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Cortisol, salivary alpha-amylase and children's perceptions of their social networks

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In recent years there has been a growing interest in the use of social network analysis in biobehavioral research. Despite the well-established importance of social relationships in influencing human behavior and health, little is known about how children's perception of their immediate social relationships correlates with biological parameters of stress. In this study we explore the association between two measures of children's personal social networks, perceived network size and perceived network density, with two biomarkers of stress, cortisol and salivary alpha-amylase. Forty children (mean age = 8.30, min age = 5, and max age = 12) were interviewed to collect information about their friendships and three samples of saliva were collected. Our results show that children characterized by a lower pre-interview cortisol concentration and a lower salivary alpha-amylase reactivity to the interview reported the highest density of friendships. We discuss this result in light of the multisystem approach to the study of children's behavioral outcomes, emphasizing that future work of this kind is needed in order to understand the cognitive and biological mechanisms underlying children's and adolescents' social perceptual biases.

Keywords: Personal social networks; Cortisol; Salivary alpha-amylase; Perceptual biases; Psychosocial stress.

Humans are inherently motivated to look for and nurture their social relationships, and the quantity and quality of these social interactions have important consequences for their physical and mental health (Baumeister & Leary, 1995; Cacioppo, Cacioppo, Capitanio, & Cole, 2015). The neuroendocrine system, with the frontal regions of the cortex, the limbic system, the hypothalamus–pituitary–adrenal (HPA) axis, and the locus coeruleus–sympathetic–adrenomedullary (LC-SAM) axis, a branch of the autonomic nervous system, seem to provide an important link between the appraisal associated with the

quality of social relationships and health outcomes (Cacioppo et al., 2015; Flinn, 2006). The typical physiological response to a social threat involves a rapid (within minutes) release of catecholamines from the LC-SAM axis followed by the slower secretion of glucocorticoids (Sapolsky, Romero, & Munck, 2000). The temporal relation between the two systems is highly adaptive, since the LC-SAM axis regulates the short-term organismal strategies to cope with the source of stress, the fight-or-flight response, while the HPA axis has long-term effects (from hours to days), aiming at reestablishing a pre-stress status or a new

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equilibrium, allostasis (McEwen & Wingfield, 2003; Munck, Guyre, & Holbrook, 1984).

There is evidence that in adolescents, middle-aged, and elderly people, chronic social isolation, as measured by either perceived loneliness or the characteristics of one's social network, is associated with a higher HPA activity (Doane & Adam, 2010; Hawkley et al., 2008; Steptoe, Owen, Kunz-Ebrecht, & Brydon, 2004), whereas data of this kind on the activity of the LC-SAM are less consistent (Cacioppo et al., 2015). Similar results have been reported by studies focusing on children's baseline and reactive cortisol levels. For example, preschool children who receive a high number of dislikes from classmates have higher baseline cortisol levels (Gunnar, Sebanc, Tout, Donzella, & Van Dulmen, 2003). Likewise, children with high cortisol reactivity during interactions with familiar peers are less outgoing and less socially competent (Gunnar, Tout, De Haan, Pierce, & Stanbury, 1997), whereas children with a better regulation of vagal tone, a measure of the parasympathetic nervous system, in the face of psychological challenges score high in peer acceptance (Graziano, Keane, & Calkins, 2007). These data demonstrate that social dynamics are associated with the activities of both the HPA axis and autonomic nervous system (Blackhart, Eckel, & Tice, 2007; Gazelle & Druhen, 2009; Stroud et al., 2009).

THE DUAL-SYSTEM APPROACH TO THE STUDY OF SOCIAL DYNAMICS

One limitation of many studies investigating the interaction between the stress system and social dynamics is that they focus only on one neuroendocrine system at a time, considering the actions of the HPA or LC-SAM as independent or merely additive. However, there are several reasons for expecting the HPA and LC-SAM activities to interact both at basal concentrations and during stress challenges (Bauer, Quas, & Boyce, 2002). The HPA and LC-SAM axes are coordinated at the level of the central nervous system where they can both, directly or indirectly, activate or inhibit each other (Chrousos, 1998). Depending on the target tissue, glucocorticoids can both enhance and inhibit the effects of the LC-SAM and this may be especially important for basal glucocorticoid concentration (Sapolsky et al., 2000). For example, glucocorticoids facilitate the effects of catecholamines on the cardiovascular system (Sapolsky et al., 2000). There are also examples showing that the depletion of glucocorticoids, through adrenalectomy or with dexamethasone treatment,

enhances the concentration of plasma catecholamines and sympathetic activity (Andrews, D'Aguiar, & Pruessner, 2012; Kvetňanský et al., 1995). Likewise, there are studies reporting that the LC-SAM axis stimulates the secretion of glucocorticoids during stressful challenges (Andrews & Pruessner, 2013).

This literature suggests that the actions of the two stress systems are interactive, not merely additive, with some evidence for a compensatory action by which, for example, a decreased activation of the LC-SAM system is compensated by an over-expression of the HPA axis (Andrews, Ali, & Pruessner, 2013). In agreement with this idea, Bauer et al. (2002) hypothesized that a multisystem approach, which requires the study of the interaction between the HPA and LC-SAM activities, helps to better explain how individual differences in physiological arousal are associated with children's behavioral outcomes. Using this approach, several studies have already demonstrated that asymmetries between the LC-SAM and HPA axes are associated with maltreatment and externalizing behaviors in children (Gordis, Granger, Susman, & Trickett, 2006, 2008), and with trauma in adolescents (Vigil, Geary, Granger, & Flinn, 2010). Adults with early life adversities show a higher overall output of salivary amylase (sAA), a biomarker of the LC-SAM axis, when compared with cortisol (Andrews et al., 2013). These data point to the importance of considering both stress systems when variation in behaviors or psychological measures needs to be explained by within- and between-individual differences in physiological arousal.

PEER SOCIAL RELATIONSHIPS AND SOCIAL ENDOCRINOLOGY IN DEVELOPMENTAL STUDIES

Developmental studies that investigate the associations between social relationships and their neuroendocrine correlates have often focused on facets of peer socialization such as social competence (popularity, temperament, antisocial behaviors) or social experiences (such as victimization), but have seldom used measures derived from social network analysis (SNA). Despite its long history as an important approach to the study of social dynamics (McPherson, Smith-Lovin, & Brashears, 2006), the use of SNA in biobehavioral research is very recent and to date has only been used in the study of adults (Kornienko, Clemans, Out, & Granger, 2013, 2014).

SNA offers two different approaches to the study of social relationships. The structuralist approach uses the whole, complete network in order to identify

specific structures of relationships that may act as behavioral opportunities and/or constraints for the individual (Marin & Wellman, 2011). In its most traditional approach, the whole-network SNA requires that a bounded group of individuals is identified and information about their relationships to other members of the group is collected. This approach is very useful when the patterns and dynamics of relationships are the main target of investigation (Marin & Wellman, 2011).

The personal (ego)-network approach is the one traditionally used in survey and psychological studies that have focused on the amount and quality of social support (Wellman, 2007). The focus is generally on the subject (ego) and all the ties directly linked to them (first-order neighbors), although at times it also includes the second-order neighbors (i.e., the persons not directly tied to ego but directly linked to one of ego's ties) (Hanneman & Riddle, 2005). With this approach the size of a subject's ego-network and the kind of ties (kinship, friendship, positive or negative) are then used to predict the subject's psychological and health outcomes (Dozier, Harris, & Bergman, 1987; Pressman et al., 2005). Historically, personal networks have been the preferred approach for researchers studying people's perceptions of social support and health outcomes. Specifically, several measures of network structure, such as size and density and the quality of ties directed toward ego, have been used to predict, among others, ego's probability of psychiatric rehospitalization (Dozier et al., 1987), immune functions (Pressman et al., 2005), and physiological biomarkers of stress (Cohen et al., 2006; Lai et al., 2012; Pressman et al., 2005).

Although with some important differences, both approaches allow the calculation of "structural" measures, such as measures of ego's centrality within the network and density of ego's relationships. Among the different measures of centrality, degree centrality measures the number of members (i.e., alters) that are directly tied to ego. Specifically, an individual with the highest degree of centrality has a graph that looks like a star (Figure 1a). Using this measure, Kornienko et al. (2013) found that people naming a lower number of friends (i.e., people with a lower number of outgoing ties and therefore a lower out-degree centrality) have higher basal cortisol, while the ones with higher in-degree centrality (the more popular) have lower basal cortisol. For personal networks, measures of centrality are inherently problematic as, for example, ego's degree centrality in an ego-network corresponds to ego-network size and cannot be compared across subjects that generate networks of different size.

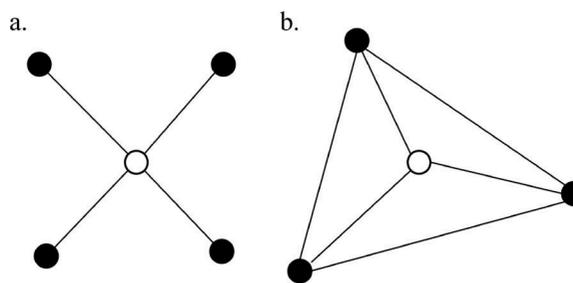


Figure 1. Two different ego-networks from children that participated in the study. White circle represents ego. In Figure 1a, the density of ego's network is 0 while ego's network size is 4. In Figure 1b, ego's network density is equal to 1 as all the possible connections between ego's alters are present. Since in ego-networks density is equal to the clustering coefficient, network closure is also equal to 1.

Density of relationships is another measure derived from SNA, and it measures the integration of the relationships between ego's neighbors (Hanneman & Riddle, 2005); density then indicates the extent ego's friends are friends of each other. In this regard, whole-network studies suggest that individuals embedded in highly cohesive groups of friends have higher basal cortisol (Kornienko et al., 2013). Network density is a measure that has been proposed as having both positive and negative influences on ego. Some scholars assume that being part of a more socially cohesive group has positive outcomes on ego, especially when high levels of trust are needed for optimal performance (Flynn, Reagans, & Guillory, 2010). It is also possible that a high density of friendships could result in ego as being more exposed to maladaptive coping behaviors, such as co-rumination (Kornienko et al., 2013), and in general to lower social support because high density results in more chances of dispersion of support toward other members of the network.

Network closure is another measure of cohesiveness that is strongly related to network density. Network closure indicates the presence of transitive, closed triads within a network (Flynn et al., 2010), and is positively associated with children's adjustment (see Fletcher, Hunter, & Eanes, 2006). Moreover, network closure may reflect the cognitive schemas used to memorize and remember social structures (Brashears, 2013; Brashears & Quintane, 2015; Flynn et al., 2010). As a consequence of the high costs associated with memorizing hundreds of dyadic relationships, people show a tendency to create clusters of relationships based on closed triads following a simple, heuristic, transitive rule: my friends are friends of each other (Freeman, 1992). Therefore, people tend to "see more" network closures than

there are in reality, creating ties where they do not exist. Importantly, how people perceive the distribution of social ties between others is context dependent. For example, experiencing social exclusion leads to a higher number of false-positive errors (i.e., reporting social ties when they do not exist), and this effect does not apply to non-social relationships (O'Connor & Gladstone, 2015).

There are no studies of the neuroendocrine mechanisms associated with individual differences in children's recall of social relationships. Furthermore, the majority of the studies that investigated a possible relation between the perception of the social environment and physiological arousal focused on the effect of chronic exposure to or perception of low levels of social support and baseline biomarkers of stress (see Cacioppo et al., 2015 for a review). However, there are reasons to expect acute, physiological responses to novelty (i.e., physiological arousal) may be associated with measures of perceived social networks. For example, several studies hypothesized a relation between children's responses to positive and negative experiences and their social competence. For instance, changes in salivary cortisol and alpha-amylase in response to psychosocial stress have been associated with externalizing behaviors (Gordis et al., 2006), and these behaviors generally result in peer rejection (Coie, Dodge, & Kupersmidt, 1990). As previously described, children with higher reactive cortisol have been reported as being less socially competent (Gunnar et al., 2003), and preschool children with better vagal regulation during a laboratory experiment were rated as more popular by their peers (Graziano et al., 2007). The assumption behind these studies is that individual differences in children's physiological reactivity and arousal reflect stable temperamental traits (e.g., emotional regulation), which then result in positive or negative social outcomes.

Moreover, given the interaction between the neuroendocrine products produced by acute stimulation of the HPA and LC-SAM axes in brain areas important for memory and cognition (Elzinga & Roelofs, 2005; Roozendaal, McEwen, & Chattarji, 2009), it seems plausible that people's perceptions of their social environment may be influenced by the acute, crossover activity of the two stress systems. For example, it is known that people, independently of their age, are extremely accurate when recreating the social ties within their social world (Freeman, 1992; Freeman, Freeman, & Michaelson, 1988). However, the accuracy of informants to correctly recall past social interactions varies as a function of

memory (Brewer, 2000), social position within the network (Cappella, Watling Neal, & Sahu, 2012; Casciaro, 1998), and cognitive (Kilduff, Crossland, Tsai, & Krackhardt, 2008) and psychological factors such as positive affect and personality (Casciaro, 1998; Casciaro, Carley, & Krackhardt, 1999). Physiological arousal may influence the way children remember their social networks. It may be that, in response to novelty, children more prone to physiological arousal, or with an asymmetric regulation of the HPA and LC-SAM axes, may either forget more social relationships or use fast and frugal cognitive strategies resulting from the cognitive load associated with the appraisal of the novel experience. The latter, as noted, will result in an overestimation of social ties.

THE PRESENT STUDY

Based on the coordination between the HPA and LC-SAM systems, a multisystem approach is a preferred method to investigate individual differences in physiological arousal as related to social behaviors (Bauer et al., 2002). However, there are relatively few such studies (e.g., Byrd-Craven, Granger, & Auer, 2011) and, to the best of our knowledge, none of these focused on children's perceptions of personal social networks. In the present work we monitored the physiological arousal of children during a video-interview designed to elicit the recall of the structure and quality of children's personal networks. Specifically, the goal was to explore whether the interaction between reactive changes in salivary cortisol and alpha-amylase was associated with two measures of personal social networks: network size and network density. However, in a previously published work we found that for the target population of children, cortisol did not increase from baseline during the interview (Ponzi, Muehlenbein, Sgoifo, Geary, & Flinn, 2014). In fact, pre-interview cortisol levels were already high as compared with samples collected at similar times but on different days, indicating an anticipatory cortisol response that likely masked the effects of the interview on the reactivity of the HPA axis. Therefore, here we focus on the interaction between this anticipatory response and reactive changes in sAA.

In line with previous research on children's physiological reactivity and social competence, we explored the possibility that children characterized by higher reactivity or by an asymmetry between the two stress systems have a smaller personal network

size, as indicated by a lower number of best friends. No clear hypotheses for network density were formulated as, based on the literature, network density has been associated with both positive and negative outcomes. However, based on cognitive studies reporting higher network closure during psychosocial challenges, we expected that children characterized by higher physiological arousal may report a higher network density.

MATERIAL AND METHODS

Study site

Bwa Mawego is a small rural village on the east coast of the island of Dominica which includes about 500 residents of mixed African, Carib, and European descent living in five different hamlets. The average annual income is \$1900US and many villagers spend months or years doing seasonal work in other islands of the Caribbean, in the USA, or in Canada. Despite poverty, children have a growth rate comparable to US standards (Flinn, Leone, & Quinlan, 1999). The kinship system is based on patrilineages (Quinlan & Flinn, 2005), however, similarly to other Caribbean regions, the social structure of Bwa Mawego is characterized by matrifocality.

Participants

Participants were 44 children (21 girls and 23 boys) of ages ranging from 5 to 12, whose parents provided consent for participation in the study, and that represent approximately 80% of the total children within this age range in the village. This study was approved by the Institutional Review Board of the University of Missouri.

Interview procedure and SNA

Data on social relationships were obtained with an ego-centered, personal network approach with alter connections (Hanneman & Riddle, 2005). The procedure involved asking children to nominate a maximum of five of their best friends and then, for each of these best friends, the child was asked to list a maximum of five of their best friends' friends. The goal of this procedure was to obtain the participants' perception of the distribution of friendship ties among their best friends, information needed to measure the participant's perceived density of social relationship

within their peer network. In personal networks with first-order neighbors, density is equivalent to the clustering coefficient, which is the number of closed triads that are present within ego's alters, an indication of network closure. Therefore, network density was also used as a measure of network closure. In Figure 1a, none of ego's alters were perceived by ego as being friends of each other, and therefore the density (and network closure) is equal to 0. In Figure 1b, the number of connected alters in ego's network is 3, the number of possible pairs between these alters is 3, and therefore the network density is equal to 1. Network density was obtained using the program E-Net (Borgatti, 2006; Borgatti & Halgin, 2012). Personal network size was indexed as the total number of best friends nominated.

Hormonal measures

Three samples of saliva were collected: before the interview, right at the end of the interview, and 15 minutes afterward. Cortisol concentrations were measured using an enzymatic immune assay (Salimetrics LLC) at the Evolutionary Physiology and Ecology Laboratory of Indiana University. Salivary alpha-amylase was measured by means of an enzymatic immune assay with kinetic reading at the University of Missouri. For cortisol, intra-assay coefficients of variation (CV) were less than 5% while inter-assay CV was 16.4%. Intra- and inter-assay CVs for sAA were 6.1 and 3.8%, respectively.

Statistical analysis

In order to reduce the effect of time of day on fluctuation in cortisol and sAA, all interviews were conducted between 2:30 and 6.00 pm. Four children were interviewed in the early morning and they were excluded from all the analyses. The distribution of values for cortisol and alpha-amylase was skewed and therefore log-transformed. Correlations and *t*-tests were used for descriptive purposes in order to explore potential covariates and to test for mean level changes of cortisol and sAA occurring during the interview. Since there are strong individual differences in hormonal reactivity to psychosocial challenges and in line with previous studies, we calculated cortisol and sAA reactivity as the difference between the log-transformed individual peak and baseline values, where the peak was chosen as the post-interview sample with the highest concentration. (Allwood, Handwerker, Kivlighan, Granger, & Stroud, 2011;

Buske-Kirschbaum et al., 2003; Oswald et al., 2006; Wust et al., 2004).

The association between the two stress biomarkers and their interaction with ego’s network size and density were investigated by means of multiple linear regression. Given the small sample size, in order to evaluate the precision of the regression coefficients we used bootstrapping with case resampling (Fox & Weisberg, 2010), and 95% bias-corrected and accelerated (BCa) confidence intervals were obtained. All analyses were conducted using the R packages MASS and CAR (CRAN.R-project.org) and SPSS 22. For descriptive purposes, the values for cortisol and sAA are reported as raw concentrations but all the analyses were carried out with their log-transformed values.

RESULTS

Descriptive statistics

Table 1 shows the descriptive statistics for the sample and Table 2 shows the zero-order correlations between the dependent variables, the predictor, and potential covariates. On average each child nominated 3.575 (SD = 1.106) best friends. Boys nominated on average more best friends than girls (boys $M = 4.000$, $SD = 0.917$; girls $M = 3.150$, $SD = 1.136$; $t(38) = 2.600$, $p = .013$). There were no sex differences in perceived density of friendship (boys $M = 0.312$, $SD = 0.321$; girls $M = 0.361$, $SD = 0.381$; $t(38) = -0.440$, $p = .663$), pre-interview cortisol levels (boys $M = -1.211$, $SD = 0.205$; girls $M = -1.194$, $SD = 0.366$; $t(38) = 0.190$, $p = .851$), and sAA reactivity (boys $M = 0.195$, $SD = 0.370$; girls $M = 0.201$, $SD = 0.455$; $t(38) = -.040$, $p = .970$).

TABLE 2
Zero-order correlations among the study variables

	1.	2.	3.	4.	5.	6.
1. Age	–					
2. Sex	.157	–				
3. Ego-net size	-.155	-.388*	–			
4. Ego-net density	.296	.071	-.220	–		
5. Cortisol T0	.367*	.030	.180	-.270	–	
6. Δ sAA	-.224	.006	.320*	-.242	.271	–

Note: * $p < .05$.

Relationship between salivary biomarkers of stress and personal network variables

To test the interactive influences of anticipatory cortisol and sAA reactivity on ego-network size and density, we used multiple linear regression with the two social network outcomes as the dependent variables and the mean-centered pre-interview cortisol, reactive sAA, and their interaction as predictors (see Table 3). The regression model for network size was not significant ($R^2 = .047$, $F(3,36) = 1.640$, $p = .197$), but was significant for perceived network density ($R^2 = .047$, $F(3,36) = 3.540$, $p = .024$), and qualified by a significant interaction between anticipatory cortisol and sAA reactivity ($\beta = .358$, $t(36) = 2.404$, $p = .021$). Simple slope analysis, which was conducted using Preacher, Curran, and Bauer (2006) web-based utilities at 1 SD above and below the mean of pre-interview cortisol, showed that for participants with lower baseline cortisol, the perception of a high density of social relationships within one’s social network depended on their low sympathetic reactivity to the interview (Figure 2; low pre-interview cortisol $b = -.497 \pm .192$, $t = -2.582$, $p = .014$; high

TABLE 1
Descriptive statistics of study variables

	Total				Boys		Girls	
	M	SD	Min	Max	M	SD	M	SD
Age	8.30	1.92	5.00	12.00	8.00	1.68	8.60	2.13
Ego-net size	3.57	1.10	1.00	5.00	4.00	.917	3.15	1.13
Ego-net density	.33	.34	.00	1.00	.31	.32	.36	.38
Cortisol T0	.07	.04	.00	.23	.06	.03	.08	.05
Peak cortisol	.09	.07	.03	.35	.08	.07	.11	.06
ΔCortisol	.02	.06	-.15	.28	.01	.07	.02	.06
sAA T0	50.96	54.15	4.10	276.94	63.47	70.20	38.44	27.57
Peak sAA	76.77	84.21	12.50	416.90	94.65	111.86	58.90	37.03
Δ sAA	25.81	59.68	-82.37	231.46	31.17	66.92	20.44	52.65

Notes: Values are presented as mean (±SD). T0 = pre-interview sample; cortisol and salivary amylase (sAA) are reported as μg/dl and U/ml, respectively. Δ = difference between peak and pre-interview (T0) concentrations. N = 40.

TABLE 3
Multiple linear regressions for ego-network size and density

	<i>B</i>	95% <i>CI</i>	<i>B</i> (<i>SE</i>)	<i>t</i>	β	R^2_{adj}
Ego-net size						
Cortisol T0	.333	[-1.131, 1.735]	.617	.541	.090	.046
Δ sAA	.831	[.106, 1.636]	.444	1.872	.308	
Cortisol T0 \times Δ sAA	-.811	[-4.103, 2.423]	1.368	-.593	-.104	
Ego-net density						
Cortisol T0	-.206	[-.528, .305]	.182	-1.135	-.174	.163*
Δ sAA	-.205	[-.485, .060]	.131	-1.571	-.242	
Cortisol T0 \times Δ sAA	.970	[.232, 2.272]	.403	2.404	.358	

Notes: * $p < .05$. Bias-corrected and accelerated confidence intervals were calculated with 5000 bootstraps.

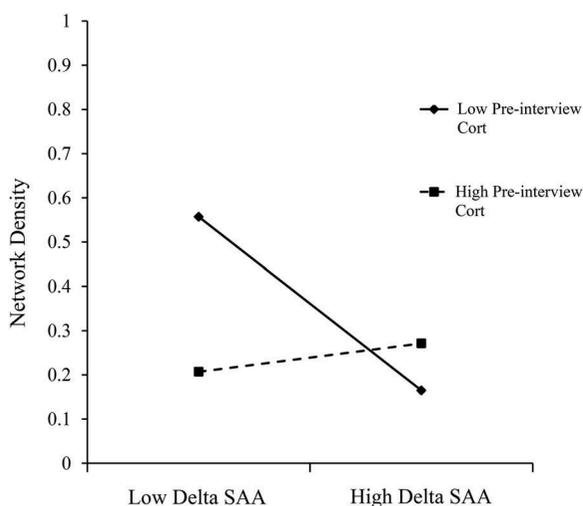


Figure 2. Relations between salivary alpha-amylase reactivity (delta sAA) and perceived network density at high and low concentrations of anticipatory cortisol. For both biomarkers, the interactions were tested at 1 SD above and below the respective means.

pre-interview cortisol $b = .085 \pm .162$, $t = .52$, $p = .601$). These results indicate that for those children with a low anticipatory cortisol response, sAA reactivity negatively predicted network density.

DISCUSSION

In this study we explored a potential relation between physiological arousal and two measures of children's perceived, personal social networks. This approach is in line with past research on stress, loneliness and social support in adults (Lai et al., 2012; Pressman et al., 2005). Our study expands on this research by focusing on children and by using the multisystem approach (Bauer et al., 2002). Specifically, we

investigated the moderating role of baseline cortisol on the relation between salivary alpha-amylase and perceived network size, a measure used in the past as a proxy for social support (Pressman et al., 2005), and perceived network density, a measure of level of social cohesion within one's peer group (Kornienko et al., 2013). The novelty of our study is that we collected this information by means of a videotaped interview and monitored hormonal changes. It is important to point out that originally the data were part of a longitudinal fieldwork study whose aim was to monitor daily variation of HPA activity in a naturalistic setting. Therefore, the interview was not designed to be stressful since we were not attempting to induce psychosocial stress. However, as we have previously reported, cortisol concentration in the samples collected before and during the interview was significantly higher than those collected at the same time but on different days (Ponzi et al., 2014). The implication is that the pre-interview cortisol concentration is an indicator of an anticipatory response of the HPA axis. Moreover, the fact that salivary alpha-amylase was affected by the video-interview with concentrations significantly higher after the interview compared with pre-interview suggests that the presence of a stranger and the videocamera were sufficient to induce physiological arousal in the children. This result is in agreement with others showing that children, adolescents, and adults under psychosocial stress during laboratory experiments experience an increase in sAA concentration (Gordis et al., 2006; Nater et al., 2006; Strahler, Mueller, Rosenlocher, Kirschbaum, & Rohleder, 2010).

Between the two measures of personal social network only the variance associated with perceived density of friendship ties was predicted by a significant interaction between the HPA and LC-SAM axes. The influence of sAA reactivity on the perceived

network density depended on children's anticipatory cortisol concentration, with low cortisol concentrations before the interview and low sAA reactivity predicting the highest density of friendship. These results show that the effects of the LC-SAM axis on perceived density of friendship depend on low, likely anticipatory, levels of cortisol.

Our results are in agreement with the Bauer et al. multisystem approach (2002), which posits that optimal behavioral outcomes should result when the arousal of the two stress systems is balanced, that is, when they are concurrently activated or deactivated. The multisystem approach advocated by Bauer and collaborators relies on the works of (Munck et al., 1984; Sapolsky et al., 2000) that suggest that one of the functions of glucocorticoids is to decrease the sympathetic response to an acute stress (i.e., to bring the body back to its homeostatic balance). On the contrary, the asymmetry of the two systems may indicate a history of allostatic load (Andrews et al., 2013; Bauer et al., 2002). Applying the multisystem approach to our result implies an assumption that the perception of a more tight-knit social environment is an optimal outcome and, as a consequence, that there is a close relationship between the experience of a more supportive group of peers and the symmetric function of the two stress systems. However, scholars have different opinions on whether network density has a positive or negative influence on a person's well-being (Dozier et al., 1987; Fletcher et al., 2006; Kornienko et al., 2013). An important difference between our study and those that use surveys to obtain measures of social network is that, by means of a videotaped interview, we asked the children to directly provide this information while measuring the neuroendocrinological changes occurring throughout the procedure. This raises the possibility of a more direct relation between a child's physiological arousal and memory recall of the personal network, especially network density. Studies on the cognitive mechanisms underlying the memorization and recall of social networks indicate that people tend to use heuristic strategies, such as triadic closure and transitivity (Brashears, 2013; Brashears & Quintane, 2015; Freeman, 1992). When reconstructing social networks, people tend to close gaps between persons instead of eliminating relationships, that is, they tend to see more social closure within the network than may actually exist (Flynn et al., 2010). Interestingly, subjects who were exposed to a social rejection paradigm tended to report a higher density of relationships when trying to remember a social network that was previously memorized (O'Connor &

Gladstone, 2015). In personal networks, density is equal to the level of clustering between ego's alters, and therefore we used network density also as a measure of network closure. Since people often use non-rational, implicit processes when under acute stress (Porcelli & Delgado, 2009), we hypothesized that children characterized by an asymmetry of the two stress systems during the interview would have reported more social closure within their personal networks. However, this was not supported by the results and instead the highest network density was reported by children with low anticipatory cortisol and low salivary alpha-amylase reactivity.

One possible explanation for this result is that high cortisol concentrations are known to impair retrieval of autobiographic memories and emotionally relevant memories (Buss, Wolf, Witt, & Hellhammer, 2004; Wolf, 2009). Additionally, the activity of the LC-SAM may be necessary in order for glucocorticoids to exert this effect, especially for emotionally related memories (De Quervain, Aerni, & Roozendaal, 2007; Roozendaal et al., 2009). If a higher network density is associated with a more detailed, accurate recall of the relationships occurring among peers, then it is possible that the lower anticipatory cortisol and low sAA reactivity of some children reflected a better mnemonic function and a more precise recall of these social relationships. However, our study was not designed to test this hypothesis since the use of personal social networks does not allow us to measure the accuracy of the subject's perception of their personal network. To accomplish this, other approaches such as complete social networks (Kornienko et al., 2013) or cognitive social structures (CSS; Krackhardt, 1987) are needed.

Our study has several important limitations. First of all, the very small sample size has reduced the statistical power to test the influence of other variables and more complex models. Furthermore, small sample sizes may also bias the findings, producing false positives (Button et al., 2013). In order to increase the accuracy of the results, in our regression models we used bootstrapping with random X resampling. Another limitation that adds to the small sample size is the relatively wide age range of the children interviewed, which makes our study unsuited for an examination of developmental differences and cohort effects. Therefore, although our results may provide groundwork for future confirmatory research, our study must be considered as exploratory. These limitations notwithstanding, our study has several aspects of novelty. For example, the interaction between anticipatory cortisol and reactive salivary

alpha-amylase may provide different prediction from the multisystem model based only on the reactive function of the HPA and LC-SAM axes, and should be explored in the future. Moreover, to the best of our knowledge, this is the first study exploring the relationship between perceptions of social networks and physiological arousal. With the aforementioned limitations in mind, our study provides important suggestions for researchers interested in the neuroendocrine mechanisms modulating social cognition and well-being.

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