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Adolescent Sympathetic Activity and Salivary C-Reactive Protein: The Effects of Parental Behavior

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Objective: This study utilized a novel multisystem approach to investigate the effect of observed parental behavior on the relationship between biological mechanisms associated with disease processes (i.e., autonomic physiology and immune response) among their adolescent children. ***Method:*** Thirty-three adolescents (23 males), aged 11–13, and their parents participated in a laboratory session in which adolescents provided baseline measures of autonomic (sympathetic) activity, and adolescents and 1 parent participated in a laboratory based dyadic conflict resolution interaction task. This included 3 male parent/male adolescent dyads, 20 female parent/male adolescent dyads, 3 male parent/female adolescent dyads, and 7 female parent/female adolescent dyads. Approximately 3 years later, adolescents provided a salivary measure of C-Reactive Protein (sCRP) to index inflammation. ***Results:*** Analyses revealed a positive association between sympathetic activity and sCRP, as well as a moderating role of positive parental behavior in this relationship, such that the association between sympathetic activity and sCRP was greater among adolescents whose parents displayed shorter duration of positive affect. ***Conclusions:*** Overall findings indicate parental behavior may influence the association between adolescent sympathetic activity and inflammatory processes. These findings have important implications for understanding the impact of psychosocial factors on biological mechanisms of disease.

Keywords: adolescence, C-reactive protein, parental behavior, pre-ejection period

Adolescence is a developmental period during which many factors that influence health and disease trajectories across the life span are established (Lupien, McEwen, Gunnar, & Heim, 2009). Two potentially important biological systems that can affect these trajectories are the sympathetic nervous system (SNS) and the immune response system (Danese & McEwen, 2012; Valkanova, Ebmeier, & Allan, 2013). Furthermore, research shows that social relationships can play a significant role in the association of these systems with poor health outcomes. For example it has been proposed that social threat (acute and chronic) up-regulates SNS activation and inflammation, leading to a dysregulated phenotype at risk for both physical and mental health problems (Slavich & Irwin, 2014), while positive social relationships may buffer against deleterious health outcomes (Chen, Miller, Kobor, & Cole, 2011; Thoits, 2011).

While previous research has examined the relationship between autonomic functioning and inflammation, as well as the connections between parenting and stress or inflammation, to our knowledge there have been no studies that have combined all these measures into a multisystem investigation (i.e., sympathetic activity, parent behavior, and inflammation) of these effects. Indeed, in general there is a dearth of research clarifying the specific mechanisms by which social relationships affect the biological systems that increase risk for poor health outcomes. Parental behavior, specifically during periods of interpersonal conflict that can provoke strong affective or stress responses, may be one important relational influence that can activate or dampen the links between sympathetic activity and inflammation (Kiecolt-Glaser, Gouin, & Hantsoo, 2010). Increased understanding that familial environmental factors influence the association between adolescent stress physiology and inflammation will help provide a deeper understanding of the ways in which the social environment can influence

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mechanisms of health and disease during this developmental period.

Sympathetic Activity and Inflammation

One noninvasive marker of SNS activity is pre-ejection period (PEP), or the time interval between the left ventricle's depolarization and the subsequent ejection of blood through the aortic valve. Shorter intervals are indicative of greater sympathetic response. It has been suggested that there is a bidirectional pathway via afferent nociceptive neurons between autonomic nervous system (ANS) activation, specifically the SNS (Nance & Sanders, 2007), and immune functioning (Jänig, 2014). By contrast, research is more sparse when it comes to the role of parasympathetic system (PNS) activity directly innervating immune organs (Nance & Sanders, 2007), although one study does indicate that PNS activity has influences on immune function (Forsythe, 2014). Moreover, the main neurotransmitter of the SNS, norepinephrine, is thought to regulate immune cell activity by influencing cytokine and antibody gene expression to increase inflammation. Finally, sympathetic innervation of the inflammatory system has been shown to be influenced by social stress in animal (Sloan et al., 2007) and human (Kemeny & Schedlowski, 2007) models. As such, the SNS is thought to regulate the inflammation response to a greater degree than the PNS (McEwen, 2008; Nance & Sanders, 2007), and this SNS–inflammation relationship is thought to serve as a critical biological pathway connecting stress (i.e., increased sympathetic tone) with inflammation (Miller & Blackwell, 2006), as has been demonstrated in animal models (Felger, Haroon, & Miller, 2015).

Parent–Adolescent Interactions and Health

The impact relationships have on psychological and physical health is well documented (Holt-Lunstad, Smith, & Layton, 2010; Jaremka, Lindgren, & Kiecolt-Glaser, 2013; Kiecolt-Glaser et al., 2010). Parent–adolescent interactions are an especially important interpersonal context that influences adolescent health (Hagan, Roubinov, Adler, Boyce, & Bush, 2016), and this may be due to the chronic environmental exposure of parental behavior. For example, research shows stability of parental behavior from infancy through adolescence (Else-Quest, Clark, & Owen, 2011), across adolescence (Sheeber, Hops, Alpert, Davis, & Andrews, 1997), as well as across generations (Neppl, Conger, Scaramella, & Ontai, 2009). For example, parents who experienced more warmth and support in their own childhood have been found to be more supporting in their interactions with their children (Belsky, Jaffee, Sligo, Woodward, & Silva, 2005), while parents who experienced more harsh and abusive parenting exhibit similar behavior with their own children (Pears & Capaldi, 2001). Therefore, conflictual family relational environments may provide a constant environmental input that heightens adolescent stress responses across time. The exact role that social relationships play in the association between sympathetic activity and inflammation is not yet clear, but these parent–adolescent interactions may serve as one psychosocial mechanism that may moderate the association between physiological stress (i.e., sympathetic activity) and later inflammation, thereby influencing the pathway from stress to disease (Kemeny & Schedlowski, 2007; Lupien et al., 2009; Priest et al., 2015).

Research shows that psychosocial mechanisms, such as supportive and positive interpersonal relationships, buffer against the negative impact of stress (Thoits, 2011). Specifically, research has separately demonstrated that supportive relationships protect against both dysregulated sympathetic activity as well as elevated inflammation (Byrne et al., 2017; Haley & Stansbury, 2003; Kiecolt-Glaser & Newton, 2001; Uchino, Cacioppo, & Kiecolt-Glaser, 1996). For example, supportive and functional relationships buffer against higher systemic inflammation (Byrne et al., 2017) as well as the deleterious effects of dysregulated physiological stress systems associated with allostatic load and psychopathology (Brooks et al., 2014; Carroll et al., 2013). Furthermore, sensitive parents, positive parental behaviors, and supportive role models for adolescents have all been shown to buffer the effect of low socioeconomic status against inflammation (Chen, Lee, Cavey, & Ho, 2013; Chen et al., 2011). Therefore, one social signal, which may act as a relational buffer (i.e., protective factor; see Hostinar, 2015) against the effects of sympathetic activity on inflammation is positive parental behavior. Specifically, positive parental behavior, especially during negative and/or challenging interactions, may buffer against the deleterious effects of sympathetic activity on inflammation (Kiecolt-Glaser, McGuire, Robles, & Glaser, 2002).

In addition, research shows that the opposite is true for those with unsupportive, stressed, and dysregulated relationships (Fagundes, Bennett, Derry, & Kiecolt-Glaser, 2011). For example, dysphoric or aggressive maternal behavior that is associated with social conflict has separately been shown to be associated with both increased sympathetic activity as well as inflammation (Fuligni et al., 2009; Kiecolt-Glaser et al., 2010; Miller, Rohleder, & Cole, 2009). Parent–adolescent relationships characterized by high conflict are associated with greater sympathetic arousal (Salomon, Matthews, & Allen, 2000), and parental harshness and stress are associated with increased current levels of adolescent inflammatory response as well as prospectively predicting, 1.5 years later, adolescent inflammatory response (Byrne et al., 2017; Miller & Chen, 2010; Wolf, Miller, & Chen, 2008). Indeed, social threat, including the absence of positive parental behavior, may influence sympathetic activity leading to up-regulated inflammation activation, which may result in a dysregulated phenotype driving disease pathogeneses (Slavich & Irwin, 2014).

The current study examined the association between sympathetic activity and salivary C-reactive protein (sCRP) in adolescents as well as the moderating role of parental behavior on this association. Body mass index (BMI; Gillum, 2003), gender (Bouman, Heineman, & Faas, 2005), socioeconomic status (SES; Pollitt et al., 2007), and pubertal status (Delany et al., 2016) were collected as potential confounders due to the previously identified associations with inflammation. Finally, although research suggests that CRP does not have a diurnal variation in healthy adults (Meier-Ewert et al., 2001; Miles et al., 2008), there is one study that has found the contrary with a medical patient population (Koc, Karaarslan, Abali, & Batur, 2010), so time of sCRP collection was also collected as well as parental gender as potential confounds.

The Current Study

The current study is the first to our knowledge to utilize a multisystem approach in order to address gaps in knowledge in the

association between SNS activity and sCRP in adolescents, and the role that the social environment plays in this relationship, by examining (1) the association between sympathetic activity and sCRP and (2) the moderating role of positive, dysphoric, and aggressive parental behavior during a negative interaction on the association between adolescent sympathetic activity and inflammation.

First, we hypothesized that shorter PEP (i.e., greater sympathetic activity) would, prospectively, be related to greater concentrations of sCRP approximately 3 years later ($M = 3.15$, $SD = .65$) such that greater sympathetic arousal would predict greater levels of inflammation. Second, we hypothesized that the duration of positive parental behavior during a negative emotion-eliciting parent–adolescent problem-solving interaction would moderate the relationship between adolescent PEP and sCRP, such that shorter duration of parental positive behavior would be associated with a stronger relationship between high sympathetic arousal (i.e., shorter PEP) and higher sCRP. In addition, we hypothesized that longer duration of parental dysphoric and aggressive behavior would be associated with a stronger relationship between high sympathetic arousal (i.e., shorter PEP) and greater sCRP.

Method

The current study included data from the Adolescent Development Study (ADS)—a large-scale longitudinal research project conducted from 2004 to 2012 at the Orygen Centre for Youth Mental Health at The University of Melbourne, Australia. Family and electrophysiology data were collected during the first assessment at Time 1 (T1) when participants were approximately 12 years old (mean age = 12.30, $SD = .68$); socioeconomic status, body mass index, and pubertal development were collected at Time 2 (T2) when participants were approximately 14.85 years old (mean age = 14.85, $SD = .51$); while adolescent sCRP data were collected from a subgroup of participants at Time 3 (T3), approximately three years after T1 ($M = 3.15$, $SD = .65$), when participants were approximately 15 years old (mean age = 15.46, $SD = .49$).

Recruitment and Screening of Participants

The recruitment and screening of participants has been reported in detail previously (Yap, Allen, & Ladouceur, 2008). Screening was conducted to identify a community sample of 10- to 12-year-old primary-school students representing the full spectrum of risk for psychopathology as a function of temperament, as measured by the Early Adolescent Temperament Questionnaire—Revised (Ellis & Rothbart, 2001). Participants were Grade 6 students enrolled in primary schools in metropolitan Melbourne, Australia. Grade 6 corresponds to the final year of primary school, with students typically aged between 10 and 12 years.

The sample was defined using a one-stage cluster sampling procedure. Primary schools were selected at random with a probability proportional to the number of persons in the target population of the schools (primary sampling units). The schools were defined within a stratified frame of Government, Catholic, and Independent Private schools. These sectors contribute 65%, 22.5%, and 12.5% respectively to the total school enrollment population in this geographic region. One hundred and seventy-five schools were selected, with 90, 44, and 24 from the Government, Catholic and Independent Private school sectors, respectively. This provided a total intended sample of 4587 students.

Ninety-seven (56%) schools approached to participate in the study did so. Of Grade 6 students enrolled in these schools, 2,453 (54% of total intended sample) became participants in the school screening, with 1,730 (71%), 501 (20%) and 222 (9%) from the Government, Catholic, and Private Independent schools, respectively. These proportions in the sampling population within each school sector were not significantly different from those found in the total intended sampling area, $\chi^2(2) = 0.81$, $p > .05$.

Schools selected within the sampling frame were visited in order to administer the Early Adolescent Temperament Questionnaire-Revised (EATQ-R) questionnaire. Research staff conducted the administration of the survey, in association with staff from the respective schools. Students completed the survey during class time in groups of approximately 18–25. Explicit consent to participate in the survey was sought from a parent or guardian and from the student himself or herself after provision of study information by letter. Any subject unavailable to complete the questionnaire during a school visit was sent the questionnaire by mail to complete at home.

A total of 2,453 students (1,168 male and 1,280 female; 5 participants did not report their gender) completed the EATQ. The mean age of the participants was 11.62 years ($SD = 0.39$), and ranged between 9.35 and 13.15 years.

Based on their scores on this measure, a smaller sample of 415 students was selected to be part of the study. Adolescents at the extreme ends of the temperamental distribution were oversampled to maximize interindividual differences in psychological well-being. Of the selected adolescents, 245 families consented to participate in the home assessment and therefore participated in the T1 data collection phase. A subset of 82 participants consented to participate in the immune analyses 2 to 3 years later at T3. The overlap between participants who completed the family interaction tasks with their parent at T1 ($n = 195$), participants who had completed a resting electrophysiology baseline at T1 ($n = 109$), and participants who had usable saliva samples at T3 ($n = 74$) were 33 participants (23 male adolescents), and this comprised the final sample. This included 3 male parent/male adolescent dyads, 20 female parent/male adolescent dyads, 3 male parent/female adolescent dyads, and 7 female parent/female adolescent dyads. Informed consent was obtained from the participants, as well as a parent/legal guardian. One-way ANOVAs showed that the subgroup of 33 dyads did not differ significantly from the larger group of 195 dyads on measures of SES, BMI, age, or parent gender. The sample did differ on adolescent gender, $F(1, 244) = 6.41$, $p = .01$, from the overall sample in that there were more males in this subsample. This study was approved by the Human Research Ethics Committee at the University of Melbourne, Australia. Participants and their parents were informed that they could cease participation at any time. Table 1 lists the percentages of ethnicity (identified by the adolescent) and household composition (identified by the parent).

Procedures and Measures

Family interaction assessment at T1. The parent–adolescent dyads completed two lab-based interaction tasks of 20 min each,

Table 1
Demographics

Demographic variables	Percentage
Ethnicity	
White/Caucasian	90.9%
More than one race	9.1%
Household composition	
Two-parent households with siblings and/or other relatives	69.7%
Two-parent households with no siblings or other relatives	9.1%
Single-parent (mother) households with siblings and/or other relatives	15.2%
Single-parent (mother) households with no other siblings or other relatives	3.0%
Relatives other than biological parents, stepparents, adoptive parents, or grandparents (e.g., aunts or uncles as parental figures)	3.0%

designed to differentially elicit positive (Event-Planning Interaction) and negative (Problem-Solving Interaction) behavior. For the current study we only focus on the Problem-Solving Interaction due to the association of conflict with both increased autonomic (Gonzalez, Moore, Garcia, Thienemann, & Huffman, 2011) and inflammatory processes (Fuligni et al., 2009; Miller et al., 2009). For the Problem-Solving Interaction, the interviewer selected up to five topics that both the parent and adolescent endorsed as occurring most frequently (participants were asked how many times they had discussed the issue in the last 2 weeks) and had the highest intensity of anger (participants filled out a Likert scale of 1–5 where 1 is *calm*, 3 is *a little angry* and 5 is *angry* for each issue) on the Issues Checklist (Prinz, Foster, Kent, & O'Leary, 1979), consisting of items representing common topics of conflict between parents and adolescents. Interactions were video-recorded with separate cameras focused on each participant.

Affective and verbal behavior were coded using the Living in Familial Environments (LIFE) event-based observational coding system that records micro changes in social behaviors (Hops, Davis, & Longoria, 1995). The LIFE system consists of 10 nonverbal affect codes (e.g., anger, dysphoric, positive) and 27 verbal content codes (e.g., validation, complaint, provoke), coded within an event-based protocol in which new codes are entered each time the affect or content of one of the participant's behavior changes. Composite Aggressive, Dysphoric, and Positive behavior constructs were derived from the affect and content codes. Aggressive behavior was defined as aggressive (e.g., raised voice; clenched teeth) or contemptuous (e.g., eye rolling; sneering) nonverbal behavior and cruel (e.g., mocking; insults; threats) or provoking (e.g., taunts; dares) statements. Dysphoric behavior was defined by sad and anxious nonverbal behavior (e.g., tearfulness, sighing) or complaining statements. Positive behavior was defined by positive nonverbal behavior and humorous statements. Trained observers were blind to psychosocial information about participants and a second observer coded 20% of interactions to provide estimates of interobserver consistency. Random pairs of observers were assigned to the interactions to minimize observer "drift." Kappa coefficients for aggressive constructs were .70 for mothers and .77 for fathers, positive constructs were .86 for mothers and .84 for fathers, and dysphoric constructs were .57 for mothers and .54 for fathers, reflecting good levels of agreement. Consistent with our prior research (e.g., Whittle et al., 2008; Yap et al., 2011), average duration of each behavior (i.e., the proportion of time during the whole interaction task that was spent engaging in a behavior) was calculated as the time between onset of one code and onset of the next code, for each person in the interaction. The validity of the LIFE coding system as a measure of family processes has been established in numerous studies of adolescents (Katz & Hunter, 2007; Schwartz et al., 2011; Sheeber, Davis, Leve, Hops, & Tildesley, 2007).

Baseline pre-ejection period assessment at T1. All data were acquired using software and equipment from the Vrije Universiteit Ambulatory Monitoring System (VU-AMS; de Geus, Willemsen, Klaver, & van Doornen, 1995; Willemsen, De Geus, Klaver, Van Doornen, & Carrofl, 1996). Simultaneous measurement of electrocardiogram (EKG) and impedance cardiography (ICG) signals were used to assess PEP (for methods see de Geus et al., 1995 and Willemsen et al., 1996).

Pubertal development at T2. The Pubertal Development Scale (PDS) was used to assess pubertal development at T2 using the self-report PDS (Petersen, Crockett, Richards, & Boxer, 1988). The PDS was collected for males and females (males: $M = 26.56$, $SD = 5.70$; females: $M = 21.80$, $SD = 4.21$). For females, this measure includes 8 items assessing the stage of breast development, hair growth, acne presence, hip width, and menarcheal status. For males, this measure includes 11 items assessing genitalia development, hair growth, acne presence, and voice change. Reliability and validity of the PDS has been well established (Brooks-Gunn, Warren, Rosso, & Gargiulo, 1987; Petersen et al., 1988). For descriptive purposes, the PDS data was coded into a 5-point scale in accordance with the Tanner stages based on prior work (Shirtcliff, Dahl, & Pollak, 2009).

Socioeconomic status at T2. A measure of socioeconomic status (SES) was calculated for participants using the Australian National University-4 (ANU4) Scale (Jones & McMillan, 2001), which provides a score between 0 and 100 based on occupation. Parents were asked about their occupation and education. For parents that had missing data or reported an occupational status that could not be coded according to ANU4 (e.g., unemployed or small business owner), data on education was used as a substitute, in number of years of education, scaled to reflect ANU4 codes. This method of measuring socioeconomic status has been recommended in Australia by the National Education Performance Monitoring Taskforce (Marks, McMillan, Jones, & Ainley, 2000).

Body mass index at T2. BMI was measured at T2 by researchers by weighing the participant on a scale and measuring height, and calculating BMI equal to the weight (kg) divided by the height (m) squared.

Immune assessment at T3. Two mL of whole, unstimulated saliva was collected from 82 participants at T3 (mean age = 15.45

years, $SD = 0.49$) using the passive drool method to analyze peripheral concentrations of sCRP. Saliva is easier and safer to collect, as compared with blood, in research studies (Granger et al., 2007). In particular, some studies show that saliva may be correlated with systemic or major sources of general inflammatory marker, C-reactive protein (CRP; Byrne et al., 2013; Ouellet-Morin, Danese, Williams, & Arseneault, 2011; Out, Hall, Granger, Page, & Woods, 2012). These studies have demonstrated that CRP can be detected in saliva as well as in blood, and the measures in these two tissues correlate with medium to large effect sizes. One study found that many inflammatory markers had higher detection rates in saliva compared with blood in an adolescent cohort (Byrne et al., 2013). However, there are two studies showing no significant correlation between these measures (Dillon et al., 2010; Kopanczyk et al., 2010). Therefore, it is not yet clear from the extant literature if sCRP is a measure of systemic inflammation or only oral inflammation. Nevertheless, several studies have found that sCRP is associated with measures of both psychological and physical health in children and adults (Cicchetti, Handley, & Rogosch, 2015; Goodson et al., 2014; Laurent, Lucas, Pierce, Goetz, & Granger, 2016; Lucas et al., 2016; Naidoo, Konkol, Biccard, DuBose, & McKune, 2012), and it may be a measure that is influenced by stressful family environments as well.

Collection time varied by participant, but research suggests that CRP does not have a diurnal variation (Meier-Ewert et al., 2001), although some research has shown diurnal variability in those with obstructive sleep apnea (Mills, Natarajan, von Känel, Ancoli-Israel, & Dimsdale, 2009). Time of day was not correlated with sCRP in our sample, $r = .04$, $p = .82$. Saliva samples were frozen immediately at −20°C after collection and stored for 24–36 months prior to analysis. After thawing to room temperature (24°C), samples were first vortexed with a protease inhibitor cocktail (PIC), "Complete, Mini" (Roche, Castle Hill, New South Wales, Australia) in order to protect the integrity of the acute-phase proteins. Samples were then centrifuged at 10,000*g* for 10 min, to isolate the precipitate and debris from the supernatant. The supernatant was extracted and divided into 3 test tubes before being snap-frozen in liquid nitrogen and stored at −80°C overnight. Samples were again thawed to room temperature the following day and centrifuged once more at 10,000*g* for 10 min, and the samples used throughout in this study therefore had a total of two freeze/thaw cycles. Pilot testing showed that a second centrifugation resulted in much lower viscosity, with less likelihood of clogging the Bio-Plex suspension array system. Furthermore, the lower viscosity enabled us to analyze the samples without further dilution with the Bio-Plex immunoassays.

Concentrations of sCRP were analyzed according to manufacturer's instructions, described elsewhere (Byrne et al., 2013), by the Bio-Plex multiplex bead array immunoassay system of human cytokine panel and plates read on Bio-Plex Array Reader (Bio-Plex 200 System and Bio-Plex Manager Version 4.0, Bio-Rad Laboratories, Inc., New South Wales, Australia). Saliva sample supernatant was assayed in duplicate, undiluted, and analyzed by the flow-based Bio-Plex suspension array system. Intraassay %CV was <20%, consistent with other studies of sCRP (Byrne et al., 2013; Ouellet-Morin et al., 2011). For the assays, the test volume was 50 μl, with a range of standards from 10 to 79560 pg/mL. The mean of recovery percentages $\left(\frac{ObservedConcentration}{ExpectedConcentration}\right)$ from standards was 99.42%, $SD = 11.31\%$, range: 75%–116%. Participants that had reported taking medication ($N = 14$) in the 24 hours prior to saliva collection were excluded from analyses to ensure that results were not due to substances that directly affect immune functioning. These medications included antihistamines, ibuprofen, and cold and flu tablets, which can affect inflammatory processes (Assanasen & Naclerio, 2002; El-Sharrawy, El-Hakim, & Sameeh, 2006; Mainous & Pearson, 2003; Nettis, Colanardi, Ferrannini, & Tursi, 2005; Vena, Cassano, Buquicchio, & Ventura, 2008). On the day of saliva collection, time of saliva collection was recorded and participants were asked to complete a "diary" that included questions about any medication or substance use in the past 24 hours prior to collection, and the type and dose.

Analyses

Analyses were performed using IBM SPSS statistical software, version 23 (SPSS Inc., Chicago, IL, U.S.). Statistical significance was set at $p < .05$, variables were centered, and heteroscedasticity consistent standard errors were used. Post hoc power analyses were run using Gpower, version 3.1 (Faul, Erdfelder, Buchner, & Lang, 2009; Faul, Erdfelder, Lang, & Buchner, 2007). All sCRP samples were log transformed.

Participants who participated in the parent–adolescent interaction and provided both PEP and sCRP were 33 subjects. Data was missing for PEP from one participant and PDS from one participant. To preserve statistical power lost through deletion methods, single imputation with the EM algorithm was used to estimate missing data (Little & Rubin, 1987). Little's MCAR test indicated that data were missing completely at random, $\chi^2 = 32.23$ ($df = 24$; $p = .12$).

Covariates. Due to the small number of subjects that had data for both PEP and sCRP, we only included theoretically determined covariates (described above) in analyses that were correlated with sCRP (so as to remove significant variance associated with these variables while preserving power as much as possible). BMI was the only covariate that emerged as significantly associated with sCRP and was therefore used as a covariate in all analyses (see Table 2).

Regression. A two-block hierarchical linear regression was run in SPSS version 23. In block one, BMI was entered as a covariate. In block two, PEP was added to test main effects.

Moderation. Moderation analyses were run using the PROCESS macro (version 2.13) for SPSS. For moderation analyses, the Johnson-Neyman Technique within PROCESS was used to derive region of significance for interpreting interaction effects (Hayes, 2013). We used PEP as the predictor, duration of parental positive, dysphoric, and aggressive behavior as the moderators in three separate models, BMI as a covariate, and sCRP as the outcome.

Results

Regression

After controlling for BMI, adolescent PEP was significantly associated with sCRP ($\beta = -.03$, $t(30) = -2.56$, $p = .02$, 95% CI [−.06, −.01]).

Table 2
Variable Correlations

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13
1. sCRP (log)	1												
2. PEP	−.38*	1											
3. BMI	.40*	.01	1										
4. Gender	.01	−.08	−.06	1									
5. Age	−.16	.26	−.05	−.14	1								
6. Family History of CVD	.17	−.08	−.12	−.02	.07	1							
7. PDS	−.09	−.02	−.04	.40*	.00	.19	1						
8. SES	−.07	.16	.13	−.04	−.26	−.41*	.06	1					
9. Time of Day	.04	−.01	−.10	.02	.13	.08	−.25	−.18	1				
10. Aggressive Behavior	.20	−.49**	−.03	.07	.27	−.06	−.03	−.39*	.13	1			
11. Dysphoric Behavior	.14	.18	−.12	.01	.08	.39*	−.28	−.26	.37*	−.06	1		
12. Positive Behavior	−.36*	.38*	−.01	.05	.27	−.03	.01	.07	.00	−.21	.13	1	
13. Parent Gender	.17	−.27	.10	.20	−.04	−.30	.03	.33	.06	.25	−.16	−.15	1

Note. sCRP = salivary C-reactive protein; PEP = pre-ejection period; BMI = body mass index; PDS = Pubertal Development Scale; CVD = cardiovascular disease; SES = socioeconomic status.
* $p < .05$. ** $p < .01$.

Moderation

The duration of parental positive behavior during parent–adolescent interaction significantly moderated the relationship between resting adolescent PEP and sCRP (β = .01, $t(28) = 3.12$, $p < .01$, 95% CI [.004, .019]; see Table 3), with the overall model significantly predicting sCRP, $R^2 = .41$, $F(4, 28) = 11.40$, $p < .001$, and the addition of the interaction term explaining significant additional variance in sCRP, $\Delta R^2 = .05$, $F(1, 28) = 9.75$, $p < .01$. Neither the duration of parental dysphoric behavior (β = −.01, $t(28) = -.80$, $p = .43$, 95% CI [-.03, .01]) nor the duration of parental aggressive behavior during parent–adolescent interaction (β = .001, $t(28) = .59$, $p = .56$, 95% CI [−.00, .01]) significantly moderated the relationship between resting adolescent PEP and sCRP.

The interaction was probed by testing conditional effects of PEP on sCRP at three levels of positive parenting behavior, one standard deviation below the mean, at the mean, and one standard deviation above the mean. As Table 4 shows, PEP was significantly related to sCRP when positive parenting behavior was one standard deviation below the mean ($p < .001$), but not when positive parenting behavior was at the mean ($p = .11$) or one standard deviation above the mean ($p = .55$). Region of significance testing showed a significant negative association between adolescent PEP and sCRP when the duration of parental positive behavior was short (values below the 49th percentile). In other words, when there was low duration of parental positive behaviors, then lower PEP (greater sympathetic activity) was significantly associated with higher levels of sCRP. In addition, when the duration of parental positive behaviors were long (values above the 97th percentile), then adolescents had significantly lower sCRP levels. Figure 1 illustrates this effect with average, +1 *SD*, and −1 *SD* duration of parents' positive behavior. This indicates that greater adolescent sympathetic activity relates to sCRP when the duration of parental positive behavior is at low levels, while adolescents' sympathetic activity is not related to sCRP when there are average or high duration of parental positive behavior.

Post-hoc power analysis. The limited sample size of the current study may have played a role in the null results for moderating effects of aggressive and dysphoric parental behavior. The observed power for the full respective models were .94 (dysphoric), .85 (aggressive), and .96 (positive), which all surpass the recommended statistical .80 level (Cohen, 1988), indicating that there was likely sufficient power to reduce chance of a Type II error, and thus providing sufficient power to reasonably detect effects for both aggressive and dysphoric behavior.

Table 3
Adolescent PEP × Parent Positive Behavior Duration Effect on Adolescent sCRP

Variable	β	*p*	95% CI
Constant	−6.19	<.001***	−7.87, −4.51
Parental positive behavior duration	−.17	<.01**	−.29, −.06
PEP	−.02	.11	−.04, .00
Parental Positive Behavior × PEP	.01	<.01**	.004, .019
BMI	.10	.01**	.03, .16

Note. sCRP = salivary C-reactive protein; PEP = pre-ejection period; BMI = body mass index.
* $p < .05$. ** $p < .01$. *** $p < .001$.

Discussion

Consistent with our hypotheses, shorter PEP (i.e., higher sympathetic arousal) was associated with higher levels of sCRP approximately 3 years later. In addition, the duration of parental

Table 4
Conditional Effects of PEP on sCRP

Positive parenting behavior (Centered)	β	*p*	95% CI
One *SD* below mean	−2.18	<.001***	−.06, −.02
At the mean	.00	.11	−.04, .00
One *SD* above mean	2.18	.55	−.02, .04

Note. PEP = pre-ejection period; sCRP = salivary C-reactive protein; *SD* = standard deviation.
* $p < .05$. ** $p < .01$. *** $p < .001$.

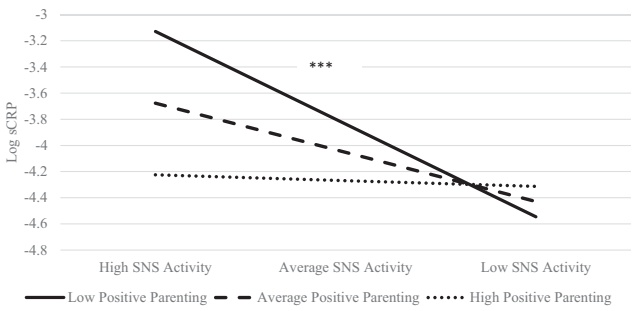

Figure 1. Adolescent pre-ejection period (PEP) relates to higher salivary C-reactive protein (sCRP) at average and lower duration of positive parental behavior. Plotted at average (dashed line), 1 *SD* (small dotted line), and 1 *SD* (solid line) duration of parental positive behavior. *** $p < .001$.

positive behavior during a negative interaction moderated the relationship between adolescent PEP and sCRP, such that adolescents with high sympathetic arousal and whose parents displayed shorter duration of positive behavior showed the highest levels of sCRP. In contrast, duration of dysphoric and aggressive parental behavior did not moderate the relationship between adolescent PEP and sCRP.

The relationship between PEP and sCRP is a notable finding given the relatively long period of 3 years between assessments. This finding is in line with previous research indicating a relationship between sympathetic nervous system activity and inflammation (Jänig, 2014; Nance & Sanders, 2007). In the current design we cannot say whether this relationship is truly prospective, given that inflammation was only measured at one time point, an issue that is further discussed below in the limitations section. However, this finding does justify future longitudinal research that examines the moderation of this relationship by social factors. Interestingly, our findings suggest that duration of positive parental behavior during parent–child interactions moderates the relationship between adolescent sympathetic activity and sCRP, such that adolescents with parents who display average and above average duration of positive behaviors show no (or weak) associations between SNS activity and sCRP, suggesting that this form of positive parenting may buffer or inhibit the biological cascade between sympathetic arousal and inflammation. In contrast, parents who display below average duration of positive behaviors have adolescents who show significant associations between SNS activity and sCRP, indicating that lack of positive parenting may exacerbate the connection between sympathetic activity and inflammation in adolescents. Interestingly, duration of parental dysphoric and aggressive behavior did not moderate the relationship between adolescent sympathetic activity and sCRP, indicating that negative parental behaviors may be less impactful than the absence of positive parental behaviors. The lack of association here may indicate that parents' depressive and angry tendencies may have less impact on their child's inflammation than a lack of positive behavior. Another possible explanation, outlined further in the limitations section, is that the kappas were lower for dysphoric and aggressive behavior than they were for positive behavior, which may have prevented significant findings from being found with these constructs. Overall, these findings indicate that resting adolescent sympathetic activity is associated with sCRP, however when adolescents experience average to higher than average levels of positive parental behavior during negatively charged parent–adolescent interactions this relationship is weakened, possibly inhibiting a biological cascade that can lead to greater susceptibility to stress related illness. Overall, these findings indicate that psychosocial processes may influence the activation and association between sympathetic activity with later inflammatory processes.

These findings have clinical implications for mental health providers working in the field of parent–child and parent–adolescent health. During treatment, focusing on enhancing the frequency and duration of positive parental behaviors that are already in use, such as those implemented through the Filming Interactions to Nurture Development (FIND) intervention (Fisher, Frenkel, Noll, Berry, & Yockelson, 2016), may capitalize on the strengths of positive feedback in order to encourage more positive, warm, and nurturing parenting behaviors. Furthermore, focusing on existent positive behaviors, rather than deficits in parenting, such as the occurrence of aggressive or dysphoric behaviors, focuses on strengths and may prevent parents from feeling discouraged, which can lead to treatment dropout.

These findings suggest that parental behaviors during parent–adolescent interactions may act as a psychosocial mechanism that can moderate the biological cascade between sympathetic arousal and immune functioning, and set off mechanisms that may increase susceptibility to stress related diseases over time (Miller, Chen, & Parker, 2011). Shonkoff, Boyce, and McEwen (2009) describe how such early negative experiences can have lagged effects over years before these experiences are expressed as disease. These data provide an empirical and mechanistic basis for examining whether treatment interventions that aim to foster positive parental behaviors during negative interaction contexts provide positive health outcomes during and after adolescence.

While this study had a number of strengths, including the utilization of a multimethod design with measures that do not contain tautological variance, there are some limitations that should be noted. First, this study contained a relatively small sample of 33 participants, which only allows for initial preliminary evidence of these effects. Therefore, there may not be great generalizability of these results. We are currently conducting a study of 200 parent–adolescent dyads, which will allow us to see if these results replicate at a larger scale as a means to provide greater generalizability. Second, the current study was limited in examining study measures at one time point, although it should be mentioned that CRP levels are relatively stable over time. For example, Deverts et al. (2010) found strong stability between CRP levels collected 5 years apart, $r = .66$, $p < .001$. Future studies should provide a prepost design to address changes in autonomic activity, parental behavior, and inflammation over the course of the study. Third, the current study only examined sCRP as the sole marker of inflammation and health. Future studies should examine multiple pro-inflammatory and anti-inflammatory biomarkers as well as other markers of disease, such as telomere length, in order to provide a more complete understanding of how the sympathetic nervous system interacts with parental behavior to influence inflammation and general health. As mentioned above, our lab is currently working on a new study to elucidate how both parent and adolescent interactions influence psychophysiology, cortisol, inflammation, and telomere length (i.e., biological aging). Fourth,

while our study excluded participants taking medication, we did not measure temperature or assess illness or dental hygiene, which could be especially relevant for salivary inflammation. Future studies should collect this information. Fifth, the positive behavior construct, the only behavior that had a significant moderation, had a higher kappa than either the aggressive or dysphoric constructs. It is possible that the lower reliability in the aggressive and dysphoric constructs may have prevented the ability to find a moderation. Future studies should use both human observers and automated facial, language, and body language analysis (De la Torre et al., 2015) to assess parental behavior as this will likely result in greater reliability. Lastly, as would be expected, CRP concentrations in the saliva samples were low compared with those found in saliva with adult samples and as is normally found in blood (Laurent et al., 2016; Lucas et al., 2016; Mohamed, Campbell, Cooper-White, Dimeski, & Punyadeera, 2012; Ouellet-Morin et al., 2011; Out et al., 2012). Our samples were stored in −20 °C, as is recommended for sCRP (Salimetrics, Inc.; http://www.salimetrics.com), and while degradation is possible at this temperature, CRP has been shown to be stable in saliva in previous research (e.g., at room temperature up to eight hours after collection; Ouellet-Morin et al., 2011). Therefore, the lower values in this sample were not likely a result of degradation, but rather is more likely due to the young age of our sample, but there is a need for more research on sCRP norms in children and adolescents.

The present study is the first to our knowledge to use a multisystem approach by looking at the interaction of adolescent electrophysiology and observed parental behavior in relation to sCRP. Overall the findings suggest that exposure to low levels of parental warmth leads to a greater coupling of sympathetic activation and sCRP, which extends prior literature examining the relationship between sympathetic activity and inflammation. More research is needed to further refine the understanding that psychosocial factors, such as close personal relationships, influence both the sympathetic nervous system and inflammatory system, which serve as two biological mechanisms in disease processes. This understanding may lead to novel prevention and intervention efforts to identify adolescent physiology and family interactions patterns that may lead to decreased risk of physical and mental health problems during adolescence and beyond.

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