these hormones in singles and individuals who began a romantic relationship within the last 3 months, to assess hormonal changes during the period of falling in love. To test mutual influences between hormones and conflict behavior, we utilized the Actor–Partner Interdependence Model, APIM (Kenny, Kashy, & Cook, 2006) and tested statistical effects of both the individual's and the partner's hormones on the two conflict dialog styles—empathy and hostility. Finally, we used mediational analysis to test whether hormonal effects on relationship breakup are mediated by their impact on behavior. Three hypotheses were proposed: (1) consistent with the *biobehavioral synchrony* model (Feldman, 2012a, 2012b, 2013) we expected mutual influences between the partners' hormones and behavior so that both the individual's and the partner's hormonal levels will shape conflict behavior; (2) affiliation hormones, such as OT and PRL, were expected to correlate with greater empathy, whereas stress- and competition-related hormones, such as CT and T to correlate with greater hostility; and (3) the effects of hormones on relationship breakup were expected to be mediated by the mutual influences of the couple's hormones on their conflict behavior.

METHODS

Participants

Participants included 120 young heterosexual adults (60 couples) who began their romantic relationship between 1.5 and 3 months prior to the first visit ($M = 2.4$ months, SD = 0.7). Men were on average 25.03 years (SD = 8.78) and women's age averaged 22.84 (SD = 4.50). Of the 54 couples whom we were able to contact 6 month after the first assessment, 36 were still together (66.6%) and 18 couples had split (6 couples could not be located). All the participants were healthy and completed at least 12 years of schooling. Exclusion criteria included age above 35 years, medication intake, physical or psychiatric condition, and self-reported health problems.

The "singles" group included 40 young adults (19 men and 21 women) who were not involved in any kind of a romantic relationship and did not separate from a former romantic partner within the past 3 months (mean age = 24.63 years, SD = 3.16 years). No differences in demographic conditions were found between new lovers and singles or between lovers who did and did not stay together.

Procedure

Participants were recruited by ads posted in the university campus and surrounding area. Experiments were conducted in a laboratory during the mid-afternoon hours to control for diurnal hormonal patterns. Participants were asked to refrain from any food intake at least 2 hours prior to lab visit. After a brief explanation and signing informed consent, participants completed self-report measures of demographic and health variables (e.g., weight, height, smoking, medication, time since last meal, and contraceptives). Next, blood was drawn by a nurse from the antecubital vein of participants into three tubes: the first was a 9-mL chilled vacutainer tube containing lithium heparin supplemented with 400 KIU Aprotinin (Sigma-Aldrich, St. Louis, MI, USA) per 1 mL

blood. The second was a 5-mL ethylenediaminetetraacetic acid (EDTA) tube. These blood samples were kept ice-chilled for up to 2 hours before being centrifuged at 4°C at 1000*g* for 15 minutes. The third 5-mL gel tube was kept at room temperature for clotting before centrifugation. Supernatants of each tube were aliquant in to several 1.5-mL tubes and stored at –80° C until the samples were assayed.

Couples were videoed in a conflict interaction paradigm adapted from Gottman (1979). Couples were asked to select an area of disagreement among them and discuss this conflict freely for 7 minutes. Length of the interaction was chosen in light of Carrere and Gottman (1999) who showed that the first 3 minutes of a conflict discussion are sufficient to predict later divorce among newlyweds. Couples received 70 USD for participation. The research was approved by the Institutional Review Board, and all participants signed informed consent.

Of the 36 couples who were still together 6 months later, we were able to get a second blood sample from 25 couples (50 individuals, 69.4%). Data for the five hormones at the second assessment and correlations with the first hormonal assessment are presented in the Supplementary Material. Apart from PRL, all hormones showed high individual stability over the 6-month period, attesting to the utility of measuring these hormones in a single plasma sample.

Hormones assays

To determine hormones concentrations, serum samples were defrosted to room temperature before analysis. T, PRL, and CT determinations were done by microparticle enzyme immunoassay (MEIA) technology, measured by AxSYM auto-analyzer (ABBOTT Diagnostics, Abbott Park, IL, USA). DHEAS determinations were done by ElectroChemiLuminescence Immunoassay (ECLIA), measured by COBAS immunoassay analyzer (Roche Diagnostics, Basel, Switzerland). These hormones were measured at AMC- medical Center Laboratories, Rishon LeZion, Israel. The inter-assays and intra-assays for T, PRL, CT, and DHEA-S were less than 16.5%, 8.3%, 4.45%, and 6.3%, respectively. Determination of nonextracted OT was performed at our lab by Dr Zagoory-Sharon using a commercial OT enzyme-linked immunosorbent assay (ELISA) kit (Assay Design, Ann Arbor, MI) consistent with previous research (Carter, 2007; Feldman, Weller, Zagoory-Sharon, & Levine, 2007; Gordon et al., 2008). Samples were diluted 1–5 in assay buffer, measurements were performed in duplicate, and the concentrations of samples were calculated by using MATLAB software (The MathWorks, Inc., Natick, MA) according to relevant standard curves (ranges from 15.6 pM to 1000 pM). The limit of detection is 11.5 pM. The intra-assay coefficients are 5.4% for the medium-high range and 12.4% for low range. The inter-assay coefficients were 8.7% for the medium-high range and 14.5% for low range. The biochemist conducting the analysis was blind to any other information. Hormonal levels were log-transformed prior to the analysis to correct for their non-Gaussian distribution and extreme values excluded.

Behavioral coding

Couple interactions were coded with the Coding Interactive Behavioral Manual (CIB) (Feldman, 1998), adult version. The CIB is a global rating system of dyadic interactions with versions for newborns, infants, children, adolescents, and adults. The CIB has good psychometric properties and has shown sensitivity to differences related to age, interactive partner, cultural background, and risk conditions and has been validated in multiple studies of children and adults (for summary of psychometric measures, see Feldman, 2012c). The adult-adult version of the CIB includes 33 codes for partners: 28 are identical codes for each partner and 5 are coded for the dyad as a whole. Each code is rated on a Likert scale of 1 = low to 5 = high (Schneiderman et al., 2012, 2013). Consistent with prior research (Levenson & Gottman, 1985; Mosek-Eilon et al., 2013) two constructs were constructed by averaging several codes into theoretically based composites:

Empathy—was the average of the following codes: *Acknowledging* the partner's feelings, perceptions, and viewpoints; *Elaborating-Broadening* partner's communications; *Expressing Empathy* through verbal and nonverbal signals; *Emotional Matching* with partner's affective states and level of communicative energy; *Dyadic Reciprocity* interactions contain give-and-receive exchanges that enhance closeness; and *Fluency* interactions are characterized by harmonious, flowing style.

Hostility—was the average of the following codes: *Expressing Anger* through verbal and non-verbal communications; *Gaze Aversion* from partner, *Hostility* expressed through scorn, hostile comments, or facial and body movement; *Assertiveness-Dominance* by "taking the floor", showing superiority, or "putting down" partner, *Constriction* interactions contain little effort to dialog conflict or express affection, and *Tension* interactions are "stuck" and filled with anxiety. Detailed description of each code are found in the Supplementary Material.

Inter-rater reliability, conducted for 15% of the sample, averaged, intraclass $r = .94$ (range = .88−.99).

Statistical analysis

Differences between singles and lovers were tested with analysis of variance (ANOVA). Differences between men and women in hormones and behavior were tested with *t*-tests, and associations between hormones were tested with Pearson's correlations. The APIM (Kenny et al., 2006) was used to test *statistical* effects of the individual's and the partner's hormones on conflict behavior, controlling for dyadic data dependency. Finally, mediation analysis (MacKinnon, 2008) was used to test the effects of hormones on relationship termination as mediated by behavior.

RESULTS

We first examined hormonal differences between new lovers and singles and between men and women. Next, we tested whether hormonal levels of individuals and their partners predict the hostile and empathic conflict interaction styles. Finally, we conducted mediation analysis assessing whether the effects of hormones on conflict behavior can predict relationship breakup 6 months later.

As a first step we examined whether variability in hormonal levels were related to the use of hormonal contraceptives or time since last menstrual cycle. Twenty-two women in the new lovers group and 13 in the singles group were using hormonal contraceptives and the number of days since last menstrual cycle was 14.10 (SD = 10.86). No associations were found between the use of hormonal contraceptives and time since last menstrual cycle with any hormonal or behavioral variable.

Hormonal differences between new lovers and singles

Means, standard deviations, and ranges for the five hormones in new lovers and singles are found in Table 1. Differences between new lovers and singles were found only in OT: F (1,155) = 41.74, $p < .000$, but not in any other hormones. During the first period of falling in love OT levels rise dramatically, nearly doubling their levels in singles.

We next examined the differences between hormonal levels of men and women at the initiation of romantic love using dependent sample *t*-tests. Results showed higher DHEAS ($t(58) = 4.07$, $p < .001$) and T ($t(54) = 12.74$, $p < .001$) in men, higher PRL ($t(58) = 3.19$, $p < .01$) and CT ($t(56) = 2.99$, $p < .01$) in women, and no gender differences in OT ($t(57) = 0.58$, NS) at the initiation of romantic love. No gender differences were found in hostility ($t(58) = 1.25$, NS) or empathy ($t(57) = 1.24$, NS). These findings are presented in Figure 1. Pearson's correlations in levels of each hormone between partners showed inter-relatedness only in PRL, $r = .29$, $p < .05$.

Pearson's correlations between hormones indicated that OT was negatively associated with DHEA in both women, $r = -.31$, $p < .05$, and men, $r = -.27$, $p < .05$. Among men, OT was positively correlated with CT, $r = .31$, $p < .05$. For women, DHEAS was positively correlated with T, $r = .44$, $p < .01$, and PRL, $r = .29$, $p < .05$. For men, CT was positively associated with, DHEAS, $r = .26$, $p < .05$, and PRL, $r = .38$, $p < .01$.

Hormones as predictors of conflict behavior: Dyadic analysis

Preliminary analysis of the correlations between hormones and behavior revealed only one significant associations between CT and hostility in women: Women with higher CT expressed more hostile behavior. However, these findings are limited since they consider only the participant's data and do not take into account the partner's. Moreover, the structure of the current data was nested, and participants were grouped within dyads. Ignoring such dyadic dependencies may bias significance tests, increase type I

TABLE 1
Hormones means, SDs, and ranges of new lovers and singles

	New lovers			*Singles*		
	M	*SD*	*Range*	*M*	*SD*	*Range*
OT (pm/mL)	459.05	140.26	180.36–1053.00	257.82	239.92	114.10–1255.03
DHEAS (mmol/L)	286.51	117.74	69.91–650.70	310.65	117.75	137.20–488.60
T (ng/mL)	2.86	2.79	0.06–10.90	2.28	1.97	0.11–5.63
PRL (ng/mL)	11.27	6.34	1.10–38.50	11.03	5.16	5.80–25.10
CT (ug/dL)	13.00	6.00	2.90–36.50	11.29	12.98	4.20–20.20

Notes: OT = oxytocin, DHEAS = dehydroepiandrosterone sulfate, *T* = testosterone, PRL = prolactin, CT = cortisol.

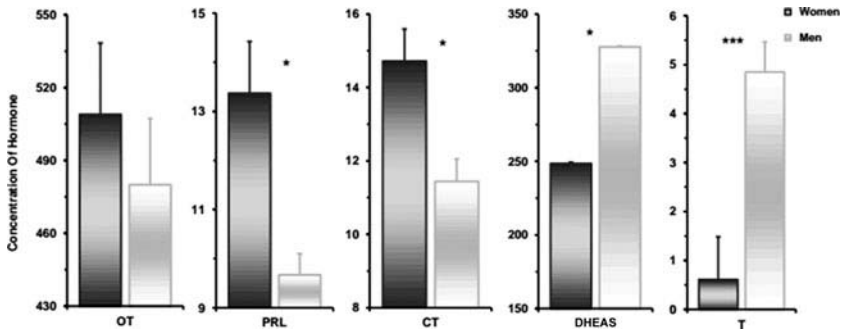

Figure 1. Hormonal levels in men and women at the initiation of romantic attachment.

errors, and undermine statistical power (Kenny, 1996). We thus used a multilevel modeling (MLM) approach to conduct a fuller and more rigorous dyadic analysis to examine the central hypothesis regarding the mutual effects between partners' hormones and conflict behavior.

The APIM (Kenny et al., 2006) was used to control for dyadic data dependency. In these analyses, an *actor effect* occurs when one's own hormonal level predicts one's own behavior on the criterion variable (i.e., hostility, empathy), and a *partner effect* is noted when the partner's hormonal level predicts the actor's score on that criterion. Gender was included to control for potential variation between men and women. Interactions between gender and hormones were included to examine whether the association between hormones and the criterion variables vary across men and women. Interactions between actor and partner effects were also included to examine how the combination of hormonal levels in the two partners predicted hostile and empathic conflict behavior. The APIM was conducted by using the MIXED procedure in SPSS (Heck, Thomas, & Tabata, 2010) with restricted likelihood estimation to estimate the coefficients (Kenny et al., 2006).

The first set of equations included the main effects for actor and partner hormones and gender. The MIXED procedure estimates coefficients for one criterion at a time, and thus hostile and empathic behaviors were examined separately. Due to limitation of sample size, analyses of actor and partner effects were conducted separately for each hormone. Prior to analysis, gender was "effect" coded (women = −1, men = 1) and the predictors were centered around the grand mean.

Main effects

Table 2 provides coefficients for actor and partner effects on hormones predicting behaviors. As seen, significant actor effects were found for DHEAS and CT in predicting hostility, indicating that individuals with higher DHEAS and CT showed greater hostility. A significant actor effect was also found for CT in predicting empathy, with individuals with higher CT displaying lower empathy. A significant *partner effect* was found for OT in predicting empathy. Participants exhibited more empathy to the extent that their *partner*s had higher OT.

Interactions

A significant actor's T by gender interaction emerged for empathy. The interaction was probed by estimating simple slopes for T in predicting empathy for men and women. Tests of simple slopes indicated

TABLE 2
APIM analysis: unstandardized MLM coefficient for actor's and partner's hormones as predictors of hostile and empathic behaviors

	Hostility					*Empathy*				
	OT	*DHEAS*	*T*	*PRL*	*CT*	*OT*	*DHEAS*	*T*	*PRL*	*CT*
Intercept	1.78	1.69	2.05	1.71	1.70	3.59	3.61	3.07	3.63	3.61
Gender	−.09*	−.09*	−.09	.01	−.03	.04	.16	.86*	.22*	.047
Actor	.00003	.00109**	.183	.011	.022**	.00105	−.00063	−.225	.026	−.037*
Partner	.00027	.00008	.156	−.016	.011	.00155*	−.00021	.092	−.012	−.009
Actor × gender	.00056	.00048	.026	.014	.001	.00009	−.00037	.348*	.031	.004
Partner × gender	−.00012	−.00040	−.052	.009	.002	−.00118	.00034	.002	.011	−.010
Actor × partner	−.00001	−.00001	.096*	−.001	.003	.00001	−.00001	.060	.001	−.007*

that women's T levels predicted less empathy ($b = -.58$, $p < .05$), whereas men's T was not associated with empathic behaviors ($b = -.12$, NS). No other actor or partner by gender interactions emerged.

Next, we tested whether the combination of the partners' hormones predicted conflict behavior. These analyses yielded several interesting findings. First, in predicting hostile behaviors, a significant interaction between actor's and partner's T emerged. In probing the interaction, we selected data points for estimating regression lines at ±1 SD for predictors of the equation (Aiken & West, 1991). Tests of simple slopes revealed that the association between actor's T and hostility was positive only when the partner also had high T ($b = .46$, $p < .05$) but was negative when the partner had low T ($b = -.10$, $p < .05$). An additional actor–partner interaction was found for CT in predicting empathy. Test of simple slopes indicated that the negative association between actor's CT and empathy was significant only when the partner also had high CT ($b = -.07$, $p < .01$), but not when the partner had low levels of CT ($b = .001$, NS).

Mediation analysis

Finally, we examined how hormonal levels of actors and partners impact the termination of the relationship through their effect on conflict behaviors. Mediation was tested by assessing the cross product of the coefficients for the hormones to behaviors during conflict (the *a* path), and the behaviors during conflict to breakup controlling for hormones (the *b* path). An *ab* cross product test is recognized as the best available method to test mediation (MacKinnon, 2008; MacKinnon, Lockwood, Hoffman, West, & Sheets, 2002).

Hormones and behaviors during conflict are considered the individual-level variables (level 1), that is, they are measured for each partner individually and can vary between romantic partners. Breakup is a dyadic level variable (level 2) as it can vary from dyad to dyad but cannot vary from person to person within each dyad. For this kind of bottom-up mediation analysis, Croon and van Veldhoven (2007) proposed a two-stage method which combines coefficients estimated with MLM for level-1 variables (the *a* path) with coefficients estimated with regression for level-2 variables adjusted for level-1 variability (the *a* path).

Here, the *a* coefficient was estimated by applying the MLM for the dyadic analysis. The *b* coefficient was estimated by logistic regression, since the dependent variable (breakup) was dichotomous. We followed MacKinnon (2008) guidelines for estimating the *ab* cross product and its 95%CI when outcome is dichotomous. The mediation effect was calculated only in cases when *a* and *b* coefficients were significant. The *a* coefficients were presented in Table 2. Results for the *b* coefficient using logistic regression yielded significant effect for empathy ($b = -1.18$, $p < .05$) but not for hostility ($b = -.61$, NS). These results suggest that partners who express more empathy during conflict dialog were less likely to separate 6 months later. Since only empathy proved a significant predictor of breakup, we tested potential mediation only for variables that showed significant effects on empathy. We first tested whether the partner's OT effect on empathy can lead to breakup. This indirect effect ($b = -.0021$) had 95%CI of –.0046, .0006 which contained zero, thus indicating no significant result. Next, we examined whether empathic behaviors can mediate the effect of women's T on breakup. This indirect effect ($b = .78$) had 95%CI of –.21, 1.75 which similarly contained zero, thereby indicating no significant effect. No significant indirect effect was also found for the actor's CT on breakup through empathic behaviors ($b = -.049$) as the 95%CI of –.104, .006 contained zero. However, the indirect effect of actor–partner CT effect on breakup through empathic behaviors ($b = -.094$) was found significant as the 95%CI of –.187, –.001 did not contain zero. This indicates that when both the partners had high CT, the couple tended to express less empathy during conflict dialog, which, in turn, increased the likelihood of breakup 9 months after the initiation of the relationship.

DISCUSSION

Despite the importance of romantic attachment to human health and well-being (Canetto & Lester, 2002; Emanuele, 2009), neuroendocrine investigations of human pair-bonding remain scant. Results of the current study are the first to demonstrate bidirectional associations of hormones and behavior between partners at the first stages of romantic love. We found that during the first 3 months of romantic attachment, hormones of individuals exert significant effects on their own behavior, their partners' behavior, but, moreover, the effects of the individual's hormones on his or her own behavior are constrained by the partner's hormonal levels so that in the context of different levels of partner's hormones, the individual's hormones exert different effects on behavior. Such dyadic influences, in turn, were associated with the consolidation or termination of the relationship, predicting relationship breakup 6 months later. A summary of the model and the results are presented in Figure 2. As seen, the individual's CT and DHEAS

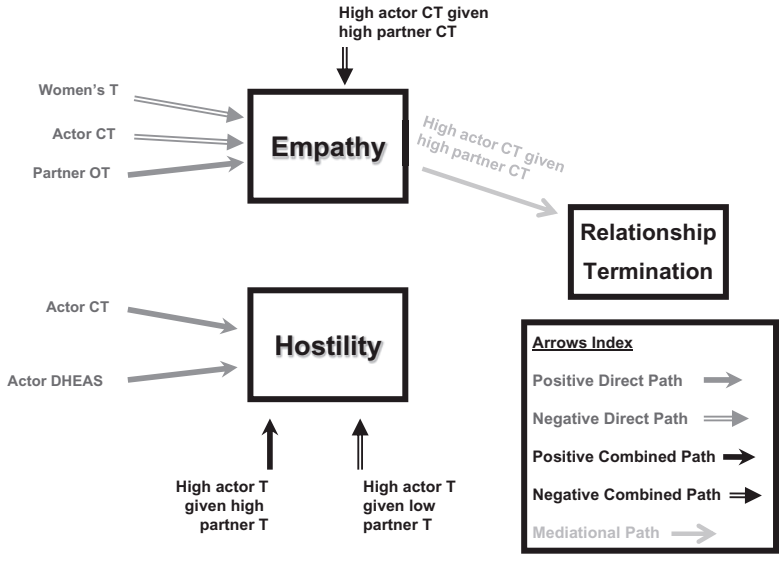

Figure 2. Mutual influences between romantic partners' hormones and conflict behavior leading to relationship termination.
Note: Arrows represent significant paths using the Actor-Partner Interactive Model (Kenny et al., 2006) and mediational analysis (MacKinnon, 2008).

predicted greater hostility. In addition, individuals with higher CT and women, but not men, with more circulating T exhibited lower empathy during conflict (actor effects). Partner effects were noted for OT: in the context of partners with higher OT, individuals expressed greater empathy. Combined effects of both the partners' hormones were also found. The individual's T levels predicted greater hostility only in the context of high partner T but were associated with lower hostility when partner's T was low. Similarly, CT predicted reduced empathy only when the partner also had high CT, but not when partner's CT was low. Such a combination of high CT in both the partners leading to low empathy charted the path to relationship breakup. Overall, these findings highlight the co-regulatory framework in which hormones and behavior shape each other within intimate relationships and underscore the dynamic, biobehavioral, and systemic nature of human social bonding Importantly, however, it should be noted that the effects described here are statistical effects and do not imply causal relationships. Research utilizing longitudinal and experimental designs is required in order to infer causality.

Our findings support and further extend the *biobehavioral synchrony* model (Feldman, 2012a, 2012b). In accordance with the model, the data show that the effects of biological processes on attachment relationships are mediated by social behavior. Moreover, the findings indicate that the effects of neurohormonal processes on bonding are not confined to the individual's hormone-behavior correlations but are also impacted by the partner's hormones and his or her hormone-to-behavior links. Thus, to further study the biological basis of attachment, there is a need to move beyond the individual and adapt the dyad as the unit of analysis for a fuller biobehavioral matrix that tests all four possible paths: hormones-to-hormones, behavior-to-behavior, hormone-to-behavior, and behavior-to-hormone under multiple hormonal contexts provided by the partner. Furthermore, while hormones are thought to shape relational behavior, it is similarly possible that behavior of both the partners shaped hormonal production. The current results further suggest that while similar processes are thought to underpin the formation of parental, pair, and filial attachment, within each bond biobehavioral synchronous processes are expressed in a unique way. Unlike the mother–infant bond, where partners share their genetic makeup, experience extended period of hormonal attunement in utero, and under all normal circumstances the bond continues, romantic attachment requires a quick and intense period of hormonal realignment. Furthermore, romantic relationships often results in breakup under no abnormal conditions, providing a unique model to address biobehavioral predictors of bond dissolution in healthy individuals. Assessing the distinct ways in which synchronous processes support each form of social bonding may thus provide an important window to study the neurobiology of human attachment.

OT has been described as the central neurohormonal system underpinning mammalian bonding (Carter,

2014; Insel, 2010; Ross & Young, 2009). Here we found that of all hormones, OT was the only one showing significant increase at the initiation of romantic love. It is thus possible that the marked increase in OT levels during this stage initiated a complex neurohormonal process that supported the emerging bond, but this hypothesis requires much further research. Interestingly, OT was the only hormone with direct partner effects: new lovers expressed higher empathy when their partners—not themselves—had higher circulating OT. Thus, OT may be the hormone that "binds" couples together by linking one partner's hormonal response to the other's empathic behavior. Since OT functions as a positive feedback loop (Carter, 2014) such partner OT-individual's empathic behavior correlations may in turn contribute to greater expression of affiliative behavior, further increasing OT release in both the partners. Extant research pointed to the relationship between OT and empathy (Gonzalez-Liencres, Shamay-Tsoory, & Brüne, 2013); yet very few studies examined empathy as measured by direct observations of behavior in real-life social contexts, not through self-reports or computer simulations, and our study contributes to this emerging research. As low empathy, not high hostility, was the factor leading to relationship breakup, such OT effects may be important at this precarious stage to support the consolidation of the romantic bond.

In addition to OT, CT played a central role in this dynamic, biobehavioral, co-regulated process of romantic bond formation and bond dissolution. CT was the only hormone that showed actor effects on both hostility and empathy in addition to a combined actor–partner effect on empathy. High CT predicted low empathy only when the partner also had high CT, but not when the partner's CT was low. Finally, the combination of both the partners' high CT was the only significant path leading from hormones to relationship breakup as mediated by behavior, in this case low empathy. These findings are consistent with previous research on the associations between emphatic communication and low CT (Torner et al., 2001), and between interpersonal hostility and elevated CT levels (van Bokhoven et al., 2005). Findings are also consistent with the hypothesis that the benefits of positive social relationships, particularly empathic couple relationships, are mediated to some degree by their effects on reducing HPA reactivity (Ditzen et al., 2007; Robles & Kiecolt-Glaser, 2003). In contrast, higher CT was associated with greater hostility during couple interactions. Interestingly, Marazziti and Canale (2004) showed high CT levels in the first 6 months of a romantic relationship. We found that although CT levels were not higher in lovers compared to singles, women had higher levels of CT at the initiation of romantic love, which possibly index higher arousal or stress at this point. Finally, our findings underscore the role of CT as a *dyadic* neuroendocrine system during bond formation and that only when both partners show high stress the individual's elevated CT leads to relationship termination. However, since only one assessment of CT was included in our model, these hypotheses require further research in longitudinal and experimental designs.

T levels were, as expected, higher in men; however, women, but not men, with high T levels were more hostile during conflict interaction. These differences in hostile communication between women with high and low T are in line with Van Honk et al. (2011) study, which showed that women with high T are more hostile. It has been shown that high T impairs cognitive empathy especially among women (Van Honk et al., 2011), and the current findings show similar associations during the first stages of romantic attachment. In addition, T was the only hormone to have a combined two-tailed effect: High T levels predicted greater hostility when the partner's T was high but predicted lower hostility when the partner's T was low. Extant literature indicates that T serves as a biomarker of the individual's readiness to enter romantic relationship and declines when relationships mature into long-term bonds (Burnham et al., 2003; Edelstein et al., 2011). Thus, T may be high when the individual is motivated to find a romantic partner and when the partner's T is low, indicating that the partner is committed to the relationship, this combination may be expressed in lower hostility within the dyad. However, when both the partners have high T, perhaps indicating that both are uncertain about the relationship, hostility may increase. These findings suggest that establishing an intimate bond is a dynamic process where two strangers must enter a close relationship in a step-by-step manner. During such a sensitive process, the delicate balance between the partners' hormones, commitment to the relationship, and their online dyadic behavior is played out to shape the course of the relationship.

To our knowledge, this is the first study to examine a panel of hormones in relation to conflict dialog in new lovers. This is also the first study in which mutual influences between these hormones are tested as mediators of relational behavior and as predictors of relationship consolidation or termination. Since social interactions between partners serve as important building blocks of intimate relationships (Cohan & Bradbury, 1997; Gottman, Coan, Carrere, & Swanson, 1998), the data suggesting hormonal involvement in the communication patterns of new lovers

point to the need for much further research on the neurohormonal basis of intimate relationships. Taken together, our findings suggest that OT, T, PRL, CT, and DHEAS may be involved in the first stages of romantic attachment in humans, although their specific roles are still largely unknown. It is possible that these hormones act together and mediate the positive effects of close relationships on health and well-being through their impact on core relational behaviors or that they act by fine-tuning one person's neuroendocrine systems to enable the inclusion of another human into close affiliation without the long period of gestation that enable maternal–infant bonding.

Limitations of the study should be considered in the interpretation of the findings. First, due to obvious constraints in human research, we did not measure hormones at the brain neurochemical level, and further research is required to assess their brain expression using imaging techniques. Second, for obvious methodological limitations, we did not follow the same individuals from a stage of being singles to bond formation, and it is possible that other factors were involved in relationship consolidation or termination. Finally, longitudinal assessment of the same couples into long-term couple relationship could have shed important light on the distinct processes involved in bond formation and bond maintenance in romantic attachment. Social isolation and social deficits play an important role in the majority of psychiatric disorders (Bora, Yucel, & Allen, 2009), and it is thus important to understand how the neurobiology of attachment affects interpersonal social behavior. Much further research is needed to integrate brain, hormonal, and behavioral analysis to further specify the neurological substrates implicated in romantic attachment and their role in supporting long-term intimate bonds, promoting health, and decreasing psychopathology.

Supplementary material

Supplementary material is available via the "Supplementary" tab on the article's online page (http://dx.doi.org/10.1080/17470919.2014.893925).

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