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Affiliation, reward, and immune biomarkers coalesce to support social synchrony during periods of bond formation in humans

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ABSTRACT

Social bonds are critical for survival and adaptation and periods of bond formation involve reorganization of neurobiological systems as mediated by social behavior. Theoretical accounts and animal studies suggest similarity between parent–infant and pair bonding, a hypothesis not yet directly tested in humans. In this study, we recruited three groups of human adults ($N = 189$); parents who had their firstborn child in the last 4–6 months, new lovers who began a romantic relationship within the past 4 months, and non-attached singles. We measured plasma oxytocin (OT), beta endorphin (β-End), and interlukin-6 (IL-6), biomarkers of the affiliation, reward, and stress-response systems, and micro-coded gaze and affect synchrony between parents and infants and among new lovers during social interaction. OT significantly increased during periods of parental and romantic bonding and was highest in new lovers. In contrast, IL-6 and β-End were highest in new parents and lowest in singles. Biomarkers became more tightly coupled during periods of bond formation and inter-correlation among hormones was highest during romantic bonding. Structural equation modeling indicated that the effects of IL-6 and β-End on behavioral synchrony were mediated by their impact on OT, highlighting the integrative role of the oxytocinergic system in supporting human social affiliation. Findings suggest that periods of bond formation are accompanied by increased activity, as well as tighter cross-talk among systems underpinning affiliation, reward, and stress management and that research on the multidimensional process of bonding may shed further light on the effects of attachment on health.

1. Introduction

Humans are fundamentally social and have a basic need to belong, connect, and bond with others. The need for social belonging has deep evolutionary roots, appears across species and taxa, and critically shapes survival and adaptation to the living ecology (Clutton-Brock, 2002; Feldman, 2012b; Silk, 2007; Wilson, 2012). In mammals, the ability to connect with conspecific is learned within the parent–infant bond through synchronous processes that reorganize the infant's brain and neuroendocrine systems toward life with others (Feldman, 2012b, 2015a,b; Hofer, 1994; Meaney and Champagne, 2001; Numan, 1985; Rosenblatt, 2003). Such fine-tuning enables young to engage in social-affiliative processes throughout life; from mating, pregnancy, and parturition to complex social interactions with close partners or group members, processes underpinned by the same neurobiological systems (Curley and Keverne, 2005; Dunbar, 2010). In particular, biobehavioral experiences within the parent–infant bond reorganize hormonal and brain systems that support three main functions critical for survival: the ability to form affiliative bonds, the capacity to draw reward from close relationships (Depue and Morrone-Strupinsky, 2005; Trezza et al., 2011), and the ability to manage the stress of daily living and buffer the wear-and-tear of stress on health (Danese and McEwen, 2012; Weaver, 2009). Theoretical accounts ranging from attachment theory (Bowlby, 1969) to biobehavioral models (Feldman, 2012b, 2015a) to evolutionary perspectives (Ross and Young, 2009) highlight the similarity between parent–infant and pair bonding and suggest that during periods of bond formation the three functions – affiliation, reward, and stress management – become more tightly coupled to ensure successful

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bonding. These models further suggest that experiences within the parent–infant bond provide a template for the formation of pair bonding in adulthood (Feldman, 2015b; Numan and Young, 2016; Sroufe, 2005).

Extant research indicates that mammalian bonding is supported by oxytocin (OT), a neuropeptide produced primarily in magnocellular neurons in the hypothalamus (Donaldson and Young, 2008; Insel and Fernald, 2004). Maternal OT is triggered by birth and increases through maternal–infant contact and the species-typical maternal behavior (Lim and Young, 2006). In rodents, maternal licking-and-grooming carries long-term effects on offspring sociality by organizing OT receptor densities in the striatum and nucleus accumbens (NAcc), structures in the dopamine reward pathway (Olazábal and Young, 2006; Liu et al., 1997), and glucocorticoids receptor densities in the hippocampus (Champagne et al., 2008). These findings highlight maternal behavior as a key social experience required for binding the affiliation-related OT with components of the reward and stress-response systems. Animal studies of the maternal brain similarly show that following childbirth, the hypothalamus, triggered by OT, projects to the reward-related ventral tegmental area (VTA) and NAcc to enhance maternal reward from her pups. In parallel, the hypothalamus projects to the amygdala to increase maternal vigilance of infant well-being. These projections coalesce to form the "mammalian maternal caregiving network" (Numan and Sheehan, 1997) by binding the OT-producing hypothalamus, the limbic dopaminergic reward pathway, and the vigilance monitoring amygdala (Aragona et al., 2006; Numan, 2006; Musser et al., 2012). A similar caregiving brain network has been shown for fathers in bi-parental rodents and humans, triggered by active paternal caregiving and exposure to infant stimuli (Abraham et al., 2014; Bales and Saltzman, 2016).

Human studies similarly point to the involvement of OT in parental bonding, its links with synchronous parent–infant interactions, and its associations with neuroendocrine systems implicated in reward and the stress response. Maternal and paternal plasma OT levels increased during the postpartum period and correlated with interaction synchrony (Gordon et al., 2010; Feldman, 2007c); parent–infant interactions including more touch induced OT release in mother and father (Feldman et al., 2010); following synchronous interactions parent and infant's OT increased in a coordinated fashion (Feldman et al., 2011); and OT administration to parent increased salivary OT and synchronous behavior (Weisman et al., 2012). Links between OT with reward and stress management systems as mediated by synchronous behavior have also been described. OT administration to parent altered parent's and infant's cortisol levels as a function of their behavioral synchrony (Weisman et al., 2013); mothers displaying greater synchrony showed higher NAcc response to their infant's video, which correlated with plasma OT (Atzil et al., 2011); activation of mother's ventral striatum was related to OT levels (Strathearn et al., 2009); and breastfeeding mothers showed greater amygdala response to their infants' cries (Kim et al., 2010). Overall, studies indicate that parental bonding, triggered by OT, is linked with high reward on the one hand but increased stress on the other. This is supported by research demonstrating changes in stress hormones and inflammatory biomarkers in the perinatal period, including cortisol, adrenocorticotropic hormone (ACTH), corticotropin-releasing hormone (CRH), monoamines, and macrophages-derived pro-inflammatory cytokines (Kammerer et al., 2006; Osborne and Monk, 2013) and its transmission to offspring via multiple pathways including breastmilk (Iyengar and Walker, 2012; Kunz et al., 1999; Minniti et al., 2014).

Human pair-bonding is similarly supported by the same affiliation, reward, and stress-response systems. During the first period of romantic attachment, OT levels increased and correlated with behavioral synchrony between partners (Schneiderman et al., 2012); variability on the *OT receptor* predicted expressed empathy (Schneiderman et al., 2014); and plasma OT levels in one partner predicted empathic behavior in the other (Schneiderman et al., 2014). Similar to the effects of touch on OT in parents and infants, warm touch among couples has been associated with higher OT and reduced cortisol levels (Hegadoren et al., 2009; Light et al., 2005). In addition, higher cortisol levels were observed during romantic bonding, indicating increased stress (Marazziti and Canale, 2004); OT administration to couples reduced cortisol during conflict discussion (Ditzen et al., 2009); and low empathy in new lovers correlated with higher cortisol (Schneiderman et al., 2014a,b). Brain imaging studies similarly showed neural response in OT- and dopamine-rich areas such as VTA and NAcc in response to romantic partner's stimuli (Aron et al., 2005; Bartels and Zeki, 2004; Scheele et al., 2013; Young and Wang, 2004). Overall, it appears that periods of parental and pair bond formation are linked not only with increased activity in affiliation, reward, and stress-response systems but also in their coupling together to supports the complex process of bonding. This hypothesis, however, has not been systematically tested.

To address this gap, in the current study we measured plasma OT, beta-endorphin (β-End), and interlukin-6 (IL-6), biomarkers of the affiliation, reward, and stress/immune systems respectively, in three groups of human adults; parents who had their first child within the last 4–6 months (new parents), romantic partners who fell in love within the last four months (new lovers), and singles not in a romantic relationship during the last six months (singles). We aimed to test whether levels of these hormones increase, become more tightly coupled, and support behavioral synchrony.

Beta-endorphin (β-End) is an endogenous opioid involved in multiple processes related to reinforcement and reward. Released into the cerebrospinal fluid, β-End is related to stress-reduction, leading to a sense of well-being by homeostatic balance (Barendregt, 2015). In several primate species, 'bond maintenance' is achieved by tactile stimulation (social grooming), which initiates the release of β-End (Keverne et al., 1989), and β-End is involved in maternal behavior, mother–infant interactions (Kalin and Shelton, 1989; Martel et al., 1993; Nelson and Panksepp, 1998; Panksepp et al., 1997), and social attachment (Panksepp, 1986).

IL-6 is a pro-inflammatory cytokine produced by a number of cell types, such as T-cells, B-cells, macrophages and adipocytes (Mika et al., 2013). IL-6 plays diverse roles in the immune system, mainly during the acute phase response. IL-6 is an important mediator of the fever response during inflammation, promotes leukocyte proliferation and differentiation, and enhances programmed cell death (Akira et al., 1990). In addition to its role as an immune mediator, evidence describes the involvement of IL-6 in the stress-response. Research demonstrates an increase in IL-6 release in response to a variety of acute lab-based psychosocial stressors, such as the Trier Social Stress Test, arithmetic tasks, mirror-tracing tasks, or public speech tasks, which are not associated with infection or inflammatory reaction (Slavish et al., 2015; Steptoe et al., 2007, 2001). Longitudinal studies suggest that chronic stress may substantially augment the typical age-related increase in IL-6 (Kiecolt-Glaser et al., 2003) and elevated IL-6 levels were also found following trauma (Wieck et al., 2014). Studies have shown that marital conflict can induce IL-6 increase (Graham et al., 2009; Heffner et al., 2004; Kiecolt-Glaser and Glaser, 2005), indicating that immune biomarkers are triggered by emotional arousal within human pair bonds. Similarly, in healthy pregnancies levels of IL-6 increased across pregnancy and remained high during the postpartum; yet, higher levels of IL-6 did not correlate with

increased risk for maternal mood disorders (Blackmore et al., 2014; Palm et al., 2013), indicating that the postpartum period may be characterized by high IL-6 levels associated with bonding.

As such, the current study focused on human adults during periods of parental and pair bond formation. We measured plasma OT, β-End, and IL-6 in new parents, new lovers, and singles and micro-coded behavioral synchrony during social interactions to understand how functioning of these systems support the expression of synchrony, a social experience critical for the establishment of new bonds. We hypothesized that (a) during periods of bond formation levels of plasma OT, β-End, and IL-6 would increase reflecting their active involvement in the dual-pronged process of bonding. (b) consistent with human and animal studies, functioning of these system would become more tightly coupled to support the formation of a new bond. And (c) β-End, and IL-6 will chart paths to greater social synchrony, both directly and as mediated by their effect on OT production.

2. Method

2.1. Participants

Participants included 200 young adults who were all physically healthy, completed at least 12 years of education, and were recruited via advertisements posted in a university campus and surrounding area. We excluded participants without high school diploma to avoid the potentially confounding effects of socioeconomic status (SES) on the findings, in light of the strong associations between low SES and high school dropout. Research shows that low SES may alter HPA-axis activity and immune response (Eklund, 2009; Morag et al., 1998). Similarly, low SES subjects were found to exhibit a more prolonged stress-induced increase in IL-6 following acute psychological stress compared to those of middle SES background (Brydon et al., 2004). Rates of high school graduation in the Israeli population are 97% (Central Bureau of Statistics data, 2013; Statistical abstract of Israel, table 8.35) and thus our sample can be considered as a representative sample of the population.

The sample included 35 singles who were not involved in a romantic relationship during the past six months (17 man and 18 women), 50 individuals (25 couples) who began a romantic relationship within the past four months (3 weeks to 4 months), and 115 new mothers and fathers (71 mothers and 44 fathers, not couples) of 4–6 month-old firstborn infants (M = 166.3 days, SD = 12.6). All fathers reported at least medium level participation in childcare (M = 3.97, SD = .75 on a 1–5 scale). Exclusion criteria included individuals who did not complete high school, were above 35 years, were taking medication for a physical or psychiatric condition, or reported not being generally healthy. Men's age averaged 26.83 (SD = 4.28) and women's age averaged 26.33 (SD = 5.46) with no difference between groups. Blood from 3 singles, 4 new lovers, and 4 new parents could not be drawn or was insufficient for analysis, resulting in a final sample of 189 participants. The study was conducted in accordance with the Declaration of Helsinki and all procedures received the approval of the Institutional Review Board. All procedures were explained to the participants before the beginning of the study and all participants signed an informed consent.

2.2. Procedure

Participants were invited to a comfortable laboratory during the mid-late afternoon hours, in light of previous research suggesting stability in the diurnal cycle of plasma OT in the afternoon and evening hours (Forsling et al., 1998). Participants were first acquainted with the lab and the study procedures, signed an informed consent, and completed self-report measures to assess demographic/ health variables (weight, height, smoking, medication, time since last meal, and use of contraceptives). To screen for mood disorders, participants also completed the Beck Depression Inventory (BDI-II) (Beck et al., 1996). BDI-II scores exceeding 10 are considered indicative of depression. Mean BDI-II scores were 5.9 (SD = 5.10). Following, blood was drawn and interactions were filmed for parents and lovers.

Parents and infants arrived at the lab (3–8 PM) and visits were coordinated to the period between 1 h following the last meal and 1 h prior to the next meal. White-Traut et al. (2009) showed that salivary OT was highest within 30 min before breastfeeding, decreased at the initiation of feeding, and increased 30 min after breastfeeding and we found no differences in baseline plasma OT between breastfeeding and non-breastfeeding mothers when OT is not sampled before or after breastfeeding (Feldman et al., 2007, 2010; Gordon et al., 2010). In the current sample, 69% of the mothers were breastfeeding and no significant differences related to hormones or behavior emerged in relation to breastfeeding status.

2.3. Social interactions

2.3.1. Videotaping interactions

Interactions were videotaped in a large observation room with cameras placed on adjacent walls that were controlled from an observation room. In the parents' group, the infant sat on an infant seat mounted on a table and the parent sat next to him/her on a chair. Parents were instructed to play with the infant for approximately 10 min "the way they play at home". Lovers were similarly videotaped in a positive interaction for approximately 10 min; they were asked to plan "the best day ever" to spend together.

2.3.2. Coding synchrony

Interactions were micro-coded on a computerized system (Noldus, The Vaggenigen, Netherlands) in a .01 s frame, consistent with our previous research on parent–infant synchrony (Feldman and Eidelman, 2004, 2007; Apter-Levy et al., 2013). Synchrony codes tap the four "human-specific" central modules of non-verbal social communications found in various constellations across all cultural communities (Feldman, 2015b), including gaze, affective facial expression, vocalization, and touch. Whereas in infancy coordination of these nonverbal modules is the sole mode of communication, among affiliated adults these four modules appear but also provide support for the verbal exchange among partners (Feldman, 2007c, 2012a, 2015a).

Micro-coding was conducted for each module and codes within each module were mutually-exclusive and coding was conducted for each partner separately (in a different viewing). Two coders, trained to 85% reliability and blind to any other information coded the interaction and reliability, conducted on 20% of the interactions in each group (parents, lovers), averaged 93% (kappa = .85, range = .80–.95). The following codes were used.

Gaze – This module addresses the direction of gaze and includes three coded; to partner, to object, gaze aversion. *Affect* – Indexes the individual's facial expression of affect- positive, neutral, negative-angry (for infant fuss/cry), negative-withdrawn (for infant-tired). *Vocalization* – Taps the tone/quality of speech or vocal production, not its content, and includes warm vocal quality (for parents: "motherese" high-pitched vocalization), neutral speech, negative/angry speech, and none. *Touch*- addresses active modes of touch, including affectionate touch (e.g., caressing, kissing), functional touch (e.g., wiping baby's face, handing over), incidental touch, and none.

Synchrony was computed as conditional probabilities (partner I in behavior A given partner II in behavior B), consistent with prior research. Two types of synchrony were computed and the mean

durations of episodes of synchrony was the outcome variable. *Gaze Synchrony* – the mean duration of time the two partners shared social gaze (both partners are in "looking at partner"). *Affect Synchrony* – the mean duration of time the two partners shared positive or neutral affect.

2.4. Hormones

2.4.1. Plasma collection and analysis

Blood was drawn from antecubital veins into a 9 mL chilled vacutainer tubes coated lithium heparin that was supplemented with 400 KIU of Aprotinin (Sigma–Aldrich) per 1 mL blood. Plasma samples were kept ice-chilled for up to 2 h before being centrifuged at 4 °C at 3000×*g* for 20 min. Supernatants were collected, divided to aliquots and stored at −80 °C until assayed.

Determination of the concentrations of the different biomarkers were performed in duplicate, diluted and run according to the relevant kit's instructions. The concentrations of samples were calculated by using MATLAB-7 software, according to appropriate standard curves. The biochemist conducting the analysis was blind to any information related to relational status (single, in relationship), sex, or behavioral and interview data.

2.4.2. Oxytocin assay

Determination of OT concentrations in plasma was performed using a commercial OT enzyme-linked immunosorbent assay (ELISA) kit (Assay Design, Ann Arbor, MI) consistent with others and ours previous research (Carter et al., 2007; Feldman et al., 2007; Gordon et al., 2008). The kit parameters were: range of calibration curve: 15.6–1000 pg/ml; and intra-assay and inter-assay coefficients are less than 14.5%.

2.4.3. IL-6 assay

IL-6 EIA kit was purchased from eBiosciences (San Diego, CA USA). This High Sensitivity ELISAs enables the analysis of Interleukin 6 in low concentrations from serum, plasma, or cell culture samples. The kits parameters are: Sensitivity 0.03 pg/mL; Range of calibration curve: 0.08–5.0 pg/mL; the intra-assay and inter-assay coefficients are 11.7% and 21.4%, respectively.

2.4.4. β-Endorphin assay

β-End EIA kit was purchased from Peninsula Laboratories International, Inc. (San Carlos, CA USA). This extraction-free EIA kit is designed to measure analyte in biological fluids. The kits parameters are: Range of calibration curve: 0–25 ng/ml; the intra-assay and inter-assay coefficients are 11.7% and 22.6%, respectively.

3. Results

Results are presented in three parts. In the first, mean-level differences between groups in biomarkers and social synchrony are presented; in the second, we test the coalescing of the three biomarkers in each group, and in the final part, we utilize structural equation modeling (SEM) to chart paths from the three biomarkers to behavioral synchrony.

3.1. Group differences in biomarkers and social synchrony

Table 1 present descriptive statistics and correlation between biomarkers. To test for group differences and to account for the potential effect of gender we conducted two-way ANOVAs. Results of the two-way ANOVA indicated significant group difference in OT, $F(2, 156) = 7.60$, $p < .01$. As seen in Fig. 2, the two groups in the process of bond formation – new lovers and new parents - had significantly higher levels of OT compared to the singles who are not currently forming a new bond. Moreover, OT levels appear to be highest during pair-bonding and new lovers showed significantly higher OT levels compared to new parents. No significant gender, $F(1, 159) = 0.02$, NS or group by gender interaction, $F(2, 159) = 0.08$, NS effects were found. Groups differences were also found for IL-6, $F(2, 156) = 5.51$, $p < .01$ (Fig. 1). Parents had higher IL-6 levels than singles, with new lovers scoring at mid-points between parents and singles. Gender, $F(1, 159) = 0.98$, NS, and gender by group interaction, $F(2, 159) = 0.27$, NS, effects were not found. Groups differences emerged also for β-End, $F(2, 181) = 3.40$, $p < .05$. Similar to IL-6, parents had higher β-End than singles and β-End levels in new lovers were at mid-point between these two groups. Again, no gender, $F(1, 159) = 0.23$, NS, or group by gender interaction, $F(2, 159) = 1.17$, NS effects were found. Descriptive statistics according to gender are presented in Supplementary Material.

Table 1
Plasma concentrations of oxytocin (OT), interlukin-6 (IL-6), and β-Endorphin and inter-correlations in each group.

	M	*SD*	1	2	3
Singles					
1. OT	280.58	91.12	–		
2. IL-6	0.75	0.94	−.21	–	
3. β-Endorphin	153.17	60.74	.15	.09	–
New lovers					
1. OT	529.41	300.27	–		
2. IL-6	1.49	2.01	.54***	–	
3. β-Endorphin	399.40	308.51	−.06	−.08	–
New parents					
1. OT	431.04	289.36	–		
2. IL-6	2.19	2.29	.20	–	
3. β-Endorphin	604.55	1120.44	.24*	.17	–

Note: OT, IL-6 and β-End levels are measured in pg/mL.
* $p < .05$.
*** $p < .001$.

As for synchrony, parents and infants engaged in significantly longer durations of both Gaze Synchrony and Affect Synchrony (Fig. 2). Mean duration of gaze synchrony was 8.96 s in new lovers (SD = 5.92) compared to 30.26 (SD = 34.24) between parents and infants, $F = 4.11$, $p < .001$. Mean durations of affect synchrony was 20.85 s in new lovers (SD = 21.45) compared to 45.33 s (SD = 41.23) in parents, $F = 96.56$, $p < .001$. For both Gaze Synchrony and Affect Synchrony no significant gender ($F = 0.12$, NS, $F = 0.39$, NS, respectively) and gender by group interaction ($F = 0.01$, NS, $F = 1.09$, NS, respectively) effects were found. The two forms of synchrony were inter-related, $r = .44$, $p < .001$ and averaged into a single construct.

3.2. Inter-relationship among Biomarkers during Periods of Bond Formation

As seen in Table 1, correlations among biomarkers differed across groups. In singles the three hormones were not significantly correlated and the magnitude of their inter-relationship was weak. For instance, oxytocin was somewhat negatively associated with IL-6 and positively associated with β-End and IL-6 and β-End were not related. Among new lovers, significant and high correlation was found between OT and IL-6. However, these two hormones were not related to β-End. Among parents, positive correlations were found between all three biomarkers.

To test the extent to which biomarkers became more tightly-coupled during periods of bond formation, we conducted three factor analyses, one for each group. In all analyses, we extracted one factor and examined the percentage of common variance as an

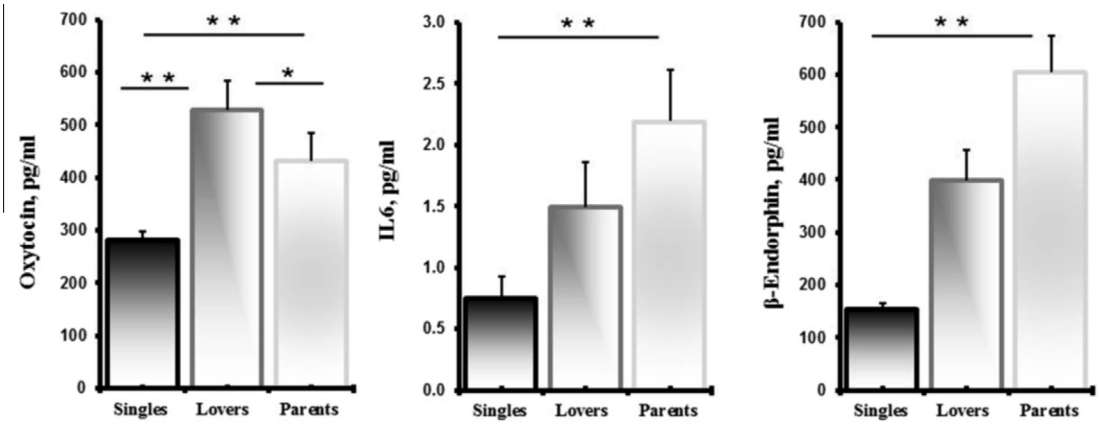

Fig. 1. Levels of plasma oxytocin (left), interlukin-6 (center) and beta endorphin (right) in singles, new lovers and new parents. *Note:* OT, IL-6 and β-End levels were measured in pg/mL. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$.

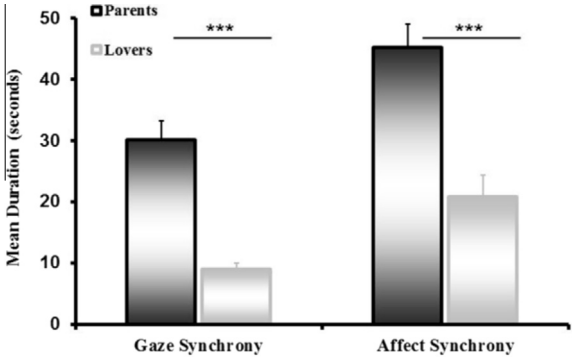

Fig. 2. Durations of gaze synchrony (left) and affect synchrony (right) between parents and infants and among new lovers. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$.

indicator of convergence among biomarkers. In singles, the common variance was 40.8%; in new lovers, the common factor explained 51.5% of the variance; and among parents, the common factor extracted from the three biomarkers explained 46.8% of the variance. These results indicate that the three biomarkers tend to converge during period of bond formation and convergence among new lovers was greater even in comparison to that observed among parents.

3.3. Structural equation modeling charting paths from biomarkers to social synchrony

Finally, we examined a structural equation model in which IL-6 and β-End charted a path to OT which, in turn, was linked to behavioral synchrony. The model also controlled for group membership and explored whether IL-6 and β-End are impacted by the individual's depressive symptoms. Analysis was conducted with AMOS.20 and results are presented in Fig. 3. Results indicated adequate fit indices ($\chi^2(6) = 4.60$, *ns*; *NFI* = .94, *CFI* = 1.00; *TLI* = 1.09; *RMSEA* = .00) suggesting the model provides a good fit for the data. As seen, depressive symptoms were positively associated with IL-6 and marginally associated with β-End. Both IL-6 and β-End were positively and uniquely linked with OT, which, in turn, was related to social synchrony.

To test the significance of the mediational paths we used Sobel's test (Baron and Kenny, 1986). Results indicated that depressive symptoms were significantly associated with OT via IL-6, $Z = 2.69$, $p < .01$, but not via β-End, $Z = 1.35$, *ns*. In addition, IL-6 was significantly associated with synchrony through its effect on OT, $Z = 3.17$, $p < .001$, and β-End was marginally associated with synchrony through its effect on OT, $Z = 1.80$, $p = .07$. These findings confirm our third hypotheses that the effects of immune and reward biomarkers on behavioral synchrony is mediated by their relations to OT.

4. Discussion

Although numerous theoretical accounts, from attachment theory to evolutionary models, underscored the centrality of social bonding for mammalian survival and adaptation and indicated that the parent–infant bond provides a neurobiological template for bonding throughout life (Bowlby, 1969; Numan and Young, 2016; Feldman, 2015b), this is the first study, to our knowledge, to directly compare human adults who are forming a bond with their infant, those forming a bond with a romantic partner, and others who are not in the process of bond formation on key neuroendocrine systems and social behaviors. Consistent with evidence in humans and animals, we found that the OT system increases its activity during periods of bond formation and OT levels were higher in new parents and new lovers compared to singles. In contrast, IL-6 and β-End levels were higher in new parents compared to singles, but not in new lovers. Furthermore, we found greater convergence between OT, IL-6, and β-End during periods of bond formation, particularly during romantic bonding, indicating that affiliation, immune, and reward biomarkers coalesce into a multifunctional system to support the complex process of bonding. Finally, path analysis indicated that the effects of IL-6 and β-End on social synchrony – the temporally-matched interactions that characterize affiliative partners who are intimately familiar with each other's cues – were mediated by their effect on OT. These findings highlight the integrative role of the oxytocinergic system in assimilating functioning of diverse systems in support of mammalian social life.

Attachment bonds provide the arena where social synchrony is learned and expressed most fully (Feldman, 2012a,b). While adults can match social behavior with any conspecifics, familiar or stranger, for instance, synchronize their social gaze during brief or extended social interactions, affiliative bonds are the context where the matching of nonverbal social signals is based on familiarity, transmits love, and fosters security (Feldman, 2015a,b). Behavioral synchrony between parents and infants during the critical period of 3–9 months carries profound effects on human sociality, predicting emotion regulation, mental health, socialization, and the capacity for empathy across childhood and adolescence (Feldman, 2007, 2012a). The current study is the first to measure non-verbal synchrony at the initial stages of romantic

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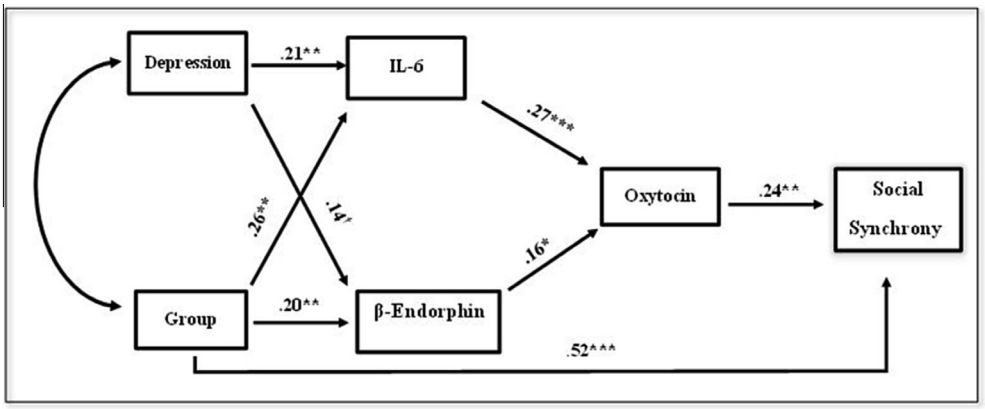

Fig. 3. Structural model describing paths leading to social synchrony. *Note:* Only significant paths are presented.

attachment, micro-coding patterns of gaze and affect coordination in new lovers. We found that durations of gaze and affect synchrony were substantially longer between parents and infants as compared to couples. This difference may chart one example of how experiences within the parent–infant bond provide a template for human social relationships throughout life, and while parents augment moments of synchrony to complement their infant's immature cognitive and affective abilities, adults can exchange brief episodes of gaze and affect matching that draw on the encoded experiences of synchrony of early infancy (Feldman, 2015b). We also found that synchrony is supported directly by the OT system, consistent with previous research (Feldman et al., 2011; Feldman, 2007a,c, 2012b), and indirectly by biomarkers of the immune and reward system via their impact on OT functioning.

β-End levels were higher in new parents compared to singles, with new lovers scoring at mid-point between these two groups. The high levels of β-End may index the increased reward associated with new parenting but may also point to the increased stress, as found in mice and monkey models (Keverne et al., 1989; Vaanholt et al., 2003). In small brained rodents maternal bonding is based primarily on odor-related cues and on hormones of pregnancy and parturition. With the evolution of the neocortex, bonding in non-human primates and humans is controlled by mutual activations of hormonal signals and the brain reward circuits (Panksepp et al., 1994). Such shift from hormonal-based to reward-based bonding allows for longer periods of parental caregiving, affords recognition of a rapidly changing infant, and enables bonding in fathers and non-biological parents (Broad et al., 2006). Our findings are consistent with models suggesting that bond formation involves a two-stage reward-related process. The first stage involves incentive motivated approach to the attachment target accompanied by feelings of wanting and desire encoded by OT and dopamine. In the next stage, stimuli from interactions with the attachment target elicit consummatory reward and a sense of liking linked with the person and context of reward, which are encoded by dopamine and β-End through μ-receptors in the NAcc (Depue and Morrone-Strupinsky, 2005; Kringelbach and Berridge, 2009; Spanagel et al., 1990; Wise, 1989). The higher durations of synchrony in parents may reflect the need to cement the bond via repeatedly-experienced stimuli that become coupled with the attachment target. This is consistent with the brain opioid theory of social attachment, which postulates that behavioral similarity between individuals within a close relationship creates mutual dependence that is mediated by the brain's reward circuits (Machin and Dunbar, 2011; Martel et al., 1993).

Consistent with our biobehavioral model on bond formation (Feldman, 2012a, 2015a,b), the higher β-End and behavioral synchrony in new parents may result from a positive biobehavioral feedback loop of parental reward from infant stimuli leading to more enjoyable caregiving and vice versa, promoting the parent–infant attachment. Research in rodents implicates β-End in the formation of maternal memory during the time-window of pregnancy to 24 h postpartum (Byrnes and Bridges, 2000). In primates, β-End increases following touch-based communications and β-End is involved in social reward (Curley and Keverne, 2005; Keverne et al., 1989). In humans, β-End increases following positive interactions (Odendaal and Meintjes, 2003). These findings are consistent with imaging studies indicating that synchronous mothers showed greater NAcc and ventral striatum activations to infant cues that were linked with plasma OT (Atzil et al., 2011; Strathearn et al., 2009). At the same time, β-End is also implicated in the stress response and in stress reduction. In mice, β-End modulates acute stress response to social conflict (Vaanholt et al., 2003) and in humans β-End is related to chronic pain (Kudielka and Wüst, 2010) and depression (Hegadoren et al., 2009). With regards to the associations of β-End and touch, studies are mixed. Whereas some studies show decreases in β-End following massage (Morhenn et al., 2012) or touch-based interventions (Weller and Feldman, 2003), others show increase in β-End following touch (Kaada and Torsteinbø, 1989), and still others report no change in β-End following touch (Day et al., 1987), possibly indicating that β-End is sensitive to the specific type of touch tested. As parenting involves heightened stress, the increased reward from infant cues may have been an evolutionary-based mechanism to support the immense investment required for infant care.

Similar to β-End, IL-6 levels were higher in new parents compared to singles and the new lovers group scored at mid-point between these groups. Our participants were all healthy; thus, the increased IL-6 was not indicative of an inflammatory process but probably indexed response to the stress involved in new parenthood. The average concentrations of plasma IL-6 in our study ranged between 0.75 pg/ml (SD = 0.94) in the singles group to 2.19 pg/ml (SD = 2.29) in the parents group. Such concentrations are within the normal physiological range of IL-6 in human serum (1–5 pg/ml) (Hunter and Jones, 2015), and also resemble IL-6 levels measured in healthy subjects after psychological stress (Brydon et al., 2004; Coussons-Read et al., 2007; Pace et al., 2006; Steptoe et al., 2001). In contrast, IL-6 levels are much higher (~10-fold or more) following viral or bacterial infection (Damas et al., 1992; Gentile et al., 1998; Hayden et al., 1998), indicating that our participants were generally healthy and differences among groups can be attributed to the stressful process of bonding.

The higher IL-6 levels in the parent group may reflect the parents' typical preoccupations and worries with regards to infant

safety and the lack of sleep in the post-partum months. The associations found between depressive symptoms and IL-6 is consistent with studies reporting correlations between IL-6, depression, and marital conflicts, which has been shown to increase at the transition to parenthood and peak at 6 months postpartum (Graham et al., 2009; Kiecolt-Glaser et al., 1996; Loving et al., 2004). Among new lovers, levels of IL-6 were not significantly higher compared to singles, suggesting that the nature of stress involved in romantic bonding may differ in substantive ways from that involved in parental attachment.

Attachment relationships and social support networks have long been known to exert a positive effect on health. Social support impacts cardiovascular reactivity and the effect is stronger when the support is provided by a close friend (Christenfeld et al., 1997). Partner support has been associated with higher plasma OT, lower systolic blood pressure, and lower heart rate, highlighting its cardio-protective effects (Ditzen et al., 2007; Grewen et al., 2005; Holt-Lunstad et al., 2008). Similarly, positive marital relationship carries a lasting effect on health (Kiecolt-Glaser and Newton, 2001). At the same time, a new romantic relationship is highly precarious and OT levels during the period of falling in love has also been linked with the anxieties and worries of new lovers regarding the future of the relationship (Schneiderman et al., 2012). This may account for the strong positive correlation ($r = 0.54$) between OT and IL-6 in the new lovers group, where the euphoria experienced during the period of falling in love goes hand in hand with increased stress reflecting the risk for the loss of the relationship, a loss not as common between parents and infant.

In addition to alterations in levels, the three hormones showed greater convergence during periods of bond formation, particularly during romantic bonding, indicating that bond formation is associated with greater coupling of the affiliation, reward, and immune systems. This multi-directional interaction between the immune and neuroendocrine systems is based on shared ligands and receptors (Wrona, 2006). Evidence suggests that neuroendocrine changes carry immuno-regulatory effects, for instance, the negative effects of stress on immunity (Haddad et al., 2002; Kiecolt-Glaser and Glaser, 2005; Pike et al., 1997). The current findings lend support to such interplay, extending it to the context of bonding and its associated stress and social reward via impact on the OT system. Interestingly, in vitro experiments showed that two cytokines, IL-1β and IL-6, are involved in the transcription regulation of the OT receptor (Schmid et al., 2001). Much further research utilizing a variety of experimental techniques is required to untangle the molecular mechanisms underlying the cross-talk between the affiliation, reward, and stress response systems.

One possible explanation of our findings is that the effects of stress on the immune system may be mediated by the OT-based attachment system. Very little human research exists on the involvement of immune components in OT-related attachment processes. Gouin et al. (2010) showed associations between OT levels and faster wound healing, and Clodi et al. (2008) found that OT reduces cytokine activation in humans following exposure to bacterial endotoxin (Clodi et al., 2008). Another link between OT and the immune system relates to CD38, a multifactorial molecule that plays an important immune function and is also implicated in OT production (Jin et al., 2007). CD38 is found on CD4+, CD8+ and natural killer (NK) cells but is also part of the downstream signaling pathway of OT. CD38 knockout mice showed decreased plasma OT levels and disruptions in maternal behavior and social recognition memory (Jin et al., 2007). Our findings on the increase in IL-6 during parent-infant bonding, the increased convergence of immune and affiliation biomarkers during bond formation, and the impact of IL-6 on social synchrony as moderated by OT may provide further evidence for the mutual influences of attachment on health during the formation of new attachments, but much further research is needed to fully understand how the formation of affiliative bonds impacts health and well-being.

Several study limitations should be noted. Our subjects reported being generally healthy; however, their health status was not directly assessed. An additional assessment of leukocyte count and function could have assisted in detecting a-symptomatic infections that may have interfered with immune- or endocrine- related processes. Another limitation is that we focused on three biomarkers, each representing a different biobehavioral system. Yet, the affiliative, reward, and stress-management systems are highly complex, each comprising numerous, mutually-influencing processes. Our findings, therefore, may provide a first step toward understanding the interrelationship among these systems during human bond formation. Much further research into these systems is required to understand the molecular mechanisms underpinning bond formation by using animal models or different sampling tools. Future research may also test different populations, for instance children or the elderly to assess whether bonding processes in younger or older individuals share the same kinetics. Additional control groups could have shed further light, including those who avoid romantic bonding for religious or other reasons (hermits, monks), non-biological adoptive or gay parents who are not impacted by hormones of pregnancy, or foster parents who are bonding with an older child or adolescent rather than an infant. Similarly, the lack of behavioral data on the singles group is a study limitation. Bonding is a defining feature of mammals. Much further research is required to understand how periods of bond formation integrate complex brain, hormonal, immune, and behavioral components in support of social life, enable adaptation to the social ecology, and afford social connection via the exchange of synchronous, temporally-matched social behaviors.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbi.2016.02.017.

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