

THE EFFECT OF SOCIOECONOMIC STATUS  
AND 5-HTTLPR ON PRESCHOOLERS' SOCIAL COMPETENCE  
AND DIURNAL CORTISOL LEVELS

REZAN NEHİR MAVİOĞLU

BOĞAZIÇI UNIVERSITY

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AND DIURNAL CORTISOL LEVELS

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## DECLARATION OF ORIGINALITY

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## ABSTRACT

### The Effect of Socioeconomic Status and 5-HTTLPR on Preschoolers' Social Competence and Diurnal Cortisol Levels

Social competence (SC) is an important contributor to children's development and influenced by genetic and environmental factors. This study investigates the associations between socioeconomic status (SES), SC and Hypothalamic-Pituitary-Adrenal (HPA) axis activity moderated by serotonin transporter polymorphism (5-HTTLPR) and sex. Preschool children (45-83 months), their mothers and teachers participated in the study. SC was assessed by teachers' report of Social Competence and Behavior Evaluation Scale – Short Form. SES was calculated as the standardized average score of parental education and income. 5-HTTLPR genotype was determined from salivary DNA as Short/Short (SS), Short/Long (SL) and Long/Long (LL). HPA-axis activity was measured as salivary diurnal cortisol levels at daycare and home through a morning and an afternoon sample. A multilevel linear model testing the association between SES and SC revealed a significant three-way interaction between sex, SES and 5-HTTLPR. For SS boys and SL-LL girls, as SES increased, SC increased, while a reverse pattern was observed in LL boys and SS girls. For HPA-axis activity, only in boys, higher SC buffered the impact of SES on daycare diurnal slope. For boys with lower SC, those with SS genotype had decreased diurnal slopes with increased SES, while LL boys had decreased slopes with decreased SES. There were no significant associations for girls or high SC boys. These findings indicate for the first time the differential effects of SES on preschoolers' SC and diurnal cortisol by sex and 5-HTTLPR. Future studies should

investigate the associations between SES, SC and HPA-axis outcomes in a longitudinal fashion.

## ÖZET

Sosyoekonomik Konum ve 5-HTTLPR'ın Anaokulu Çocuklarında

Sosyal Yetkinlik ve Günlük Kortizol Seviyelerine Etkisi

Sosyal yetkinlik (SY) çocukların gelişimi için önemli bir etmendir ve genetik ve çevresel faktörler tarafından etkilenir. Bu çalışma, sosyo-ekonomik konum (SEK), SY ve Hipotalamik-Hipofiz-Adrenal (HPA) eksenini etkinliğinin arasındaki ilişkileri, serotonin transporter gen polimorfizmini (5-HTTLPR) ve cinsiyeti düzenleyici olarak kullanarak araştırmaktadır. Çalışmaya anaokulu çocukları (45-83 ay arası), anneleri ve öğretmenleri katıldı. SY, öğretmenler tarafından doldurulan Sosyal Yetkinlik ve Davranış Değerlendirme Ölçeği kullanılarak değerlendirildi. Ailenin SEK'i ebeveynlerin eğitim düzeyleri ve ailenin gelir düzeylerinin standardize edilip ortalamasının alınması yoluyla hesaplandı. 5-HTTLPR genotipi tükürük DNA'sından, Kısa/Kısa (KK), Kısa/Uzun (KU) ve Uzun/Uzun (UU) gruplarına göre belirlendi. HPA eksenini etkinliği bir gündüz bir öğleden sonra olmak üzere kreşte ve evde alınan tükürük diurnal kortizolü seviyelerine göre ölçüldü. SEK ve SY arasındaki ilişkiyi ölçen çok düzeyli doğrusal model, cinsiyet, SEK ve 5-HTTLPR genotipi arasındaki anlamlı bir üçlü etkileşimi açığa çıkardı. Buna göre, KK erkekler ve KU-UU kızlarda SED arttıkça SY'nin de arttığı gözlemlendi. UU erkekler ve KK kızlarda ise bu etkinin tam tersi gözlemlendi. SEK ve HPA eksenini etkinliği arasındaki ilişkiye yönelik sonuçlar, yalnızca erkek çocuklarında yüksek SY'nin, SEK'nin günlük kortizol eğrisi üzerindeki etkisinde tampon vazifesi gördüğünü gösterdi. Düşük SY'ye sahip erkek çocuklarında, KK genotipe sahip olanların SEK'lerindeki artış günlük eğrilerindeki düşüşle ilişkiliydi. Fakat UU genotipe sahip erkeklerin SEK'lerindeki artış, günlük eğrilerindeki artışla ilişkiliydi. Kızlarda veya yüksek

SY'ye sahip erkeklerde herhangi bir anlamlı iliřki bulunamadı. Bu bulgular SEK'in, 5-HTTLPR genotipi ve cinsiyete baęlı olarak, anaokulu çocuklarının SY ve HPA eksenini etkinliklerini farklı olarak etkileyebileceęini göstermektedir. Gelecekteki çalıřmalar, SEK, SY ve HPA eksenini arasındaki iliřkileri uzun dönemli olarak arařtırmalıdır.

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*To my life mentor and amazing mom,*

*Fatma Meral*

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## ABBREVIATIONS

5-HTTLPR: Serotonin Transporter Linked Polymorphic Region

ACTH: Adrenocorticotropic Hormone

CBCL: Child Behavior Checklist

CRH: Corticotropin Releasing Hormone

DNA: Deoxyribonucleic Acid

HPA: Hypothalamic-Pituitary-Adrenal

LL: Log Likelihood

PCR: Polymerase Chain Reaction

PVN: Paraventricular Nucleus

SAM: Sympathetic-Adrenomedullary

SC: Social Competence

SCBE – 30: Social Competence Behavior Evaluation-Preschool Edition, Short Form

SD: Standard Deviation

SES: Socioeconomic Status

SNP: Single Nucleotide Polymorphism

SPSS: Statistical Package for the Social Sciences

## CHAPTER 1

### INTRODUCTION

Social competence (SC), being effective in social interactions (Waters & Sroufe, 1983; Rose-Krasnor; 1997; Rose-Krasnor & Denham, 2009), has been a widely studied concept among developmental and clinical psychologists due to its impact in predicting children's behavioral adjustment, social functioning, and mental health outcomes (Waters & Sroufe, 1983). Peer acceptance and friendship, the two important indices of SC, were found to make significant contributions to children's performance and attitudes in kindergarten (Ladd, 1990). On the other hand, deficiencies in SC, as indicated by peer rejection and aggressiveness, was associated with childhood depression (Panak & Garber 1992), social problems and other psychopathology later in life (Ladd, 1999). Researchers also reported associations between SC and Hypothalamic-Pituitary-Adrenal (HPA) axis stress response (Gunnar, Tout, de Haan, Pierce, & Stanbury, 1997; Gunnar & Donzella, 2002; Gunnar, Sebanc, Tout, Donzella & van Dulmen, 2003; Lengua, Zalewski, Fisher & Moran, 2013), which might lead to differences in vulnerability to psychopathology. However, these studies are very few, use a wide range of assessment methods for SC and HPA-axis activity, allow a broad time range for cortisol sampling, and usually do not take sex differences into account. Therefore, additional studies are needed to understand the complex interactions between SC and HPA-axis activity.

Effectiveness in social interactions is mainly shaped by demographic, genetic and environmental factors. Among demographic factors, age may impact SC differently across developmental periods due to changes in the characteristics of SC. For instance, children may become more socially competent and obtain complex social skills and abilities over time due to their cognitive and motivational

development (Waters & Sroufe; 1983). Indeed, a recent study conducted in Turkish preschool children reported increased levels of SC from age 31 to 73 months (Çorapçı, Aksan, Arslan-Yalçın & Yağmurlu, 2010). Considering the development of SC skills, one important group to investigate in particular is the preschoolers that encounter different social interactions primarily through their experiences in daycare centers. Apart from age, sex also alters SC, possibly due to differences in the attitudes of girls and boys in daily social interactions (Rose-Krasnor, 1997) and behaviors, such as aggression styles (Crick & Grotpeter, 1995). Moreover, previous studies consistently report higher SC in girls compared to boys when SC is measured in the form of a collection of positive social skills (Barbu, Cabanes & Le Maner-Idrissi, 2011). Besides age and sex, family socioeconomic status (SES) is an important environmental factor that alter children's SC (Hosokawa & Katsura, 2017), as well as HPA-axis activity (Evans & Kim, 2007; Chen, Cohen, & Miller, 2010). In terms of genetic factors, although very few studies consider SC in particular, many studies emphasize changes in social behavior by polymorphisms of the serotonergic system (Duman & Canli, 2010). Among these, a genetic polymorphism in the serotonin transporter gene (i.e. 5-HTTLPR) has been investigated widely in relation to differences in social behavior under various adverse environmental conditions (Canli & Lesch, 2007). Considering these studies, it is important to examine the collective role of these different factors on SC and HPA-axis activity.

According to previous literature, the first aim of this study is to investigate how sex, SES and 5-HTTLPR affect preschoolers' SC. The second aim is then to further investigate whether differences in SC would moderate the effects of these factors on HPA-axis reactivity.

## 1.1 Social competence: theory and perspectives

Almost three decades ago, Dodge (1985) stated that the number of definitions of SC were nearly equal to the number of SC researchers, which still holds true today. There is so far very little consensus among researchers about what exactly SC is (Stump, Ratliff, Wu & Hawley, 2009). However, there are two common perspectives with different interpretations of SC. As Waters and Sroufe (1983) pointed out, there are “molar” and “skills” approaches to SC. Molar, or “bottom-up” approaches aim to explain the roots of the actual construct of SC (Stump et al., 2009). Instead of providing lists of skills, these approaches define SC as the effectiveness in social environment (Waters and Sroufe, 1983). Therefore, according to this approach, socially competent behavior may change under different circumstances (Waters & Sroufe, 1983; Stump et al., 2009). Despite the molar approaches’ efforts in explaining the construct of SC, their assessment ability is poor. Consequently, Waters and Sroufe (1983) attain to the molar root and defined a socially competent individual as “*one who is able to make use of environmental and personal resources to achieve a good developmental outcome*” (p. 80), providing some developmentally appropriate assessment formulations. Another bottom-up model that is definitely prominent in the field is Rose-Krasnor’s (1997) framework of *SC prism*. The top level of the prism is *the theoretical level*, which defines SC as the effectiveness in interaction, similar with Waters & Sroufe (1983). The middle level is *the index level*, which consists of “summary indices” of the construct. It includes self and other domains, which is related to “individual’s own needs”, and “interpersonal connectedness”, respectively. According to this model, a socially competent individual is the one that can successfully balance self- and others-oriented needs. The last level of the SC prism is *the skills level*. The skills level consists of

motivations, skills, and goals that are needed and related with the index level, and social behavior. Although this bottom-up model explains the nature of SC thoroughly, it was again criticized as only including “positive” aspects of SC such as prosocial behavior, but neglecting some other self-related goals such as being “socially elite” or “dominant” (Stump et al., 2009). Stump and colleagues also criticized models like SC prism as being highly focused on group cohesion and culturally appropriate values, thus dismiss aggression as a strategy to reach goals. Relying on resource control theory (Hawley, 1999), Stump and colleagues (2009) state that children who are both coercive and prosocial controllers are the ones that are most successful in social interactions (Hawley, 2003a; Hawley, 2003b), thus aggression might be an adaptive strategy in certain environments, and should be included in the SC framework.

In contrast to molar approaches, skills, or “top-down” approaches provide certain skill sets or values as “competencies” that a socially competent individual has, including personal maintenance habits, prosocial behavior, and presence of positive relationships (Anderson & Messick, 1974; LaFreniere & Dumas, 1996). In terms of skills approach, a group of developmental psychologists published a list of skills that a socially competent child should possess (Anderson & Messick, 1974). These skills were not only limited to social skills (e.g. prosocial behavior), but included also cognitive (e.g. control of attention) and coordination (e.g. fine motor dexterity) skills. The problem here, as authors reported, was that these skills might not be generalizable across different contexts and cultures. For instance, McFall (1982) argued that skills, social ones in particular, cannot be conceptualized as SC, since SC is more of a broad and evaluative term. It means that an adaptive social skill might be different across different situations and cultures, such that a child

competent in one social situation might fail in another. Thus, social competence might be composed of different social skills depending on the situation (McFall, 1982; Stump et al., 2009). However, compared to the molar approaches, skills approach is much more superior in terms of the assessment process, since children's SC skills might be easily and consistently evaluated through parent and teacher reports suited to children's age. Therefore, in this study, a skills approach to SC is attained by utilizing the Social Competence and Behavior Evaluation Scale – Short Form (SCBE-30; LaFreniere & Dumas, 1996) suitable for children 3-6 years of age.

## 1.2 Sex and social competence

Children's sex is an important factor influencing SC, since girls and boys tend to behave differently in daily social interactions. For instance, boys are reported to be more interested in games that involve direct competition than girls (Rose-Krasnor, 1997). On the other hand, during play times, girls are shown to spend more time with adults, have smaller circle of friends, and have more intimate friendships than boys, allowing them to learn different social skills (Crombie, 1988). In addition, girls are reported to be more prosocial than boys (Eisenberg, Fabes & Spinrad 2006) and express mostly relational aggression, such as gossiping, while boys express more of physical aggression, such as hitting and pushing (Crick & Grotpeter, 1995). In addition, peer rejection, an index of poor SC, has been found to predict different behavioral maladjustments by sex, such that in boys, it mainly predicts aggression whereas in girls, it predicts withdrawal, underachievement and anxiety (French, 1990; Ladd, 1999). Taken together, it was proposed that girls and boys may have separate social relationships and behaviors, and thus SC should be considered

separately for boys and girls (Crombie, 1988). Skills-based assessments generally confirm this difference between boys and girls, with girls having higher SC than boys (Barbu et al., 2011), as measured by SCBE-30 (LaFreniere & Dumas, 1996; Tout, Haan, Campbell & Gunnar, 1998). This result was also replicated for the Turkish version of the scale (Çorapçı, Aksan, Arslan-Yalçın & Yağmurlu, 2010). This sex difference might be related to the pursuit of different social goals (Walker, 2005), or sex-typed behavior (Rose & Smith, 2009).

Girls and boys also differ in terms of how they are affected from certain environmental conditions. For instance, compared to boys, girls are reported to be more resilient in social interactions with various people starting from out-of-home daycare centers (Gamble & Zigler, 1986). Family risk factors might also affect boys and girls differently. Boys react to stressful situations in the family more than girls, such that these kinds of circumstances affect boys' behavioral and emotional outcomes more than of girls' (Rutter, 1982). For example, boys tend to express more disruptive behavior than girls after divorce of parents (Emery, 1982). These sex differences are suggested to be unstable across development (Barbu et al., 2011) and may change by how SC is measured (Volling, Mackinnon-Lewis, Rabiner & Baradaran, 1993). Therefore, although there is a common pattern on girls having high SC in the literature, this may change by developmental period and assessment of SC. Therefore, it is important to investigate sex differences at particular age groups by the use of particular SC measures.

### 1.3 Hypothalamic-Pituitary-Adrenal (HPA) axis

Environmental factors, while affecting children's behavior, also interact with various physiological changes, such as changes in stress response systems. These changes in early life are especially important, since development and biological dysregulations of these systems are important predictors of mental and physical health problems later in life (Cohen, Kessler & Underwood, 1995).

Stress response is the physiological reaction of the body, in response to aversive, unfamiliar or menacing situations. These reactions prepare individuals to deal with these situations, by running away from a threat, or fighting or threatening conditions (Carlson, 2013). Although most humans are not faced with primitive circumstances daily, such as the urgency to run away from a predator, or obligatory direct competition with other beings for food anymore, we still encounter situations that are unexpected, unpleasant or challenging to us. Because humans live dependent to each other in a group setting, those situations might arise from social interactions, or familial environment. Apart from the immediate response of the sympathetic system, the HPA-axis is activated at the paraventricular nucleus (PVN) of hypothalamus, leading to the release of corticotropin-releasing hormone (CRH). CRH then triggers the anterior pituitary to release adrenocorticotrophic hormone (ACTH) that leads to release of glucocorticoids, mainly cortisol in humans, from the adrenal cortex. Release of cortisol prepares the body in a stressful situation, leading to digestion of proteins and fats to provide glucose and energy, provoke behavioral responses, and increase blood flow (Carlson, 2013). This increased cortisol in response to stress is brought back to baseline levels by negative feedback loops. In addition to changes in cortisol in response to stressors, cortisol also follows a diurnal rhythm, such that it increases within the first thirty minutes after awakening and

decrease throughout the day, reaching the lowest levels before bedtime, leading to a diurnal slope (Miller et al., 2016; Figure 1). The difference between evening and morning cortisol levels, called as the diurnal cortisol slope, is associated closely with mental and physical health (Adam et al., 2017). For instance, a recent systematic review and meta-analysis revealed that flatter diurnal cortisol slopes are associated with poorer mental and physical health outcomes (Adam et al., 2017).

Diurnal cortisol slope is influenced by various factors in children, such as age, sex, season of the year, and sleep (Watanura, Donzella, Alwin & Gunnar, 2003; Watanura, Donzella, Kertes, & Gunnar, 2004; Miller et al., 2016). For young children attending daycare, one additional factor altering cortisol levels is the influence of the daycare environment. For instance, the diurnal slope is less observed in these daycare environments for preschool children, while it is commonly observed at home settings (Watanura, Kryzer & Robertson, 2009). Furthermore, this difference between daycare and home can be more pronounced according to the characteristics of the daycare centers, such as the quality of the daycare provided (Watanura et al., 2009). Therefore, a children's diurnal cortisol levels may change depending on the characteristics and environment of the daycare centers (Watanura et al., 2009). Therefore, in studies with preschoolers, it is important to investigate the cortisol levels at home and daycare separately. Consequently, in this study, we address this issue by measuring diurnal cortisol twice at daycare centers and home, separately.

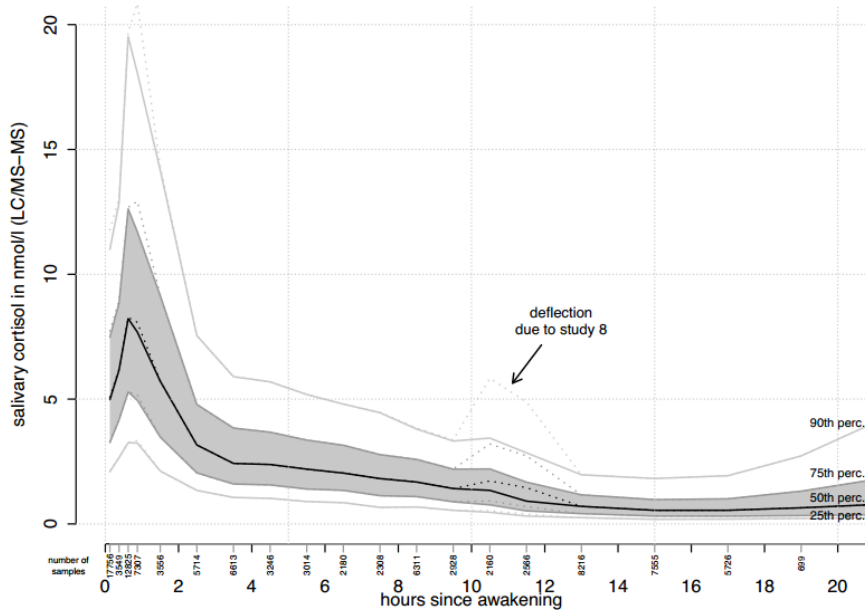


Figure 1. Diurnal salivary cortisol rhythm throughout the day. The figure is adapted from the CIRCORT database which consists of different studies, samples from in a total of 18,698 individuals in different age ranges. The arrow indicates the alteration in the averaged values due to the unusual concentrations in one of the studies. From “The CIRCORT database: Reference ranges and seasonal changes in diurnal salivary cortisol derived from a meta-dataset comprised of 15 field studies,” by Miller et al., 2016, *Psychoendocrinology*, 37, p. 20. Copyright 2016 by Elsevier. Reprinted with permission.

#### 1.4 Social competence and Hypothalamic-Pituitary-Adrenal axis

Problems in SC may lead to activation of stress response systems in the body, commonly studied in the context of changes in cortisol levels. Investigation of this change by SC is especially critical in preschoolers, since first peer relationships are built at these ages and success in these relationships are important. Therefore, researchers have been studying the associations between SC and stress response systems, especially cortisol levels. These studies suggest associations between SC and cortisol in preschoolers.

One of the first studies examining the SC-cortisol relationship reported a positive correlation, especially for maltreated children, with cortisol reactivity operationalized as “the tendency to produce cortisol levels markedly higher than

one's typical basal level" (Hart, Gunnar & Cicchetti, 1995, p. 17). Also, SC was positively associated with cortisol only in high conflict days in the classroom. Another study reported that the association between SC and cortisol reactivity might differ by adaptation to the preschool environment, such that children with high SC were reported to have lower cortisol reactivity after adapting to their preschool environment, compared to when they newly started (Gunnar, Tout, de Haan, Pierce, & Stanbury, 1997). Sex differences were also reported for the relationship between SC and HPA-axis reactivity. Tout, Haan, Campbell, and Gunnar (1998) showed a positive association between externalizing behavior and cortisol reactivity, and a negative association between internalizing behavior and cortisol specific to boys only. Researchers also observed a trend of a negative association in girls between cortisol reactivity and SC, which suggested that SC might buffer HPA-axis activity. Other studies with SC measures different than teacher reports also reported similar results in the association between SC and HPA-axis activity. A study which operationalizes SC as sociometric status, a measure of popularity among peers, reported that children who were rejected by their peers had higher cortisol concentrations than popular, neglected, or average children (Gunnar, Sebanc, Tout, Donzella & van Dulmen, 2003). Another study with 7-year olds made use of perceived SC of children themselves. They measured the change in cortisol concentrations in response to a presentation task. Their results suggested that, children with higher perceived SC had a larger decrease, thus a healthier and more adaptive response, in cortisol concentrations from the 20 to 35 minutes following the task (Schmidt et al., 1999).

Later studies used diurnal cortisol slope as a measure of HPA-axis activity, and showed that the association between SC and HPA-axis activity exists also for

diurnal slopes. For instance, Gunnar and Donzella (2002) argued that the constantly reported increase of cortisol in childcare throughout the day gets smaller as SC increases, especially with age. This association was also consistent for younger children. In a study with 3-year olds, Lengua, Zalewski, Fisher and Moran (2013) reported that low SC was related to a flatter diurnal cortisol rhythm. They also reported an indirect effect of SES on SC, through some marginal associations between SES and diurnal cortisol, and SES and effortful control. Similarly, Watamura and colleagues (2003) reported that in toddlers, higher playing with peers predicted less cortisol concentrations in the morning and afternoon, but it was not related with the difference in between.

SC indices were shown to be useful in explaining the associations between certain environmental factors and HPA-axis activity. For instance, in a recent longitudinal study by Alink, Cicchetti, Kim and Rogosch (2012) examined the relationships between childhood maltreatment, social functioning, and cortisol response. They found that maltreated children were less prosocial, more aggressive, and thus less socially competent compared to children without maltreatment. They also found that prosocial behavior mediated the relationship between maltreatment and cortisol response. Specifically, if maltreated children had lower prosocial behavior, and they were more prone to have lower morning cortisol a year later. This was also the case for maltreated children with higher aggression. Moreover, they found that girls had a steeper diurnal slope. They suggested that lack of positive peer interactions, and difficulty in relationships might lead to disrupted cortisol regulations.

Overall, studies consistently report a negative association between SC and HPA-axis activity in general. SC might also serve as a factor in explaining the

relationships between environmental factors and HPA-axis activity. In this study, we use SES as an environmental factor, which has been shown to be related to both SC and HPA-axis activity which is further explained in the next section of this thesis. Since SC was shown to mediate the relationship between childhood maltreatment and HPA-axis activity (Alink et al., 2012), we examine its role in the association between SES and diurnal cortisol slopes in daycare and home, and expect a buffering effect in daycare.

## 1.5 SES as a predictor of social competence and HPA-axis activity

### 1.5.1 SES and social competence

Family environment and parents are important contributors to children's SC and peer relationships (Parke & Ladd, 1992; Guralnick & Neville, 1997; Ladd, 1999; Ross & Hove, 2009). According to Parke and Ladd (1992), parents influence their children's peer relationships directly and indirectly. Direct parental influences include parents' direct roles in peer relations, such as arranging play dates, teaching the child how to behave, and monitoring the child's behavior in peer settings (Ladd, Profilet & Hart, 1992; Guralnick, 1999). On the other hand, indirect parental influences, such as SES, parental attitudes and parenting styles, consist of parents' conception of their children's SC, parent-child interactions, and family risk factors (Guralnick, 1999; Ladd, 1999; Ross & Howe, 2009).

Among the indirect influences of parents on child's development, a widely studied factor is SES. It represents the social and financial positions of parents within the society and is commonly assessed by parental education, occupation, and income;

individually or as a combination (Guidubaldi & Perry, 1984; Bradley & Corwyn, 2002). Family SES has a wide impact on children's development, performance and health. Children with lower family SES were reported to have lower growth in utero and birth weight, and higher birth defects and risk of developing a disease in childhood (Bradley & Corwyn, 2002). SES also impacts cognitive performance and academic achievement in children (McCall, 1981; White, 1982). For instance, determinants of lower SES, such as less parental education and increased poverty, were associated with lower IQ and lower school success (Masten et al., 1988; Alexander, Entwisle & Dauber, 1993; Duncan, Brooks-Gunn & Klebanov, 1994). Finally, SES also alters vulnerability to mental health problems. Children with low SES exhibit higher tendency to social dysfunction and psychiatric problems, such as childhood depression (Bradley & Corwyn, 2002).

In terms of its relation to SC, in general, indices of high SES are associated with better outcomes in children. For example, Masten et al. (1988) reported that higher SES, conceptualized as better parental occupation, was related to higher engagement and lower disruptive behavior of children in school. Another study by Guidubaldi and Perry (1984) evaluated the distinct roles of different SES indices, such as paternal occupation, paternal education, and maternal education, on children's SC assessed as peer status rating and social maturity. It was reported that paternal occupation predicted children's peer status, whereas maternal education predicted both peer status and social maturity. Other studies investigating the role of family income on children's SC reported a positive relationship, with lower income predicting lower SC (Assis, Avanci & Oliveira, 2009; Lengua et al., 2013). Importantly, the influence of SES on children's SC may have a long-term impact as suggested by a recent longitudinal study with 11-13 years old teenagers (Brody et al.,

2013). Researchers showed that lower SES was linked to lower teacher-rated SC in preadolescence, and this lower SC predicted higher stress- and health-related problems at age 19. Another recent longitudinal study, conceptualizing SC as social skills, reported that components of SES (parental education and family income) positively and individually predicted preschoolers' SC (Hosokawa & Katsura, 2017). They also reported that children's preschool levels of SC, which were influenced by SES, predicted less behavioral problems when they become first graders, so high SC might protect children from the adverse effects of family risk factors that may lead to behavioral dysfunction (Hosokawa & Katsura, 2017).

Considering these studies with different indices of SES and measurements of SC, in this study we investigated a combination of these SES indices (parental education and income) on children's SC, in order to represent the family SES as thorough as possible.

### 1.5.2 SES and HPA-axis

SES not only influences individual differences like SC, but it also changes individuals' physiology, such as their stress response, possibly through its effects on childhood neurodevelopment (Hertzman, 1999; Hackman & Farah, 2009; Hackman, Farah & Meaney, 2010). The effects of low SES may stem from living in a riskier environment with plenty of stress exposure due to factors such as maltreatment, malnutrition, and adverse parenting (Lupien, King, Meaney & McEwen, 2001). This increased exposure to stressful conditions by low SES in turn can influence hyperactivity of the stress response systems, mainly the HPA-axis, causing disturbances in multiple systems of the body. These disturbances, in turn lead to increased allostatic load, the "wear and tear that results from either too much stress

or from inefficient management of allostasis” (McEwen, 2016, p. 57), leading to various health problems in the long run (Lupien et al., 2001, Miller et al., 2009). For example, Lupien and colleagues (2001) associated lower SES with higher morning cortisol in 6 to 10 year old children. But after the school transition, this effect was gone. Moreover, a longitudinal study with 9-18 years old children revealed that, when children were from lower SES backgrounds, they had increased cortisol response over two years (Chen, Cohen & Miller, 2010). Another longitudinal study with children found a positive association between the number of years lived in poverty (i.e. low SES) and elevated overnight cortisol levels (Evans & Kim, 2007). A study with adults reported a similar result such that as SES decreased, total cortisol output increased (Cohen, Doyle & Baum, 2006). Overall, studies performed with different age groups consistently show an impact of SES on HPA-axis activity. Therefore, in this study, we test this association in a sample of preschoolers.

The relationship between SES and HPA-axis activity might also be influenced by some other factors. For example, Cohen and colleagues (2006) reported that the aforementioned relationship between SES and cortisol was mediated by health practices (e.g. smoking behavior), and psychosocial factors such as diversity of the social network, depression, and social support. Surprisingly, when the mediators, especially diversity of the social network and smoking status were introduced, SES had no direct effect on cortisol. Therefore, according to that study, the effect of SES on cortisol might be through other psychosocial factors. Finally, a longitudinal study by Brody and colleagues (2013) examined the interrelations between SC, SES, and allostatic load, measured by SAM markers and cortisol. They reported that SES in preadolescence positively predicted allostatic load at the age of 19. Interestingly, they also found that in children from low SES backgrounds, the

relationship between SC and allostatic load might be positive such that children with higher SC had higher levels of allostatic load. These findings indicate that the relationship between SES and HPA-axis activity may not be stable, and might be altered by other factors such as SC.

Overall, SES is an important factor in predicting HPA-axis activity. In preschoolers, it can increase allostatic load, and disrupt HPA-axis activity such that it can lead to flatter diurnal slopes. This association might also be influenced by children's SC, which might moderate the relationship between SES and HPA-axis activity.

## 1.6 Genetic factors, social competence and HPA-axis

### 1.6.1 Genetic factors and social competence

In addition to environmental factors, social behavior is also shaped by genetic factors. For instance, twin studies indicate significant heritability of children's social behavior (Scourfield, Martin, Lewis, & McGuffin, 1999; Fedko et al., 2017). In relation to SC, heritability studies so far utilized different measures related to SC, such as the competence scale of Child Behavior Checklist (CBCL; Achenbach, 1991), perceived popularity among peers, and parent – teacher reports of SC. Competence scale of CBCL consists of subscales for school competence, SC, and activities. In one of these twin studies with 7-15 year old twins, heritability of school competence was 21% utilizing the CBCL subscale (Edelbrock, Rende, Plomin, & Thompson, 1995). Interestingly, this heritability was suggested to be sex-specific in a later study with adolescents (Kuo, Lin, Yang, Soong, & Chen, 2004). For boys, genetic factors explained 54% of the variation in SC as measured by CBCL, while

contribution of genetic factors was not significant for girls. However, genetic factors accounted for 64% of the variance in total competence scores in girls. These results indicate that for adolescent and preadolescent twins, SC might be a trait with a significant genetic contribution for boys, whereas it is mainly influenced by environmental factors in girls.

Another study with adolescent twins using perceived popularity among peers as a measure of SC suggested nearly 50% heritability (McGuire et al., 1999).

Furthermore, a study adopting teacher-rated popularity as a measure of SC reported that intraclass correlations of biological siblings' popularity were twice as high as those of adopted siblings; therefore popularity had a genetic component suggesting heritability (O'Connor, Jenkins, Hewitt, DeFries, & Plomin, 2001).

Some studies also suggested that the heritability of SC can be influenced by covarying factors such as parenting quality. For instance, a study using parental and teacher reports of SC assessed by Preschool and Kindergarten Behavior Scales–Second Edition (Merrell, 2003) and the Social Skills Rating System (Gresham & Elliott, 1990), suggested 47% heritability when a bivariate analysis for the covariation between parenting quality and SC was performed (Roisman & Fraley, 2012).

Considering research related to the heritability of SC, there is evidence for the contribution of genetic factors to individual differences in SC, which can be further influenced by sex and related environmental factors. Variation in heritability among studies may also be accounted by differences in age, sex, culture and genetic characteristics between the samples, as well as in assessment techniques.

### 1.6.2 Theoretical models

One important genetic factor that might be related to differences in SC and HPA-axis is a genetic polymorphism in serotonin transporter gene (*SLC6A4* or *5-HTT*), called the serotonin transporter-linked polymorphic region (5-HTTLPR). The polymorphism leads to a Short (S) and a Long (L) allele, with the S-allele associated with less transcriptional efficiency of the gene (Heils et al., 1996). 5-HTTLPR was repeatedly shown to be related to differences in social behavior (Canli & Lesch, 2007; Duman & Canli, 2010), environmental sensitivity (Caspi, Hariri, Holmes, Uher, & Moffitt, 2010), HPA-axis reactivity (Wüst et al., 2009; Miller, Wankler, Stalder, Kirschbaum, & Alexander, 2012; Duman & Canli, 2015), and amygdala activity (see Murphy et al., 2013 for a meta-analysis). In these studies, S-allele carriers were generally associated with less favorable outcomes, such as higher HPA-axis and amygdala activation, in case of exposure to adverse environments. Therefore, studies usually follow the dominant model of 5-HTTLPR, and group SS and SL individuals together and compare them with LL homozygotes. Additive model of 5-HTTLPR groups SS, SL and LL separately, and look for additive effects of the S-allele. Recessive model of 5-HTTLPR groups SL and LL individuals together, and compare them with SS homozygotes (Goldman, Gleib, Lin, & Weinstein; 2010). This difference in grouping genotypes thus makes it harder to compare findings of similar studies, especially when considered together with environmental factors in Gene-Environment Interaction (GxE) studies.

Indeed, GxE studies play a substantial role in determining social behavior (Moffitt, Caspi, & Rutter, 2005). GxEs occur when the same environmental condition affects individuals differently as a function of their genetics. Some models were proposed by developmental psychologists about the role of personal factors,

including genetic factors, in susceptibility or vulnerability to changes in the environment, leading to certain developmental outcomes. These models (see Figure 2) were also implemented to GxEs.

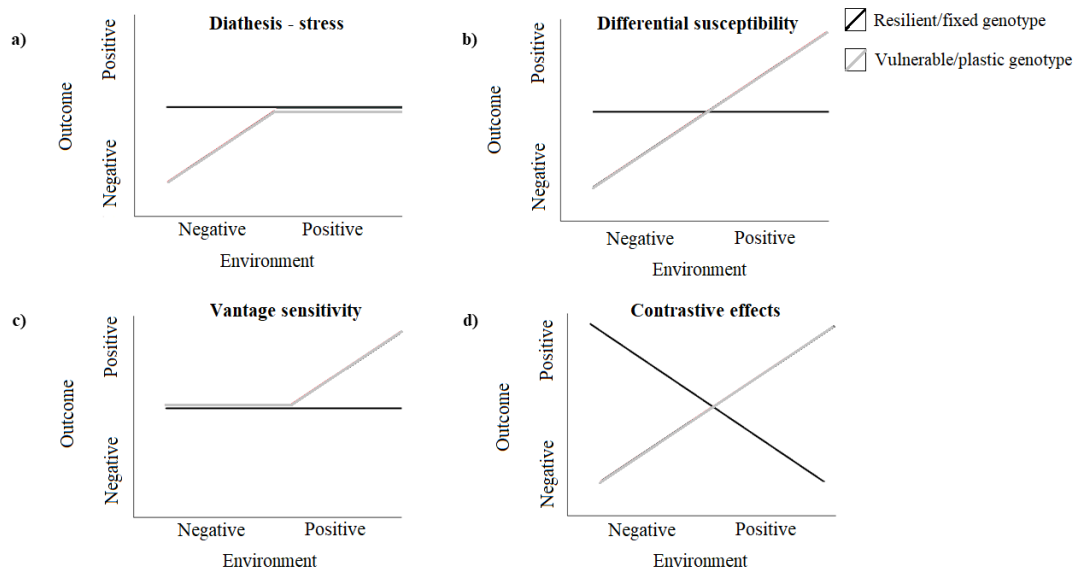


Figure 2. Models of gene-environment interactions in developmental psychology. Black lines indicate resilient (for dual-risk) or fixed (for differential susceptibility and vantage sensitivity) individuals in terms of their genotypes. Gray lines indicate vulnerable (for dual-risk) or plastic (for differential susceptibility and vantage sensitivity) individuals in terms of their genotypes. Adapted from Belsky & Pluess, 2016.

One of the first proposed models is diathesis-stress, or dual-risk model (Figure 2a). According to this model, some individuals, depending on individual factors, are more prone to negative outcomes such as psychopathology or behavioral problems, when they are faced with adverse experiences or environments (Sameroff, 1983; Monroe & Simons, 1991). In the case of GxEs research, this model states that individuals with certain ‘risky’ genotypes are affected more from negative environmental conditions than individuals without risky genotypes, thus ‘vulnerable’ individuals are more likely to experience negative behavioral outcomes than ‘resilient’ individuals (Belsky & Pluess, 2016). In positive environments, vulnerable

and resilient individuals are not expected to differ in terms of their behavioral outcomes.

The second model is the differential susceptibility model (Figure 2b) that follows an evolutionary approach to environmental sensitivity. It states that individuals with ‘plastic’ genetic factors might be affected by the changes in the environment ‘for better or for worse’, meaning that these individuals will develop more negative outcomes in negative environments, while they will exhibit more positive outcomes in positive environments, compared to ‘fixed’ genotypes (Belsky & Pluess, 2009; Belsky & Pluess, 2016).

The third model is the recently proposed vantage sensitivity model (Figure 2c), which states that some individuals with ‘plastic’ genetic factors might be exclusively prone to being positively affected only by positive environments than individuals with ‘fixed’ genetic factors (Pluess & Belsky, 2013; Pluess, 2017). These groups are suggested to be similar under negative environments.

Finally, the last model is the contrastive effects model (Figure 2d; Belsky, Bakermans-Kranenburg, & van IJzendoorn, 2007). When applied to GxEs, this model suggests that individuals with different genetic factors may be influenced by the environment differently. For some individuals with a certain genotype, negative environments may increase negative outcomes, whereas the opposite pattern can be observed in individuals with another genotype for that genetic factor.

When the interaction between serotonin transporter polymorphism and different environmental factors are considered, there is evidence supporting more than one of these models. For instance, a line of studies supporting the diathesis-stress model associated S-allele carriers from adverse environmental backgrounds with higher adverse mental health outcomes and social maladjustment (see Caspi et

al., 2010 for a review; Pluess et al., 2011; Bakermans-Kranenburg, Dobrova-Krol, & van IJzendoorn, 2012). On the other hand, additional studies provide evidence for the differential susceptibility model, report that the S-allele is not a risk, but a plasticity allele (van IJzendoorn, Belsky & Bakermans-Kranenburg; 2012). In this meta-analysis, S-carrier Caucasian children and adolescents were shown to have higher emotional problems and depression than L-homozygotes in adverse environments, whereas they were better off than L-homozygotes in supportive environments (van IJzendoorn et al., 2012). This view was also supported in another recent GxEs review, considering 5-HTTLPR as a plasticity marker together with some other polymorphisms (Belsky & Pluess, 2016). In a recent study, Brock, Kochanska and Boldt (2017) composed a cumulative biobehavioral plasticity measure that involved being an S-carrier, having high levels of skin conductance, inhibition and sadness, and low levels of joy. It was reported that this biobehavioral plasticity measure interacted with interparental relationship on predicting internalizing behaviors in children. Children with high plasticity had higher internalizing problems over time than low plasticity children, if the interparental relationship was acrimonious, whereas they had lower internalizing problems over time than low plasticity children if the interparental relationship was harmonious. However, there was a trend towards significance in low plasticity children ( $p = .076$ ) such that the pattern was the opposite of high plasticity children, which is congruent with contrastive effects model in Figure 2d.

### 1.6.3 5-HTTLPR and social competence

In relation to GxEs on SC, Kochanska, Kim, Barry and Philibert (2011) reported that 5-HTTLPR genotype moderated the relationship between maternal responsivity and

SC, such that maternal responsivity positively predicted SC only in S-carriers, but not in L-homozygotes. In case of lower maternal responsivity, L-homozygotes had higher SC than S-carriers. In case of higher maternal responsivity, there was not a significant difference between S-carriers and L-homozygotes, supporting the diathesis-stress model. Similar interactions were reported in a study with low SES children investigating the interaction between 5-HTTLPR and maltreatment history on resiliency, with resiliency reflected in an aggregate measure of SC, prosocial behavior, aggression and withdrawal. Researchers found that SS children with a maltreatment history had significantly lower resiliency than children without a maltreatment history. For L-homozygotes, the finding was also significant in the same direction as S-homozygotes, but was not as strong. These findings partially support the differential susceptibility model (Cicchetti & Rogosch, 2012). Considering these GxE studies, there is not a consistent pattern of how 5-HTTLPR influences SC in case of adverse environments, requiring additional studies.

#### 1.6.4 5-HTTLPR and HPA-axis activity

HPA-axis activity is influenced by genetic factors as well, including the serotonin transporter polymorphism. In a meta-analysis, individuals with SS genotype were shown to have higher HPA-axis activity in response to laboratory stressors, compared to other genotype groups (Miller, Wankerl, Stalder, Kirschbaum, & Alexander, 2012). However, this effect is not replicated in all studies and changes extensively when investigated together with environmental factors, similar to studies of SC (Kochanska et al., 2011; Cicchetti & Rogosh; 2012). According to these findings related to the interaction between 5-HTTLPR and stressful life events on HPA-axis activity, there is ample evidence in support of the contrastive effects

model. Indeed, studies investigating this interaction at the level of intermediate phenotypes, phenotypes between the genetic and disorder levels, often yield similar findings, such as in the case of HPA-axis activity and neural activation (Canli et al., 2006, Alexander et al., 2009, Mueller et al., 2011; Duman, 2012). For instance, in a study with adult men, Alexander and colleagues reported that SS individuals with high stressful life events had higher salivary cortisol in response to a laboratory stressor than those with low stressful life events. In contrast, other genotype groups (SL and LL) exhibited the opposite pattern such that individuals with low stressful life events had higher cortisol levels than those with high stressful life events (Alexander et al., 2009). A similar result was shown in men by Duman (2012) such that S-carriers with higher childhood maltreatment had higher cortisol response than L-homozygotes, while in case of lower childhood maltreatment, L-homozygotes had higher cortisol than S-carriers, supporting the contrastive effects model. Mueller et al. (2011) reported a similar interaction in young adults for stressful life events specific to the first five years of life. Apart from HPA-axis reactivity to laboratory stressors, recently Duman (2016) replicated these findings for Cortisol Awakening Response (CAR) in young adults from Turkey.

Considering studies of GxEs on behaviors and intermediate phenotypes, it is clear that there is not a consistent model that captures the effect of 5-HTTLPR and adverse environment. However, consistent findings mainly for HPA-axis studies suggest that contrastive effects model might work the best, at least in the context of 5-HTTLPR. Therefore, in this study, we expect to find results resembling contrastive effects for the interaction between SES and 5-HTTLPR genotype on HPA-axis activity.

### 1.6.5 5-HTTLPR and SES

SES is another factor that interacts with genetic factors in predicting social behavior and adjustment. Kim-Cohen, Moffitt, Caspi, and Taylor (2004) found in a twin sample, children's outgoing behavior, stimulating activities, and maternal warmth provided resilience, operationalized by having a higher than predicted IQ score or less than predicted antisocial behavior, for children against the adverse effects of SES, and this resiliency is heritable such that it is influenced by genetic and environmental factors. A study about the heritability of adolescent antisocial behavior reported that the role of heritability on antisocial behavior tended to change in terms of SES: genetic factors accounted for more of the variance of antisocial behavior in adolescents from high SES backgrounds, versus those from low SES backgrounds (Tuvblad, Grann, & Lichtenstein, 2006), which might be due to 'social push perspective' that in high SES environments, children might be influenced more by their genetics whereas in low SES environments, they might be 'pushed' to behave antisocially due to certain social risk factors (Raine, 2002).

The interaction between SES and 5-HTTLPR genotype was examined in terms of its influence on mental health outcomes and social adjustment. A 2004 study performed with adults stated that 5-HTTLPR S-carriers with low SES had lower serum prolactin levels, an indicator of serotonergic responsivity, than individuals with high SES. However, L-homozygotes had similar prolactin levels regardless of their SES. The result of this study provides support for differential susceptibility, and gives a clue about the possible effect of SES on central nervous system functioning, moderated by 5-HTTLPR genotype (Manuck, Flory, Ferrell, & Muldoon, 2004). However, as the case with SC and HPA-axis activity, there is not a consistent GxE model for the interaction between SES and 5-HTTLPR. For example,

in a 2010 study examining the effects of SES on psychopathic traits in youth, researchers reported that as SES increased, psychopathic traits (i.e. callous-unemotional and narcissistic traits) decreased only in LL individuals (Sadeh et al., 2010). Although these findings were also consistent with differential susceptibility, the 'plastic' genotype was LL but not SS, which is contrary to common findings (Caspi et al., 2010, Miller et al., 2012). Considering the variety of findings supporting different models, similar to the case with 5-HTTLPR and HPA-axis activity, there is not a single model that is agreed to fit the findings about the interaction between SES and 5-HTTLPR.

Studies also report sex-specific effects on behavioral outcomes resulting from the interaction between SES and 5-HTTLPR. A study with adults reported an interaction indicating sex specific effects on depression. In females with low childhood SES, operationalized by paternal education, S-allele was associated with more depressive symptoms than females with high childhood SES. On the other hand, for LL females, high and low SES individuals did not differ in terms of their depressive symptoms. However, in males, the pattern was opposite, such that the L-allele carrier males were associated with more depressive symptoms in low SES backgrounds than high SES backgrounds. But this pattern was not observed for SS males (Brummett et al., 2008). These findings support diathesis-stress model for both males and females. Sex specific effects were also reported in a 2013 study about delinquent behavior in adolescents. Åslund and colleagues (2013) reported a curvilinear interaction between SES and 5-HTTLPR genotype. In SL and LL boys with high SES, and in LL boys with low SES, delinquent behavior was highest. However in girls, highest delinquent behavior was observed in SS and SL individuals with high SES, and SL individuals with low SES. These studies illustrate that SES

might impact individuals with different genetic backgrounds, specifically individuals with different 5-HTTLPR genotypes. Also, sex might be an important determinant in the interaction between 5-HTTLPR genotype and SES on social behavior. Studies reviewed above are also important in showing that S-allele is not the sole ‘plasticity’ or ‘risk’ allele, and L-homozygotes might also be affected by environmental factors.

### 1.7 Present study

The goal of this study was to examine the relationships between SES, SC, and HPA-axis activity, as a function of sex and 5-HTTLPR. This is the first study to analyze the interaction between SES, sex and 5-HTTLPR genotype on SC, and the interaction between SES, SC and 5-HTTLPR genotype on HPA-axis activity.

In order to accomplish this goal, two main research questions were investigated in this study. The first research question examined the associations between SES and SC, as a function of sex and 5-HTTLPR. In this part of the study, SES was the independent variable and 5-HTTLPR genotype and sex were moderators. It was expected that girls would have higher SC, replicating the literature about sex differences in skills approaches to SC (Barbu et al., 2011), and studies performed with SCBE-30 (LaFreniere & Dumas, 1996; Çorapçı et al., 2010). In addition, no differences were expected in SC by 5-HTTLPR genotype for boys or girls. Since boys were reported to be more sensitive to environmental challenges than girls (Emery, 1982; Rutter, 1982; Gamble & Zigler; 1986), and that the S-allele of 5-HTTLPR was associated with vulnerability (Caspi et al., 2010) and environmental susceptibility (van IJzendoorn et al., 2012); a three-way interaction between sex, 5-HTTLPR genotype and SES was predicted; and S-carrier boys were expected to have a positive association between SES and SC, such that as SES increased, SC

increased. LL boys were not expected to differ in SC by SES. The abovementioned pattern for S-carrier boys was expected to resemble a differential susceptibility model rather than diathesis-stress model, based on findings about 5-HTTLPR being a plasticity marker (van IJzendoorn et al., 2012). For boys, it was expected that S-carriers with higher SES would have higher SC than L-homozygotes with similar SES. For girls, no significant difference in SC was expected by SES, 5-HTTLPR or their interaction.

The second question examined the associations between SES and HPA-axis activity as a function of sex, SC and 5-HTTLPR. No differences in terms of sex or 5-HTTLPR genotype were expected in HPA-axis activity, measured by diurnal slope, morning, and afternoon cortisol. It was expected that the findings would replicate the literature associating a healthier diurnal cortisol rhythm, calculated as the diurnal slope, with SC and SES. A positive association between diurnal slope and social competence in daycare (Gunnar & Donzella, 2002; Lengua et al., 2013), and positive association between diurnal slope and SES (Lupien et al., 2001; Chen, Cohen & Miller, 2010) were expected. Separate analyses were performed for boys and girls, since they might differ in terms of the nature of SC (Crombie, 1988), and changes in SC might affect boys and girl differently in terms of their HPA-axis responses. For boys, diurnal cortisol slopes throughout the day were expected to have a positive association with SES, such that as SES increased, diurnal cortisol slope increased both at home and daycare. Also, SC was predicted to moderate this relationship between SES and diurnal cortisol slope only in daycare samples. This moderation was expected to be present especially in S-carrier boys which were expected to be more susceptible to the environment. It was predicted that depending on their SC levels, the association between SES and diurnal cortisol slope in daycare might be

different, such that higher SC levels might prevent the relationship between SES and diurnal slope. Considering the previous findings using intermediate phenotypes that support contrastive effects (e.g. Alexander et al., 2009; Mueller et al., 2011), for LL boys, it was predicted that there might be a negative relationship between SES and diurnal cortisol. No predictions were made for the moderator effect of SC in LL boys. For girls, no SES-5-HTTLPR-SC interactions on HPA-axis response were expected. The three-way interaction was not expected to be present in diurnal cortisol slopes at home since peer SC was used in this study. Therefore it was hypothesized that there would not be any buffering effects of SC at home. The influences of SES and SC on average morning and afternoon cortisol values were also explored. No predictions were made about the interactions on morning and afternoon cortisol values at daycare. However, no interactions were expected on those at home.

## CHAPTER 2

### METHODS

#### 2.1 Participants

Participants were preschool children attending daycare centers from 3 different districts of Istanbul, Turkey. Mothers and teachers of 340 children agreed to take part in the study. Please see Table 1 for participant details by district, daycare center, classroom, and biological samples provided. In exchange of participation, families were given a personalized report indicating their children's language and socioemotional development. For children providing the HPA-axis measures, they were also given information on whether their cortisol levels are within normal ranges or not. All families completed written informed consent forms before participation.

Table 1. Participant Details by District, Daycare Center, Classroom and Biological Sample

District	Daycare centers	Classrooms	Participants	Total samples for genotyping	Total samples for cortisol analysis
Kağıthane	1	2	11	10	-
Bakırköy	6	24	179	146	-
Kartal	10	40	150	114	110
<i>Total</i>	3	17	66	340	110

Out of 340 children from 3 districts, 266 children's DNA samples were successfully extracted and 5-HTTLPR genotypes were determined. From these children, 210 of them had full information on age, sex, SES, and SC. Sampling for cortisol analysis took place only in Kartal district. Saliva collection for measurement of diurnal cortisol concentration was possible from 110 children, at least for one

sampling day, either at home or daycare. Among these 110 children, 81 of them had full information on age, sex, SES, SC and 5-HTTLPR genotype. Please see Table 2 for details about these characteristics.

Table 2. Participant and Family Characteristics by Different Analyses

	Participant number	Mean age in months (SD)	Sex (girls)	Monthly income above 3000 TL	Mothers with at least high school degrees	Fathers with at least high school degrees
Genotype analysis	210	62.42 (7.50)	49%	55%	79%	69%
Cortisol analysis	81	61.70 (7.54)	43%	52%	85%	73%

Twenty children were not eligible for diurnal cortisol measurements due to illness or medicine use that may affect HPA-axis activity. In order to be eligible, children should not 1) have a chronic health condition that may affect central nervous system and/or hypothalamic-pituitary-adrenal axis activity (e.g. autism, chronic heart conditions, familial mediterranean fever) 2) have a recent surgery that may affect child's mood 3) regularly use medicine that may affect central nervous system and/or hypothalamic-pituitary-adrenal axis activity (e.g. sedative antihistamines with cetirizine as the active ingredient), psychoactive drugs (e.g. methylphenidate as the active ingredient). Any other situation was evaluated case-by-case by a trained graduate student. Please see Figure 3 for a flowchart depicting the number of participants at different stages of the study.

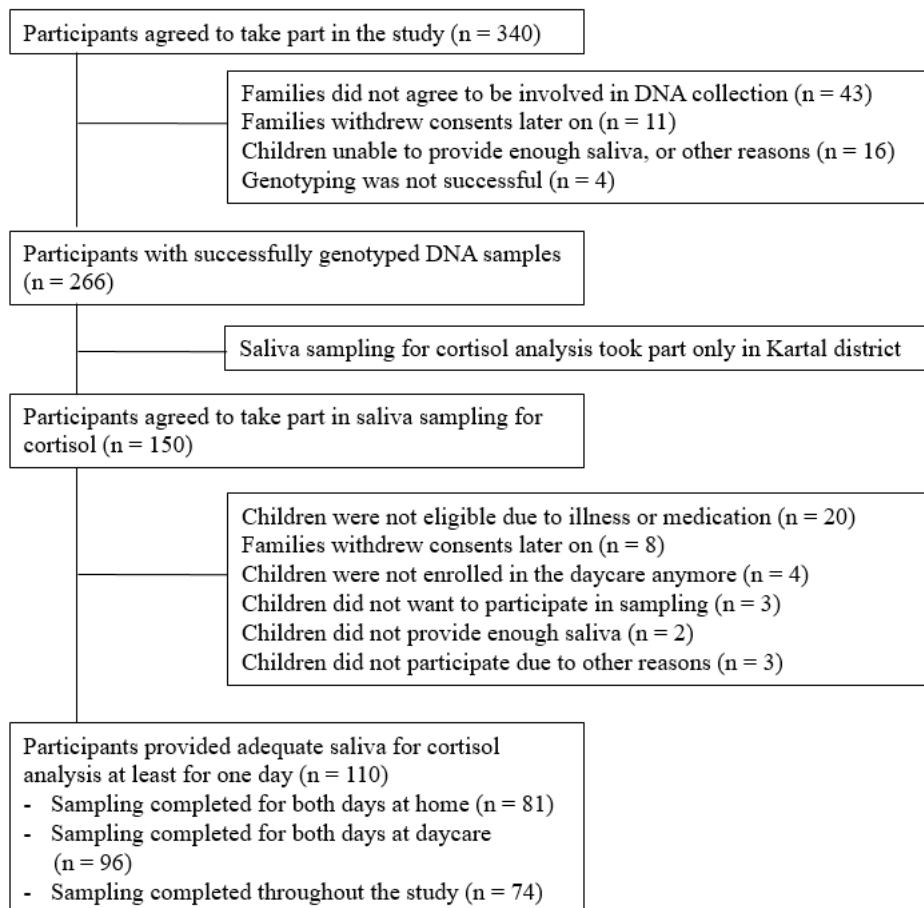


Figure 3. Flowchart depicting changes in participant numbers throughout the study.

## 2.2 Measures

### 2.2.1 Demographic variables

Mothers and teachers were given separate paper-pencil surveys to complete.

Mothers' survey included questions about demographics (e.g. maternal and paternal age, education status, family income, physical conditions of the house), children's and mothers' health, children's behavior, and parenting behavior.

### 2.2.2 SES

Socioeconomic status (SES) of the family was calculated by standardizing mother's education level, father's education level and monthly income level. Families were asked to select the best fitting education level on a scale from 1 (primary school dropout) to 10 (graduate degree), and the best fitting monthly income level on a scale from 1 (below 1000 Turkish Liras) to 6 (above 10000 Turkish Liras). Each family's responses for those SES variables were first converted to Z-scores. Maternal education was positively correlated with paternal education ( $r_s(232) = .50, p < .01$ ) and family income ( $r_s(234) = .48, p < .01$ ). Family income was also positively correlated with paternal education ( $r_s(225) = .47, p < .01$ ). All these scores correlated significantly with each other, so they were averaged in order to calculate the standardized SES score.

### 2.2.3 Social competence

SC was measured by the Turkish translation of SCBE-30 (LaFreniere & Dumas, 1996; Çorapçı, Aksan, Arslan-Yalçın, & Yağmurlu, 2010), which is the short form of Social Competence and Behavior Evaluation Scale (SCBE; LaFreniere, Dumas, Capuano, & Dubeau, 1992). The scale is composed of three sub-scales named SC, anger-aggression, and anxiety-withdrawal, and only the SC scale is utilized in this study. Each subscale consists of 10 items and rated typically on a 6-point Likert-type scale (1: never, 2-3: sometimes, 4-5: often, 6: always) by preschool teachers on the frequency of children's behavior. Items in the SC subscale are designed to evaluate children's joyful, socially integrated, calm, prosocial, and cooperative behaviors in classroom through items such as "comforts or assists another child in difficulty", "negotiates solutions to conflicts", and "attentive toward younger children". SC

subscale scores range from 6 to 60 and higher scores mean higher SC. In this sample, Cronbach's alpha was .87.

#### 2.2.4 Genotyping

Children provided saliva samples in Oragene DNA collection tubes by spitting into an empty tube (DNA Genotek, Ottawa, Canada). The DNAs were then extracted from these saliva samples by prepIT L2P reagent (DNA Genotek, Ottawa, Canada) according to manufacturer's instructions. DNA extraction took place in the Psychoepigenetics Laboratory as the following: Saliva samples in Oragene tubes were kept at 50°C for 14 hours in an incubator. 0.5 ml of each sample was transferred to a microcentrifuge tube, and 20 µl of prepIT L2P reagent was added on top of the sample. After 10 minutes on ice, the mixture was centrifuged at 15000 RPM at 20°C for 15 minutes. The supernatant was transferred to a clean microcentrifuge tube, and 600 µl of absolute ethanol was added into the new tube. After a wait period of 10 minutes, the mixture was centrifuged at 15000 RPM at 20°C for 2 minutes. The supernatant is removed and the DNA extracted is collected as the pellet. In order to clean the DNA pellet, 250 µl of 70% ethanol was added into the tube and removed from the tube shortly after, and the DNA was let dry on the bench by leaving the caps of the tubes open. Finally, 50 µl of 1X Tris-EDTA buffer (TE) was added into the tube. DNA is homogeneously dissolved in the TE. After the DNA samples concentration was measured using Thermo Scientific Multiskan GO spectrophotometer (Thermo Fisher Scientific, Waltham, USA), the concentration of the sample was adjusted to 25 ng/µl with double distilled water. All DNA samples were kept at -20°C until genotyping experiments.

Children's DNA samples were genotyped for the 5-HTTLPR polymorphism (Heils et al., 1996) by polymerase chain reactions (PCRs) utilizing previously designed primers (Wendland, Martin, Kruse, Lesch, & Murphy, 2006). The primer sequences were: Forward: 5'-TCCTCCGCTTTGGCGCCTCTTCC-3', Reverse: 5'-TGGGGGTTGCAGGGGAGATCCTG-3'. The PCRs were performed with the HotStarTaq DNA Polymerase Kit (Qiagen, Hilden, Germany), using Applied Biosystems-Veriti™ 96-Well thermal cycler (Foster City, California, USA). PCR conditions were: initial enzyme activation at 95°C for 15 minutes, continued by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 63°C for 30 seconds, and extension at 72°C for 45 seconds. The PCR ended with a final extension step for 8 minutes at 72°C. PCR products were then used to determine 5-HTTLPR genotypes through 1.5% agarose gel electrophoresis. Short allele (S) was 469 base pairs (bps) long, while the Long allele (L) was 512 bps long. According to these gel results, children's genotypes were determined as SS, SL and LL.

In addition to 5-HTTLPR, another polymorphism within the serotonin transporter gene was genotyped as well, considering its influence on gene expression. The G-allele of this A/G Single Nucleotide Polymorphism (SNP; rs25531) was shown to lower gene expression of the L-allele to the level of the S-allele (Hu et al., 2005). In order to determine the genotypes for this SNP, PCR products were digested with *HpaII* restriction enzyme (Thermo Fisher Scientific, Waltham, USA) for 3 hours at 37°C alongside with an uncut control without the enzyme. Afterwards, the enzyme is inactivated by incubation at 65°C for 20 minutes. Since *HpaII* recognizes the G-allele, only the PCR products that have at least one G-allele were digested into

multiple products. Genotypes were determined again by 1.5% agarose gel electrophoresis, with the G-allele leading to digestion of the S-allele into 402 and 67 bp-long products, and the L-allele into 402 and 110 bp-long products. As a result of the A/G SNP genotyping, children were divided into genotype groups of AA, AG and GG. Due to low allele-frequency, no children possessed the G-allele in the S-allele of the 5-HTTLPR. For all children possessing the G-allele in the L-allele of the 5-HTTLPR ( $L_G$ ), L-allele is converted to S-allele and grouped accordingly. As a result of this triallelic categorization (S,  $L_A$ ,  $L_G$ ), final genotypes of children were categorized as SS (SS,  $SL_G$ ,  $L_GL_G$ ), SL ( $SL_A$ ,  $L_AL_G$ ) or LL ( $L_AL_A$ ).

#### 2.2.5 Salivary cortisol

In this study, HPA-axis activity is measured from cortisol levels in the saliva. Synthetic cotton-like rolls called Salivettes (Sarstedt, Germany) were used to collect saliva from children. In order to prevent the risk of choking, a thread was passed through the Salivettes by a needle and knotted at the end, so that children/mothers could hold the thread to secure the location of Salivettes during sample collection. Each child provided 8 Salivettes in total: morning and afternoon samples for two days at the daycare center by project assistants and two days at home by caregivers. Please see Table 3 for average sampling times at home and daycare.

Each saliva sample was analyzed in duplicates with Roche Cobas Cortisol assay in an automatized system (Roche Diagnostics, Indianapolis, USA) at Centro Laboratories, Istanbul. If there was more than .10  $\mu\text{g/dL}$  difference between the duplicates, that sample was reanalyzed. Intra-assay coefficient of variation (CV) was 10%, and inter-assay CVs were less than 5%. All cortisol values were converted from  $\mu\text{g/dL}$  to nmol/L prior to analyses by multiplying the value by 27. After this

conversion, the average of each duplicate was taken as the cortisol value for that sample.

Table 3. Average Saliva Sampling Times at Home and Daycare

	Morning			Afternoon		
	Mean	SD	Range (min - max)	Mean	SD	Range (min - max)
Home	10.23	.32	9.18 - 11.16	15.57	.28	14.88 - 16.42
Daycare	10.43	.38	9.88 - 11.58	15.37	.30	14.88 - 15.85

Note. Sampling times were converted to numbers (e.g. 10.50 represents 10:30)

Diurnal slope for each day was calculated by subtracting the afternoon value from the morning value. Since diurnal slopes for the two collection days were correlated for both home ( $r = .26, p < .05$ ) and daycare samples ( $r = .27, p < .01$ ), average scores of the two days were used in the analyses. For some children, samples for only one day was available. For those samples, cortisol values from that day was used ( $n = 20$ ).

### 2.3 Procedure

All experimental procedures were approved by Bogazici University Institutional Review Board, and permissions were obtained from Kağıthane, Bakırköy and Kartal municipalities and individual daycare centers. For families interested in participating in the study, signed consent forms were collected from mothers. Afterwards, mothers and teachers were given separate paper-and-pencil surveys to complete. In the mother's survey, in addition to the demographic information summarized in measures section, there were questionnaires about child's behavior, life events, and

home environment. In the teacher's survey, there were questionnaires about children's behavior such as SC, and the classroom environment. Saliva collection procedures for genotyping and cortisol analysis are explained in detail in the next sections.

### 2.3.1 Saliva collection for genotyping

Saliva samples for DNA extraction were collected through Oragene DNA collection tubes (DNA Genotek, Ottawa, Canada). In order to provide saliva for DNA extraction, children were to refrain from eating or drinking for at least 30 minutes prior to sample collection. Sampling times were determined according to daycare center's meal times. Daycare centers were also notified about these times and not providing children with additional snacks. Saliva sampling was conducted in groups of 1 to 5 children in available rooms of the daycare centers. Project assistants stimulated salivation in children by asking them questions, such as their favorite food, and collected saliva samples via the children spitting into the Oragene DNA collection tubes. The procedure took about 5 minutes. For children having a hard time spitting, project assistants facilitated salivating further by massaging children's cheeks and instructing them to move their tongues inside their mouths. After sampling, children were given a sticker of their choice.

### 2.3.2 Saliva collection for cortisol analysis

Saliva samples for cortisol analysis were collected through Salivette cotton rolls (Sarstedt, Germany) for two weekdays at the daycare centers by project assistants and for two days at the weekends at home by caregivers. Sampling for cortisol

analysis was only conducted in Kartal municipality daycare centers. Prior to sampling, consenting families were called and children's health conditions were asked. If the children were eligible (see eligibility criteria), sampling was performed at home and the daycare center. If children were eligible but had an ongoing acute illness, such as the flu, or were on medication for a short time, such as antibiotics, the sampling was postponed until the children recovered. The sampling was also postponed if the children had unusual plans for the weekend that might overly excite or stress them, such as having a doctor's appointment or birthday.

Mothers were given an appointment at the daycare centers for a one-on-one training session by a trained graduate student about saliva sampling. If the mother was working and unavailable to come to the daycare center, a trained graduate student gave her the training over the phone. After the training session, mothers were provided with a saliva collection package that included a saliva sampling guideline (see Appendix A for English translation, and Appendix B for Turkish version), a form with questions about child's sleeping patterns on the sampling days (see Appendix C for English translation, and Appendix D for Turkish version), and a total of 5 Salivettes labeled according to subject ID, collection day and time. One of the Salivettes was labeled differently and served as a backup, in case one of the 4 Salivettes could not be used.

Saliva collection procedures at home and at the daycare centers were similar and mostly adopted from Watamura et al. (2009). Collection times were set as 10:00-10:30 for the morning sample, and 15:30-16:00 for the afternoon sample. Children did not eat or drink (except water), brush their teeth, or perform excessive exercise or play for 30 minutes prior to collection. Children did not consume caffeinated food or drinks (including chocolate, tea and coffee), or take an afternoon nap 2 hours prior to

saliva collection. The Salivette was placed in the child's mouth, next to one of the cheeks for at least 3 minutes. If the Salivette was not soaked with saliva enough, it was kept in the mouth for longer. For sampling at home, mothers left a missing call to the project phones after sampling and took notes about sampling times, awakening times and sleeping times of the children on the saliva sampling forms provided. After sampling, the samples were placed into the refrigerator (at 4°C) as soon as possible. All forms and samples in the saliva sample collection package were returned to the daycares after sampling is completed. The two days of sample collection at home and at daycares were usually consecutive, with the average difference between sampling days being 1.47 (SD = 1.92) for home and 1.87 (SD = 1.86) for daycare. In case there was not enough saliva for analysis, the procedure was repeated.

#### 2.4 Statistical analyses

SPSS v22.0 was used for descriptive and inferential statistics together with the multilevel linear models. In order to test if children's SES was associated with children's SC as a function of sex and 5-HTTLPR genotype, a multilevel linear modeling approach was utilized. Children were nested within classrooms (teachers), and classrooms were nested within daycare centers. Therefore, the data was hierarchical; and children in the same classrooms had similar teacher ratings of SC than children in other classrooms. A random effect was included in the model, such that the children were nested within classrooms. The model was incorporated using a linear mixed models approach (Figure 4). For analysis, it was first tested if the model with random parameters significantly improved the first model with fixed parameters using maximum likelihood estimation. The significance of change in  $-2LL$  (log

likelihood) between fixed models and random models was significant in chi-square tests, and therefore random models were used.

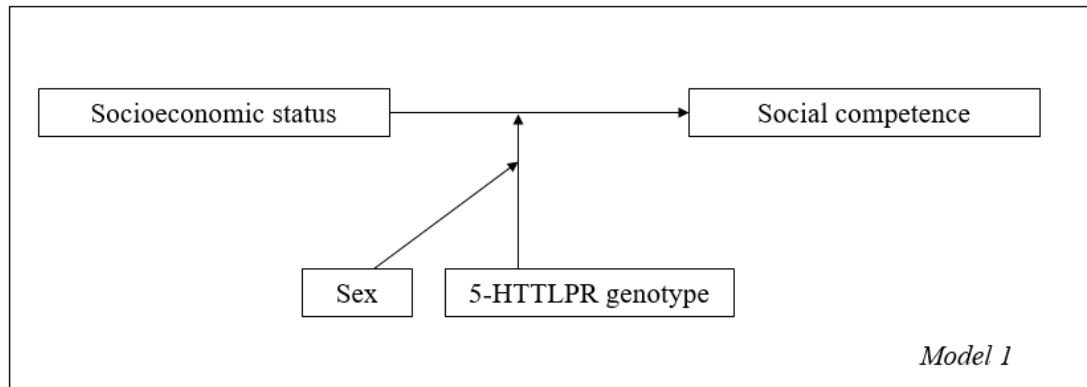


Figure 4. Multilevel moderation model testing the association between SES and SC, moderated by sex and 5-HTTLPR genotype

One aim of this study was to test the effect of SES on cortisol, and see if SC exerts any buffering on the effects of SES on HPA-axis activity. Another aim was to observe if sex and 5-HTTLPR genotype had any impact on this relationship. Therefore, a simple moderation model was used, allowing for two moderators and their interaction. Awakening time, sleep duration, and collection time were used as covariates only if they were significantly associated with the outcomes. The same model was conducted with morning and afternoon cortisol values and diurnal slopes at home and at daycare centers. All analyses were conducted for boys and girls separately. The model is given in Figure 5, testing the association between SES and HPA-axis activity, moderated by SC and 5-HTTLPR genotype. For the analyses, a multilevel approach was not used because there was not enough children per class and sex, since cortisol analyses were performed only in a subsample of the participants. Multilevel linear models were advised to be used in a sample of at least 100 participants (at least 10 groups, minimum 10 subjects in each group; see Hoyle

& Gottfredson, 2015). Therefore, “Model 3” option of PROCESS v2.0 plug-in of SPSS (Hayes, 2013) was used, via a bootstrapping approach for a moderated regression model. Since SC was a continuous variable, it was divided into three levels as -1 *SD* below the mean, the mean, and +1 *SD* above the mean, while calculating the conditional effects of the predictor (SES) on outcomes due to different levels of moderators.

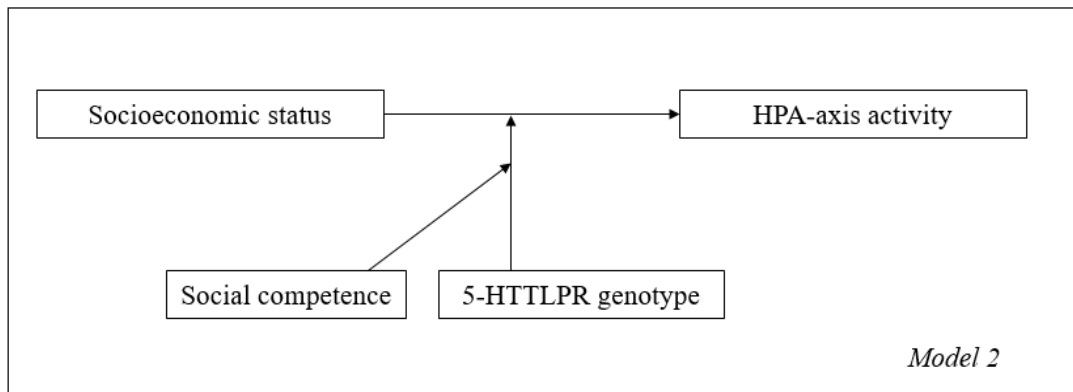


Figure 5. Moderation model testing the association between SES and HPA-axis activity, moderated by SC and 5-HTTLPR genotype

## CHAPTER 3

### RESULTS

#### 3.1 Interactions between SES, sex and 5-HTTLPR on social competence

In this model, we aimed to test the hypothesis related to the associations between SES and SC, moderated by sex and 5-HTTLPR. Descriptives of children's age, SES, and SC by sex are summarized in Table 4. Children's age ranged from 45 to 83 months. A series of *t*-tests revealed that boys and girls did not differ in terms of their age or SES ( $ps > .46$ ,  $ts < -.04$ ). Boys' SC ranged from 8 to 60, while girls' ranged from 21 to 60. Overall, girls had significantly higher SC than boys ( $t(224) = -2.60$ ,  $p = .01$ ). There were no significant correlations between age, SES and SC in either boys or girls ( $p > .05$ ). Therefore, age is not included as a covariate in any of the analyses.

The distribution of 5-HTTLPR genotypes ( $n = 263$ ) with and without the A/G SNP is shown in Table 5 separately for boys and girls. Allelic distribution was in Hardy-Weinberg equilibrium according to both triallelic (HWE;  $\chi^2(1, N: 257) = 2.80$ ,  $p > .05$ ) and biallelic classification (HWE;  $\chi^2(1, N: 263) = 0.64$ ,  $p > .05$ ). Age, SES and SC ( $ps > .61$ ), as well as distribution of sex ( $\chi^2(2, N: 257) = 2.42$ ,  $p = .30$ ) did not differ by triallelic classification of 5-HTTLPR genotype.

Table 4. Descriptive Statistics of Age, SES and SC by Sex

	Boys		Girls		<i>p</i>
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	
Age (months)	61.72	7.74	62.42	7.18	.47
SES	-.06	.85	-.05	.78	.97
SC	49.65	9.92	52.77	7.89	.01
Education (%)	Mothers		Fathers		
Less than high school	14		21		
High school	31		28		
Some higher education	55		51		
Income (%)	Below 3000 TL	3000 – 5000 TL	Above 5000 TL		
	37	36	27		

Table 5. Distribution of 5-HTTLPR Genotypes with and without the A/G SNP by Sex and Available Cortisol Data

		5-HTTLPR		5-HTTLPR + A/G SNP	
		Genotype analysis	Cortisol analysis	Genotype analysis	Cortisol analysis
SS	Boys	43	18	50	21
	Girls	28	10	34	11
	Total	71	28	84	32
SL	Boys	64	26	56	25
	Girls	61	18	58	20
	Total	125	44	114	45
LL	Boys	37	20	34	17
	Girls	30	16	25	13
	Total	67	36	59	30

A series of multilevel analyses were conducted in order to test the effect of fSES, 5-HTTLPR genotype, sex, and their interaction in a nested data structure. The assumptions for multilevel linear models, such as linearity, normality of residuals, homoscedasticity were met for all analyses.

In order to test if the data represented a hierarchical structure or not, first, the analysis with basic model with fixed parameters was performed, ignoring the nested data structure. Then, the intercepts were allowed to vary over the classrooms, introducing a random intercept. Since, this treatment improved the model as shown by the Chi-Square test results ( $-2LL_{\text{change}} = 47.44$  and  $df_{\text{change}} = 1$ ); it was established that the data significantly represented a hierarchical structure. Therefore, the random intercept model was selected. This model revealed a main effect of sex ( $F(1, 178.81) = 5.68, p = .018$ ), indicating that girls had higher SC than boys. There was also a significant three-way interaction between sex, 5-HTTLPR genotype, and SES (Figure 6,  $F(2, 181.96) = 3.37, p = .037$ ). Estimates of fixed effects revealed that in LL boys, there was a negative association between family SES and SC (Coef = -8.10,  $SE = 3.13, t(183.91) = -2.58, p = .011$ ).

In order to better explain the three-way interaction between family SES, 5-HTTLPR genotype and sex, the analysis was repeated for different genotype and sex groups, separately. In all analyses, random intercept model improved the fixed effect model, and thus only models with random intercepts are reported. First, the interaction was divided in terms of genotype, and different analyses for different genotype groups were performed. In SL or LL genotype, no significant main effects or interactions between SES and sex were present. However, in children with SS genotype, there was a main effect of sex ( $F(1, 58.10) = 6.65, p = .012$ ). This result suggested that for children with SS genotype, boys had lower SC than girls (Coef = -

4.86,  $SE = 1.88$ ,  $t(58.10) = -2.58$ ,  $p = .012$ ). There was also an interaction between SES and sex ( $F(1, 60.50) = 5.14$ ,  $p = .027$ ), which suggests that the association between SES and SC was opposite for SS boys and girls; such that higher SES was related to higher SC in boys, but lower SC in girls (Coef = 5.11,  $SE = 2.26$ ,  $t(60.50) = 2.27$ ,  $p = .027$ ).

When the three-way interaction was divided in terms of sex, in boys, no main effects or interaction were observed. There was a marginal main effect of 5-HTTLPR genotype, which might explain the marginal interaction between sex and 5-HTTLPR in the first analysis ( $F(2, 91.81) = 2.40$ ,  $p = .098$ ). However, in girls, there was an interaction between 5-HTTLPR genotype and SES ( $F(2, 89.24) = 3.47$ ,  $p = .035$ ). Estimates of fixed effects revealed that in LL girls, there was a positive relationship between SES and SC (Coef = 6.06,  $SE = 2.45$ ,  $t(93.04) = 2.47$ ,  $p = .015$ ). This effect was also significant for SL girls (Coef = 4.18,  $SE = 2.09$ ,  $t(85.01) = 1.99$ ,  $p = .049$ ). Please see Figure 6 for a depiction of the interaction by sex, and Table 6 for a summary of the results of the interaction.

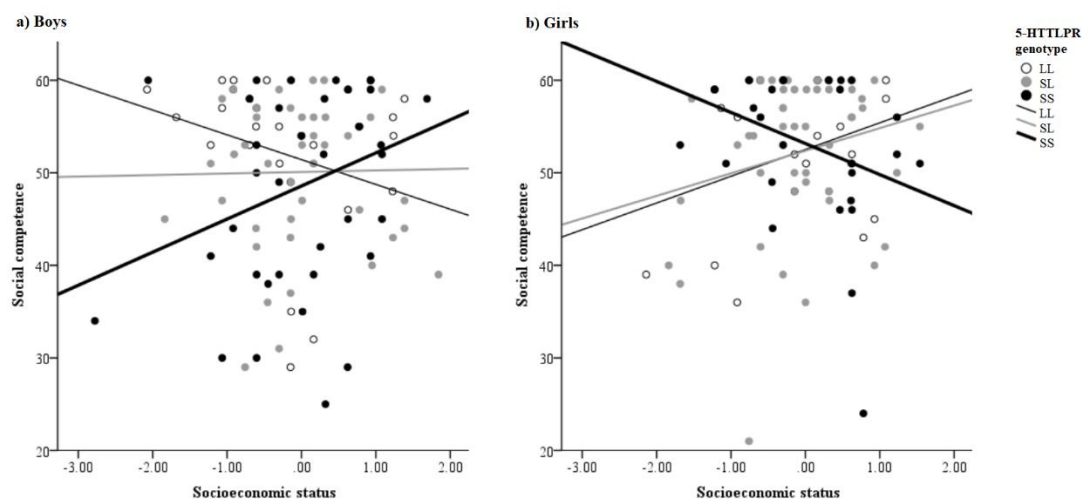


Figure 6. Scatterplot with best fit lines depicting the relationship between SES and SC in children, by 5-HTTLPR genotype. The figure is divided into two for a) boys and b) girls. The data points represent raw scores.

Table 6. Summary of the Three-Way Interaction between SES, 5-HTTLPR, and Sex on SC

		Social competence	
		Boys	Girls
Socioeconomic status ↑	SS	↑	↓
	SL	↔	↑
	LL	↓	↑

*Note.* Arrows pointing up and down indicate increases and decreases in SC, respectively. Sideway arrow indicates no significant change.

### 3.2 Interactions between SES, social competence and 5-HTTLPR on HPA-axis activity in boys and girls

In this model, we aimed to test the hypothesis related to the associations between SES and HPA-axis activity, moderated by SC and 5-HTTLPR separately for boys and girls. Descriptives of home and daycare cortisol measures and sleep-related factors for each sampling day are summarized in Table 7. For cortisol values at home and daycare, there were 5 outliers in total that were more than 3 SD above or below the mean. These values were excluded from all analyses. All cortisol values for Day 1 and Day 2 were significantly correlated with each other ( $r_s > .26, p_s < .05$ ). Paired sample t-tests revealed that for each sampling day, except for the second sampling day in daycare, afternoon cortisol was significantly lower than morning cortisol, indicating a decreased cortisol pattern during the day ( $t(95) = .15, p = .88$  for second sampling day in daycare,  $t_s(70-100) > 2.90, p_s < .01$  for other days). This decrease, measured by average difference scores, was more pronounced in home samples, than in daycare samples ( $t(93) = -5.83, p < .001$ ).

Table 7. Descriptives of All Cortisol Values at Home and Daycare by Sampling Days Together with Inter-Day Correlations

		Day 1		Day 2		<i>r</i>
		<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	
Home	Morning	4.92	3.12	5.72	4.07	.53***
	Afternoon	3.60	2.74	3.17	1.91	.71***
	Difference	1.35	3.56	2.56	3.92	.26*
Daycare	Morning	3.46	2.35	3.53	2.45	.63***
	Afternoon	3.10	2.12	3.55	2.35	.71***
	Difference	.38	1.63	.01	2.12	.27**

Unit for all cortisol measures is nmol/L. \*  $p < .05$ , \*\*  $p < .01$ , \*\*\*  $p < .001$ .

In order to test whether sampling location (home vs. daycare) and time of the day (morning vs. afternoon) influences cortisol levels, a 2x2 repeated measures ANOVA was conducted. The results revealed a main effect of location ( $F(1, 95) = 19.18, p < .01$ ) and time ( $F(1, 95) = 66.87, p < .01$ ), indicating that home cortisol was higher than daycare cortisol, and morning cortisol was higher than afternoon cortisol. There was also a significant interaction between sampling location and time ( $F(1, 95) = 32.57, p < .01$ ), such that morning cortisol was higher than afternoon cortisol only at home. Also, morning cortisol at home was higher than cortisol levels at daycare. However, awakening times ( $t(79) = 7.79, p < .01$ ) and sleeping durations ( $t(70) = 3.34, p < .01$ ) at home were significantly higher than the ones at daycare.

Among children with cortisol data, 39 % had an increase in cortisol from the morning sample to the afternoon sample at daycare, whereas 21 % had such a pattern at home. Please see Table 8 for a distribution of children's daily cortisol patterns from morning to afternoon at home, daycare, and both locations divided by sex. Chi square tests revealed no significant difference between boys and girls in terms of

their diurnal cortisol patterns at home ( $\chi^2 (1, N: 97) = 2.10, p = .21$ ), daycare ( $\chi^2 (1, N: 105) = .06, p = .84$ ), or both locations combined ( $\chi^2 (2, N: 94) = 4.28, p = .12$ ).

Table 8. Distribution of Changes in Children’s Cortisol Patterns from Morning to Afternoon by Sampling Location and Sex

Morning to afternoon (n)	Home		Daycare		Both	
	Boys	Girls	Boys	Girls	Boys	Girls
Increase	14	6	23	17	5	0
Decrease	40	37	39	26	23	20

In order to explore the relationships between demographic, psychological, genetic and cortisol measures, a series of Pearson correlations were run. The associations between psychological measures and cortisol measures at daycare and at home are summarized in Tables 9 and 10, respectively. As seen in Table 9, morning daycare cortisol was positively associated with afternoon daycare cortisol ( $r (104) = .68, p < .01$ ), diurnal slope ( $r (102) = .46, p < .01$ ) and morning sampling time ( $r (105) = .34, p < .01$ ). Similarly, afternoon daycare cortisol was negatively associated with the diurnal slope ( $r (102) = -.29, p < .01$ ) and positively associated with afternoon sampling time ( $r (80) = .26, p = .021$ ). As a result, sampling time is taken as a covariate in all subsequent analyses. In terms of the relationships between SES, SC and daycare cortisol measures, the only significant association was between SES and daycare diurnal slope, such that as SES increased, daycare diurnal slope increased ( $r (99) = .20, p = .045$ ), indicating a steeper slope. None of the cortisol related variables had a significant correlation with sex. A series of ANOVAs revealed that none of the cortisol-related variables differed by 5-HTTLPR genotype ( $ps > .05$ ). As seen in Table 10, morning home cortisol was positively associated

with afternoon home cortisol ( $r(96) = .60, p < .01$ ) and home diurnal slope ( $r(95) = .81, p < .01$ ). Awakening time at home had a positive correlation between morning home cortisol ( $r(82) = .39, p < .01$ ) and home diurnal slope ( $r(80) = .35, p < .01$ ), thus it was a covariate in analyses with those outcomes. Afternoon cortisol did not have any associations with the possible covariates. Therefore, covariates for cortisol-related analyses were slightly different for home and daycare values. SES positively predicted morning cortisol values at home ( $r(95) = .23, p = .021$ ). None of the cortisol related variables differed by sex or 5-HTTLPR genotype.

Table 9. Correlations between Study Variables with Daycare Related Cortisol Measures

	2	3	4	5	6	7	8	9	10	11	12
1. Age (months)	-.17*	.07	-.16	-.15	-.06	-.08	-.28*	-.15	.09	0.17	.05
2. SES	-	.06	.16	0	.20*	.09	.06	0.11	-.14	-.14	0
3. Social competence		-	-.06	.10	-.17	-.15	.07	-.13	-.07	.01	.17*
4. Morning cortisol			-	.68**	.46**	.16	.07	.34**	0	-.26**	.03
5. Afternoon cortisol				-	-.29**	.26*	.03	.27**	-.01	-.22*	-.05
6. Diurnal slope					-	-.10	.04	-.05	-.05	.03	-.03
7. Awakening time						-	.38**	.09	-.06	-.12	-.04
8. Sleeping duration							-	.14	.01	-.10	.08
9. Morning sampling time								-	-.01	-.79**	-.09
10. Afternoon sampling time									-	.62**	-.08
11. Sampling difference										-	.03
12. Sex											-

All cortisol related measures are averages of Day 1 and Day 2. Boys were coded as 0, girls were coded as 1. \*  $p < .05$ , \*\*  $p < .01$

Table 10. Correlations between Study Variables with Home Related Cortisol Measures

	2	3	4	5	6	7	8	9	10	11	12
1. Age (months)	-.17*	.07	-.04	0	-.03	-.01	-.03	-.18	-.14	.06	.05
2. SES	-	.06	.23*	.13	.19	-.07	-.21	-.02	.21*	.18	0
3. Social competence		-	-.11	.09	-.21	.05	-.04	-.07	-.02	.04	.17*
4. Morning cortisol			-	.60**	.81**	.39**	.10	.10	0	-.08	.04
5. Afternoon cortisol				-	.01	.21	.03	.11	-.10	-.17	-.17
6. Diurnal slope					-	.35**	.10	.05	.07	.02	.16
7. Awakening time						-	.55**	.47**	.14	-.27*	.08
8. Sleeping duration							-	.21	.16	-.04	.21
9. Morning sampling time								-	.21*	-.70**	-.10
10. Afternoon sampling time									-	.56**	.10
11. Sampling difference										-	.16
12. Sex											-

All cortisol related measures are averages of Day 1 and Day 2. Boys were coded as 0, girls were coded as 1.  $p < .05$ , \*\*  $p < .01$

In order to test the hypothesis related to the relationship between SES and HPA-axis activity, moderated by SC and 5-HTTLPR, moderated regressions were conducted for each HPA-axis outcome (morning cortisol, afternoon cortisol and diurnal slope at home and daycare), for boys and girls separately. Since the number of children with cortisol data were low for running models with nested designs (Hoyle & Gottfredson, 2015, see Statistical Analyses), multilevel linear models were not used. All analyses were performed using 10000 bootstrapped re-samples. The assumptions for moderated regression analyses, such as linearity, multicollinearity, normality of residuals, and homoscedasticity, were checked and fulfilled.

### 3.2.1 Interactions between SES, social competence and 5-HTTLPR on daycare diurnal slope

Moderated regression analyses were conducted in order to test the associations between SES and daycare diurnal slope, moderated by SC and 5-HTTLPR. For boys, the model was significant, indicating that the model was valid ( $F(7, 37) = 4.11, p < .01$ ). The model explained 44% of the variance in daycare diurnal slope (Table 11). Results indicated that SES positively predicted daycare diurnal slope ( $B = 13.30, SE = 4.68, p = .007, CI = 3.83, 22.78$ ), such that as SES increased, daycare diurnal slope increased. There was also an interaction between SES and SC ( $B = -22.40, SE = .08, p = .01, CI = -.39, -.05$ ), indicating the effect of SES on daycare diurnal slope differed as a function of SC. The interaction between SES and 5-HTTLPR genotype was also significant ( $B = -11.54, SE = 2.84, p < .001, CI = -17.29, -5.79$ ), together with a significant three-way interaction between SES, SC and 5-HTTLPR genotype ( $B = .20, SE = .05, p < .001, CI = .10, .30$ ). This three-way interaction significantly increased the explained variance of the model by 22%. Please see Table 12 for the

conditional effects of SES on daycare diurnal slope at different levels of the moderators. Also see Figure 7 for a depiction of the results for boys.

Table 11. SES Predicting Daycare Diurnal Slope, Moderated by 5-HTTLPR Genotype and SC in Boys

	<i>B</i>	<i>SE</i>	<i>t</i>	<i>p</i>
Constant	2.45	2.02	1.22	.23
Social competence	-.04	.04	-1.07	.29
SES	13.30	4.68	2.84	.01
SES x Social competence	-.22	.08	-2.67	.01
5-HTTLPR genotype	-.38	.99	-.38	.70
SES x 5-HTTLPR genotype	-11.54	2.84	-4.07	< .01
Social competence x 5-HTTLPR genotype	.01	.02	.50	.62
SES x 5-HTTLPR x Social competence	.20	.05	3.89	< .01

Table 12. The Effect of SES on Daycare Diurnal Slope at Different Levels of 5-HTTLPR Genotype and SC in Boys

5-HTTLPR genotype	Social competence	Effect	<i>SE</i>	<i>t</i>	<i>p</i>	Lower CI	Higher CI
LL	-1 SD	2.38	.84	2.82	.01	.67	4.09
LL	Mean	1.18	.43	2.77	.01	.31	2.04
LL	+1 SD	< .001	.48	.01	.99	-.97	.98
SL	-1 SD	.20	.47	.42	.67	-.76	1.16
SL	Mean	.24	.26	.94	.35	-.28	.77
SL	+1 SD	.29	.33	.87	.39	-.38	.96
SS	-1 SD	-1.97	.49	-4.06	< .01	-2.96	-.99
SS	Mean	-.69	.29	-2.36	.02	-1.28	-.10
SS	+1 SD	.57	.39	1.47	.15	-.22	1.36

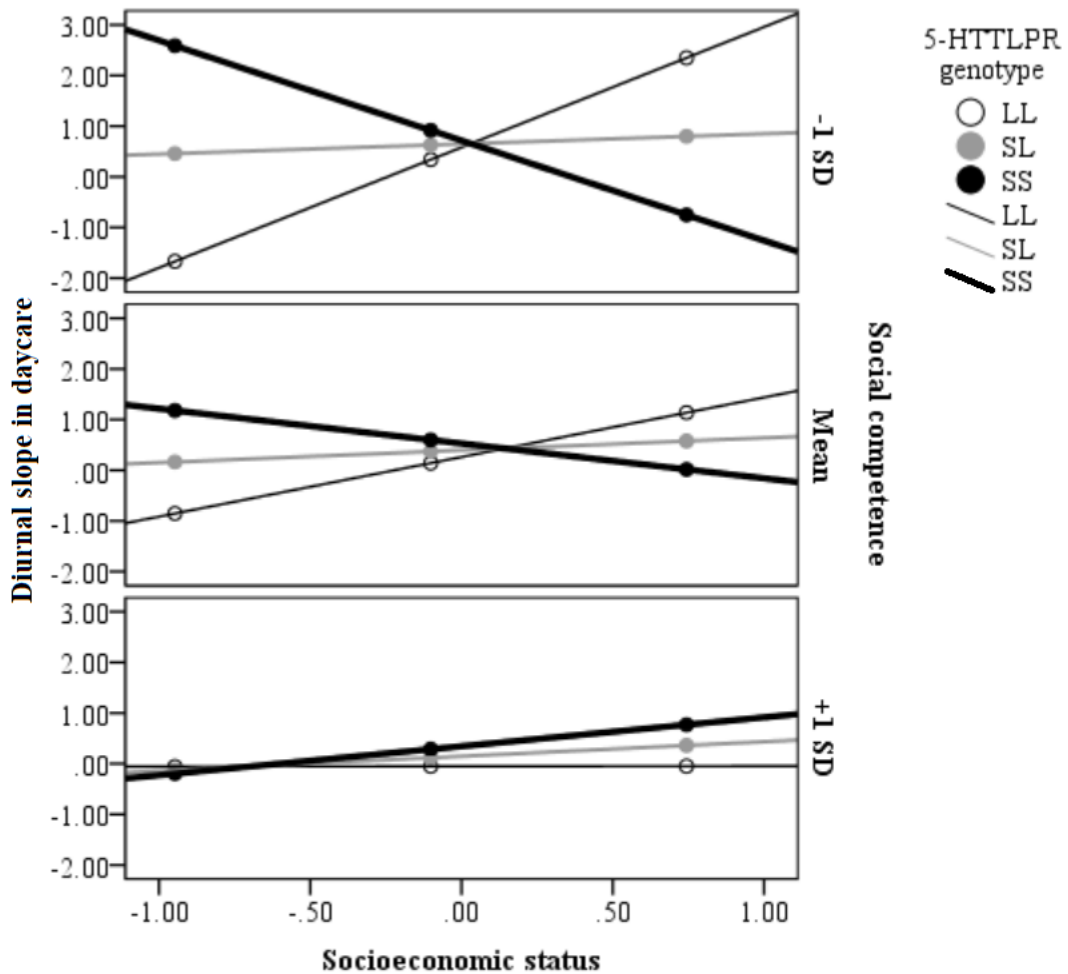


Figure 7. Line graph depicting the association between SES and daycare diurnal slope by 5-HTTLPR genotype in boys

The three-way interaction revealed that for boys with SC levels around and below the mean, there was a positive relationship between SES and daycare diurnal slope only in LL boys. However, this effect was reversed in SS boys of similar SC, indicating a negative association between SES and daycare diurnal slope. There were no significant associations between SES and daycare diurnal slopes in SS and LL boys with +1 SD SC, or boys with SL genotype ( $ps > .15$ ).

For girls, the model predicting the daycare diurnal slope from SES, with 5-HTTLPR genotype and SC as moderators, was not significant, indicating that the

model was not valid ( $F(7, 27) = .29, p = .95$ ). Thus, for girls, fSES did not have an effect on daycare diurnal slopes.

### 3.2.2 Interactions between SES, social competence and 5-HTTLPR on home diurnal slope

Moderated regression analyses were conducted in order to test the associations between SES and home diurnal slope, moderated by SC and 5-HTTLPR. For boys, the model was not significant, indicating that the model was not valid ( $F(8, 26) = 2.03, p = .08$ ).

For girls, the same model was valid, explaining 18% of the variance in home diurnal slope ( $F(8, 22) = 2.84, p = .02$ ). However, there were no significant effects of the predictor or the moderators on the home diurnal slope. Thus, SES did not have any associations with the home diurnal slope for both boys and girls.

### 3.2.3 Interactions between SES, social competence and 5-HTTLPR on daycare morning cortisol

Moderated regression analyses were conducted in order to test the associations between SES and daycare morning cortisol, moderated by SC and 5-HTTLPR. For boys, the model predicting the morning cortisol values from SES with 5-HTTLPR genotype and SC as moderators was significant, indicating that the model was valid ( $F(8, 37) = 2.94, p = .01$ ), explaining 47% of the variance in morning cortisol values in daycare. However, none of the moderators or the predictor had a significant contribution to the model, suggesting that the explained variance was mostly related

to the covariate, morning sampling time ( $B = 3.57$ ,  $SE = 1.20$ ,  $p < .01$ ,  $CI = 1.14$ ,  $5.99$ ).

For girls, the model predicting the morning cortisol from SES with 5-HTTLPR genotype and SC as moderators was not significant, indicating that the model was not valid ( $F(8, 25) = 2.09$ ,  $p = .08$ ). Therefore, SES did not have an association with morning daycare cortisol in both boys and girls.

#### 3.2.4 Interactions between SES, social competence and 5-HTTLPR on home morning cortisol

Moderated regression analyses were conducted in order to test the associations between SES and home morning cortisol, moderated by SC and 5-HTTLPR. For boys, the model was not significant, indicating that the model was not valid ( $F(8, 27) = 1.11$ ,  $p = .39$ ).

For girls, the same model was valid, explaining 26 % of the variance in diurnal slope ( $F(8, 22) = 3.34$ ,  $p = .01$ ). However, there were no significant effects of the predictor or the moderators on the diurnal slope at home. The model might be significant due to marginal effect of the covariate, awakening time ( $B = 1.55$ ,  $SE = .93$ ,  $p = .11$ ,  $CI = -.38$ ,  $3.48$ ). Thus, SES did not have any association with the morning cortisol values at home for both boys and girls.

#### 3.2.5 Interactions between SES, social competence and 5-HTTLPR on daycare afternoon cortisol

Moderated regression analyses were conducted in order to test the associations between SES and daycare afternoon cortisol, moderated by SC and 5-HTTLPR. The models were not significant for either boys ( $F(8, 27) = 1.07$ ,  $p = .42$ ) or girls ( $F(8, 21) = 1.50$ ,  $p = .22$ ), so they were not valid. Therefore, SES did not

have any association with the afternoon cortisol values at daycare for both boys and girls.

### 3.2.6 Interactions between SES, social competence and 5-HTTLPR on home afternoon cortisol

Moderated regression analyses were conducted in order to test the associations between SES and home afternoon cortisol, moderated by SC and 5-HTTLPR. The models predicting afternoon cortisol values in home were not significant for either boys ( $F(7, 33) = .37, p = .91$ ) or girls ( $F(7, 26) = 1.16, p = .36$ ), and thus, were not valid. Therefore, SES did not have any association with the afternoon home cortisol, neither for boys or girls.

## CHAPTER 4

### DISCUSSION

The aim of this study was to investigate primarily the associations between SES and SC in preschoolers aged 4-6, depending on their sex and 5-HTTLPR genotype. Secondly, it aimed to examine the relationships between SES and HPA-axis activity in daycare and home, how SC effects these relationships in boys and girls, moderated by 5-HTTLPR genotype. Although there were studies about the influence of SES on SC (Guidubaldi & Perry, 1984; Assis et al., 2009) and HPA-axis activity (Lupien et al., 2001; Chen et al., 2010), the relationships between these variables were not entirely understood, the sex differences were not examined, and the role of 5-HTTLPR as a moderator was not studied. In addition, although some studies hinted that indices of SC might buffer the relationship between adverse environment and mental health outcomes such as HPA-axis (Alink et al., 2012), no study so far examined the possible buffering role of SC in the association between SES and HPA-axis activity. In this study, the associations between SES, SC and HPA-axis were tested for the first time, considering the possible differential effects for boys and girls, with 5-HTTLPR genotype as a moderator.

#### 4.1 Role of sex and 5-HTTLPR genotype on the relationship between SES and social competence

It was hypothesized that there would be a three-way interaction between sex, 5-HTTLPR genotype and SES; such that S allele carrier boys' SC would be altered as a function of SES, since boys were reported to be affected from environmental challenges more than girls (Emery, 1982; Rutter, 1982; Gamble & Zigler; 1986), and

S allele of 5-HTTLPR was repeatedly associated with susceptibility to environment (Caspi et al., 2010). Since a recent meta-analysis reported that for Caucasian children, S allele might be a plasticity, rather than vulnerability marker (van IJzendoorn et al., 2012), it was expected that the aforementioned association would be in a ‘for better or for worse’ manner, or would support the contrastive effects model. As predicted, the results revealed a significant three-way interaction, in the exact same pattern for SS boys. For SS boys, as SES increased, SC increased. However, this pattern was not observed for SL boys, and the exact opposite pattern was observed for LL boys. These surprising results indicated that, as for boys, contrastive effects, rather than differential susceptibility or diathesis-stress, was suitable as a model in explaining environmental susceptibility of boys, in terms of their 5-HTTLPR genotype. Because no association between SES and SC in boys with SL genotype was observed, and the effect of SES on SC was opposite for SS and LL boys, there is probably an additive effect of S allele. Thus, dominant view of S allele (i.e. grouping SS and SL genotypes together) in studies with 5-HTTLPR genotype do not fit with the results of this study, at least in boys. These results suggest that increases in SES might improve “effectiveness in social interactions”, i.e. SC, in SS boys but might deteriorate SC in LL boys. Therefore, increases in SES might not be adaptive for all genotype groups in boys, at least in terms of social interactions.

As for girls, it was predicted that there would not be any GxEs on SC. This prediction failed such that for SL and LL girls, there was a positive association between SES and SC, but a negative association was present in SS girls. This finding was unexpected, since girls in such ages were reported to be more resilient to environmental difficulties than boys at home and daycare conditions (Emery, 1982;

Rutter, 1982; Gamble & Zigler; 1986). The finding also appears to be counterintuitive for several reasons. First of all, in SL girls, SES was associated with SC in exactly the same pattern as LL girls. This result does not match with either additive or dominant model of 5-HTTLPR, and supports the recessive model. Moreover, S-allele was commonly reported to be a ‘vulnerability’ or ‘plasticity’ marker. In studies supporting vulnerability, it was reported that S allele carriers function worse in adverse environments (dual risk; Caspi et al., 2010). In studies supporting plasticity, it was shown to generally behave in a ‘for better or for worse’ manner, according to changes in environmental adversity or support (van IJzendoorn et al., 2012). According to the results reported in this thesis, the expected ‘risk’ of genotype was observed in L-allele carrier, but not SS girls. Thus, our results do not support plasticity or vulnerability view for the 5-HTTLPR genotype. Although not as frequently studied as diathesis-stress and differential susceptibility, findings supporting contrastive effects are common in GxE research (Canli et al., 2006, Alexander et al., 2009, Mueller et al., 2011, Duman, 2012, Duman, 2016). Contrastive effects are rarely reported in developmental psychobiology. One such study is conducted by Brock, Kochanska and Boldt (2017), reporting a trend towards significance for children with low plasticity having LL genotype, as interparental relationship satisfaction increased, children’s internalizing problems over time also increased. The pattern was opposite for high plasticity children, having S-allele. Therefore, although speculative, our findings add to the literature supporting this model, and emphasizing its importance in explaining individual differences in developmental psychobiology.

The role of 5-HTTLPR genotype seems to be different for boys and girls in moderating the association between SES and SC, and this kind of a sex specific

effect might be controversial, but it is not one of a kind. For example, sex specific effects on the moderating role of 5-HTTLPR in the association between childhood SES and depression (Brummet et al., 2008), and delinquent behavior (Åslund et al., 2013) were reported before. In those studies, in adverse environments, different genotypes were associated with different outcomes in males and females. Thus, this study adds to the literature reporting sex specific SES - 5-HTTLPR interactions.

Moreover, it was predicted that girls would have higher levels of SC than boys, since a skill-set based assessment tool (SCBE-30) was used in this study, and girls were repeatedly shown to have higher SC with such measurements (LaFreniere & Dumas, 1996; Çorapçı et al., 2010; Barbu et al., 2011). As expected, this was also the case with our sample of preschoolers. There were not any significant differences in SC in between different genotype groups, as expected. However, contrary to expectations, age did not have any effect on SC. This might be due to the age range in our sample. Although there is some difference between children's ages, they are all preschoolers, thus in the same developmental period and expected to have similar social skills (Waters & Sroufe, 1983).

Overall, the results supported contrastive effect models, but not diathesis-stress or differential susceptibility models, for both boys and girls. The behavior of genotypes due to environmental changes were particularly distinct for boys and girls. These results suggest that boys and girls, as well as all children with different 5-HTTLPR genotypes, might be affected from the environment differently. Also, decreases in SES might not always lead to decreases in SC levels, especially for LL boys and SS girls. Moreover, increases in SC might not be the same for boys and girls, in terms of adaptability to environment. In higher SES backgrounds, low SC might indeed be adaptive for LL boys, but maladaptive for SL and LL girls.

#### 4.2 Role of social competence, sex and 5-HTTLPR genotype on the relationship between SES and HPA-axis activity

It was previously reported that, a healthier diurnal response, meaning larger difference between morning and evening levels, was observed in cortisol values at home than in those at daycare (Watanura, Kryzer & Robertson, 2009). The results of this study was in line with previous findings, meaning that the demand of frequently being in social interactions in daycare, and other daycare stressors might disrupt daily cortisol rhythms (Watanura et al., 2009).

Furthermore, it was predicted that none of the HPA axis activity markers measured at home or daycare (morning cortisol, afternoon cortisol, and the diurnal slope) would differ by 5-HTTLPR genotype or sex. According to the study results, this prediction was accurate. However, contrary to expectations, no main effect of SC was found on any of the cortisol measures in daycare, either in boys or girls. Thus, the findings reporting a positive association between SC and diurnal slope was not replicated (Gunnar & Donzella, 2002; Lengua et al., 2013). However, it should be noted that participants' age, and assessment technique for SC were different between this study and theirs, such that Lengua and colleagues performed their study with 3 year olds, while Gunnar and Donzella reported findings using peer popularity as a measure of SC, which may contribute to differences in results.

As expected, SES positively predicted daycare diurnal slope in boys, which is in line with other studies (Lupien et al., 2001; Chen, Cohen & Miller, 2010). This indicates that decreases in SES might be related to dysfunction in HPA-axis activity. Flatter diurnal cortisol slopes were associated with poorer mental and physical health outcomes (Adam et al., 2017). SES was also previously shown to be related to poorer mental health outcomes in children, such as childhood depression (Bradley &

Corwyn, 2002). This association between SES and daycare diurnal cortisol slopes might be important in explaining the detrimental effects of SES in boys' mental health. This association was not present in girls, as expected. This result indicates that boys might indeed be more sensitive to environmental challenges, than girls. It was also hypothesized that in boys, the relationship between SES and diurnal slope in daycare might be moderated in terms of SC levels. It was expected that SC might buffer the possible deleterious effect of SES on HPA axis activity. The results indicated that as SC increased, the effect of SES on diurnal slope disappeared, such that there was no significant association between SES and diurnal slope in boys with high SC; thus expectations were confirmed

It was expected that SC might buffer the possible adverse effects of SES on diurnal slope, especially in S allele carrier boys. Contrary to expectations, in SS boys with lower SC levels (around the mean and -1 SD), SES had a negative association with diurnal slope, such that as SES increased, diurnal slope decreased. Interestingly, the opposite pattern was observed in LL boys, such that increases in SES predicted higher diurnal slope. Also, SC levels did not have any impact on the association between SES and diurnal slope in SL boys. As mentioned above, in high SC levels (+1 SD), there was no association between SES and diurnal slope in any of the genotype groups. These results did not match with the expectations. The pattern was exactly opposite to expectations in SS boys. The expected pattern for SS boys was observed in LL boys, and no pattern was observed in SL. These results also do not match with the dominant model for S-allele, such that additive effects are supported.

It was hypothesized that the possible association between SES and diurnal slope in daycare, moderated by 5-HTTLPR and SC, would not be present for girls due to girls being more resilient to adverse environment than boys, which was indeed

the case. This supports the previous studies reporting girls might be more resilient to challenges of daycare (Gamble & Zigler, 1986), and other overall environmental challenges (Emery, 1982; Rutter, 1982; Gamble & Zigler; 1986); at least in terms of HPA-axis activity in daycare.

There were not any significant associations between SES and other HPA axis markers. No effect of SC was expected in the relationship between SES and HPA axis markers at home, since in this study, peer SC was used. We did not expect increases in peer SC to buffer the deleterious effects of SES at home levels of cortisol, which was exactly the case.

Finally, in this study, we preferred to use SC as a moderator in the relationship between SES and HPA-axis activity. However, one cannot be sure about the direction of the relationship between SC and HPA-axis activity. It might also be the case that, increased HPA-axis activity due to constant stress (i.e. lower SES) might affect children's SC. In this study, we tested the buffering factor of SC in SES – HPA-axis relationship, but it is possible for healthy cortisol responses to moderate the relationship between SES and SC, as tested in Lengua et al., 2013. In their study with 3-year olds, they reported that SES predicted SC indirectly, through its marginal associations with diurnal cortisol slope and effortful control. However, it was a trend ( $p = .10$ ) and the authors report that the associations do not add much to explaining the relationship between SES and SC. In order to fully understand the nature and direction of this relationship, longitudinal studies measuring preschool children's SC and HPA-axis activity over time should be performed, and analyses should reveal the actual direction. Most probably, SC and HPA-axis influence each other, and there is not a single direction.

These results are in line with other studies suggesting a buffering effect of SC in the effect of an adverse environmental factor on HPA axis activity (Alink et al., 2012). Results also suggest that boys might be more susceptible to environmental factors than girls, at least about the impact of SES on diurnal slope. In lower SC values, for boys with LL and SS genotypes, the effect of SES on diurnal slope was opposite. Meaning that, the impact of SES on HPA-axis activity might be different for LL and SS boys. This pattern is congruent with contrastive effects model, as seen in our previous finding about SC. A similar pattern was observed in studies using HPA-axis reactivity as an outcome, exploring the effects of stressful life events and childhood maltreatment (Alexander et al., 2009; Mueller et al., 2011; Duman, 2016). Together, our results suggest that adaptations of different genotype groups to the environment might be distinct from each other. In order to better understand if adaptability to the environment is actually different or not in different genotype groups, one should adopt a longitudinal approach and see changes in diurnal slope over time, as a function of SES and SC. We reported our finding only in boys, but we cannot compare the studies in terms of sex differences, because in Alexander et al. (2009), all participants were male, and in Mueller et al. (2011), sex was introduced as a covariate.

#### 4.3 Strengths, limitations and future directions

The present study has several strengths. First of all, this is the first study to consider sex and 5-HTTLPR genotype related differences in the associations between SES, SC, and HPA-axis activity. It presents a meaningful contribution to the literature of sex differences in the development of social behavior, and environmental

susceptibility. It also takes A/G SNP rs25531 into account instead of genotyping participants' DNA only for 5-HTTLPR, in order to be biologically accurate.

Another strength of the study was the careful timing of cortisol sampling. Most of the sampling times fall within the designated time intervals. Also, we were careful about children's school adjustment period, and did not perform sampling between the beginning of the school year (September) and December, which is also congruent with the suggestions of Miller et al., 2016. In their paper, they warn researchers about the possible changes in cortisol concentrations according to the changes in seasons. We were careful about performing the sampling around the same months for both sampling years. These considerations are important in the accuracy of our cortisol sampling.

An important strength of our study was the sample size of the children participating in saliva sampling for DNA analyses. This sample size allowed us to conduct a multilevel linear model, which is appropriate for the nested structure of our data.

There are some limitations of this study that should be addressed. First of all, although the variable was normally distributed, the variability in SES in this sample might not be representative of the whole distribution of family SES in the general population. This study was performed only in municipality daycare centers, and children were usually from neighborhoods with similar socioeconomic factors. As can be seen in Table 2, most parents had at least high school education, and more than half of families had an income higher than 3000 TL. It might be the case that, parents with higher SES agreed to participate in our study. Future studies investigating similar research questions may benefit from collecting data from a

better representative sample, including neighborhoods with better and worse SES, and also from private daycare centers.

Furthermore, in this study, a skill-set based assessment of SC was performed, and it is widely accepted that girls outscore boys in those measurements. For a lot of girls, teachers reported the maximum possible SC score, which might affect the results of this study. In addition, it was also argued that girls and boys have ‘different cultures’ (Crombie, 1988), and the actual criteria for being a socially competent child might be different for girls and boys. Therefore, other studies using different assessment methods for SC, such as sociometrics, might not replicate our results since boys and girls do not differ in SC based on sociometrics measures (Volling et al., 1993). If sociometrics measures were to be used in this study, the results might not be drastically different between boys and girls. Future studies should also use other SC measures such as peer acceptance, and try to replicate our results in order to find if adaptation of different methods would influence the results. It would be also useful in order to understand if these measures actually represent different aspects of SC at a biological level. For example, it is possible that one can find significant interactions with one measure of SC, but fail to find an interaction using another. Also, one measure of SC might be associated with HPA-axis activity while the other might not.

Moreover, in this study, we followed a careful procedure to track cortisol sampling times at home. Caregivers were asked to note down the sampling time, and call the research assistants right after sampling. These two sampling times were cross-checked later. However, there might be unreported differences between denoted and actual sampling times. Although sampling time affected only some of the cortisol measures, it can still affect the reliability of the results. However, the

high correlations between Day 1 and Day 2 samples reveal that the cortisol values were mostly consistent across two days, adding to the reliability of our data. Therefore, in addition to what we did, objective methods, such as caps with time stamps, can also be used in cortisol sampling as suggested before (Stalder et al., 2016). Also, for some children, consecutive sampling at daycare and home could not take place, due to the children being sick, or caregivers being unavailable. This also might affect the reliability of the results. Another problem that might be generalized to all similar studies is that, children have particularly different habits at home and daycare. For example, their eating and sleeping schedule is usually different at home than daycare.

Another limitation of the study was the sample size of children participated in cortisol sampling. Although we had the data of approximately 100 children, the study with cortisol levels aimed to find interactions between 5-HTTLPR genotype, SES and SC, and sample size per classroom was not enough to perform a multilevel linear model for three genotype groups. Therefore, a random intercept was not introduced to any of the models using a cortisol measure as an outcome. Because SC scores of children from the same classrooms might be similar to each other than those from other classrooms, this might be a problem for the accuracy of the results. Therefore, these results should be replicated with a bigger sample, acknowledging the possible nested structure of the data, using multilevel models.

Finally, results of this study did not match with most commonly studied environmental sensitivity models, namely diathesis – stress and differential susceptibility. There is a gap in the literature such that no possible theoretical explanation about contrastive effects model is present. Future studies should focus more on this model.

#### 4.4 Contributions and implications

The results of this thesis indicate that SC levels of boys and girls from the same genotype groups were influenced by SES levels in an exact opposite way. This provides another example that boys and girls might be affected from the same environmental condition differently. This opposite pattern might also mean that the nature and implications of SC might indeed be different for boys and girls.

Moreover, for diurnal slope values in daycare, an association was observed for boys, but no impact of SES was found in girls. This finding is important in explaining how SES might affect mental and physical health in boys, and adds to the literature about sex differences in susceptibility to environment. In their recent paper, Del Giudice and colleagues hypothesized that males might show more developmental plasticity than females, due to higher exposure to androgens in early life; and this can be an explanation for the observed effect (Del Giudice et al., 2018).

This is the first study to show that the association between SES and SC is moderated by 5-HTTLPR genotype and sex. Our findings are important in explaining the underlying relationship between SES and SC. It shows that SES and SC are not positively associated in all sex and genotype groups. It is also the first study to suggest that the impact of SES on HPA axis activity might be buffered by SC, in males. It provides another clue about 5-HTTLPR genotype might not be a plasticity or vulnerability marker, especially in the case of HPA-axis activity. It also emphasized the need to perform more research on contrastive effects, also in developmental psychobiology. Our results are also important in evaluating the appropriate pattern of inheritance. Studies usually use the dominant view such that they group S-allele carriers and LL homozygotes separately. However, our overall results did not match with either pattern of inheritance (dominant, recessive or

additive) Thus, treating the three genotype groups separately is suggested for future studies using 5-HTTLPR as a genetic marker.

The findings are important in explaining the relationship between SES and SC, and SES and HPA axis activity in different sex and genotype groups. The findings also show that the development of social behavior might be different for boys and girls, carrying certain genotypes. In the future, the results might lead to constructing different, personalized intervention methods for the sake of a healthier diurnal cortisol rhythm and increased social adjustment in daycare environments that might lead to better mental and physical health outcomes for children, in the future.

APPENDIX A  
SALIVA SAMPLING GUIDELINE

**Guideline for saliva sampling from children at weekends at home**

This guideline summarizes the important points that need attention while sampling saliva twice a day on Saturdays and Sundays. Please read carefully.

**On the night prior to sampling and the sampling day:**

Please make sure that your child

1. Is not ill (e.g. common cold)
2. Is not on any medication
3. Does not wear face or lip cream
4. Does not have any bleeding in his/her gums, does not have any wound or infection in his/her mouth

If any of the abovementioned conditions occur, please do not perform saliva sampling, and inform the experimenter.

**PRIOR TO 2 HOURS of saliva sampling, please:**

- Do not give your children any caffeinated drinks and foods such as cola, tea, coffee, and chocolate.
- Make sure that your child is awake from his/her afternoon nap.

**PRIOR TO 30 MINUTES of saliva sampling, please:**

- Do not let your child consume any foods or drinks.
- Do not let your child brush his/her teeth.

- Be careful that your child does not perform excessive physical activity (e.g. running, jumping, going up the hill).

### **Saliva sampling procedure on the weekends**

1. Please collect the sample as close as possible to 10:00 IN THE MORNING, and 15:30 IN THE AFTERNOON.
2. Sampling tube consists of a small tube, a big tube, a cotton roll and a blue cap.
3. Please collect the sample while your child is properly seated, and wash your hands before starting.
4. Take out the appropriate sampling tube which been labelled in advance, and shows the exact time and hour for the sampling from the zip lock bag.
5. Remove the blue cap and place it on a clean tissue. Do not remove the small tube inside. If it comes off, please place it back.
6. Place the cotton roll inside the tube into your child's cheek in such a way that the thread placed at the end of the cotton roll should from your child's mouth.
7. In order for the cotton roll to absorb the saliva thoroughly, keep the cotton roll in your child's mouth for at least 3 minutes. Keep waiting if the cotton roll is not wet enough. Meanwhile, you can control the cotton roll by holding its thread.
8. After the cotton roll is thoroughly wet, by holding the thread, remove it from your child's mouth. Place the cotton roll back into its tube and make sure that it does not have contact with your hand or any surface, and close the blue cap TIGHTLY.
9. Please keep the samples in the fridge (not in deep freezer or freezer), in their zip lock bag.

10. Write down the sampling time in the date-time chart below.
11. Please give us a missed call from the phone number you have been previously called as soon as you finish the procedure. You will be notified that your call has been received when we see your missed call. Please follow this procedure exactly in the same way for both the morning and the afternoon samples.

**Delivering the saliva samples**

On Monday morning, take out the samples from the fridge, and put them into the envelope with all the documents you have filled out, right before your child leaves the house. After tightly closing the envelope, send it to daycare center with your child so that s/he hands it to their teacher.

We always receive several questions from participating families. If you have a question that is not answered in this guideline, please do not hesitate to contact us.

Our contact numbers: **0535 739 3626**                      **0533 739 3656**

**Please do not forget to fill out these charts below.**

**For saliva samples collected at DAYCARE CENTER ON WEEKDAYS**

	<b>Date</b>	<b>Sleeping time in the previous night</b>	<b>Awakening time in the morning</b>
<b>Day 1</b>			
<b>Day 2</b>			

**For saliva samples collected at HOME ON WEEKENDS**

	Date	Sleeping time in the previous night	Awakening time in the morning	Sampling time in the morning	Sampling time in the afternoon	Time of placing the samples in the fridge (morning / afternoon)	
<b>Saturday</b>							
<b>Sunday</b>							

Please write down any problems occurred during sampling below:

**Frequently Asked Questions**

- 1- Why do we collect saliva samples? Can any of the sampling materials cause harm to my child?

Physiological measurements about your child’s mood are performed using saliva samples. The cotton roll for absorbing the saliva is used in this kind of studies all over the world and does not have any harmful effects on your child’s health.

- 2- I realized that I am not able to / I could not collect the samples for 1 or 2 days on/at the weekend. May I perform the sampling on any weekday instead?

The samples are required to be collected on weekends. If you realize that you are unable to perform sampling on the arranged sampling dates, please contact us in order to rearrange the sampling procedure date.

- 3- We plan to do an activity (e.g. trip, picnic, playing football etc.) together with my child on this weekend. Can we perform the sampling outside?

If this is an activity that will be excessively tiring to your child, please do not collect the samples on that day and contact us beforehand. If it is an activity

that is not physically tiring, you may collect the samples. However, please place the samples to the fridge as soon as possible.

4- I forgot to perform the sampling on the required time. What should I do?

If there has been less than 30 minutes from the required time, you can IMMEDIATELY collect the samples. If more time has passed, please inform us so we can guide you. Please remember that it is important to follow the required sampling times for us to obtain reliable data.

5- My child removed the cotton roll from his/her mouth while sampling. The cotton roll fell down or contacted with a surface. What should I do?

Please do not proceed sampling with the same cotton roll. In such a case, you may use the orange tube labelled as spare collection and repeat the sampling procedure. Please write down sampling date and time (such as Saturday Morning) on the spare tube. If you had to use the spare tube before, please contact us.

6- My child ate/ drank something/ brushed his-her teeth/ performed excessive physical activity half an hour prior to sampling time, without me noticing. What should I do?

If you realized this situation after sampling, please note it down in the provided space below the date and time chart. If you have not performed sampling yet, please wait half an hour for sampling and note the sampling time again.

7- After collecting the morning sample, I realized that my child is ill (showing symptoms such as sore throat, fever, and runny nose). What should I do?

Please do not perform the next sampling and contact us.

## APPENDIX B

### SALIVA SAMPLING GUIDELINE

(TURKISH)

## Çocuklar için Hafta Sonu Evde Tükürük Örneği Toplama Kılavuzu

Bu kılavuz Cumartesi ve Pazar günleri ikişer tükürük örneği toplarken dikkat etmeniz gereken noktaları özetlemektedir.  
Lütfen dikkatlice okuyunuz.

### **Toplamadan Önceki Gece ve Toplanılan Gün İçinde:**

Lütfen çocuğunuzun

1. Hasta olmadığından (soğuk algınlığı vb.)
2. Hiçbir ilaç almadığından
3. Yüz ya da dudak kremi sürmediğinden
4. Dişetinde kanama, ağızda yara ya da iltihaplanma olmadığından



emin olunuz.

Eğer yukarıda belirtilenlerden herhangi biri gerçekleştiyse, lütfen tükürük toplama işlemi yapmayınız ve deney yürütücüsünü bilgilendiriniz.

### **Lütfen tükürük örneğini toplamadan**

#### **ÖNCEKİ 2 SAAT boyunca:**

- Çocuğunuza kola, çay, kahve gibi kafein içeren içecekler içirmeyiniz, çikolata yedirmeyiniz.
- Çocuğunuzun öğle uykusundan uyanmış olduğuna emin olunuz.

### **Lütfen tükürük örneğini toplamadan**

#### **ÖNCEKİ 30 DAKİKA boyunca:**

- Çocuğunuza hiç bir şey yedirip içirmeyiniz.

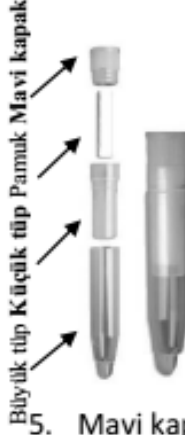


- Dişlerini fırçalamayınız.

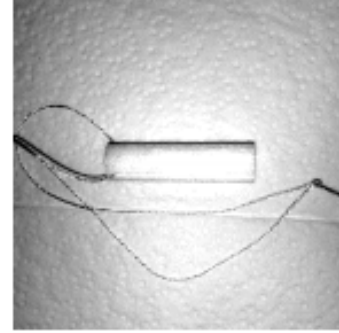
- Çocuğunuzun fiziksel olarak çok yorucu hareketler (koşmak, yokuş çıkmak, zıplamak vb.) yapmamasına dikkat ediniz.

## Hafta sonu tükürük örneği toplama işlemi

1. Lütfen örnekleri **SABAH 10:00** ve **ÖĞLEDEN SONRA 15:30**'a olabildiğince yakın zamanlarda toplayınız.
2. Tükürük toplama tüpü yandaki şekildeki gibi bir küçük tüp, bir büyük tüp, pamuk ve mavi kapaktan oluşmaktadır.
3. Lütfen tükürük örneğini çocuğunuz otururken alınız, örneği toplamadan önce ellerinizi yıkayınız.
4. Kilitli poşetten gün ve saate göre işaretlenmiş tüplerden uygun olanını (Cumartesi Sabah gibi) çıkarınız.



5. Mavi kapağı açınız ve temiz bir peçetenin üzerine koyunuz. İçerisindeki küçük tüpü çıkarmayınız, eğer çıkarsa lütfen geri takınız.
6. Tüpün içerisindeki pamuğu çocuğunuzun yanağına ip dışarı sarkacak şekilde yerleştiriniz.



7. Tükürüğü iyice emmesi için pamuğu **en az 3 dakika** boyunca çocuğunuzun yanağında bekletiniz. Pamuğun ıslanmadığını fark ederseniz bekletmeye devam ediniz. Bu süre boyunca pamuğu ipten tutarak kontrol edebilirsiniz.
8. Pamuk iyice ıslandıktan sonra, pamuğu çocuğunuzun ağzından ipten tutarak çıkarınız. Pamuğu mümkün olduğunca elinize ya da başka bir yüzeye değdirmeden tüpün içine geri yerleştiriniz ve mavi kapağı **SIKICA** kapatınız.
9. Lütfen topladığınız örnekleri buzdolabının içinde (derin dondurucu ya da buzlukta değil) poşeti içerisinde saklayınız.



10. Örneğin toplandığı saati aşağıdaki saat-tarih çizelgesine yazınız
11. Sizi aradığımız numaradan bizi uzun uzun çaldırınız. Çağrınızı görünce size çağrınızı aldığımıza dair bir mesaj göndereceğiz.
12. Lütfen bu aşamaları sabah ve öğleden sonraki örnekler için aynı şekilde tekrarlayınız.

**Tükürük Örneklerinin Teslim Edilmesi**

Önceden buzdolabına kaldırdığınız tüpleri Pazartesi sabahı çocuğunuzu anaokuluna göndermeden hemen önce buzdolabından çıkarıp doldurduğunuz bütün belgelerle birlikte yeniden zarfın içine koyunuz. Zarfın ağzını sıkıca bantladıktan sonra çocuğunuzla öğretmenine teslim edilmek üzere okula gönderiniz.

Her zaman katılımcı ailelerden gelen çeşitli sorularla karşılaşyoruz. Bu kılavuzda değinmediğimiz ancak sizin sormak istediğiniz bir soru olursa lütfen bizimle iletişime geçmekten çekinmeyiniz.

Bize ulaşabileceğiniz telefon numaraları:

**0553 739 3626**

**0533 739 3656**

**Lütfen aşağıdaki çizelgeleri doldurmayı unutmayınız.**

**HAFTA İÇİ OKULDA toplanan tükürük örnekleri için:**

	Tarih	Önceki gece yatma saati	Sabah kalkma saati
<b>1. Gün</b>			
<b>2. Gün</b>			

**HAFTA SONU EVDE toplanan tükürük örnekleri için:**

	Tarih	Önceki gece yatma saati	Sabah kalkma saati	Sabah toplama saati	Öğleden sonra toplama saati	Buzdolabına konma saati (sabah / öğleden sonra)
<b>Cumartesi</b>						
<b>Pazar</b>						

Toplamada bir sorun olduysa lütfen aşağıda belirtiniz:

### Sıkça Sorulan Sorular

- 1- Niçin tükürük örneği topluyoruz? Bu örnekleri toplarken kullandığımız materyallerin çocuğuma herhangi bir zararı dokunur mu?  
Çocuğunuzun duyu durumu ile ilgili fizyolojik ölçümler tükürük örnekleri kullanılarak yapılmaktadır. Tükürüğün emilmesi için kullanılan pamuk tüm dünyada bu tarz çalışmalarda kullanılan bir üründür ve çocuğunuzun sağlığına hiçbir zararı yoktur.
- 2- Hafta sonunda 1 ya da 2 gün örnekleri toplayamadım/toplayamayacağımı fark ettim. O günlerin yerine hafta içi herhangi bir gün örnekleri toplayabilir miyim?  
Örneklerin hafta sonunda toplanması gerekmektedir. Önceden ayarlanan tarihlerde örnekleri toplayamayacağınızı fark ettiğiniz takdirde yeni bir tarih belirlemek için lütfen bizimle iletişime geçiniz.  
Telefon numaralarımız: 0553 739 3626 / 0533 739 3656
- 3- Bu hafta sonu çocuğumla birlikte bir etkinlik (gezi, piknik, top oynamak vb.) yapmayı planlıyoruz. Örnekleri gittiğimiz yerde toplayabilir miyiz?  
Eğer bu çocuğunuzun fiziksel olarak aşırı yorulacağı bir etkinlikse lütfen örnekleri o gün toplamayınız ve öncesinde bizimle iletişime geçiniz. Eğer fiziksel olarak yorulacağı bir etkinlik değilse, örnekleri toplayabilirsiniz; ancak en kısa sürede buzdolabına koymanızı rica ediyoruz.
- 4- Belirtilen zamanda örneği toplamayı unuttum, ne yapmalıyım?  
Eğer belirtilen saatin üzerinden (10:00 veya 15:30) 30 dakikadan fazla geçmediyse örneği HEMEN toplayabilirsiniz. Lütfen örneği ne zaman topladığınızı bu kılavuzdaki tarih ve saat çizelgesine yazınız. Eğer daha fazla bir gecikme olduysa lütfen bizi bilgilendiriniz, biz sizi yönlendireceğiz. Unutmayın, örnekleri zamanında toplamanız güvenilir veri elde edebilmemiz açısından çok önemlidir.
- 5- Tükürük örneğini alırken çocuğum pamuğu ağızından çıkardı. Pamuk yere düştü/herhangi bir yere değdi, ne yapmalıyım?  
Lütfen aynı pamukla örnek almaya devam etmeyiniz. Böyle bir durumda vakit kaybetmeden size verilen turuncu etiketli yedek tüpü kullanarak yeniden örnek alabilirsiniz. Lütfen yedek tüpün üzerine gün ve zamanı yazınız (Cumartesi Sabah gibi). Eğer yedek tüpü daha önceden kullanmak durumunda kaldıysanız lütfen bizimle iletişime geçiniz.
- 6- Örnek toplamadan yarım saat öncesinde çocuğum ben fark etmeden bir şeyler yemiş içmiş/dişini fırçalamış veya kendini yoracak bir aktivitede bulunmuş. Ne yapmalıyım?  
Bu durumu örneği topladıktan sonra fark ettiyseniz, lütfen tarih ve saat çizelgesinin sonundaki "örnek toplarken yaşanan sorunlar" ile ilgili kısma not ediniz. Henüz örneği almadıysanız lütfen yarım saat daha bekledikten sonra örneği toplayınız ve yine not alınız.
- 7- Sabah ilk örneği topladıktan sonra gün içinde çocuğumun hasta olduğunu fark ettim (boğaz ağrısı, ateş yükselmesi, burun akıntısı gibi belirtilere bakarak). Ne yapmalıyım?  
Lütfen fark ettiğiniz andan itibaren bir sonraki örneği almadan bizimle iletişime geçiniz.

APPENDIX C

SLEEP INFORMATION QUESTIONNAIRE FOR WEEKENDS

Participant ID: \_\_\_\_\_

**FILL OUT THIS FORM AFTER SALIVA SAMPLING ON**  
**SATURDAY**

**Please answer the questions below completely and correctly.**

1- In this morning, did your child wake up by himself/herself or did you wake him/her up?

- Woke up by himself/herself                       I woke him/her up

2- How would you assess the light level of the room your child slept in last night?

- Very light       Light       Dark       Very dark

3- How would you assess the sound level of the room your child slept in last night?

- Completely quiet       Somewhat noisy       Noisy       Very noisy

4- In your opinion, how well did you child get enough sleep last night?

- Very well       Well       Fair       Poor       Very poor

5- Did anything happen today that could cause anxiety to your child? If so, how anxious your child is about this situation? (Such as doctor's appointment.

You can also ask this question to your child after sampling.)

- No  Yes, but it did not cause anxiety  
 Yes, it caused some anxiety  Yes, it caused excessive anxiety

6- Did your children take an afternoon nap before afternoon sampling?

- No  Yes, he/she went to bed at \_\_\_\_\_, and woke up at \_\_\_\_\_ .

7- Do you have anything to add about your child's sleep last night that might be important? (Such as he/she went to bed later than usual, he/she woke up harder than usual, he/she woke up frequently during the night, and any situation that is not usual for your child.)

**FILL OUT THIS FORM AFTER SALIVA SAMPLING ON SUNDAY**

**Please answer the questions below completely and correctly.**

1- In this morning, did your child wake up by himself/herself or did you wake him/her up?

- Woke up by himself/herself                       I woke him/her up

2- How would you assess the light level of the room your child slept in last night?

- Very light             Light             Dark             Very dark

3- How would you assess the sound level of the room your child slept in last night?

- Completely quiet             Somewhat noisy             Noisy             Very noisy

4- In your opinion, how well did you child get enough sleep last night?

- Very well             Well             Fair             Poor             Very poor

5- Did anything happen today that cause anxiety to your child? If so, how anxious your child is about this situation? (Such as doctor's appointment.

You can also ask this question to your child after sampling.)

- No                                               Yes, but it did not cause anxiety
- Yes, it caused some anxiety             Yes, it caused excessive anxiety

6- Did your children take an afternoon nap before afternoon sampling?

No                       Yes, he/she went to bed at \_\_\_\_\_, and woke up at \_\_\_\_\_ .

7- Do you have anything to add about your child's sleep last night that might be important? (Such as he/she went to bed later than usual, he/she woke up harder than usual, he/she woke up frequently during the night, and any situation that is not usual for your child.)

APPENDIX D

SLEEP INFORMATION QUESTIONNAIRE FOR WEEKENDS

(TURKISH)

Katılımcı Numarası: \_\_\_\_\_

**BU KISIM  
CUMARTESİ TÜKÜRÜK TOPLADIKTAN SONRA  
DOLDURULACAKTIR**

**Lütfen aşağıdaki soruları eksiksiz ve doğru cevaplayınız.**

1- Çocuğunuz bu sabah kendiliğinden mi uyandı yoksa siz mi kaldırdınız?

- Kendiliğinden uyandı  Ben uyandırdım

2- Çocuğunuzun dün gece uyuduğu ortamdaki ışık miktarını nasıl değerlendirirsiniz?

- Çok aydınlık  Aydınlık  Karanlık  Çok karanlık

3- Çocuğunuzun dün gece uyuduğu ortamdaki ses miktarını nasıl değerlendirirsiniz?

- Tamamen sessiz  Biraz sesli  Gürültülü  Çok gürültülü

4- Çocuğunuzun dün gece uykusunu ne kadar iyi aldığını düşünüyorsunuz?

- Çok iyi  İyi  Orta  Kötü  Çok kötü

5- Bugün çocuğunuzu kaygılandırarak bir durum oldu mu? Varsa bu durum onu ne kadar kaygılandırıyor? (Doktor randevusu vb. Örnekler toplandıktan sonra bu soruyu çocuğunuza da sorabilirsiniz)

- Yok  Var, ama kaygılandırmıyor.

- Var, biraz kaygılandırıyor.  Var, çok kaygılandırıyor.

6- Çocuğunuz bugün öğleden sonra örneğini almadan önce öğle uykusu uyudu mu?

- Uyumadı  Uyudu, saat \_\_\_\_\_'de yattı ve saat \_\_\_\_\_'de kalktı.

7- Çocuğunuzun dün geceki uykusuyla ilgili eklemek istediğiniz, önemli olduğunu düşündüğünüz bir şey var mı? (Örneğin çok daha geç uyudu, sabah çok daha zor uyandı, gece çok daha sık kalktı, gibi diğer gecelerden farklılık var mıydı?)

**BU KISIM**  
**PAZAR TÜKÜRÜK TOPLADIKTAN SONRA**  
**DOLDURULACAKTIR**

**Lütfen aşağıdaki soruları eksiksiz ve doğru cevaplayınız.**

- 1- Çocuğunuz bu sabah kendiliğinden mi uyandı yoksa siz mi kaldırdınız?  
 Kendiliğinden uyandı  Ben uyandırdım
- 2- Çocuğunuzun dün gece uyuduğu ortamdaki ışık miktarını nasıl değerlendirirsiniz?  
 Çok aydınlık  Aydınlık  Karanlık  Çok karanlık
- 3- Çocuğunuzun dün gece uyuduğu ortamdaki ses miktarını nasıl değerlendirirsiniz?  
 Tamamen sessiz  Biraz sesli  Gürültülü  Çok gürültülü
- 4- Çocuğunuzun dün gece uykusunu ne kadar iyi aldığını düşünüyorsunuz?  
 Çok iyi  İyi  Orta  Kötü  Çok kötü
- 5- Bugün çocuğunuzu kaygılandırarak bir durum oldu mu? Varsa bu durum onu ne kadar kaygılandırıyor? (Doktor randevusu vb. Örnekler toplandıktan sonra bu soruyu çocuğunuza da sorabilirsiniz)  
 Yok  Var, ama kaygılandırmıyor.  
 Var, biraz kaygılandırıyor.  Var, çok kaygılandırıyor.
- 6- Çocuğunuz bugün öğleden sonra örneğini almadan önce öğle uykusu uyudu mu?  
 Uyumadı  Uyudu, saat \_\_\_\_\_'de yattı ve saat \_\_\_\_\_'de kalktı.
- 7- Çocuğunuzun dün geceki uykusuyla ilgili eklemek istediğiniz, önemli olduğunu düşündüğünüz bir şey var mı? (Örneğin çok daha geç uyudu, sabah çok daha zor uyandı, gece çok daha sık kalktı, gibi diğer gecelerden farklılık var mıydı?)

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