

THE RELATIONSHIP BETWEEN ENVIRONMENTAL CHAOS
AND DIURNAL CORTISOL LEVELS OF PRESCHOOLERS

NİHAN SÖNMEZ

BOĞAZIÇI UNIVERSITY

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Nihan Sönmez

Boğaziçi University

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DECLARATION OF ORIGINALITY

I, Nihan Sönmez, certify that

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ABSTRACT

The Relationship Between Environmental Chaos and Diurnal Cortisol Levels of Preschoolers

Cortisol is a widely used biomarker of the hypothalamic-pituitary-adrenal (HPA) axis of stress system. The aim of the present study was to investigate the main, additive and interactive role of chaotic home and daycare environment on preschoolers' diurnal cortisol levels, above and beyond socioeconomic status (SES). Participants were 75 preschoolers (47-to-83-months old), their mothers and teachers. Chaos was measured by CHAOS and LECP scales for home and daycare, respectively. SES was calculated as the standardized average of income and parental education. HPA-axis activity was measured as diurnal salivary cortisol. Samples were collected in the morning and afternoon, for two days at home and daycare. Diurnal slopes were calculated by subtracting afternoon values from morning values. Replicating previous research, diurnal slopes were higher at home compared to daycare, indicating a steeper decrease from morning to afternoon. Hierarchical linear regression analyses were conducted to test the main hypotheses. No main contributions of home or daycare chaos on relevant cortisol measures were observed. Contrary to expectations, daycare chaos was positively associated with home diurnal slope, whereas home chaos was uniquely and positively associated with daycare diurnal slope, over and above SES. An interaction was observed on individual daycare cortisol levels, suggesting a dual risk. Findings were discussed in terms of the possible adaptive role of mild stressors across different microsystems for low-risk samples and other factors in light of previous research.

ÖZET

Çevresel Kaos ve Okul Öncesi Çocukların Günlük Kortizol Seviyeleri Arasındaki İlişki

Kortizol, hipotalamik-hipofiz-adrenal (HPA) eksenini etkinliğinin yaygın olarak kullanılan bir göstergesidir. Bu çalışma kaotik ev ve okul öncesi ortamının çocukların bazal kortizol seviyeleri üzerinde, sosyoekonomik statüsünün (SES) de ötesindeki ana, artırıcı ve etkileşimli rolünü araştırmayı amaçlamıştır. Çalışmaya 75 (47-83 ay) çocuk, aileleri ve okul öncesi öğretmenleri katılmıştır. Çevresel kaos, ev için CHAOS ve sınıf için LECP ölçekleri ile ölçülmüştür. SES ebeveynlerin gelir ve eğitim durumunun standardize ortalaması olarak hesaplanmıştır. HPA eksen etkinliği diurnal tükürük kortizolu ile ölçülmüştür. Numuneler ev ve okul öncesi kurumlarda, sabah ve öğleden sonra olmak üzere ikişer gün toplanmıştır. Diurnal kortizol eğrileri, gündüz ve öğleden sonra ölçümlerinin farkı olarak hesaplanmıştır. Önceki çalışmaları da replike ederek, ev kortizol eğrilerinin kreştekilerden daha yüksek olduğu, kortizol değerlerinin sabahtan öğleden sonraya doğru daha dik bir şekilde azaldığı gözlenmiştir. Temel hipotezler çok katmanlı doğrusal regresyon analizi ile test edilmiştir. Ev ve kreş ortamındaki kaosu ilgili ortamda ölçülen kortizol seviyeleriyle anlamlı bir ilişkisi görülmemiştir. Beklenenin aksine, kreş ortamındaki kaosu evde ölçülen kortizol eğrisiyle, ev ortamındaki kaosu ise kreşte ölçülen kortizol eğrisiyle, SES'in de ötesinde, özgün ve pozitif bir şekilde ilişkili olduğu görülmüştür. Kreş kortizol değerlerinde çevresel kaosu etkileşimli rolü saptanmış, sonuçlar ev ve kreş ortamındaki kaosu ikili risk yaratabileceğini önermiştir. Bulgular hafif ve orta dereceli kaosu düşük risk örneklemelerinde mikrosistemler arası olası adaptif etkisi ve literatür verileri ışığında incelenmiştir.

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To my mother Ayşegül Nuray Sönmez,

May she rest in peace...

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ABBREVIATIONS

ACTH: Adrenocorticotropic Hormone

GR: Glucocorticoid Receptors

CHAOS: Confusion, Hubbub, and Order Scale

CRH: Corticotropin Releasing Hormone

DNA: Deoxyribonucleic Acid

ECERS-R: Early Childhood Environment Rating Scale-Revised

HPA: Hypothalamic-Pituitary-Adrenal

NICHD: National Institute of Child Health and Human Development

LECP: Life in Early Childhood Programs

PVN: Paraventricular Nucleus

SAM: Sympathetic-Adrenomedullary

SES: Socioeconomic Status

CHAPTER 1

INTRODUCTION

Early and ongoing life adversities are shown to have lingering detrimental effects on psychological and physical well-being (Lupien, McEwen, Gunnar & Vazquez, 2001; Gunnar, & Heim, 2009; Repetti, Taylor, & Seeman, 2012). Emerging evidence points to the importance of stress systems, especially the hypothalamic-pituitary-adrenal (HPA) axis, as one important mechanism of the long-term effects of childhood adversity on poor health outcomes in adulthood (de Kloet, Joëls, & Holsboer, 2005; Gunnar & Vazquez, 2001; Lupien et al., 2009; Sapolsky, Romero, & Muck, 2000). Hence, investigating the role of environmental factors on HPA activity in childhood would provide valuable information in terms of identifying risk factors for concurrent and future well-being (Blair & Raver, 2012; Boyce & Ellis, 2005; Del Giudice, Elis, & Shirtcliff, 2011; Gunnar & Vazquez, 2006; Hostinar, Stellern, Schaefer, Carlson, & Gunnar, 2012).

Cortisol is the hormonal ‘end-product’ of the HPA axis, and increases in salivary cortisol levels are considered as a biological marker of psychological distress (Kirschbaum & Hellhammer, 1989; 1994; Hellhammer, Wüst, & Kudielka, 2009). Aside from stress-reactivity, cortisol normally follows a circadian rhythm; characterized by a peak after waking up and a decline throughout the day, reaching the lowest levels in the evening and midnight (Gunnar & Donzella, 2002; Gunnar & Quevedo, 2008).

Daily change of basal cortisol fluctuations from morning to evening, namely the diurnal cortisol slope, was associated with psychological and physical health (Adam et al., 2017). In children, flatter diurnal cortisol slopes were also associated with adverse

health conditions and maladjustment (Adam et al., 2017; Gunnar & Vazquez, 2001; Salis, Bernard, Black, Dougherty, & Klein, 2016). Further, children's diurnal cortisol levels were observed to be context-dependent, as children generally showed decreasing cortisol patterns at home whereas increased or flatter patterns at daycare, from morning to afternoon (for reviews, see Geoffroy, Cote, Parent, & Seguin, 2006; Gunnar & Donzella, 2002; Vermeer & van IJzendoorn, 2006). Such differences in diurnal cortisol slopes with respect to home and daycare measurements were discussed in terms of household factors (e.g. SES, poverty, chaos), daycare factors (e.g. daycare quality, group characteristics) and child-related factors (e.g. temperament). However, most research has mainly focused on one group of environmental factors while saliva samples were generally collected only in one environment or only in the mornings.

Ecological systems theory by Bronfenbrenner (2005), suggest that while home and daycare are two important microsystems, the mesosystem, which refers to the interactions between different microsystems, is important to understand the role of environment on child development. Therefore, while investigating the role of environmental factors on children's HPA activity, we must consider both home and daycare environment (Bronfenbrenner, 2001; 2005; McCartney, 2006).

Of particular importance to the present study are the chaotic environmental features of children's two principal microsystems, namely home and daycare, in relation to children's cortisol levels. Environmental chaos, characterized by noise, crowding, lack of stability and structure, is suggested to influence the HPA activity given the unpredictable and uncontrollable aspects of chaos (Evans & Wachs, 2010; Wachs, 2010). Although SES and poverty were repeatedly associated with HPA activity and children from lower-SES and low-income backgrounds are likely to experience greater chaos,

chaos was shown to have its unique contributions to child adjustment, over and above SES (Deater-Deckard et al., 2009; Dumas et al., 2005; Evans & English, 2002; Evans et al., 2005). Despite an increasing number of research suggesting the impact of early chaotic home environment on preschoolers' increased salivary cortisol levels, daycare chaos was overlooked in the previous studies. Furthermore, additive and interactive contributions of home and daycare chaotic characteristics on diurnal cortisol have not been studied systematically.

To fill in the gap in the literature, the present study aims to investigate the relationship between chaotic environmental characteristics of home and daycare, and their combined role on children's cortisol levels, over and above SES. By examining and identifying these dynamic environmental effects on children's HPA-axis, certain preventive and protective measures can be developed for children, who are vulnerable for later psychological and physical health problems.

1.1 Children's stress system, HPA axis, and cortisol

Stress can simply be defined as a physiological response to a stressor (e.g., novel, unpredictable or uncontrollable situations, negative experiences or expectations) (Lupien, Maheu, Tu, Fiocco, & Schramek, 2007; Ulrich-Lai & Herman, 2009). The main function of the human stress system is to prepare the organism for reacting and adapting to potential stressors and possible threats (Gunnar & Quevedo, 2007; McEwen, 2000b; Sapolsky et al., 2000). Appropriate activation and deactivation of the physiological stress response is adaptive for the organism (Jansen, Beijers, Riksen-Walraven, & de Weerth, 2010; Sapolsky et al., 2000). However, increased or constant

stimulation of the system might have adverse effects (Hunter, Minnis, & Wilson, 2011; McEwen, 2000a, 2000c; Sapolsky et al., 2000).

Early childhood is an important period due to the development of stress systems (Blair & Raver, 2012; Boyce & Ellis, 2005; Del Giudice, Elis, & Shirtcliff, 2011; Gunnar & Quevedo, 2007; 2008; Hostinar, Sullivan, & Gunnar, 2014). Adversities experienced in early childhood were repeatedly associated with various mental and physical health problems later in life, and the HPA axis has been proposed as an important biological intermediary of these effects (Bremner & Vermetten, 2001; Danese & McEwen, 2012; de Kloet, Joëls, & Holsboer, 2005; Felitti et al., 1998; Gunnar & Vazquez, 2001; Hunter et al., 2011; Lupien et al., 2009; Sapolsky, Romero, & Muck, 2000; Widom, Czaja, Bentley, & Johnson, 2012). Thus, HPA activity has become one of the major interests for developmental researchers in the last decade, and its end-product cortisol is used as a reliable biomarker of stress (Dallman & Hellhammer, 2011; Fisher, van Ryzin, & Gunnar, 2011; Hellhammer, Wüst, & Kudielka, 2009; Hunter et al., 2011; Kirschbaum & Hellhammer, 1989; Sapolsky et al., 2000). Non-invasiveness of saliva collection via salivettes (i.e., cotton rolls) allows researchers to obtain and study objective measures of stress even from very young children (Gunnar & Quevedo, 2006; Hunter, 2011; Vermeer & van IJzendoorn, 2006). Granger, Susman, Gunnar, & Laird, 1998).

1.1.1 HPA axis and cortisol diurnal rhythm

The physiological stress system consists of the dynamic interactions between two main pathways. The first one, Sympathetic-Adrenomedullary (SAM) axis, reacts rapidly to internal and external stressors through projections to adrenal glands, which secrete

norepinephrine and epinephrine (de Kloet, 2003; Gunnar & Quevedo, 2007, Ulrich-Lai & Herman, 2009). The second system is the HPA axis, which responds slower, yet have more prolonging effects through a cascade of complicated genetic, neurological and hormonal activations, including the secretion of cortisol (de Kloet, 2003; Chrousos, 1998; Gunnar & Quevedo, 2007, Sapolsky et al., 2000).

The HPA activation in response to stress and SAM activity starts in the paraventricular nucleus (PVN) of the hypothalamus and the nucleus releases corticotropin-releasing hormone (CRH), which, then activates the production of adrenocorticotrophic hormone (ACTH) by the pituitary. ACTH then travels towards the adrenal glands, where it starts the production and secretion of cortisol (Chrousos, 1998, de Kloet, 2003; Gunnar & Quevedo, 2007; Ulrich-Lai & Herman, 2009). After reaching a certain level, cortisol molecules bind to glucocorticoid receptors (GRs) throughout the brain. Once bound with cortisol, GRs start the cascade of reactions to inhibit the production of CRH, ACTH, and cortisol, to restore the system to the baseline levels (Chrousos, 1998; de Kloet, 2003; Lupien et al., 2009; Sapolsky et al., 2000). This process is referred as the negative feedback loop, and through this negative feedback mechanism, HPA axis down-regulates and maintains the balance (de Kloet, 2003; de Kloet et al., 2005; Lupien et al., 2009). In addition to stress reactivity, cortisol is also produced normally throughout the day, following a circadian rhythm, often referred as the diurnal slope (de Weerth, Zijl, & Buitelaar, 2003; Edwards, Evans, Hucklebridge, & Clow, 2001; Sapolsky et al., 2000).

1.1.2 Diurnal slope: Basal fluctuations of daily cortisol

Although stress reactivity offers valuable information in terms of how children cope with short-term challenges in the forms of acute stress, daily fluctuation of cortisol (i.e. diurnal slope) is important in terms of how environmental factors interact with child characteristics to influence their stress levels during the day (Gunnar & Quevedo, 2007).

Generally, cortisol levels peak in the morning, following approximately 30 minutes after awakening (i.e. cortisol awakening response), then decreases throughout the day, reaching lowest levels at midnight (Watamura, Donzella, Kertes, & Gunnar, 2004; Watamura, Kryzer, & Robertson, 2009). While newborns do not possess a stable circadian fluctuation of cortisol levels through the day, children acquire a relatively stable diurnal pattern within their first year of life that continues through adolescence (de Weerth, Zijl, & Buitelaar, 2003; Price, Close, & Fielding, 1983; Rotenberg, McGrath, Roy-Gagnon, & Tu, 2012; Watamura et al., 2004; 2009).

The peak of the cortisol level in the morning is suggested to be more influenced by genetic factors, whereas mid-day and evening cortisol levels are suggested to be less genetically influenced and more related to daily stressful activities (Bartels, van den Berg, Sluyter, Boomsma, & de Geus, 2003; Kunz-Ebrecht, Kirschbaum, Marmot, & Steptoe, 2004). Differences of cortisol patterns between measurements at home and daycare settings also suggest that the daily changes in the diurnal slopes are affected by environmental factors and situations (for a review, see Geoffroy et al., 2006; Vermeer & van IJzendoorn, 2006). Since diurnal cortisol levels are associated with physical and physiological health, it is important to investigate the links between environmental factors and basal HPA-axis activity to identify potential risk factors (Adam et al., 2017; Gunnar & Vazquez, 2001; Salis, Bernard, Black, Dougherty, & Klein, 2016).

1.1.3 Importance of cortisol on child development

Atypical diurnal slopes and repeated increases of cortisol levels were linked to internalizing and externalizing problems (Alink et al., 2008; Granger, 1998; Scerbo & Kolko, 1994), anxiety (Bremner & Vermetten, 2001; Chorpita & Barlow, 1998; Quirin, Pruessner, & Kuhl, 2008; van West, Claes, Sulon, & Deboutte, 2008), depression (Chrousos & Gold, 1992; Dougherty, Klein, Olino, Dyson, & Rose, 2009; Goodyer, Tamplin, Herbert, & Altham, 2000; Halligan, Herbert, Goodyer, & Murray, 2004; Holsboer, 2001; Parker, Shalzburg, & Lyons, 2003), and post-traumatic stress disorder (Yehuda, 2002; 2009). In addition to psychological problems, repeated increases in cortisol levels were linked to detrimental effects on the immune system and physical health in both children (Boyce & Jeremin, 1990; Boyce et al., 1995; Flinn, 1999; Hertzman, & Boyce, 2010; Jessop & Turner-Cobb, 2008; Watamura, Coe, Laudenslager, & Robertson, 2010), and adults (Essex et al., 2011; Seeman, Singer, Rowe, Horwitz, & McEwen, 1997; Widom et al., 2012).

Recent research has begun to examine the mechanisms that might explain the disruption of stress systems. Allostasis and allostatic load have been identified as one explanatory framework (McEwen, 1998; 2000a). Allostasis refers to maintaining equilibrium through active processes of change in an attempt to adapt stressful challenges, while the allostatic load is the ‘wear and tear’ of the system in response to allostasis (Danese & McEwen, 2012; Gunnar & Quevedo, 2007; McEwen, 1998; 2000a; Sapolsky, 2004). Several models that focus on early life effects on later psychological and health problems are based on allostasis and allostatic load perspective, such as the experiential canalization model (ECM; Blair & Raver, 2012), adaptive calibration model (ACM; Del Giudice et al., 2011) and the Risky Families Model (FRM; Repetti, Taylor,

& Seeman, 2002; Repetti et al., 2007). Despite their differences, all of these models agree that early adverse conditions and unpredictable threatening environment act to shape and modulate stress response systems, in accordance with inadequate emotion regulation skills and negative emotional states, and contribute to future mental and physical health problems (Evans, 2003; Evans et al., 2005; Evans & Kim, 2012).

Considering the research presented above, and early childhood as an important period for the development of the brain and neurobiological systems, it is important to identify the environmental and individual factors that might contribute to later problems, so certain preventive and protective measures and protocols might be developed for vulnerable groups.

1.2 Household factors that contribute to children's cortisol levels

Risky family environments might have detrimental effects on children's stress systems (Blair & Raver, 2012; Evans, 2003; 2004; Evans & English, 2002; Evans & Kim, 2007; Lupien, King, Meaney, & McEwen, 2000; 2001; Repetti et al., 2012). Family-related factors suggested to influence HPA-axis activity of children can be listed as SES and poverty (e.g. low income and maternal education), chaos (e.g. household instability, noise, density), parental characteristics (e.g. positive parenting, parenting stress), and other possible risk factors (e.g. neighbourhood safety, environmental toxins) (Blair & Raver, 2012; Ellen, Mijanovich, & Dillman, 2001; Evans, 2003; 2006; Evans & Kim, 2007; Lupien et al., 2001).

1.2.1 Poverty and socioeconomic status

Poverty and low socioeconomic status (SES) are shown to have detrimental effects on children's physical and psychological health (Evans & Kantrowitz, 2002; Evans & Kim, 2007; McEwen, 2003; Reiss, 2013). Further, flattened diurnal cortisol levels, compared to a normative declining pattern, were observed in children who live in low-SES and poverty conditions (Blair et al., 2011b; Blair & Raver, 2012; Evans, 2003; Evans & English, 2002; Lupien, et al., 2000; 2001; Miller, Chen, & Zhou, 2007) and adults (Cohen, Doyle, & Baum, 2006; Kunz-Ebrecht et al., 2004).

A cumulative risk index consisting of low family income, low maternal education, high hours of employment, low occupational prestige, poor safety, high household density and neighborhood noise, was associated with increased morning cortisol levels measured at home, at the age of 7, 15 and 24 months (Blair et al., 2011a; 2013). In part of the same longitudinal project, Blair and colleagues (2011b; 2015) showed that children in poverty had higher concentrations of salivary cortisol at three years of age. Furthermore, the duration of poverty exposure was associated with increased or flatter cortisol responses over 90 minutes of assessment of stress-reactivity at four years of age.

Regarding morning cortisol levels, Lupien, King, Meaney, and McEwen (2000; 2001) compared 6-to-16-year-old children from low and high SES backgrounds in two studies. Their results suggested low SES children showed increased morning cortisol levels, compared to their higher SES counterparts. However, this effect was diminished after school transition at the age of 10, which was explained in terms of equalization process of school, by education, peers, youth culture and resilience.

1.2.2 Home chaos

Home chaos is characterized by high ambient noise, increased crowding and family traffic, in combination with lack of stability and structure (Evans & Wachs, 2010; Martin, Razza, & Brooks-Gunn, 2012; Matheny, Wachs, Ludwig, & Phillips, 1995; Shamama-tus-Sabah & Gillani, 2011). Children from low-SES and low-income backgrounds are likely to experience higher chaos (Evans & English, 2002; Evans et al., 2005; Moore, Vandivere, & Ehrle, 2000), however, chaos was shown to have its unique contributions to child adjustment, over and above SES (Deater-Deckard et al., 2009; Dumas et al., 2005; Evans et al., 2005; Hart, Petrill, Deater-Deckard, & Thompson, 2007).

There is emerging evidence that household chaos and its sub-components relate negatively to children's morning and diurnal cortisol levels (Berry et al., 2016b; Blair et al., 2011b; 2013; Evans & English, 2002; Laurent et al., 2014; Suor et al., 2015). In a longitudinal study, Chen, Cohen, and Miller (2010) collected saliva samples at home, from morning to evening for every six months over two years from children between 9 and 18 years of age. Their results suggested that low SES children had increased daily cortisol levels over two years than their higher SES counterparts. More importantly, home chaos predicted increased cortisol levels and partially mediated the role of SES on cortisol, beyond children's perceptions of threat. Further, Laurent and colleagues (2014) showed that increase of home chaos within a year and a half, along with marital instability, was associated with increased and more stable morning cortisol levels in adopted children between 4.5 and 6 years of age, over and above financial need and other cumulative adversity index factors. Increased and more stable morning cortisol, in turn, was associated with internalizing problems (Laurent et al., 2014).

A recent study by Brown, Anderson, Garnett, and Hill (2019) showed that family chaos and financial instability mediated the relationship between financial adversity and increased morning cortisol levels of children between three and five-year-olds attending Head Start daycares. Also with the children who previously attended Head Start centers, Doom and colleagues (2018) showed that family chaos measured at the preschool period was associated with flatter diurnal cortisol slopes at middle school. Overall, the results of previous studies suggest that home chaos have direct and indirect contributions to children's HPA-axis activity.

1.3 Daycare factors that contribute to children's cortisol levels

As mentioned above, children are exposed to multiple ecologies during their early years, and the daycare setting is another microsystem where children spend a considerable amount of time (Geoffroy et al., 2006; Phillips & Lowenstein 2011; Shpancer, 2006; Vandell & Wolfe, 2000). There is increasing research showing that daycare environment is stressful for many children, as their diurnal slopes were flattened or more increased at daycare, compared to declining diurnal slopes at home (for reviews see Geoffroy et al., 2006; Vermeer & van IJanzendoorn, 2006). There are inconsistent findings, however, regarding stress levels of children in accordance with daycare characteristics, such as structural and process quality factors of the daycare setting, density, and caregiving environment as reviewed in the next section.

1.3.1 Daycare quality

Daycare quality consists of structural (e.g., physical aspects, such as size of the groups, staff-child ratio, and expertise of caregivers) and process (e.g., aspects of child's

experiences, such as child-caregiver interactions, and learning activities) factors (Clifford, Harms, Pepper & Stuart, 1992; Lamb, 1998; NICHD 2000a; Marshall, 2004; Vandell & Wolfe, 2000). Both process and structural factors are suggested to influence child development through dynamic interactions (Cryer, Tietze, Burchinal, Leal, & Palacios, 1999; NICHD, 2000a; 2000b; 2002; Phillipsen, Burchinal, Howes, & Cryer, 1997; Vandell & Wolfe, 2000).

Quality of the daycare environment was shown to influence children's cortisol fluctuations (Vermeer & van IJzendoorn, 2006). Especially low-quality daycare settings are associated with elevated cortisol levels (Geoffroy et al., 2006; Lisonbee, Mize, Payne, & Granger, 2008; Sims, Guilfoyle, & Parry, 2006). Yet, even at very good to excellent quality daycare centers, children's cortisol levels were higher than home (Suor et al., 20015; Tout, de Haan, Campbell, & Gunnar, 1998; Watamura et al., 2001; 2003; 2010). On the other hand, Vermeer and colleagues (2010) did not find any statistically significant differences between home and daycare cortisol levels. However, when daycare centers were divided into two groups by a median split of ECERS-R ratings, a global measure of daycare quality, below-median quality centers were associated with lower cortisol, compared to above-median quality centers (Vermeer et al., 2010).

Structural features, such as high group size, low density (i.e., space per children) and high child-to-caregiver ratio were associated with higher cortisol in daycare settings (Dettling et al., 2000; Legendre, 2003), yet, these factors also overlap with daycare chaos as mentioned below.

1.3.2 Daycare chaos

Similar to household chaos, daycare chaos is characterized by increased environmental traffic, noise, crowding, and disorganization (Corapci, 2010). Daycare chaos is suggested to individually contribute children's adjustment measures, and inclusion of daycare chaos, in addition to home chaos, is important to assess all possible chaotic factors as in early childhood (Corapci, 2010; Evans, 2006; NICHD, 2000a; 2002; Wachs, Gurkas, & Kontos, 2004).

While effects of daycare chaos are not fully explored in terms of children's cortisol levels, sub-components of daycare chaos, such as noise (e.g. Evans, Bulinger, & Hygge, 1998; Evans et al., 2001) and density (Legendre, 2003; Rappolt-Schlichtmann et al., 2009) were associated negatively with children's physiological stress responses.

Few studies investigated the effects of density on children's cortisol levels. Legendre (2003) found that children from higher density groups, in terms of lower space per children, showed increased morning cortisol levels. Further, for children in groups with a staff-to-child ratio above four, salivary cortisol levels were higher; which was discussed in terms of interns and part-time teachers' unpredictable schedules (Legendre, 2003). Rappolt-Schlichtmann and colleagues (2009) showed that transition from large to smaller group settings within high-quality daycare center resulted in lower morning cortisol levels, for children from low-income families. However, their small-group setting manipulation included two children spending 35 minutes as free playtime in a novel classroom environment with two adults; hence, the effects can also be attributed to factors regarding novelty, increased adult-to-child ratio, and possible therapeutic effects of free play, besides crowding.

Chronic exposure to noise (e.g., airport or railroad traffic) was also associated with increased neuroendocrine responses and elevated cortisol levels in children, in addition to poor attention and cognitive performance (for a review see Evans, 2006). However, Groeneveld and colleagues (2010) failed to find any effects of noise on children's cortisol levels measured in home- and center-based daycare setting, which was argued as a result of low variability in noise levels. Notably, one recent study by Sjödin and colleagues (2012) investigated the effects of noise on the psychological and physiological well-being of daycare personnel in Sweden. While there were no significant associations between objective noise and cortisol levels, subjective noise exposure was linked to the well-being of the staff. However, the authors also suggested that the lack of findings can be due to the low variability of noise recordings in daycare facilities (Sjödin et al., 2012).

1.4 Comparison of home and daycare cortisol levels

Ecological systems theory by Bronfenbrenner (2001; 2005) suggests that examination of interrelated microsystems (i.e., activities and relations of the child) is of particular importance to understand the interactions between the child and his or her environment. During early childhood, home and daycare environments are the two most prominent microsystems contributing to the socio-emotional and cognitive development of the child (Bronfenbrenner, 2005; McCartney, 2006). These microsystems include social (e.g., child-caregiver relations and social interactions between peers) and physical (e.g. setting of these interactions, background noise, crowding) components, both of which can influence stress levels of children (Coley, Lynch, & Kull, 2015; Evans, 2006; Evans

& Kim, 2007; Evans & Wachs, 2010; Phillips & Lowenstein, 2011, Vernon-Feagans et al., 2011).

Up to date, three meta-analyses contributed to early childhood stress research by calculating and comparing effect sizes for children's cortisol levels at home and daycare. Vermeer and van Ijzendoorn (2006) concluded that children's diurnal slopes were higher at daycare compared to home ($r = .18$). Geoffroy, Cote, Parent, and Seguin (2006) included nine studies in their meta-analysis, with a total number of 541 children. They found that diurnal cortisol slope at daycare was higher than home ($d = .72$). Eliassen (2012) also compared home and daycare cortisol increase by including 14 studies with a total number of 738 children in meta-analysis in her unpublished master thesis. Her results were similar to Geoffroy and colleagues (2006), albeit with a much lower effect size ($d = .31$). In addition, comparison of daycare and home cortisol levels at an absolute level, in terms of average cortisol for each context, yielded no significant differences between home and daycare cortisol levels ($d = .06$) (Eliassen, 2012).

All of these meta-analyses emphasize the varying effect sizes between studies and elaborated further to determine other factors at play. Geoffroy and colleagues (2006) showed that cortisol increase at daycare was more pronounced for children attending lower quality daycare facilities ($d = 1.17$) than high-quality daycare ($d = .11$). Vermeer and van Ijzendoorn (2006) suggested that even in the highest quality daycare facilities, cortisol levels were increased, compared to home, but did not compute effects sizes due to the insufficient number of studies. It must be noted, however, children attending high-quality daycare are also very likely to be from high SES families, and children from low-SES backgrounds are likely to attend low-quality daycares, except for children who

attend to government-funded prevention programs such as Head Start. Thus, assessment and controlling for each relevant home and daycare factor is important.

Authors of both meta-analyses reviewed above, also suggested variances might be due to other factors at play, and family background was not known for most of the samples. This is important because children from high-risk environments (i.e., low SES, chaotic homes), are exposed to a variety of stressors which might influence their stress systems. Several studies suggested that cortisol levels at daycare showed different patterns for children from high-risk family contexts, yet their results were generally based on cortisol samples collected only at home or daycare (Berry et al., 2013; 2016a; 2016b; Blair et al., 2011a; 2013; Doom et al., 2018; Rappolt-schlichtmann et al., 2009). Table 1 shows an overview of previous studies on cortisol levels of children in terms of sampling time, location and environmental factors of interest.

1.5 Present study

To date, some research investigated the role of environmental factors by collecting salivary cortisol samples both at home and daycare, yet, the results were inconsistent. In most of the cases, saliva samples were collected only at home (e.g., Berry, Blair, & Granger, 2016, Blair et al., 2013; Laurent et al., 2010; Smider, 2002) or only at daycare (e.g. Groeneveld et al., 2010; Rappolt-Schlichtman et al., 2009; Sims et al., 2006; Tout et al., 1998; Wagner et al., 2015). In some cases, diurnal slopes increased or stayed flat in daycare through the day, compared to decline at home during weekends as expected (Dettling, Gunnar, & Donzella, 1999; Sumner, Bernard, & Dozier, 2010; Watamura et al., 2003), yet, two recent studies showed no differences between home and daycare diurnal slopes (Oullet-Morin et al., 2010; Vermeer et al., 2010).

Table 1. An Overview of Previous Studies on Home and Daycare Cortisol for Comparison

Study and Sample Details						Cortisol Collection			Factors Tested	
First Author	Date	Design	Sample	Age(y)	N	Home	Daycare	Sampling Time	Home	Daycare
Berry	2013	Longitudinal	Head Start	1 to 4	1235	Yes		Morning	Yes	Yes
Berry	2016a	Longitudinal	Head Start	1 to 2	1155	Yes		Morning	Yes	Yes
Blair	2011	Longitudinal	Head Start	1 to 2	1292	Yes		Afternoon	Yes	
Brown	2019	Longitudinal	Head Start	3 - 5	374		Yes	Morning	Yes	
de Haan	1998	Longitudinal	Middle-to-upper class	2	24	Yes	Yes	Morning		Yes
Dettling	2000	Cross-sectional	Middle-to-upper class	3-5.8	61	Yes	Yes	Morning, afternoon		Yes
Doom	2018	Longitudinal	Head Start	4 to 7-10	242	Yes		Diurnal pattern	Yes	
Groeneveld	2010	Cross-sectional	Middle-to-upper class	1.5-3.5	115	*Home based care	Yes	Diurnal pattern		Yes
Gunnar	2010	Cross-sectional	Middle-to-upper class	3-4.5	Yes	Yes	Yes	Morning, afternoon		Yes
Laurent	2013	Longitudinal	Adopted children	4.5 to 6	200	Yes		Waking, Bedtime	Yes	
Legendre	2003	Cross-sectional	Middle-to-upper class	1.5-3.5	113	Yes (n = 10)	Yes	morning		Yes
Lupien	2001	Cross-sectional	Mixed SES	6-16	307		*Lab	Morning	Yes	
Oullet-Morin	2010	Longitudinal	Middle-to-upper class	2 to 3	116	Yes	Yes	Morning, afternoon	Yes	
Rappolt-Sch.	2009	Cross-sectional	Low income	2-4	60		Yes	Morning		Yes
Sajainemi	2011	Cross-sectional	Middle-to-upper class	4-6	146	Yes	Yes	Diurnal pattern	Yes	Yes
Sajainemi	2014	Longitudinal	Middle-to-upper class	6	91	Yes	Yes	Diurnal pattern		Yes
Sims	2006	Cross-sectional	Middle-to-upper class	3-5	117		Yes	Morning, afternoon		Yes
Sumner	2010	Cross-sectional	Mixed SES	1.5-2	42	Yes	Yes	Diurnal pattern		
Vermeer	2010	Cross-sectional & cross-country	Basque & Netherlands	1.5-3.5	85	Yes	Yes	Morning, afternoon		Yes
Wagner	2015	Cross-sectional	Mixed SES	3-5	101		Yes	Morning	Yes	Yes
Watamura	2002	Cross-sectional	Middle-to-upper class	1-2.5	55	Yes (n = 8)	Yes	Morning, afternoon		
Watamura	2003	Cross-sectional	Middle-to-upper class	3-6	55	Yes	Yes	Morning, afternoon		
Watamura	2009	Cross-sectional	Middle-to-upper class	1-3	69	Yes	Yes	Diurnal pattern		Yes

No study, to my knowledge, investigated the role of both home and daycare chaotic features on children's cortisol levels, by collecting samples from both environments at different time points.

The aim of the present study is to systematically investigate the main, additive, and interactive effects of chaotic home and daycare on preschoolers' salivary cortisol levels above and beyond the effect of SES.

First, replicating past research on home and daycare cortisol levels, it is hypothesized that diurnal slopes of children at daycare are expected to increase more from morning to afternoon, compared to home.

Second, the main effects of each chaotic environment were tested. Children from more chaotic homes are expected to show higher morning and afternoon cortisol as well as flatter diurnal cortisol slope compared to children from less chaotic homes, above and beyond the effect of SES. Similarly, children from more chaotic classrooms are expected to show higher morning and afternoon cortisol as well as flatter diurnal cortisol slope compared to children from less chaotic homes, above and beyond the effect of SES.

Third, in terms of additive effects, it is expected that home chaos and daycare chaos would directly and independently predict flatter diurnal slopes and overall morning and afternoon cortisol levels of preschoolers.

Finally, interactive effects were also expected. For children living in a family environment with higher chaos, attending a non-chaotic daycare is expected to serve a protective role and predict decreasing cortisol levels at daycare setting from morning to afternoon. However, whether these effects will be carried on to home measurements cannot be determined pre-hoc, as previous studies show mixed findings.

CHAPTER 2

METHOD

2.1 Participants

A total number of 150 preschoolers, their families, and teachers from municipality preschools took part in the study. Families of 8 children withdrew their consents afterwards. Twenty children were not eligible for sampling, due to a chronic health condition or regularly using drugs that might influence the central nervous system and HPA axis activity. Of the remaining children, 4 was not enrolled in the daycare center at the time of sampling, 3 did not want to take part in the sampling, 2 did not provide a sufficient amount of saliva for analyses, 3 did not participate due to various reasons (e.g. family vacation, weekends at a different home). Salivary concentrations of cortisol were collected at home or daycare for at least one day for 110 children. Among 110, 10 of the children did not provide any home samples, 1 of the children did not provide any daycare samples. Finally, family demographics were not available for 3 children, classroom demographics in addition to daycare chaos were not available for 21 children.

Therefore, the final sample of the present study consisted of 75 (35 girls, 40 boys) preschoolers, their mothers, and teachers. Children were between 47 and 83 months of age ($M = 62.30$, $SD = 7.45$). Please see Table 2 for a summary of the relevant child and family characteristics.

Boys represented 53.3% of the sample. The majority of children (65.8%) had at least one sibling, and the majority of the families (90.7%) were intact. Of the families in the study, 46.7% reported a change of residence at least one time since the child was born. Income was below 3000 TL for 39.7% of the families, between 3000 TL and 5000

TL for 34.2% of the families, between 5000 TL and 7000 TL for 19.2% of the families, and above 7000 TL for 6.9% of the families. Regarding employment, 82.4% of fathers worked full-time, 17.6% was working part-time, whereas 1.5% was unemployed. Among mothers, 44.4% was working full-time, 8.3% was working part-time and 47.2% was unemployed.

Table 2. Descriptive Statistics for Child and Family Demographics

Demographic Variable	<i>M</i>	<i>SD</i>	Min.	Max.
Child age (months)	62.30	7.45	47	83
Mother age (years)	34.16	4.06	25	43
Number of siblings	0.75	0.64	0	3
Number of family members	4.30	1.38	3	10
Number of rooms in the house	3.73	0.55	2	6
Household density	1.18	0.44	0.67	3.33
Years in current residence	7.49	7.97	0	41
Hours at preschool	36.89	5.51	8	56
Education (%)			Mothers	Fathers
Less than high school			13.3	18.3
High school			34.7	35.2
At least 2-year college			46.7	40.8

Note. $N = 75$

A total number of 23 different classrooms from 9 different municipality preschools and their teachers participated in the study. Convenience sampling was used. All teachers were female and 27.7% had high school degrees, 22.7% had vocational high school degrees, and 45.5% had a university or at least two-year college degrees. As reported by teachers, 30.4% of the classrooms had at least one child with behavior problems, 8.7% had at least one child with special needs. Please see Table 3 for a summary of the relevant classroom and teacher characteristics.

Table 3. Descriptive Statistics for Daycare Classroom and Teacher Demographics

Demographic Variable	<i>M</i>	<i>SD</i>	Min.	Max.
Number of children in the classroom	12.17	2.42	9	17
Child-to-adult ratio	6.16	2.63	2.6	15
Experience level of teacher (years)	8.96	6.72	2	27

Note. *n* = 23

2.2 Measures

2.2.1 Salivary cortisol

Salivary cortisol concentrations were obtained via Salivette saliva collection kits (Sarstedt, Germany). Salivette kits included a small synthetic cotton roll 3/8" in diameter and 1.5" in length, within a plastic tube. A thread was sewn through the salivettes using a sterilized needle, for mothers and children to be able to securely hold while collecting

saliva. The cotton roll was kept in the child's mouth for a couple of minutes until it was sufficiently saturated with saliva.

Saliva samples were collected at daycare by trained research assistants and graduate students in two days, in the morning and mid-afternoon. Parents collected weekend samples in two consecutive days at home. Overall, eight saliva samples were collected from each child; four samples for home and four samples for daycare. Morning saliva samples were collected between 10-to-10:30 a.m. and afternoon samples were collected between 3-to-3:30 p.m. across all contexts. Parents filled two short forms to control for possible confounds that might affect cortisol secretion. The first form included items regarding the child's birth, overall health and development, chronic illness, medication use, and such. The second form included questions according to sleep and napping, in addition to possible worrisome events (e.g., doctor's visit) experienced by the child during the weekend of saliva collection at home.

Saliva samples were analyzed for cortisol concentrations in an automatized system (Roche Diagnostics, Indianapolis, USA) at Centro Laboratories, Istanbul, Turkey. The sample was reanalyzed if the difference between duplicates was above .10 $\mu\text{g}/\text{dL}$. Overall, the intra-assay coefficient of variation was 10% and the inter-assay coefficient of variation was less than 5%.

Cortisol concentration values were converted from $\mu\text{g}/\text{dL}$ to nmol/L , and the average of each duplicate was taken as the cortisol value for that sample. Cortisol values were log-transformed for normality, prior to analyses, as recommended in the field (Miller & Plessow, 2013). Morning and afternoon cortisol at home and daycare were calculated as the average of two days for each measure separately. Diurnal slopes, differences between morning and afternoon measures, were calculated by subtracting

afternoon cortisol value from morning cortisol value for each day separately and averaged for each environment as a composite score. Therefore, higher diurnal slopes indicated a steeper decrease, from morning to afternoon.

2.2.2 Home environment

2.2.2.1 Home chaos

The Confusion, Hubbub, and Order Scale (CHAOS), developed by Matheny, Wachs, Ludwig, and Phillips (1995), was used to obtain parent reports of household chaos. The scale is consisted of 15 items in a 6-point Likert scale format, from 1 as “*definitely untrue*” to 6 as “*definitely true*”. The scale was shown to have satisfactory internal consistency (Cronbach’s $\alpha = .79$) and stability over twelve months ($r = .74$) (Matheny & Phillips, 2001; Matheny et al., 1995). Turkish version of the scale (Sumer, Solak, & Harma, 2003) was shown to have satisfactory internal consistency (Cronbach’s $\alpha = .82$). With the present sample, Cronbach’s alpha was .79, representing a good internal consistency.

2.2.2.2 Family demographics

Mothers filled demographics form regarding child and home environment and demographics of parents; such as age, gender, physical characteristics, weekly number of hours child spends in daycare, number of siblings and the total number of household members currently living with children, along with parents’ age, education, occupation, marital status, employment schedule, household income for both parents, and the number of residency changes since the child was born in addition to the years spent in

the same home. SES was computed by averaging the standardized values of family income, maternal and paternal education levels, as all measures were highly inter-correlated ($r_s > .36, p < .001$). Household density was calculated by dividing the number of people living in the house to the number of rooms in the house.

2.2.3 Daycare environment

2.2.3.1 Daycare chaos

The Life in Early Childhood Programs Scale (LECP), developed by Kontos and Wachs (2000), was used to obtain teacher reports of daycare chaos in a class setting. The scale was found to have moderate reliability ($\alpha = .67$) and consisted of 16 items, in a 6-point Likert scale format ($1 = "definitely untrue"$ and $6 = "definitely true"$) (Wachs et al., 2004). The scale is adapted to Turkish, and the Turkish version is shown to have satisfactory reliability ($\alpha = .62$) (Eroğlu Ada, 2016). With the present sample, Cronbach's alpha was .61.

2.2.3.2 Classroom and teacher demographics

Demographic information regarding teacher's qualifications (i.e., training and expertise), classroom setting (i.e., the total number of teachers and interns) and group specifications (i.e., age and number of children in the group, children with behavior problems, children with special needs) were obtained by a teacher demographics form. Child-to-adult ratio was calculated by dividing the number of children in the classroom to the number of caretakers (i.e. both teachers and interns).

2.2.3.3 Daycare quality

The Early Childhood Environment Rating Scale-Revised (ECERS-R), developed by Harms, Clifford, and Cryer (1998), was used as an initial check for daycare quality. The scale is a widely used observational measure, depicting both structural and process daycare quality. It consists of 7 subscales and a total number of 43 items. The scale is adapted to Turkish with satisfactory reliability ($\alpha = .97$) (Tovim, 1996). In the present study, the scale is shown to possess satisfactory internal consistency ($\alpha = .79$).

2.3 Procedure

Data collection continued as part of an ongoing research project by Çorapci and Duman, which was approved by Boğaziçi University Ethics Committee and funded by Bogaziçi University Scientific Research Projects Commission.

Kartal municipality preschools in Istanbul were contacted for participation in the project and directorates' permissions were obtained, followed by class teachers. Once daycare centers and teachers gave permission, parents were informed about the study and only children's and their mothers agreed to participate with signed consent forms, were included in the study. Consenting mothers were sent and asked to complete the Confusion, Hubbub, and Order Scale in addition to the family demographic scale.

Teachers completed the LECP and teacher demographic scale. Daycare quality was measured by observations of trained graduate students, upon two to three hours of daily visits to classes. Observations began in the mornings, and observers did not interfere with activities in any way, only observed and rated environmental characteristics in accordance with ECERS-R criteria. If any indicators were not observed during the visit, they were asked to teachers in brief. As mentioned in the above

sections, daycare quality was measured to control for daycare environmental factors and inclusion of municipality preschools in the study provided similar daycare structure characteristics. ECERS-R ratings represented low variability in municipality preschools included in the municipal preschools included in the study, and daycare quality measures were not included in further analyses.

Saliva samples at daycare centers were collected from children by trained research assistants and graduate students in a group setting, in a playful manner for two days, at 10:00 - 10:30 am and 15:00 – 15:30 pm. Saliva samples at home were collected by parents for two days from 10:00 - 10:30 am and 15:00 – 15:30 pm. Prior to saliva collection, parents were contacted through phone and asked about the health conditions of their child and any medications they use. If any children were sick during the saliva collection days, their collection time was postponed until child regains health. If the child had a chronic condition and regularly used any medication that might affect HPA activity, the child was excluded from the study. Also, if the child were going to experience a novel event during the weekend (e.g., attending to a new amusement park, wedding, travel, visits from distant relatives, etc.), saliva collection was postponed to a weekend which would present a normative environment for the child.

Daycare staff and parents were informed, and children did not eat or drink anything besides water, or take part in intense physical activity for half an hour prior to saliva collection. They did not drink any caffeinated beverages (e.g., tea, coffee, coke) or eat chocolate, which might disrupt their hormonal trajectories, two hours prior to saliva collection. Generally, daycare schedule of meals and exercise hours were compatible with sampling time; however, if necessary, adjustments were made in the timing of the daycare schedule with the assistance of daycare directors and teachers.

During saliva collection, children kept cotton rolls in their mouths for several minutes until the salivettes are fully saturated with saliva. If the collected sample did not include a sufficient amount of saliva for analysis at least for one time-point, the whole day was repeated for those children in the following week. Children were compensated with small toys and stickers for their participation in the study. Salivettes were stored in 4°C until assayed in a local laboratory.

Parents were invited to daycare for a brief informing and demonstration session. If parents could not attend information sessions, they were informed in detail over the phone. Weekend packages, consisted of five salivette kits individually labeled for days and time-points (two for saturday, two for sunday, and one spare kit), a short brochure that explains the procedure and requirements, with forms to be filled with information regarding timing of the sampling, children's sleep and illness, were sent to children's homes. Parents were informed and demonstrated about the procedure, also assisted over the phone during weekends if necessary. To increase compliance, they were called to remind saliva collection at the weekend upon their approval of doing so. Parents kept the samples in the fridge after collection and sent packages to daycare centers, and they were collected to be assayed in a local laboratory.

CHAPTER 3

RESULTS

In this section, results of descriptive analyses, tests of the hypotheses, and supplementary analyses are presented. First, descriptive statistics and correlations among study variables are documented. Secondly, differences between cortisol levels are compared by time and environment. Hierarchical linear regression analyses were conducted to test the main, additive and interactive role of chaotic environmental factors on morning and afternoon cortisol levels, as well as diurnal slope, controlling for SES. Exploratory analyses for home and daycare demographic variables on home and daycare cortisol measures were conducted as supplementary analyses. For all analyses, SPSS (Version 24.0; IBM Corp., 2016) was used.

3.1 Descriptive statistics among the study variables

Descriptive statistics for raw cortisol values at home and daycare, as well as relevant factors for each day are presented in Table 4. Overall, five cortisol values which were three standard deviations above or below the mean were excluded from further analyses as recommended in the field (Gunnar & White, 2001). Since the first and the second-day salivary cortisol concentrations were highly correlated with each other, an average of both days was used as composite scores. However, 20 children ($n = 14$ for home, $n = 9$ for daycare) had complete morning and afternoon cortisol samples for only one day, hence, the available score was used in the composite scores. Descriptive statistics for cortisol composite scores at home and daycare, as well as home and daycare chaos variables are presented in Table 5.

Table 4. Descriptive Statistics for Raw Cortisol Concentrations and Relevant Factors

Cortisol Variable		Day 1		Day 2		<i>r</i>
		<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	
Home	Morning Cortisol	4.38	2.57	5.08	3.08	.52**
	Afternoon Cortisol	3.03	1.86	2.87	1.61	.72**
	Diurnal Slope	1.36	2.52	2.23	2.73	.27*
	Awakening Time	8.44	.87	8.67	.99	.66**
	Sleep Duration	10.40	2.00	10.39	1.19	.23*
	Morning Sampling Time	10.22	.32	10.22	.37	.51**
	Afternoon Sampling Time	15.55	.25	15.62	.43	.37**
	Sampling Time Difference	5.34	.35	5.40	.50	.35**
Daycare	Morning Cortisol	3.02	1.90	3.27	2.38	.21*
	Afternoon Cortisol	2.47	1.62	3.13	2.15	.71**
	Diurnal Slope	0.61	1.47	1.23	1.96	.25*
	Awakening Time	7.80	.59	7.82	.60	.77**
	Sleep Duration	9.86	.97	10.07	1.07	.24*
	Morning Sampling Time	10.25	.35	10.31	.22	.22*
	Afternoon Sampling Time	15.38	.34	15.34	.33	.53**
	Sampling Time Difference	5.13	.55	5.03	.45	.64**
Change from morning to afternoon (%)		Home	Daycare	Both		
Decrease		78.7	66.2	48		
Increase		21.3	33.8	6.7		

Note. Raw cortisol concentrations values are (nmol/L). Minutes were recoded as percentile values (e.g., 10.50 is 10:30 a.m.).* $p < .05$, ** $p < .01$

Table 5. Descriptive Statistics for Environmental Chaos and Cortisol Variables

	<i>M</i>	<i>SD</i>	<i>Min.</i>	<i>Max.</i>
SES	-0.03	.85	-2.15	1.92
Home Chaos	2.36	.59	1.27	4.00
Daycare Chaos	1.99	.52	1.13	3.19
Daycare Quality	2.89	.31	2.65	3.15
Home Morning Cortisol	.59	.28	-.30	1.03
Home Afternoon Cortisol	.39	.28	-.29	.78
Home Diurnal Slope	.20	.24	-.34	.81
Daycare Morning Cortisol	.41	.28	-.25	1.01
Daycare Afternoon Cortisol	.35	.31	-.25	.88
Daycare Diurnal Slope	.07	.20	-.40	.64

Note. $N = 75$. For daycare quality, $n = 65$. Cortisol variables are log-transformed.

3. 2 Correlations among study variables and demographic variables

3.2.1 Bivariate correlations between environmental factors and cortisol measures related to home context

Relationship between environmental chaos indicators and relevant cortisol measures for home are presented in Table 6. Home awakening time was correlated with morning cortisol ($r = .28, p < .01$) and had a marginally significant correlation with home diurnal slope ($r = .24, p = .05$). As expected, diurnal slope, morning and afternoon cortisol measures were significantly correlated with each other ($r_s > .42, p_s < .01$).

Table 6. Correlations Between Environmental Factors and Cortisol Measures Related to Home Context

	1	2	3	4	5	6	7	8	9	10	11	12
1. Child Age (months)	-	-.11	-.20	.11	-.02	.09	-.12	-.30*	.18	-.26*	-.22	.04
2. Sex (Boys = 0)		-	-.15	-.06	.00	-.15	.18	.13	-.07	-.15	.03	.15
3. SES			-	-.12	.06	.14	-.09	-.12	.04	-.01	.23*	.20
4. Home Chaos				-	-.11	-.19	.09	-.22	.16	-.04	.03	.06
5. Morning Cortisol					-	.63**	.42**	.28*	.15	.20	.03	-.15
6. Afternoon Cortisol						-	-.44**	.07	.21	.16	-.02	-.15
7. Diurnal Slope							-	.24"	-.08	.05	.05	.00
8. Awakening Time								-	-.50**	.23	.18	-.04
9. Sleep Duration									-	.25*	.00	-.20
10. Morning Sampling Time										-	.29*	-.62**
11. Afternoon Sampling Time											-	.56**
12. Sampling Time Difference												-

Note. * $p < .05$, ** $p < .01$, " $p < .06$, two tailed.

3.2.2 Bivariate correlations between environmental factors and cortisol measures related to daycare context

Relationship between environmental chaos indicators and relevant cortisol measures for daycare are presented in Table 7. Daycare chaos was only correlated with awakening time ($r = .25, p < .05$). Daycare diurnal slope was positively correlated with SES ($r = .24, p < .05$). Morning sampling time was positively correlated with morning cortisol ($r = .45, p < .01$) and afternoon cortisol ($r = .48, p < .01$). Afternoon cortisol was significantly correlated with morning cortisol ($r = .78, p < .01$), and diurnal slope ($r = -.44, p < .01$) at daycare.

3.2.3 Bivariate correlations between environmental and demographic factors

Correlations among environmental variables are presented in Table 8. None of the chaos indicators, as well as SES, were correlated with each other. Only daycare quality was negatively correlated with daycare chaos, $r = -.32, p < .05$. In addition, age and gender differences were not detected for home chaos ($t(73) = .54, p > .5$), daycare chaos, ($t(73) = -1.66, p > .5$), and SES ($t(73) = 1.29, p > .5$). Further, morning cortisol ($t(73) = -.03, p > .5$), afternoon cortisol ($t(73) = 1.29, p > .5$), and diurnal slopes ($t(73) = -1.55, p > .5$) as measured at home, were not significantly different from each other according to sex. Finally, no gender differences for daycare morning ($t(73) = 1.09, p > .5$) and daycare afternoon cortisol ($t(73) = .40, p > .5$), as well as daycare diurnal slopes ($t(73) = .91, p > .5$) were observed.

Table 7. Correlations Between Environmental Factors and Cortisol Measures Related to Daycare Context

	1	2	3	4	5	6	7	8	9	10	11	12
1. Child Age (months)	-	-.11	-.20	-.17	-.16	-.16	.01	-.12	-.21	-.22	.12	.19
2. Sex (Boys = 0)		-	-.15	.19	-.13	-.05	-.11	-.05	.21	.00	-.03	-.02
3. SES			-	-.18	.15	-.02	.24*	-.05	-.05	.16	-.11	-.16
4. Daycare Chaos				-	.08	.11	-.06	.25*	.10	.20	-.04	-.13
5. Morning Cortisol					-	.78**	.22	.13	.10	.45**	-.01	-.23*
6. Afternoon Cortisol							-	-.44**	.18	.08	.48**	.03
7. Diurnal Slope								-	-.08	.02	-.11	-.07
8. Awakening Time									-	.22	.21	-.11
9. Sleep Duration										-	.06	-.05
10. Morning Sampling Time											-	-.45**
11. Afternoon Sampling Time												-
12. Sampling Time Difference												

Note. * $p < .05$, ** $p < .01$, two tailed.

Table 8. Correlations Between Environmental Chaos and Child-related factors

	1	2	3	4	5	6
1 Child age (months)	-	-.11	-.20	.11	-.18	.10
2 Child sex (B = 0)		-	-.15	-.06	.19	-.03
3 SES			-	-.12	-.18	.02
4 Home Chaos				-	.04	.09
5 Daycare Chaos					-	-.32*
6 Daycare Quality						-

Note. * $p < .05$, ** $p < .01$, two tailed.

3.3 Comparison of cortisol levels within and across home and daycare environment

To investigate the role of time and environment on cortisol levels, in order to replicate previous findings, a 2 (time) x 2 (environment) repeated measures ANOVA was conducted. The first within-subject factor, namely time, had two levels: morning and afternoon. The second within-subject factor, environment, also had two levels: home and daycare. Results revealed a significant main effect of time, such that morning cortisol was significantly higher than afternoon cortisol $F(1, 74) = 3.985, p < .001$, both at home ($t(74) = 7.288, p < .001$) and daycare ($t(74) = 2.904, p < .01$). There was also a significant main effect for the environment, as home cortisol was higher than daycare cortisol, $F(1, 74) = 14.165, p < .001$. Also, a significant interaction of time and the environment was observed, $F(1, 74) = 14.195, p < .001$, indicating that morning cortisol was significantly higher at home than daycare, $t(74) = 5.139, p < .001$. But afternoon cortisol did not significantly differ for the environment, $t(74) = -.556, p = .58$. See Figure 1 for the interaction of time and location on cortisol levels.

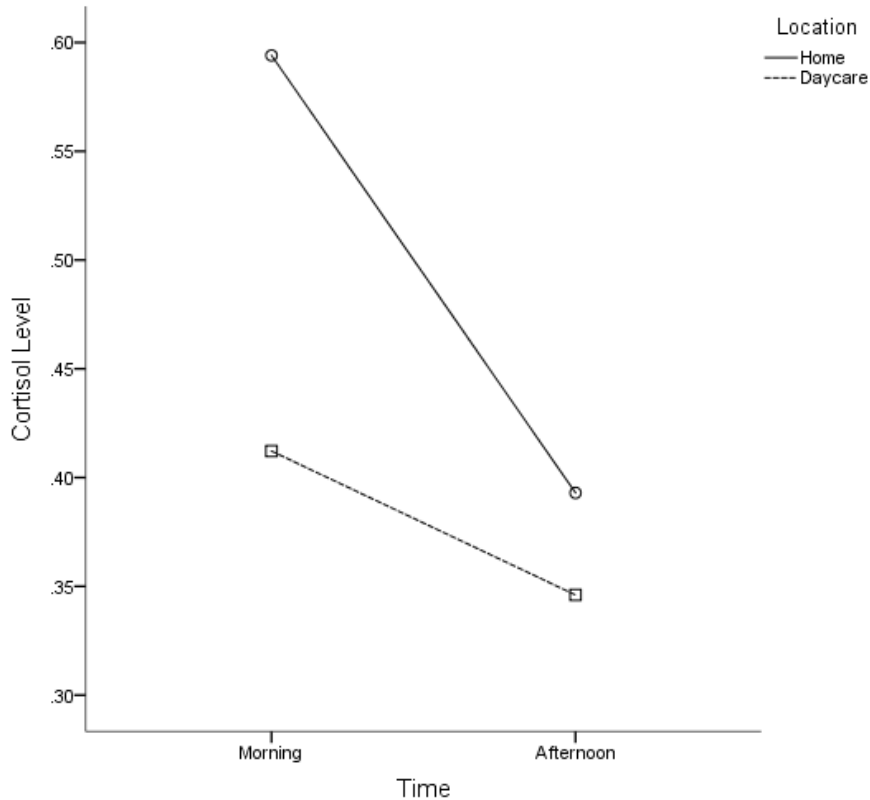


Figure 1. Relationship between time and location on home and daycare cortisol levels

Regarding diurnal slopes, a paired samples t-test showed that home diurnal slope was significantly higher than daycare diurnal slope ($t(74) = 3.727, p < .001$), such that children were showing a steeper decrease of cortisol from morning to afternoon at home.

3.4 Main, additive and interactive role of environmental chaos on diurnal slopes and individual cortisol measures

Hierarchical linear regression analyses were conducted to test the main, additive and interactive roles of home and daycare chaos above and beyond SES. Assumptions for regression analyses were satisfied in terms of independence, linearity, normality of residuals, multicollinearity, and homoscedasticity.

The initial analyses included all of the time-relevant factors (i.e., awakening time, sleep duration, morning and afternoon sampling time, sampling time difference) as potential covariates. Further, child age and sex were entered in the second step after covariates, followed by test variables. However, the step including age and sex did not significantly predict any of the cortisol variables, therefore age and sex were dropped from further analyses since the results did not change. As listed in more detail in each analysis, most of the potential covariates were not significantly associated with the outcome variables, therefore they were dropped from the further analyses as well.

In the final analyses, variables that were significantly associated with the outcome variable entered in the first step as a covariate. Afterwards, SES was entered in the second step, followed by the chaos scores for each context. Finally, the interaction term was entered in the last step (i.e., the multiplicative product of home and daycare chaos).

3.4.1 Predicting home cortisol levels from home and daycare chaos

To investigate the main, additive and interactive role of home and daycare chaos on the home cortisol over and above SES, three hierarchical regression analyses were conducted with diurnal slope, morning cortisol and afternoon cortisol as outcome variables. Among all of the potential covariates (i.e. awakening time, sleep duration, morning sampling time, afternoon sampling time and sampling time difference), only awakening time was significantly associated with home morning cortisol and diurnal slope, hence other covariates were dropped from the subsequent analyses as the results did not change. The results are presented in Tables 9, 10, 11, respectively. The same order of entry was used in each regression. The first step included covariate, the second

step included SES, the third step included home chaos, the fourth step included daycare chaos, and the last step included the interaction term.

The first hierarchical regression analysis was conducted with home diurnal cortisol slope as the outcome variable. As shown in Table 9, awakening time in the first step made a marginally significant contribution to the diurnal slope $R^2 = .057$, $F(1, 66) = 3.978$, $p = .05$. SES and home chaos were not significant predictors, home chaos did not contribute to the overall model ($\Delta R^2 = .015$, $F(1, 64) = 1.016$, $p = .32$.) Daycare chaos made a uniquely significant contribution to home diurnal slope over and above SES, home chaos and awakening time in the fourth step ($\Delta R^2 = .015$, $F(1, 63) = 5.867$, $p = .018$). Results indicated that higher daycare chaos was associated with higher diurnal slope at home ($b = .14$, $t = 2.42$, $p = .02$), indicating a steeper slope from morning to afternoon. The interaction term in the final step did not contribute to the overall model, $\Delta R^2 = .002$, $F(1, 62) = .147$, $p = .70$.

The second hierarchical regression analysis was conducted with home morning cortisol as the outcome variable. Since sleep duration and morning sampling time were not significantly associated with home morning cortisol, only awakening time was included in the analysis as a covariate. Variables were entered in the same order as above. As shown in Table 10, only the first step accounted for significant variance $R^2 = .078$, $F(1, 66) = 5.606$, $p = .021$, such that home awakening time predicted home morning cortisol ($b = .058$, $t = 2.368$, $p = .021$). No main ($F(1, 64) = .20$, $p = .66$), additive ($F(1, 63) = .075$, $p = .68$), or interactive role ($F(1, 62) = .416$, $p = .52$) of home and daycare chaos was observed on home morning cortisol levels.

Table 9. Hierarchical Regression Analyses Predicting Home Diurnal Slope from Home and Daycare Chaos

Predictors	<i>b</i>	<i>SE b</i>	β	<i>T</i>	<i>p</i>	95% <i>CI</i>	<i>R</i> ²	ΔR^2	<i>p</i>
Step 1							.06	.06	.05
Awakening Time	.05	.02	.24	2.00	.05	.00, .09			
Step 2							.06	.00	.77
Awakening Time	.04	.02	.23	1.93	.06	.00, .09			
SES	-.01	.04	-.04	-.30	.77	-.08, .06			
Step 3							.07	.02	.32
Awakening Time	.05	.02	.26	2.12	.04	.00, .10			
SES	-.01	.04	-.02	-.13	.89	-.08, .07			
Home Chaos	.05	.05	.13	1.01	.32	-.05, .16			
Step 4							.15	.08	.02
Awakening Time	.04	.02	.22	1.80	.08	-.01, .09			
SES	.01	.04	.04	.33	.75	-.06, .08			
Home Chaos	.05	.05	.12	.95	.34	-.05, .15			
Daycare Chaos	.14	.06	.29	2.42	.02	.02, .25			
Step 5							.15	.00	.70
Awakening Time	.04	.02	.21	1.74	.09	-.01, .09			
SES	.01	.04	.04	.35	.73	-.06, .09			
Home Chaos	-.04	.23	-.09	-.16	.87	-.49, .42			
Daycare Chaos	.04	.26	.09	.15	.88	-.49, .57			
Home X Daycare Chaos	.04	.11	.30	.38	.70	-.18, .26			

Note. *SE*: Standard Error. *CI*: Confidence interval.

Table 10. Hierarchical Regression Analyses Predicting Home Morning Cortisol from Home and Daycare Chaos

Predictors	<i>b</i>	<i>SE b</i>	β	<i>T</i>	<i>p</i>	95% <i>CI</i>	<i>R</i> ²	ΔR^2	<i>p</i>
Step 1							.08	.08	.02
Awakening Time	.06	.03	.28	2.37	.02	.01, .11			
Step 2							.08	.01	.54
Awakening Time	.06	.03	.29	2.41	.02	.01, .11			
SES	.02	.04	.07	.62	.54	-.06, .10			
Step 3							.09	.00	.66
Awakening Time	.06	.03	.28	2.22	.03	.01, .11			
SES	.02	.04	.07	.54	.60	-.06, .10			
Home Chaos	-.03	.06	-.06	-.45	.66	-.14, .09			
Step 4							.09	.00	.78
Awakening Time	.06	.03	.27	2.14	.04	.00, .11			
SES	.02	.04	.07	.57	.57	-.06, .11			
Home Chaos	-.03	.06	-.06	-.45	.65	-.14, .09			
Daycare Chaos	.02	.07	.03	.27	.79	-.12, .15			
Step 5							.09	.01	.52
Awakening Time	.06	.03	.28	2.18	.03	.01, .11			
SES	.02	.04	.07	.53	.60	-.06, .11			
Home Chaos	.14	.26	.29	.53	.60	-.38, .66			
Daycare Chaos	.21	.30	.39	.69	.49	-.40, .82			
Home X Daycare Chaos	-.08	.13	-.52	-.65	.52	-.33, .17			

Note. *SE*: Standard Error. *CI*: Confidence interval.

The third regression analysis was conducted with home afternoon cortisol as the outcome variable. Variables were entered in the same order as above, except for the covariate, as none of the potential covariates (i.e. awakening time, sleep duration, afternoon sampling time, and sampling time difference) were associated with the afternoon cortisol measured at home. The first step included SES, the second step included home chaos, the third step included daycare chaos, and the last step included the interaction term. As shown in Table 11, SES ($\Delta R^2 = .019$, $F(1, 73) = 1.380$, $p = .24$) and home chaos ($\Delta R^2 = .031$, $F(1, 72) = 2.351$, $p = .13$) did not contribute to the overall model. The third step, which included daycare chaos, contributed to marginally significant variance ($\Delta R^2 = .05$, $F(1, 71) = 2.915$, $p = .05$) such that daycare chaos was associated with lower levels of afternoon cortisol at home ($b = -.12$, $t = -1.975$, $p = .05$). No additive and interactive contributions of home and daycare chaos on home afternoon cortisol levels were observed.

3.4.2 Predicting daycare cortisol levels from home and daycare chaos

To investigate the main, additive and interactive role of home and daycare chaos on the daycare cortisol over and above SES, three hierarchical regression analyses were conducted with diurnal slope, morning cortisol and afternoon cortisol as outcome variables. Among all of the potential covariates (i.e. awakening time, sleep duration, morning sampling time, afternoon sampling time and sampling time difference), only morning sampling time was significantly associated with daycare morning cortisol and only the sampling time difference was associated with daycare afternoon cortisol, hence other covariates were dropped from the subsequent analyses. The results are presented in Tables 12, 13, 14, respectively. The same order of entry was used in each regression.

Table 11. Hierarchical Regression Analyses Predicting Home Afternoon Cortisol from Home and Daycare Chaos

Predictors	<i>b</i>	<i>SE b</i>	β	<i>T</i>	<i>p</i>	95% <i>CI</i>	<i>R</i> ²	ΔR^2	<i>p</i>
Step 1							.02	.02	.24
SES	.05	.04	.14	1.18	.24	-.031, .12			
Step 2							.05	.03	.13
SES	.04	.04	.12	.99	.33	-.038, .113			
Home Chaos	-.08	.06	-.18	-1.53	.13	-.192, .025			
Step 3							.10	.05	.05
SES	.02	.04	.07	.64	.52	-.051, .099			
Home Chaos	-.08	.05	-.17	-1.53	.13	-.189, .025			
Daycare Chaos	-.12	.06	-.23	-1.98	.05	-.241, .001			
Step 4							.11	.01	.47
SES	.02	.04	.07	.60	.55	-.053, .098			
Home Chaos	.10	.25	.21	.39	.70	-.4, .593			
Daycare Chaos	.09	.29	.17	.31	.76	-.491, .669			
Home X Daycare Chaos	-.09	.12	-.56	-.73	.47	-.325, .15			

Note. *SE*: Standard Error. *CI*: Confidence interval.

The first step included covariate, the second step included SES, the third step included daycare chaos, the fourth step included home chaos, and the last step included the interaction term.

The first hierarchical regression analysis was conducted with daycare diurnal cortisol slope as the outcome variable. Since there was no covariate significantly associated with the diurnal slope, the first step included SES, the second step included daycare chaos, the third step included home chaos and the fourth step included the interaction term. As shown in Table 12, the first step accounted for significant variance in the outcome variable ($R^2 = .060$, $F(1, 73) = 4.693$, $p = .034$). SES predicted diurnal slope in a positive direction ($b = .057$, $t = 2.166$, $p = .034$), indicating that higher SES was associated with higher diurnal slope in daycare. Daycare chaos in the next step did not account for significant variance. The third step significantly predicted 6% of the variance ($R^2 = .12$, $F(1, 71) = 4.723$, $p = .033$). SES ($t = 2.38$, $\beta = .027$, $p = .02$) such that home chaos uniquely predicted increases in daycare diurnal slope ($t = 2.17$, $\beta = .24$, $p = .03$). No additive and interactive effects of home and daycare chaos on daycare diurnal slope were observed.

The second hierarchical regression analysis was conducted with morning cortisol as the outcome variable. Morning sampling time was entered in the first step as a covariate, SES was entered in the second step, daycare chaos was entered in the third step, home chaos was entered in the fourth step and the interaction term was introduced to the model in the final step. As shown in Table 13, the first step accounted for significant variance in the outcome variable ($R^2 = .199$, $F(1,73) = 18.120$, $p = .00$). Morning sampling time significantly predicted morning cortisol ($b = .542$, $t = 4.257$, $p =$

.00). SES, home and daycare chaos did not account for significant variance in morning cortisol level in the second, third, and fourth steps.

Table 12. Hierarchical Regression Analyses Predicting Daycare Diurnal Slope from Home and Daycare Chaos

Predictors	<i>b</i>	<i>SE b</i>	β	<i>T</i>	<i>p</i>	95% <i>CI</i>	<i>R</i> ²	ΔR^2	<i>p</i>
Step 1							.06	.06	.03
SES	.06	.03	.25	2.17	.03	.005, .11			
Step 2							.06	.00	.89
SES	.06	.03	.24	2.09	.04	.003, .11			
Daycare Chaos	-.01	.04	-.02	-.14	.89	-.094, .081			
Step 3							.12	.06	.03
SES	.06	.03	.27	2.38	.02	.01, .116			
Daycare Chaos	-.01	.04	-.02	-.18	.86	-.093, .077			
Home Chaos	.08	.04	.24	2.17	.03	.007, .157			
Step 4							.12	.00	.86
SES	.06	.03	.27	2.35	.02	.01, .116			
Daycare Chaos	.03	.21	.07	.14	.89	-.382, .437			
Home Chaos	.11	.18	.33	.64	.53	-.239, .463			
Home X Daycare Chaos	-.02	.08	-.13	-.18	.86	-.182, .153			

Note. *SE*: Standard Error. *CI*: Confidence interval.

Table 13. Hierarchical Regression Analyses Predicting Daycare Morning Cortisol from Home and Daycare Chaos

Predictors	<i>b</i>	<i>SE b</i>	β	<i>T</i>	<i>p</i>	95% <i>CI</i>	<i>R</i> ²	ΔR^2	<i>p</i>
Step 1							.20	.20	.00
Morning Sampling Time	.54	.13	.45	4.26	.00	.29, .80			
Step 2							.21	.01	.46
Morning Sampling Time	.53	.13	.43	4.07	.00	.27, .79			
SES	.03	.04	.08	.75	.46	-.04, .10			
Step 3							.21	.00	.96
Morning Sampling Time	.53	.13	.43	3.91	.00	.26, .79			
SES	.03	.04	.08	.74	.47	-.05, .10			
Daycare Chaos	.00	.06	.01	.05	.96	-.12, .12			
Step 4							.22	.01	.34
Morning Sampling Time	.55	.14	.45	4.02	.00	.28, .82			
SES	.02	.04	.06	.57	.57	-.05, .10			
Daycare Chaos	.00	.06	.00	.02	.99	-.12, .12			
Home Chaos	-.05	.05	-.11	-.96	.34	-.15, .05			
Step 5							.26	.04	.05
Morning Sampling Time	.54	.13	.44	3.98	.00	.27, .80			
SES	.02	.04	.06	.50	.62	-.05, .09			
Daycare Chaos	.54	.27	1.01	1.99	.05	.00, 1.08			
Home Chaos	.41	.23	.86	1.77	.08	-.05, .88			
Home X Daycare Chaos	-.23	.11	-1.44	-2.04	.05	-.45, .00			

Note. *SE*: Standard Error. *CI*: Confidence interval.

The final step including the product term also significantly contributed to the prediction of daycare morning cortisol and explained 5% of the variance ($R^2 = .26$, $F(1, 69) = 4.14$, $p = .046$). In the final step, daycare chaos ($\beta = 1.011$, $t = 1.99$, $p = .05$) and home chaos ($\beta = .86$, $t = 1.77$, $p = .08$) uniquely and positively contributed to morning cortisol levels at daycare as well. The interaction term significantly contributed to the overall model, $\beta = -1.44$, $t = -2.04$, $p = .046$, albeit close to marginal significance. The interaction indicated that at lower levels of home chaos, an increase of daycare chaos was associated with higher morning cortisol. At higher levels of home chaos, however, the increase of daycare chaos was associated with lower morning cortisol at daycare.

The third hierarchical regression analysis was conducted with daycare afternoon cortisol as the outcome variable. Since only sampling time difference accounted for significant variance, among all other potential covariates (i.e., awakening time, sleep duration, afternoon cortisol sampling time), it was included in the analyses as covariate. Variables were entered in the same order as above. As shown in Table 14, the first step accounted for marginally significant variance in the outcome variable ($R^2 = .04$, $F(1, 73) = 3.73$, $p = .06$). Sampling time difference marginally significantly predicted morning cortisol ($b = -.15$, $t = -1.93$, $p = .06$). SES and home chaos did not account for any variance on morning cortisol levels in the second step ($\Delta R^2 = .0$, $F(1, 72) = .23$, $p = .63$), and the third step ($\Delta R^2 = .0$, $F(1, 71) = .40$, $p = .53$). The fourth step with the inclusion of home chaos made a marginal contribution to the overall model, accounting for 4% of the variance ($R^2 = .05$, $F(1, 70) = 3.32$, $p = 0.07$). Home chaos ($\beta = -.21$, $t = -1.82$, $p = .07$) marginally predicted afternoon cortisol at daycare, whereas contribution of sampling time difference was significant ($\beta = -.25$, $t = -2.11$, $p = .04$). The final step including the interaction term, also suggested a marginally significant contribution to the

Table 14. Hierarchical Regression Analyses Predicting Daycare Afternoon Cortisol from Home and Daycare Chaos

Predictors	<i>b</i>	<i>SE b</i>	β	<i>T</i>	<i>p</i>	95% <i>CI</i>	<i>R</i> ²	ΔR^2	<i>p</i>
Step 1							.04	.05	.06
Sampling Time Difference	-.15	.08	-.22	-1.93	.06	-.30, .01			
Step 2							.03	.00	.63
Sampling Time Difference	-.16	.08	-.23	-1.97	.05	-.31, .00			
SES	-.02	.04	-.06	-.48	.63	-.10, .06			
Step 3							.02	.01	.53
Sampling Time Difference	-.15	.08	-.22	-1.83	.07	-.31, .01			
SES	-.02	.04	-.04	-.34	.74	-.10, .07			
Daycare Chaos	.04	.07	.08	.63	.53	-.10, .18			
Step 4							.05	.04	.07
Sampling Time Difference	-.17	.08	-.25	-2.11	.04	-.33, -.01			
SES	-.03	.04	-.07	-.60	.55	-.11, .06			
Daycare Chaos	.04	.07	.07	.62	.54	-.09, .18			
Home Chaos	-.11	.06	-.21	-1.82	.07	-.23, .01			
Step 5							.08	.05	.06
Sampling Time Difference	-.17	.08	-.26	-2.21	.03	-.33, -.02			
SES	-.03	.04	-.09	-.73	.47	-.12, .05			
Daycare Chaos	.63	.32	1.08	1.99	.05	.00, 1.26			
Home Chaos	.39	.27	.75	1.45	.15	-.15, .93			
Home X Daycare Chaos	-.25	.13	-1.4	-1.90	.06	-.50, .01			

Note. *SE*: Standard Error. *CI*: Confidence interval.

overall model ($\Delta R^2 = .05$, $F(1, 69) = 3.60$, $p = .06$). Sampling time difference ($\beta = -.26$, $t = -2.21$, $p = .03$) and daycare chaos ($\beta = 1.08$, $t = 1.99$, $p = .05$) significantly predicted the overall model, whereas the contribution of the interaction term was marginally significant ($\beta = -1.44$, $t = -1.90$, $p = .06$). The interaction trend indicated a similar relationship as the above, such that at lower levels of home chaos, an increase of daycare chaos was associated with higher afternoon cortisol. At higher levels of home chaos, however, the increase of daycare chaos was associated with lower cortisol levels in the afternoon.

3.5 Supplemental analyses

As shown in Table 15, exploratory analyses were conducted for available household factors other than chaos and SES; such as number of residents and household density as measures of crowding, number of residency changes since the child was born as a measure of instability, years of residency in the current home as a measure of stability, television on time as a measure of noise, weekly hours of outside caretaker assistance (e.g., babysitters or external family members); yet no relationship was observed with diurnal, morning or afternoon cortisol levels at home ($r_s > 100$, $p_s > .72$). Only weekly hours of caretaker assistance negatively correlated with home diurnal slope ($r = -.24$, $p < .05$), yet, when entered in the hierarchical linear regression, presence or duration of caretaker assistance did not significantly contribute to any of the home cortisol variables.

Table 15. Bivariate Correlations Among Supplementary Household Factors and Home Cortisol Levels

	1	2	3	4	5	6	7	8	9	10
1. Morning cortisol	-	.63**	.42**	.04	.04	-.22	.20	-.15	.04	-.22
2. Afternoon cortisol		-	-.44**	-.10	-.07	-.17	.08	-.11	.12	-.01
3. Diurnal slope			-	.17	.13	-.06	.13	-.04	-.09	-.24*
4. Number of family members				-	.88**	.15	.17	-.15	.10	-.02
5. Household density					-	.22	.17	-.13	.13	-.09
6. Number of siblings						-	.16	-.12	.18	.07
7. Weekly hours TV is on							-	.10	-.02	.03
8. Number of residence changes								-	-.37**	-.08
9. Years at the current house									-	.00
10. Weekly caretaker assistance										-

Note. * $p < .05$, ** $p < .01$

As shown in Table 16, bivariate correlations among daycare cortisol and classroom factors were also investigated. Results showed that number of children in the classroom was significantly and positively correlated with both morning ($r = .41, p < .001$) and afternoon ($r = .40, p < .001$) daycare cortisol. In addition, the child-to-adult ratio was positively correlated with daycare morning cortisol, ($r = .27, p < .05$).

However, neither classroom size nor child-to-adult ratio significantly contributed to the cortisol levels measured at daycare, once entered in hierarchical regression analyses.

Table 16. Bivariate Correlations Among Supplementary Classroom Factors and Daycare Cortisol Levels

	1	2	3	4	5	6	7	8
1. Morning cortisol	-	.78**	.22	.27*	.41**	.18	.03	.00
2. Afternoon cortisol		-	-.44**	.20	.40**	.14	.08	-.04
3. Diurnal slope			-	.08	-.04	.05	-.08	.05
4. Child-to-adult ratio				-	.37**	.23*	.03	.07
5. Classroom size					-	.34**	-.03	-.06
6. Experience of teacher (years)						-	.16	-.62**
7. Weekly hours at daycare							-	.23
8. Daycare quality								-

Note. * $p < .05$, ** $p < .01$

As mentioned earlier, differences in daycare quality were not expected due to the municipality preschools' characteristics. Likewise, daycare quality was not associated with any of the cortisol measures when all previous analyses were repeated with daycare quality entered in the second step.

CHAPTER 4

DISCUSSION

The aim of the present study was to investigate the main, additive and interactive effects of chaotic environmental factors on preschoolers' salivary cortisol levels above and beyond the effect of SES. Although an increasing number of studies focused on differences between home and daycare measures of salivary cortisol, results portrayed inconsistencies according to environmental factors. Hence, the first aim was to examine the differences between salivary cortisol levels of children at home and daycare contexts in order to replicate previous findings. The relationship between home chaos and cortisol has been studied repeatedly, yet daycare chaos was overlooked, and cortisol measures were generally collected only at home or daycare. Therefore, the second aim of the study was to investigate the main, additive and interactive effects of environmental chaos on preschoolers' morning, afternoon, and diurnal cortisol levels at home and daycare, over and above SES. Significant and non-significant findings of the present study are discussed in the following sections.

4.1 Replication of past research on differences between home and daycare cortisol levels

It was hypothesized that children's diurnal slopes would show a decreasing, more normative pattern from morning to afternoon at home as compared to daycare. Results of the present study supported this hypothesis. Diurnal slopes indeed showed a steeper decrease from morning to afternoon at home than daycare. These findings are consistent with previous research, along with three meta-analyses summarized in previous sections

(Geoffroy et al., 2006; Eliassen, 2012; Vermeer & van Ijzendoorn, 2006). The difference between home and daycare cortisol levels was previously attributed to several factors, such as the social demands of daycare, group dynamics, child-to-adult ratios, interaction with multiple caregivers, adaptation to daycare setting, and daycare quality, in addition to individual characteristics of the child (Eliassen, 2012; Vermeer & van Ijzendoorn, 2006).

However, it is important to note that morning cortisol was higher at home than daycare in the present study. When comparing absolute levels of cortisol, Eliassen (2012) did not report any differences between home and daycare concentrations. Further, researchers comparing home and daycare cortisol of children between two-to-five-years-old, did not find any statistically significant differences on morning cortisol levels sampled between 9:00 and 11:00 a.m. (de Haan, 1998, Lundberg et al., 1993; Gunnar et al., 1997; 2010; Watamura et al., 2003). Oullet-Morin et al. (2000) however, showed that three-year-old children had higher morning cortisol levels at home, compared to their two-year-old counterparts. In a comparative study by Vermeer et al. (2010), two-to-three-year-old children from Basque country had higher morning cortisol levels at home than daycare, measured at 11:00, whereas children from the Netherlands did not differ in terms of morning cortisol. This difference was more pronounced for children who attended half-day preschools, compared to children attending full-time preschools. In our study, children's weekly hours at daycare were not associated with cortisol levels.

Since cortisol fluctuation follows a circadian rhythm, one reason regarding higher morning cortisol might be that children slept for longer hours and woke up later during weekends at home sampling days, compared to daycare. While previous studies suggest that longer sleep durations were associated with steeper diurnal slopes, higher

morning cortisol was associated with shorter sleep duration in infants and school-aged children (Lemola et al., 2015; Rotenberg et al., 2012; Saridjan et al., 2017). In the present study, results did not change when the difference between awakening time and morning sampling time was controlled for. Further, sleep durations at home and daycare did not significantly predict relevant cortisol measures. Therefore, individual factors, in addition to environmental factors, should also be taken into consideration when interpreting the results.

Among two separate meta-analyses, one suggested that difference between home and daycare cortisol levels were more pronounced for children between 39-to-59 months of age compared to younger children (Geoffroy et al., 2006), whereas other one suggested cortisol levels were less pronounced for children between 41 and 60 months ($r = .08$), compared to younger children (Vermeer & van Ijzendoorn, 2006). Participants of this study were between 47 and 80 months of age, and age was not associated with children's cortisol levels measured in any environment. None of the meta-analyses reported any gender differences, and this study was in line with their findings.

4.2 Main effects of home and daycare chaos on children's salivary cortisol levels

The second aim of the study was to test the main role of environmental chaos on cortisol levels measured in the given environment, over and above SES. Children from more chaotic homes were expected to show increased morning cortisol, as well as flatter diurnal slope at home, compared to children from less chaotic homes. A similar pattern was expected for children from more chaotic daycares. Results of the present study failed to support these hypotheses. Home chaos was not associated with any individual cortisol measures or diurnal cortisol slope at home. Similarly, daycare chaos did not

predict any individual cortisol measures or diurnal cortisol slope at daycare. Only SES was uniquely associated with diurnal cortisol slope at daycare, indicating that higher SES children were more likely to show a normative, decreasing pattern from morning to afternoon at daycare.

However, as studies suggested, environmental chaos might not have immediate effects but might alter HPA activity in the long run, since stress systems are still developing. Further, timing, duration, and intensity of environmental factors are important to take into consideration. Several studies suggested that previous experience of chaos, not the concurrent experience, was associated with elevated cortisol levels and flatter diurnal cortisol (Blair et al., 2011; 2013; Brown et al., 2019; Chen et al., 2010, Doom et al., 2018; Laurent et al., 2014). Results of Laurent et al. (2013) suggested the increase of adversity from four-to-six years of age was associated with increased morning cortisol. Chen et al. (2010) also showed an increase of chaos over two years was associated with an increase of daily cortisol, for lower SES children. However, it must be noted that their sample was between 9-to-18, the measure of SES was an average of family savings and homeownership, and only family savings predicting cortisol increase.

In a recent study, Doom et al. (2018) showed that children who experienced higher levels of household chaos in the preschool period were more likely to show flatter diurnal cortisol fluctuations during middle childhood. And finally, Suor et al. (2015) suggested that family instability (i.e. an index of disorganizing family events occurred within the last 3 years, such as change of caregiver, resident, or loss of a family members), was associated with elevated or low levels of diurnal cortisol fluctuations for low-income children between two-to-four years of age (Suor et al., 2015).

Other than the CHAOS scale, which was shown to have high stability over twelve months, only variables we had for the previous life of the child were the total number of residence and daycare changes the family had since the child was born, yet none of them was associated with cortisol levels. It is not surprising since our sample was not comparable to the studies mentioned above in terms of chaos, SES or poverty. It is important to note that chaos levels as measured by parent and teacher reports were rather low. Mean scores were around two, and the maximum score was between three and four, out of six. Also, most of our sample was middle class, 44% with an income between 1000-3000 and the majority with an income above 3000 Turkish liras. Our results, therefore, reflect relatively low-risk families. Hence, for the present sample, results might be interpreted as mild-to-moderate levels of concurrent chaos did not predict cortisol trajectories of preschool children.

4.3 Additive effects of home and daycare chaos on children's salivary cortisol levels

The third hypothesis of the present study was in terms of additive effects of home and daycare chaos. It was expected that both home chaos and daycare chaos would directly and independently predict flatter diurnal slopes as well as higher morning and afternoon cortisol levels. However, no additive effects of home and daycare chaos were observed in the present study and results failed to support these hypotheses.

The most interesting finding of this study was that home chaos predicted cortisol levels measured at daycare, and daycare chaos predicted cortisol levels measured at home. Specifically, higher home chaos was associated with steeper diurnal slopes from morning to afternoon and lower afternoon cortisol levels at daycare. This finding suggested that daycare setting was less stressful for children who previously experienced

higher levels of chaos at home, than for children who experienced lower levels of chaos at home, as shown by their diurnal cortisol levels. Similarly, higher daycare chaos was associated with a higher diurnal slope, as well as lower afternoon cortisol. This result also suggests that experiencing higher levels of daycare chaos during the week, contributed to a steeper decrease of cortisol throughout the day during the weekend at home.

When interpreting this result, it is important to keep in mind that the level of chaos in the present sample was relatively low as mentioned above. Higher chaos in this study might still be considered as mild-to-moderate levels of chaos. Given that the main function of the stress systems is to prepare and adapt potential threats (Gunnar & Quevedo, 2007; McEwen, 2000b; Sapolsky et al., 2000), and flatter diurnal slope was associated with adverse outcomes (Adam et al., 2017; Gunnar & Vazquez, 2001; Salis, Bernard, Black, Dougherty, & Klein, 2016), it is possible that mild-to-moderate levels of chaos, by presenting minor challenges through unpredictable and uncontrollable circumstances in another microsystem, might be adaptive for another microsystem (Brown et al., 2019; Doom et al., 2018; Evans & Wachs, 2010; Gunnar & Vazquez, 2006; Tarullo & Gunnar, 2006; Wachs, 2010).

While no studies investigated the relationship between mild-to-moderate levels of chaos on children's diurnal cortisol levels across different microsystems to my knowledge, it was suggested that lower-levels of childhood adversity may buffer against proximal stress and promote an adaptive stress response (Ellis & Boyce; 2008). It was also shown that the relationship between childhood adversity and stress-reactivity are curvilinear; such that children from moderately stressful environments show lower stress-reactivity, whereas children from low and high-risk environments show increased

reactivity (Ellis & Boyce; 2008; Ellis, Essex, & Boyce, 2005). Similarly, Gunnar and colleagues (2009) investigated the stress-reactivity of adopted children between 10 and 12 years of age, and their results showed that children with moderate levels of early life stress showed diminished cortisol activity, while there was no difference between stress-reactivity of children who experienced severe and low early life stress. In a study by Shapero and colleagues (2015), adolescents who reported moderate levels of early life stress were shown to report less depressive symptoms against proximal stressful events, compared to their counterparts who experienced lower levels of early stress. Adaptive Calibration Model (Del Guice et al., 2011) suggests that buffered phenotypes that experience moderate levels of environmental stress, would usually balance between the ‘costs and benefits’ of stress and show less anxiety as well as higher social sensitivity to the context, compared to the sensitive phenotype experiencing a secure environment and the vigilant phenotype experiencing higher levels of stress. Of course, without taking individual differences along with biological, neurological and genetic sensitivity to the context, we cannot conclude such an argument (Boyce & Ellis, 2005; Del Guice et al., 2011; Obradovic et al., 2010). Further, children’s diurnal cortisol levels were associated with total cortisol levels in response to stress but failed to show any significant associations with stress-reactivity measures (Simons, Cillessen & Weerth, 2016). Hence, more research is required to conclude whether the changes in diurnal cortisol across environmental factors are associated with children’s stress-reactivity in terms of adaptive responses. Further, as stress systems are still developing in early childhood, even mild-to-moderate levels of chaos might have exponential influences in the long run (Doom et al., 2018; Gunnar & Vazquez, 2006; Ulrich-Lai & Herman, 2009). Overall, while the results suggest a possible adaptive role of lower levels of environmental chaos

on children's diurnal cortisol levels across two different microsystems, we can only speculate without further research.

4.4 Interactive effects of home and daycare chaos on children's cortisol levels

The final hypothesis of the present study was in terms of the interactive effects of home and daycare chaos. For children living in a family environment with higher chaos, attending a non-chaotic daycare was expected to serve a protective role and predict decreasing cortisol levels at the daycare setting. No interaction was observed in terms of diurnal cortisol slopes. However, results for morning and afternoon cortisol measurements at daycare suggested an interactive role of home and daycare chaos. The pattern of the interaction revealed that children from more chaotic homes who attended a less chaotic daycare were more likely to show elevated cortisol levels in the morning and afternoon. This result suggested that less chaotic daycare environment does not buffer children from the negative role of high home chaos. However, children who experience lower levels of chaos at both home and daycare were more likely to show blunted cortisol in the morning and afternoon, as measured in daycare. For children living in more chaotic homes, however, attending a chaotic daycare was more likely to be associated with lower morning and afternoon cortisol levels. This finding suggested a dual risk model and is partially in line with the previous findings of Berry and colleagues (2013; 2016a). Their results suggested that high-risk children who spent more time at daycare facilities had decreased morning cortisol, whereas cortisol levels were increased for children from low-risk contexts. It must be noted that, in both studies, saliva samples were collected only at home and in the morning, which prevents further comparisons with regard to daycare cortisol. For the present study, no link of daycare

hours on diurnal slopes or morning cortisol was found, which might be due to lower levels of risk factors our participants were facing, compared to the Head Start sample (Berry et al., 2013; 2016a).

Apart from the hypotheses, daycare quality did not significantly vary or influence child measures as expected, due to characteristics of municipality preschools. Although daycare centers were above average for the local standards, initial ECERS-R ratings were around three, representing minimal quality for international standards due to the local structure (i.e. art, music and such activities taking place in a weekly schedule compared to daily planning since there are separate teachers for each subject), procedures (e.g., not allowing parents inside daycare centers) and educational standards (e.g., worksheet based techniques). Therefore, our results partially reflect cortisol levels measured in low-to-adequate quality daycare centers.

Overall, considering the characteristics of our sample, our results suggest that concurrent experiences of mild-to-moderate chaos in one microsystem, in terms of unpredictable and uncontrollable challenges in addition to noise and crowding, might be adaptive for experiences in another microsystem, suggested by preschoolers' steeper decrease of baseline cortisol levels from morning to afternoon at home and daycare.

4.5 Limitations, contributions and future directions

The present study had several strengths and limitations. One of the main strengths was that salivary cortisol samples were collected both at home and daycare, both in the morning and afternoon, predominantly for two days. Most of the samples were within the designated time intervals, and possible novel or stressing events child might experience were controlled for. Since participants were 47 months old and above, they

did not take naps at daycare like their younger counterparts, hence, effects of napping on HPA-axis activity were not apparent for in the present study (Ward, Gay, Alkon, Anders, & Lee, 2008; Watamura, Donzella, Kertes, & Gunnar, 2004; Watamura, Sebanc, & Gunnar, 2002). Samples were collected after children's adjustment period to daycare, within the same months over two years to avoid seasonal differences in cortisol levels, hence possible effects of school-transition were not apparent in the present study (Turner-Cobb, Rixon, & Jessop, 2008; Miller et al., 2016). Parents were asked to note down sampling times on the forms and call research assistants right after sampling. Comparison of sampling times as reported by the parents and as recorded by the research assistants, along with high correlations among cortisol values, suggest sampling times were consistent. However, there might be differences between the reported and actual sampling times. When asked to collect saliva samples from their 3-to-5-year-old children four times a day, self-report compliance was shown to be 83%, whereas electronic monitoring of timing with MEMS track cap yielded 68.8% compliance (Smith & Dougherty, 2014). Therefore, inclusion of electronic monitoring of sampling times, such as caps with time stamps, would be advantageous for portraying more accurate results. Further, there is a possibility that despite the initial training and guiding, sampling process, as well as recording awakening and sleeping times, might not have processed accurately by the parents. Future studies with controlled home saliva collection, in addition to daycare, would provide more reliable data for more accurate results.

Secondly, the present study investigated the role of home and daycare chaos, focusing on multiple ecologies. One limitation was that home and daycare chaos scores relied on self-report measures. Although more objective measures which were obtained

through demographic forms did not relate to the cortisol levels, future studies including self-report and objective chaos measures, along with observational methods would provide more accurate results for comparison of home and daycare chaos. An index of chaos with all sub-components would be a more accurate way of assessing chaotic environments.

Sample of the present study did not represent children living in high risk, rather, middle-class families experiencing mild-to-moderate levels of chaos participated in the study. Although it was not the intention, this can be interpreted as both a limitation and strength. Our results failed to replicate some of the previous findings, yet, presented a meaningful contribution to the literature by introducing the role of chaotic environment on low-risk children's cortisol levels. However, the sample size is a limitation of the study and future studies with a larger sample size would provide more accurate results.

Also, many studies mentioned above presents longitudinal data, whereas the present study was cross-sectional. Since stress systems are still developing in childhood, chaos might have different influences on children at short and long periods. Timing, duration, and intensity of chaos are important for more accurate comparison; therefore, future longitudinal studies would make valuable contributions to the field.

Diurnal cortisol slopes are measured in various ways (Adam et al., 2017; Miller et al., 2016) and morning-to-afternoon difference was used in the previous study. Measurement of cortisol levels from awakening to sleeping with more sampling across the day might provide more reliable results. Although providing valuable information, diurnal cortisol patterns are not fully associated with cortisol increases due to stress-reactivity towards a psychological or social stressor (Simons, Cillessen & Weerth,

2016). Hence, future studies with more saliva samples across the day and contexts, along with measurements of stress-reactivity, would be valuable.

Further, children with different temperamental characteristics might be sensitive to environmental stressors at different thresholds or process the same stimuli at different levels (Bush, & Boyce, 2014; Stansbury, & Henker, 1994). Cortisol secretion is also shown to be influenced by individual, genetic and epigenetic factors (Kim-Cohen, Moffitt, Caspi, & Taylor, 2004; Murgatroyd & Spengler, 2011). For a more comprehensive comparison and interpretation of the role of environmental chaos, individual factors such as temperament, self-regulation, and social skills, should be examined.

Home chaos has distal effects on parent-child interaction and parent-related factors were shown to be associated with elevated cortisol levels of children, such as parenting stress (Wagner et al., 2015), maternal unresponsiveness (Suor et al., 2015), along with positive parenting as a buffer (Blair et al., 2011). In addition, parental conflict was shown to influence child cortisol (Doom et al., 2017; Slatcher & Robles, 2012). Future studies including parent-related variables along with family conflict would be valuable to investigate how environmental and child characteristics interact to influence children's stress responses.

It must be noted that since only municipality preschools were included study for more homogenous daycare and classroom factors, daycare quality was similar and children were living in the same district. Apart from daycare quality, teachers' interaction with children and caregiving quality was shown to influence children's cortisol levels in previous studies (Gunnar et al., 2010; Lisonbee et al., 2008; Vermeer & van IJzendoorn, 2006). Hence, the inclusion of a variety of preschools and measures for

teacher-related factors would contribute to the literature to compare different environmental characteristics on children's cortisol levels for further studies.

Most research to date has addressed the role of home chaos, along with several daycare factors, in high-risk samples. The present study contributes to the literature by investigating the role of home and especially daycare chaotic environment, on children's diurnal cortisol levels, measured at multiple environments and multiple time points. Secondly, little was known about the relationship between mild-to-moderate levels of environmental chaos and diurnal cortisol levels of children from low-risk samples. This study contributes to the literature by suggesting a possible adaptive value of mild levels of stressors across environments. Further, to my knowledge, the present study is unique in terms of investigating Turkish preschoolers' basal cortisol levels and chaos in two separate microsystems.

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