Correlates of
Physiological Stress Reactivity
in Middle Childhood

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ISBN 978-94-6259-018-2

Cover and layout
Promotie In Zicht, Arnhem

Print
Ipskamp Drukkers, Enschede

Correlates of Physiological Stress Reactivity in Middle Childhood

Proefschrift

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus prof. mr. S.C.J.J. Kortmann,
volgens besluit van het college van decanen
in het openbaar te verdedigen op dinsdag 11 februari 2014
om 10.30 uur precies

door

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geboren op 24 juni 1985
te Leidschendam

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General Introduction

1.1. Stress

Stress is an everyday phenomenon that many people can relate to. The first definition of stress, posited by Hans Selye in the twentieth century, considered stress to be a non-specific bodily response to any demand (Fink, 2009). Later definitions often distinguish between a *stressor*, and a *stress response*. A stressor has been defined as a stimulus that threatens homeostasis, a term that refers to the stability of the organism's internal environment. A stress response in turn refers to the organism's attempt to re-establish homeostasis (Chrousos, 2009). Initially, stress was investigated in the context of injuries, intoxication, and other physical stressors that will consistently elicit a response. However, for more common daily life events the response will depend on how a person evaluates the threat level of the potential stressor (Dantzer, 2007). Novelty, uncertainty, uncontrollability, and social-evaluative threat are important factors in this evaluation process (Dantzer, 2007; Dickerson & Kemeny, 2004).

As the definition in the previous paragraph implies, stress responses are in principle adaptive responses. Among other things, they promote bodily changes that operate to protect the stressed organism and promote its survival (McEwen, 2007). In humans, as in other animals, such bodily changes include the activation of the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal axis (HPA axis). The SNS responds to stress with rapid changes in epinephrine and norepinephrine levels, thereby allowing fast mobilization of metabolic resources and initiating the fight/flight response (Gunnar & Quevedo, 2007). Activation of the HPA axis leads to a cascade of events that results in the production of glucocorticoid hormones (Gunnar & Quevedo, 2007), whose actions are diverse, ranging from energy mobilization to aiding the organism in stress recovery (Sapolsky, Romero, & Munck, 2000).

Besides physiological responses, individuals also exhibit changes in behaviour and emotions in face of a stressor (Steptoe, 2007). Much is still unknown about how these interact, because few studies have investigated stress reactivity in a comprehensive manner that incorporates physiological, behavioural and emotional changes in response to an acute stressor. Such knowledge is important in obtaining a clear picture of the mechanisms involved in human stress responses. Information about these mechanisms is important for clinical practice, as certain patterns of physiological, behavioural, and emotional responses to stress have all been found to be related to psychopathology (e.g. Charmandari, Tsigos, & Chrousos, 2005; Hughes, Gullone, & Watson, 2011; McEwen, 2003; Mogg, Philippot, & Bradley, 2004). As such, knowing how different types of responses to stress are interrelated can provide important information that could build towards a better understanding of the origins and treatment of psychological disorders.

Although it is often assumed that physiological, behavioural and emotional responses to stress are coherently activated, support for this assumption is still fragmented and results of studies with adults do not always point in the same direction. For example, Cohen et al. (2000) did not find an association between participants' physiological reactivity to a public speaking task and their stress-induced anxiety. However, Hellhammer and Schubert (2012) found that more stress perception during a stress task was weakly but significantly related to higher cortisol reactivity to the task. Avero and Calvo (1999) did not find any relation between physiological indices of stress and nonverbal behaviour during a stress task involving a motor test and speech in front of a video camera. In the same study, what the authors termed 'cognitive anxiety' (e.g. "I am worrying about my performance") was not associated with physiological indices of stress, and only moderately associated with behaviour during the stress task. In a different study, Mauss et al. (2005) found that although emotion experience was highly correlated to behaviour, correlations with physiological reactivity were only moderate. The results of these studies indicate that there is by no means consistent evidence for the postulate that stress results in the coherent activation of response domains.

As most studies that have investigated how different responses to stress are interrelated were conducted with adults, even less is known about how physiological, behavioural, and emotional responses to stress are associated in children. However, knowledge about how children respond during stress is important, as how individuals respond to stress in childhood at least partly determines their responses to stressors later in life (Heim & Nemeroff, 2002), and is thus related to children's development into adolescence and adulthood. In addition, as patterns of coping with stressors are still developing in childhood they may be easier to change than in adulthood, which would make them potentially effective targets for interventions. Therefore, *the first aim of this thesis was to investigate whether children's physiological responses to a stressor are related to their behavioural and emotional responses to the same stressor.* Paragraph 1.2 provides more information about emotional responses to stress. Paragraph 1.3 provides more details about behavioural responses to stress.

As mentioned above, physiological responses are generally adaptive in terms of coping with the stressor at hand. This is also reflected in the consequences that physiological stress responses have on cognitive functioning. For example, memory encoding and consolidation is enhanced during stress, especially for emotional stimuli, while at the same time retrieval is impaired (e.g. Wolf, 2009). It has been suggested that these effects are the combined result of physiological stress responses that switch the brain into a state that allows for strong consolidation of the stressful event and that compromises the retrieval of unrelated information, perhaps in order to limit retroactive interference of this unrelated material (Joëls, Pu, Wiegert, Oitzl, & Krugers, 2006; Roozendaal, 2002). Such effects may be very useful in the context of severe stressors that pose an immediate threat for survival. However, in the case of more moderate daily life stressors, these types of reactions may also lead to undesirable decreases in cognitive performance. For example, during an extremely busy period at work you might forget going to a dentist appointment that you made several months earlier. Although there is quite an extensive body of literature on the effects of stress on learning and memory in adults, little is known about effects of stress on cognitive functioning in children, especially with regard to the interactive effects of the SNS and HPA axis. This is surprising considering the obvious relevance of such knowledge in the context of children's school performance. As such, *the second aim of this thesis was to investigate whether children's physiological responses to a stressor are related to their performance on cognitive tasks during the same stressor.* Paragraph 1.4 elaborates on the effects of stress on cognitive functioning.

1.2. Emotion, Emotion Regulation, and Stress

Emotions can be seen as integrated packages of response tendencies that help coordinate adaptive behaviour in challenging situations (Levenson, 1994), and as such they are likely to change in response to a stressor. A recent meta-analysis shows that research investigating emotional changes in response to a moderate stressor has focused on anger, anxiety, confusion, distress, depression, happiness, calmness, and shame, among others. Although the experience of these emotions was often found to change in the expected direction following stress exposure, a vast majority of studies did not find a relation between these changes in emotion and changes in physiological parameters (Campbell & Ehlert, 2012). This might in part be due to methodological issues. Correlations between emotional and physiological stress responses seem to be highest when assessment focuses either on how the stressor is appraised (e.g. as challenging, threatening, novel, or intense), or on a specific affective response, as opposed to a global mood state (Denson, Spanovic, & Miller, 2009). In addition, correlations have been found to be higher when assessment takes place during stress induction, as opposed to before or after stress (Hellhammer & Schubert, 2012).

These findings raise the question whether the extent to which people try to regulate their appraisals and emotions during a stressor, for instance by using emotion regulation strategies, might also be related to their physiological responses to stress. Emotion regulation has been defined as the processes that influence which emotions a person has, when that person has these emotions, and how these emotions are experienced and expressed (Gross, 1998b). In infancy and early childhood most emotion regulation takes place through behaviourally oriented approaches (e.g. gaze aversion, hiding emotions). Between 6 and 10 years of age, these approaches gradually shift towards more cognitively based strategies (e.g. mental distraction, reappraisal; Meerum Terwogt & Stegge, 1995). The availability of both behavioural and cognitive strategies of emotion regulation during middle childhood allows for the study of two emotion regulation strategies that have received ample attention in the adult literature over the last years: *reappraisal* and *suppression* (Gross, 1998a).

Reappraisal is a strategy where the meaning of a situation is reinterpreted in such a way that the emotional impact of the situation is changed, whereas suppression is a tactic that involves inhibiting the expression of emotions that are already being experienced (Gross, 1998a). In adults, the use of more reappraisal is related to better outcomes in terms of emotion experience, interpersonal functioning, and well being, whereas more use of suppression is associated with worse outcomes on these factors (Gross & John, 2003). A study that investigated the use of reappraisal and suppression throughout middle childhood and early adolescence found that reappraisal use was relatively stable, whereas the use of suppression gradually decreased over time (Gullone, Hughes, King, & Tonge, 2010). Research into the relation between the use of reappraisal and suppression, and physiological responding has focused on adult populations. Moreover, these studies primarily used experimental paradigms (e.g. Gross, 1998a; Gross & Levenson, 1993; Steptoe & Vögele, 1986) or used a trait measure of the overall use of reappraisal and suppression (Lam, Dickerson, Zoccola, & Zaldivar, 2009), as opposed to a state measure that reflects the use of reappraisal and suppression in a specific situation. However, in light of the findings that physiological stress responses showed the strongest relation with emotional responses assessed *during* stress (Hellhammer & Schubert, 2012), a state measure for emotion regulation might prove to be more strongly related to individual differences in physiological responses to stress. The present thesis therefore investigated whether children's use of the emotion regulation strategies reappraisal and suppression during a stress task (i.e. state emotion regulation) is related to their physiological stress responses to the task.

1.3. Behavioural Responses to Stress

Behavioural coping strategies are the earliest available means to voluntarily regulate emotions and deal with stressful situations (Zimmer-Gembeck & Skinner, 2011), and gaze aversion is one of the first behaviours that can be used for this purpose. Infants already use gaze aversion in order to diminish stimulation. For example, 6-month-old infants use gaze aversion as a regulatory strategy when confronted with a stranger, and especially if this stranger is controlling and insensitive (Mangelsdorf, Shapiro, & Marzolf, 1995). Also at older ages gaze aversion seems to play a role in the regulation of psychosocial stress. For example, 8-year-olds show more gaze aversion during face-to-face questioning than during questioning across a live video link (Doherty-Sneddon & Phelps, 2005). In adults, it has been found that during social stress, individuals high in social anxiety looked at emotional faces for less time than individuals low in social anxiety. This indicates that anxious individuals might use gaze aversion as a strategy to reduce their discomfort (Garner, Mogg, & Bradley, 2006). These findings in different age groups point towards the importance of gaze aversion as a behavioural coping strategy throughout development.

It has been suggested that avoidance of stress inducing stimuli, as is the case in gaze aversion, serves to reduce threat perception and perhaps even physiological reactivity (e.g. Applehans & Luecken, 2006). Research using attentional bias tasks has indeed found a relation between attentional avoidance and physiological reactivity to a moderate stressor (e.g. Roelofs, Bakvis, Hermans, van Pelt, & van Honk, 2007). However, much less is known about how gaze aversion during a moderate stressor is related to subsequent physiological reactivity to this stressor. In a study with adult participants that used a public speaking task to induce stress, displaying more gaze aversions from the audience was related to lower cortisol reactivity to the task (Sgoifo et al., 2003). However, the relation between gaze aversion and cortisol reactivity seems different in a younger population. In a sample of healthy 6-to-15-year-old children, cortisol responses to a social challenge stress test were related to the use of gaze during the test: increased cortisol reactivity was found in children who displayed "poor quality" gaze behaviour, i.e. either continuous staring or total gaze aversion, while lower reactivity was found in children with "good quality" gazing, who kept eye contact with the jury without staring or avoiding (Hessl, Glaser, Dyer-Friedman, & Reiss, 2006). The relation between gaze avoidance and cortisol reactivity thus seems to be U-shaped in childhood, with intermediate levels of gaze aversion being associated with lower cortisol reactivity. The present thesis aimed to shed more light on this issue by relating ethological observations of children's gaze aversion during a psychosocial stress task to their cortisol reactivity to the task.

1.4. Stress and Cognitive Functioning

Research into the effects of stress on cognitive functioning often focuses on declarative long term memory and working memory (WM). Declarative long term memory refers to "the explicit storage of facts and events, which can later be intentionally retrieved" (Wolf, 2007, p. 167). WM, on the other hand, refers to the temporary storage and manipulation of information required for task performance (Baddeley, 1992). Studies in adults have assessed the effects of stress on these memory processes through the use of stress paradigms and glucocorticoid administration. For declarative memory, the resulting effects are dependent on the stage of memory processing under investigation. That is, findings indicate that high levels of glucocorticoids during encoding or consolidation of information enhance subsequent memory for this information (e.g. Andreano & Cahill, 2006; Buchanan & Lovallo, 2001; Cahill, Gorski, &

Le, 2003; Smeets, Otgaar, Candel, & Wolf, 2008), whereas high levels of glucocorticoids during retrieval impair memory for information unrelated to the stressor (e.g. Smeets, et al., 2008; Tollenaar, Elzinga, Spinhoven, & Everaerd, 2008; Tollenaar, Elzinga, Spinhoven, & Everaerd, 2009). Rodent studies have indicated that these opposing effects are the result of noradrenergic activity in the basolateral amygdala and its interaction with the hippocampus and prefrontal cortex (Roozendaal, 2002; Roozendaal, McEwen, & Chattarji, 2009), pointing towards the need for concurrent glucocorticoid and noradrenergic activation in these effects. This notion is endorsed by findings in human adults that effects of glucocorticoids on memory are most pronounced for emotional stimuli (e.g. Kuhlmann, Kirschbaum, & Wolf, 2005; Smeets, et al., 2008), or limited to a condition were participants were emotionally aroused (Kuhlmann & Wolf, 2006). Taken together, these findings indicate that during stress, concurrent activation of the HPA axis and SNS has enhancing effects on declarative memory encoding and consolidation, while reducing declarative memory retrieval capacities.

The results of studies investigating the effects of stress on WM in human adults are less consistent, as some found decreased performance (Elzinga & Roelofs, 2005; Schoofs, Preuss, & Wolf, 2008; Schoofs, Wolf, & Smeets, 2009), whereas others reported no effects (Kuhlmann, Piel, & Wolf, 2005; Smeets, Jelicic, & Merckelbach, 2006), or even enhanced performance (Lewis, Nikolova, Chang, & Weekes, 2008). A study in rodents suggests that noradrenergic activity in the amygdala is essential for enabling glucocorticoid effects in the prefrontal cortex on working memory (Roozendaal, McReynolds, & McGaugh, 2004). In line with this, Elzinga and Roelofs (2005) found in a study with adult humans that impairments in WM were only present when both cortisol levels and adrenergic activity were elevated. This suggests that, as with effects of stress on declarative memory, negative effects of stress on WM are also dependent on concurrent activation of the HPA axis and SNS.

Developmental changes have been observed in the structure and activation of brain areas involved in declarative memory and WM, like the hippocampus, amygdala, and prefrontal cortex (Casey, Giedd, & Thomas, 2000). Such changes, or changes in the susceptibility of the prefrontal cortex to glucocorticoids over the course of development (Perlman, Webster, Herman, Kleinman, & Weickert, 2007), might lead to differential effects of stress on cognitive functioning in children versus adults. Although the body of literature about the neurobiology of learning and memory in adults is quite extensive, much less is known about these processes in children. Moreover, most research in children to date has focused primarily on the effects of stress during encoding of declarative memories (e.g. Quas, Yim, Edelstein, Cahill, & Rush, 2011; Quas, Yim, Rush, & Sumaroka, 2012). Studies that did investigate the relation between physiological stress responses and memory retrieval or WM did not assess the interaction effect of HPA axis and SNS activation on performance

(Nathanson & Saywitz, 2003; Quas & Lench, 2007; Quesada, Wiemers, Schoofs, & Wolf, 2012). The present thesis therefore sought to test the interactive effects of the HPA axis and the SNS on both the delayed retrieval (DR) of declarative memories, as well as on WM.

1.5. The Present Research Project

The present thesis investigated how children's physiological stress responses are related to their emotional responses to stress in terms of emotion regulation strategy use, their behavioural responses to stress in terms of gaze aversion, and their cognitive functioning during stress in terms of DR and WM. The targeted age group was 9- to 11-years of age, as this is considered the earliest age at which children can independently participate in a laboratory session, and reliably report on their use of emotion regulation strategies. Also, for this age there is an age-appropriate stress test that has repeatedly proven to be effective in inducing a physiological stress response (Buske-Kirschbaum et al., 1997).

All children were tested twice: once during a control condition in a mobile laboratory in front of their home, and approximately one week later during a stress condition in the laboratory of the Behavioural Science Institute of the Radboud University Nijmegen (the Netherlands). To induce stress during the stress condition we used the Trier Social Stress Test for Children (TSST-C; Buske-Kirschbaum, et al., 1997), which consists of a public speaking task and a mental arithmetic task that are performed in front of a jury of two confederates in white lab coats. Table 1 (p. 16) provides an overview of the data used in the present thesis. We refer to the relevant chapter number in the last column.

Saliva samples were collected during both sessions, and analyzed for cortisol and alpha-amylase (sAA). This allowed both the assessment of children's physiological responses to the stressor within the stress condition, as well as the comparison of physiological activation between conditions.

Measures of children's emotional and behavioural regulation during stress were obtained in the stress condition. State and trait use of emotion regulation strategies were assessed with an adapted version of the Emotion Regulation Questionnaire (ERQ; Gross & John, 2003). For use in the current sample, the formulation of the items from the Dutch translation of the ERQ (Koole, 2004) was simplified, and the instructions were extended. Gaze aversion was assessed by ethological observations of the children's gaze behaviour during the stress task.

Measures for DR and WM were obtained in both conditions, to allow comparison of memory performance across conditions in addition to relating physiological responses in the stress condition to memory performance in the stress condition. Two

Table 1 Overview of the data used in the present thesis.

Measure	Collected in condition		Used in chapter
	Control	Stress	
Saliva samples			
Cortisol and sAA	3 samples[a]	7 samples	2, 3, 4, 5
Questionnaires (child self report)			
Trait ERQ		x	5
State ERQ		x	2
Perceived stress[b]		x	3
Pubertal stage[c]		x	2, 3, 4
Video observations			
Gaze aversion		x	3
Memory tasks			
Declarative memory	x	x	4
Working memory	x	x	4
Questionnaire (parental report)			
Personality[d]	x[e]		5

[a] Due to practical constraints, we only analyzed two out of three saliva samples in the control condition, and only in a subset of $n = 53$ participants. [b] Manipulation check to the TSST-C (Buske-Kirschbaum et al., 1997). [c] Tanner criteria (Marshall & Tanner, 1969, 1970). [d] Big Five Bipolar Rating Scales (B5BBS-25; Mervielde, 1992). [e] Parents were handed the questionnaire to rate their child's personality at the end of the control condition, and were requested to bring the completed form along to the stress condition one week later.

versions of a new task using verbal stimuli were created to assess DR. The WM tasks were digit span tests based on that from the Wechsler Intelligence Scale for Children (Wechsler, 1991).

1.6. Thesis Outline

The present thesis consists of four empirical studies, which are described in four separate chapters. All four studies are based on the same sample of 158 children (83 girls) that were recruited from 31 primary schools in Nijmegen and surrounding areas (The Netherlands). As a result, there is considerable overlap in the Method sections of the studies. For a detailed description of the sample, procedures, and measures, the reader is referred to the separate studies.

After this introductory chapter, the thesis continues in *Chapter 2* with a study that examined whether children's spontaneous use of the emotion regulation strategies reappraisal and suppression during a psychosocial stress task was related to their physiological stress responses to that task.

The study outlined in *Chapter 3* investigated the relation between ethological observations of children's gaze aversion from the jury during a psychosocial stress task and their cortisol reactivity to that task. In addition, perceived stress was explored as a potential moderator of the relation between gaze aversion and cortisol reactivity.

Chapter 4 presents a study that examined whether children's performance on working memory and delayed retrieval tasks decreased during stress exposure, and how children's physiological stress responses were related to working memory and delayed retrieval performance under stress.

The fourth and final study, described in *Chapter 5*, aimed to answer a question that emerged during the course of the project, based on a publication that appeared after the present data had been collected (Balodis, Wynne-Edwards, & Olmstead, 2010). This fourth study investigated whether the changes in cortisol and sAA over a one hour pre-stress period in the laboratory were related to subsequent cortisol and sAA reactivity to a psychosocial stress task, trait emotion regulation strategy use, and personality traits.

Finally, Chapter 6 presents a summary of the results of the four studies, followed by the main conclusions and a general discussion.

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2

The Relation between Emotion Regulation Strategies and Physiological Stress Responses in Middle Childhood[1]

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Summary

The current study sought to examine whether children's spontaneous use of the emotion regulation strategies suppression and reappraisal during a psychosocial stress task was related to their cortisol and alpha-amylase responses to that task.

Salivary cortisol and alpha-amylase responses to a psychosocial stress task were assessed in 158 10-year-old children (83 girls). The children completed a self-report questionnaire measuring use of reappraisal and suppression during the task. Results showed overall increases in cortisol and alpha-amylase in response to the stressor, with higher cortisol reactivity in girls than in boys. With regard to emotion regulation, more use of suppression was related to lower cortisol reactivity in girls, and lower alpha-amylase reactivity and quicker alpha-amylase recovery in all children. The use of reappraisal was not related to the children's cortisol or alpha-amylase responses.

The current study is the first to investigate the relation between the spontaneous use of reappraisal and suppression, and physiological stress responses to a psychosocial stressor in children. Our results indicate that reappraisal and suppression are used and can be measured even in 10-year-olds. At this age reappraisal appears ineffective at down-regulating physiological responses, while suppression was related to lower physiological responses. For cortisol reactivity there was a sex difference in the relation with suppression, indicating the importance of including sex as a moderator variable in research studying stress reactivity and its correlates in this age group.

2.1. Introduction

In face of a stressor, humans respond with changes in emotions and physiology. Two systems are central to human peripheral physiological stress responses: the autonomic nervous system (ANS), and the hypothalamic-pituitary-adrenocorticol (HPA) axis. The sympathetic branch of the ANS, the sympathetic nervous system (SNS), is a fast responding system involved in initiating the fight/flight response through the release of epinephrine and norepinephrine (Gunnar & Quevedo, 2007). The HPA axis works through the release of glucocorticoids, a type of steroid hormones, the production of which takes some time. As a result, the HPA axis responds slower to stressors and takes longer to return to baseline (Gunnar & Quevedo, 2007).

There are individual differences in the regulation of physiological stress responses (Kudielka, Hellhammer, & Wust, 2009; Rohleder & Nater, 2009). Adequate regulation of physiological responses to stress is important, as previous research has indicated that repeated and long-lasting activation of the stress-system is related to adverse effects on the immune system (e.g. Sapolsky, 1998), and to the development of physical and psychological problems (Charmandari, Tsigos, & Chrousos, 2005; McEwen, 1998). As such, it is important to determine the origins of the differences in physiological stress regulation. Therefore, the current study investigated whether the extent to which people try to regulate their appraisals and emotions in the face of a stressor, for instance by using emotion regulation strategies, might also affect the strength of their physiological responses to stress.

According to classical theories on stress, differences in physiological responding may follow from differences in cognitive appraisals of the stressful situation, and emotional responses associated with those appraisals (Frijda, 1986; Lazarus & Folkman, 1984). A recent paper investigated this notion for the HPA axis, with a meta-analysis of 54 studies that experimentally manipulated social stress or induced emotions (Denson, Spanovic, & Miller, 2009). Nine judges rated the likelihood that participants experienced certain appraisals and emotions. Results showed that higher appraisal ratings for challenge, threat, novelty, and intensity predicted larger effect sizes in terms of cortisol responses. For the experienced emotions, only surprise showed a positive relation with cortisol responses. The results of this meta-analysis show how appraisals and emotions are related to cortisol reactivity.

Activation of the SNS also appears to be related to experienced emotions. Salivary alpha-amylase (sAA) is considered a non-invasive biomarker of this system (Nater & Rohleder, 2009; but see also Bosch, Veerman, de Geus, & Proctor, 2011). A recent study showed that levels of sAA were related to participants' self-reported emotional state following the completion of a 'Fear Challenge Course' museum exhibit. Specifically, participants that indicated they were 'negatively aroused' showed significantly elevated levels of sAA, whereas participants that reported to be 'positively aroused' or 'positively calm' showed a significant reduction in sAA levels (Buchanan, Bibas, & Adolphs, 2010).

Research relating children's use of specific emotion regulation strategies to their physiological stress responses is limited. Investigating this is nonetheless important, as reactions to stressors in childhood at least partly determine how individuals respond to stressors later in life (Heim & Nemeroff, 2002). Also, both emotion regulation and physiological response systems are developing during childhood. For example, previous research found that children's HPA axis reactivity to a psychosocial stress task changed from a significant response at age nine, to blunted reactivity at age 11, back to a significant response at age 13 (only in girls) and at age 15 (Gunnar, Wewerka, Frenn, Long, & Griggs, 2009). As a result, findings regarding the relation between emotion regulation strategies and physiological responding for older age groups might not generalize to children. In the current study, we investigated whether individual differences in the way 10-year-old children regulate their emotions are related to their physiological reactions to a psychosocial stressor.

There is ongoing debate as to how the concept of emotion regulation should be defined (see e.g. Eisenberg & Spinrad, 2004; Thompson, Lewis, & Calkins, 2008). Gross (1998b) defines it as the processes that influence which emotions a person has, when that person has these emotions, and how these emotions are experienced and expressed. Across the early years of life, children gradually learn how to regulate their emotions. Infants and toddlers greatly rely on the help of adults in regulating their emotion experience and expression. During the preschool years, the understanding emerges that expressed emotions do not need to reflect current emotion experience (Zeman, Cassano, Perry-Parrish, & Stegall, 2006). Between six and ten years of age, children's repertoire of emotion regulation strategies expands rapidly, and shifts from an external, behaviourally oriented approach (e.g. gaze aversion, hiding emotions), towards the use of more cognitively based strategies (e.g. mental distraction, reappraisal; Meerum Terwogt & Stegge, 1995). This availability of both behavioural and cognitive strategies of emotion regulation makes middle childhood a good age at which to study the use of two strategies that have recently gained a lot of attention in the adult literature: *reappraisal* and *suppression* (Gross, 1998a).

Reappraisal is a strategy where the meaning of a situation is reinterpreted in such a way that the emotional impact of the situation is changed. As this strategy is used prior to the activation of emotional response tendencies, it is considered antecedent-focused. Suppression, on the other hand, is a tactic that involves inhibiting the expression of emotions that are already being experienced, and as such it is a response -focused strategy (Gross, 1998a). In adults, reappraisal has been related to the experience and expression of more positive emotions and less negative emotions, better interpersonal functioning, and greater well-being (Gross & John, 2003). Suppression has been associated with less experience and expression of positive emotions, more experience of negative emotions, worse interpersonal functioning, and lower well-being in adults (Gross & John, 2003). In relation to the larger repertoire of emotion regulation strategies that is available in middle childhood, reappraisal is considered a cognitive strategy, whereas suppression is considered a behavioural strategy (Meerum Terwogt & Stegge, 1995). As such, reappraisal could be considered a more mature strategy, and suppression a more immature strategy.

The use of reappraisal and suppression in middle childhood and early adolescence has been researched by Gullone et al. (2010). They found that use of reappraisal seems to be relatively stable across middle childhood and early adolescence, while the use of suppression gradually decreases. In relation to adaptive functioning it has been found that children and adolescents reporting high levels of depressive symptoms used less reappraisal, and more suppression than matched controls with low levels of depressive symptoms (Hughes, Gullone, & Watson, 2011). Also, a school refusal sample of children and adolescents with a primary diagnosis of an anxiety disorder reported fewer use of reappraisal, and more use of suppression than matched controls (Hughes, Gullone, Dudley, & Tonge, 2010).

Previous research on adults that investigated the relation between the use of these emotion regulation strategies and physiological responses consisted primarily of experimental studies that related the use of reappraisal and/or suppression to sympathetic nervous system (SNS) activation (e.g. Gross & Levenson, 1993; Steptoe & Vögele, 1986). For example, in a study by Gross (1998a) physiological responses to watching a disgust inducing film clip were comparable for participants in a reappraisal and control condition. However, participants in a suppression condition showed heightened SNS activity.

Although experimental studies provide a good impression on what the use of a certain type of emotion regulation *can* do, they do not provide information on how day-to-day spontaneous use of these strategies influences physiological responses. An individual differences approach could shed more light on these types of questions. To accommodate this type of research, Gross and John (2003) devised a trait measure of suppression and reappraisal. In adults, this measure has been used to investigate the relation between the tendency to use reappraisal or suppression, and cortisol reactivity to a speech task (Lam, Dickerson, Zoccola, & Zaldivar, 2009). Results showed that higher trait use of both suppression and reappraisal was related to higher cortisol reactivity to the speech task.

Although the use of a trait measure provides information about more naturally occurring use of emotion regulation strategies, it is limited to participants' overall indication of strategy use. The pattern of results from a study assessing both trait use of suppression and reappraisal, and state use in five different scenarios indicated that state strategy use arises from both dispositional and situational factors (Egloff, Schmukle, Burns, & Schwerdtfeger, 2006, Study 1). As such, the use of a state measure for emotion regulation might be an even more adequate measure to explain variance in individual differences in acute physiological responses to stress.

Egloff et al. (2006, Study 3) investigated how state use of reappraisal and suppression during an evaluative speech task was related to physiological responses to the same task. Suppression was positively related to SNS activation. No relations were found for reappraisal or with heart rate.

In sum, research into the relation between the use of reappraisal and suppression, and physiological responding has focused on adult populations. Also, use of state measures to assess emotion regulation strategies is scarce, thereby limiting our knowledge on how the spontaneous use of emotion regulation strategies is related to acute physiological stress reactions. In the current study, we used a state measure to assess the extent to which 10-year-old children had used the emotion regulation strategies reappraisal and suppression during their performance on a psychosocial stress task, and related these scores to their cortisol and sAA responses to the task. As the emotion regulation strategies may differentially relate to initial physiological reactivity versus subsequent recovery, we included both physiological reactivity and recovery as measures in our study. As reappraisal is an antecedent-focused emotion regulation strategy, and has been associated with greater experience of positive emotions, we expected to find that more use of reappraisal would be related to less physiological reactivity to our stress task. And also given the antecedent-focused nature of reappraisal, we expected it to relate to reactivity only, and not to subsequent recovery. For suppression, as a response-focused strategy, we expected that more use would be related to higher physiological reactivity, based on findings indicating greater experience of negative emotions and heightened physiological arousal. With regard to the relation between suppression and recovery, we had different expectations for cortisol recovery and sAA recovery. For sAA, which responds and recovers relatively fast as compared to cortisol, we expected that more use of suppression would be related to slower sAA recovery. Given the slowly responding nature of the HPA axis, we did not expect suppression to be related to cortisol recovery.

Because the age of our sample is close to the onset of puberty, and at least one study found a marginally significant positive correlation between puberty and cortisol reactivity (Gunnar, et al., 2009), we took pubertal stage into account as a possible confounder. Also, as onset of puberty is slightly different for boys and girls, we incorporated sex as a moderator variable in all analyses.

2.2. Method

2.2.1. Participants

Parents and children were invited through 31 primary schools in Nijmegen and surrounding areas (the Netherlands) to participate in a study on different aspects of responses to stress and their consequences for cognitive functioning. Schools handed out information packages to the children in grades 4 and 5 (age 9 to 11). Each package contained information about the research project and an application form. Parents of children willing to participate sent in the application form. Inclusion criteria were: birth date between 1 February 1998 and 1 September 2000, and proficiency in the Dutch language. Exclusion criteria were: stuttering, a diagnosis of a developmental disorder, and the use of centrally acting corticosteroid medication. A total number of 183 applications were received from 27 schools. Of this group, seven children did not participate because they did not meet the inclusion criteria. An additional eleven children did not participate due to scheduling problems (n = 4), and personal reasons (n = 7). This resulted in a sample of 165 participants.

For the current study, five additional children were excluded because they did not complete the entire data collection protocol, and two children were excluded because during data collection it was discovered they met one of the exclusion criteria. Thus, the sample for the current study consisted of 158 children (83 girls; M_{age} = 10.61 years, SD = 0.52). The majority of the participants was Caucasian (94%), and had at least one parent with a college or university degree (79%).

The study was approved by the ethics committee of the Faculty of Social Sciences of the Radboud University Nijmegen. All parents provided written informed consent prior to their child's participation.

2.2.2. Procedure

As part of the larger study, all children were first visited at home with a mobile lab, where they completed questionnaires and memory tasks. As this visit was not relevant for the current study, it will not be discussed further.

Testing took place after school in the laboratory of the Behavioural Science Institute of the Radboud University Nijmegen (for an overview of the procedure, see Figure 1, p. 31). Upon arrival, children were taken to a separate room, where the experimenter told them that they would be asked to do some tasks and fill out several questionnaires. After this introduction, children provided a saliva sample (S1; within 5 min after arrival), filled out several questionnaires, and performed a memory task. This was followed by a 30-min relaxation period during which children could read a magazine or make puzzles, and listened to relaxing music. Right after relaxation they filled in a short questionnaire, provided a second saliva sample (S2), and chose a favourite and least preferred present out of six small items. After this, children were led to an adjacent room where a stress task took place (adapted and extended TSST-C; see Section 2.2.3). During this procedure, a third saliva sample was taken (S3). Afterwards, the children were escorted back to the first room, where they provided another saliva sample (S4), and completed two questionnaires. This was followed by a fifth saliva sample (S5), and the completion of a state emotion regulation questionnaire (see Section 2.2.3). Upon completion of this questionnaire the children received positive feedback on their performance during the stress task, followed by a short questionnaire. Then, a 25-min post-stress relaxation period was initiated. 10 Min into this relaxation period, a saliva sample was obtained (S6). After relaxation, children completed several questionnaires, performed a memory task, provided a last saliva sample (S7), and completed a pubertal stage measure (see Section 2.2.3). The entire procedure took approximately 2.5 h.

2.2.3. Instruments and Measures

Stress task

To induce psychosocial stress, an adapted and extended version of the Trier Social Stress Test for Children (TSST-C; Buske-Kirschbaum et al., 1997) was administered. This task consists of a public speaking task in which children provide the ending to a story, and a mental arithmetic task in which children count backwards from 758 to zero by repeatedly subtracting seven from the most recently acquired number. Both tasks are performed in front of a jury of two confederates in white lab coats. To increase motivation, children were asked to pick a favourite and least preferred present out of six small items (Jones et al., 2006), and told that a favourable judgement by the jury would earn them their favourite present, whereas in case of an unfavourable judgement they would get the least preferred present. After the TSST-C, children were seated in front of the TSST-C jury. There they performed a working memory task, supplied a saliva sample (S3), filled out a short questionnaire, and performed an additional memory task. This entire procedure took approximately 34 min (see Figure 1).

State emotion regulation strategies

Children's use of emotion regulation strategies during the stress task was assessed with an adapted version of the Emotion Regulation Questionnaire (ERQ; Gross & John, 2003). The ERQ is a 10-item questionnaire assessing the use of both suppression and reappraisal. The four-item suppression scale includes items such as "I keep my emotions to myself". The reappraisal scale contains six items such as "I control my emotions by changing the way I think about the situation I am in". Responses are indicated on a seven-point Likert scale (1 = strongly disagree, 7 = strongly agree).

For use in the current study, the Dutch translation of the ERQ (Koole, 2004) was adapted for the use in 10-year-old children by simplifying the formulation of the items, and extending the instructions. To reflect emotion regulation strategy use during the stress task, the questionnaire was adapted to a state measure. For example, the original item "I control my emotions by not expressing them" was changed into "I controlled my emotions by not showing them". The instructions explained that emotions describe how you feel, and that in this case we wanted to know how the child had dealt with his or her emotions during the time spent in front of the jury. To

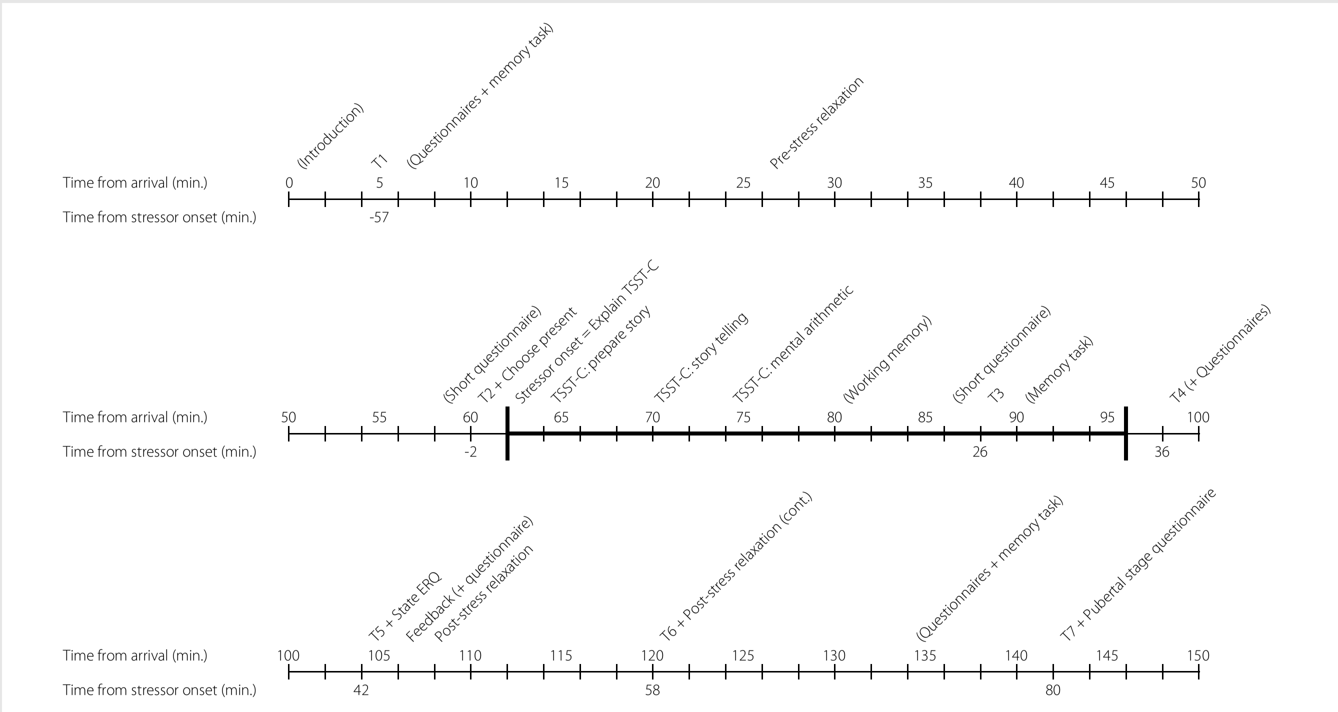

Figure 1 Overview of the procedure expressed in minutes from arrival and minutes before/after stressor onset (indicated by the first thick black vertical bar). Underlined tasks were done in the presence of the TSST-C jury. The thick black horizontal bar indicates the stress task. Tasks in parentheses are not relevant to the current study.

make referrals to positive and negative emotions in the questions less abstract, instructions were supplemented with a box containing exemplars of positive emotions (e.g. happy, proud), and a box with exemplars of negative emotions (e.g. angry, sad). Mean item scores for each scale were computed as indices for state use of reappraisal and suppression.

Principal components analysis revealed a two-factor solution, corresponding to the original factor structure reported by Gross and John (2003). Reliability in the current sample was sufficient for both scales (Cronbach's alpha .67 for suppression, and .84 for reappraisal).

Pubertal stage

To assess pubertal stage, children reported their physical development using Tanner criteria (breast development and pubic hair for girls, genital development and pubic hair for boys; Marshall & Tanner, 1969, 1970). For both physical attributes, each child indicated which one out of five pictures (Lee, 2001) best corresponded to how his/her body currently looked. Mean item scores were then computed as an index of pubertal stage.

Cortisol and sAA

To obtain reliable cortisol measures, participants were asked to only drink water in the 2 h before arrival in the lab, to limit physical exercise in the hour prior to arrival, and to abstain from meals at least 45 min before arrival.

Seven saliva samples were obtained throughout the course of the procedure, at -57, -2, 26, 36, 42, 58, and 80 min from the onset of the stressor. Participants swallowed all saliva in order to empty their mouths, and collected all subsequently secreted saliva in their mouths for 2 min, after which they used a short straw to spit the saliva into a small tube. This procedure was repeated until at least 0.25 ml of saliva was collected, with a maximum total collection time of 5 min. Samples were kept frozen at -20°C until their shipment to the analysis lab.

Cortisol concentrations were determined at the Endocrinology Laboratory of the University Medical Center Utrecht, using an in house competitive radio-immunoassay employing a polyclonal anticortisol-antibody (K7348). [1,2-3H(N)]-hydrocortisone (Amersham TRK407) was used as a tracer. The lower limit of detection was 1 nmol/L and inter-assay and intra-assay variations were below 10%.

sAA concentrations were determined from the same saliva samples as were used to determine cortisol concentrations. Analysis was performed at the Endocrinology Laboratory of the University Medical Center Utrecht. Alpha-amylase was measured on the DxI analyzer (Beckman Coulter Inc., Fullerton, CA, USA). Saliva samples were diluted 500x with 0.2% BSA in 0.01 M Phosphate buffer pH 7.0. Interassay variation was <2.2%.

Table 1 Overview of number of outliers, number of participants with lowest pre-stress value (Baseline), and number of participants with highest post-stress value (Peak) at each sample for cortisol and sAA. A dash indicates that this sample was not considered in determining baseline and/or peak.

	S1	S2	S3	S4	S5	S6	S7
Cortisol							
Outliers	3	3	5	3	3	3	3
Baseline	17	141	-	-	-	-	-
Peak	-	-	35	60	54	9	-
sAA							
Outliers	4	4	4	3	3	3	3
Baseline	28	129	-	-	-	-	-
Peak	-	-	49	74	34	-	-

2

All physiological data were screened for outliers, which were defined within each assessment point as values greater than 3 SD above the mean. See Table 1 for the number of outliers per sample for each measure. All outliers were winsorized[2] by replacing their values with the value of 3 SD above the mean (Tukey, 1977).

To compute the strength of children's physiological responses to the stressor, we first determined a baseline value for cortisol and sAA by selecting the lowest pre-stress value for each participant. Then, we determined peak reactivity for each measure. To capture inter-individual differences in timing of the peak cortisol reactivity, this was done by selecting the highest post-stress cortisol concentration from samples S3 through S6. For sAA, being a faster-responding measure, peak reactivity was defined as the maximum concentration from samples S3, S4, and S5.[3] See Table 1 for the number of children that had their baseline and peak at each sample. Peak reactivity and baseline variables were lg10 (cortisol) or sqrt (sAA) transformed to normalize their distributions.

A recovery measure for cortisol was computed by subtracting the baseline value for cortisol from cortisol concentrations at S7. For sAA, recovery was computed by subtracting the baseline sAA value from sAA at S6. Lower recovery scores thus indicate quicker recovery.

2 We also analyzed the data without participants whose cortisol or sAA values had been winsorized. For both cortisol and sAA, and both reactivity and recovery, this yielded significant results that were comparable to the ones presented below.

3 The fact that children show the peak of their physiological responses at different times does not influence the results; for both cortisol and sAA controlling for time from baseline to peak yielded results that are comparable to the ones presented below.

2.2.4. Statistical Analyses

Square root (sqrt) and logarithm (lg10) transformations were applied where necessary to correct skewed data. To assess whether there was a significant increase in cortisol and sAA to the stressor, we used repeated measures ANOVA with Time as a within subject factor. In case of a violation of the sphericity assumption, multivariate statistics are reported.

To test whether reappraisal and suppression were associated with cortisol and sAA reactivity and recovery, two hierarchical regression analyses were performed for all dependent variables. In the first model, all possible confounders and predictors were entered in separate steps. These first models are presented in a footnote to the tables with the final models (see Results). The second and final model contained only variables that individually explained at least 1% of the variance in the first model (calculated as (part correlation)²*100), thus eliminating irrelevant confounders and predictors, and increasing power.

2.3. Results

2.3.1. Preliminary Analyses

Descriptives and correlations of the study variables for the whole sample are presented in Table 2. Cortisol reactivity was significantly higher for girls than for boys (M_{girls} = .17, M_{boys} = -.19, $t(156) = 2.31$, $p < .05$, *Cohen's d* = .37), and cortisol recovery was significantly less for girls than for boys (M_{girls} = 1.27, M_{boys} = .48, $t(156) = 2.37$, $p < .05$, *Cohen's d* = .38). There were no sex differences for the other variables. When correlations were computed separately for girls and boys, girls showed some significant correlations that were not significant for boys. This was the case for the correlations between sAA reactivity and cortisol recovery ($r = .26$, $p < .05$), suppression and cortisol reactivity (r = -.30, $p < .01$), suppression and cortisol recovery (r = -.24, $p < .05$), reappraisal and suppression (r=.44, $p < .01$), and age and pubertal stage (r = .48, $p < .01$). The correlation between suppression and sAA recovery was significant only in boys (r = -.26, $p < .05$).

Because there was a significant correlation between the baseline and peak values for both measures ($r = .32$, $n = 158$, $p < .001$ for cortisol, and $r = .76$, $n = 157$, $p < .001$ for sAA), reactivity was recalculated for both measures by saving the standardized residuals from a regression of the peak reactivity variable on the baseline values (Schuetze, Lopez, Granger, & Eiden, 2008). This resulted in two peak residualized reactivity variables, one for cortisol and one for sAA, that were used as the dependent variables in the subsequent regression analyses.[4]

4 For both cortisol and sAA regression analyses using an autoregressive model predicting peak reactivity while controlling for baseline in Step 1 yielded results that are comparable to the ones presented below.

Table 2 Descriptives and correlations for the study variables.

	Descriptives		Correlations							
	N	*M (SD)*	1.	2.	3.	4.	5.	6.	7.	8.
1. Cortisol reactivity	158	0.00 (1.00)	-							
2. Cortisol recovery	158	0.89 (0.18)	.84***	-						
3. sAA reactivity	157	0.00 (1.00)	.28***	.24**	-					
4. sAA recovery	157	41.17 (5.17)	.15	.14	.55***	-				
5. Suppression	158	4.35 (1.16)	-.16	-.13	-.16*	-.22**	-			
6. Reappraisal	158	3.91 (1.26)	-.02	-.12	.02	.06	.33***	-		
7. Pubertal stage	158	1.98 (0.64)	-.09	-.11	-.08	-.08	.07	.10	-	
8. Age	158	10.61 (0.52)	-.06	-.06	.02	.07	.05	.01	.27***	-
9. Parental education	158	6.30 (1.63)	.00	.02	-.03	.00	.11	.17*	.09	.10
10. Sex	83 girls 75 boys									

* $p < .05$, ** $p < .01$, *** $p \leq .001$

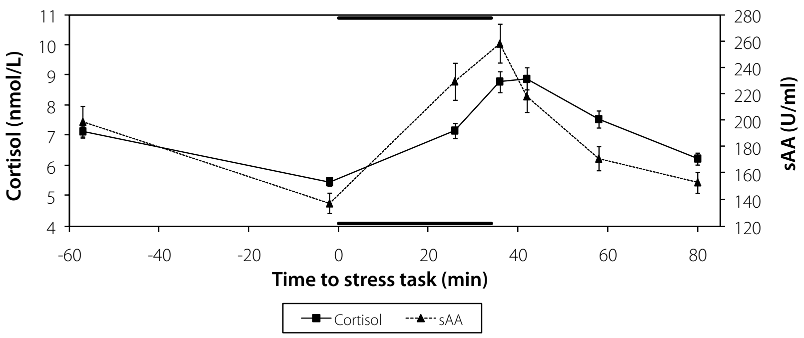

Figure 2 Cortisol and sAA responses to the stress task. Duration of the stressor is indicated with the dark grey bars.

2.3.2. Cortisol Stress Response

Data for each assessment point were first lg10 transformed to normalize the distribution. A repeated measures ANOVA with Time as a within subject factor showed a significant effect of time, Wilks' Lambda = .19, $F(6, 152) = 105.02$, $p < .001$, multivariate partial eta squared = .81. As shown in Figure 2, the significant effect of Time was due to an increase in cortisol in response to the stressor.

2.3.3. sAA Stress Response

Data for each assessment point were first sqrt transformed to normalize the distribution. A repeated measures ANOVA with Time as a within subject factor showed a significant effect of time, Wilks' Lambda = .41, $F(6, 148) = 36.24$, $p < .001$, multivariate partial eta squared = .60. The significant effect of Time was due to an increase in sAA in response to the stressor (see Figure 2).

2.3.4. Associations between Cortisol Reactivity and Emotion Regulation Strategies

The final regression model for the prediction of cortisol reactivity from emotion regulation strategies was significant, and is summarized in Table 3. There was a significant main effect of sex, indicating that girls showed a stronger cortisol response than boys. In addition, there was a significant Suppression * Sex interaction. For girls, more use of suppression was associated with lower cortisol reactivity, whereas there was no relation between suppression and cortisol reactivity in boys (see Figure 3).

Table 3 Final regression model for the prediction of cortisol reactivity.

	B	*SE B*	*β*	*Part²*	R^2_{model}	F_{change}	R^2_{change}
Cortisol reactivity[a, b]							
Step 1					.02	3.46⁺	
Time of day	.20	.13	.12	.01			
Step 2					.07	2.39⁺	.04
Suppression	-.10	.03	-.38**	.06			
Reappraisal	.05	.03	.21⁺	.02			
Sex (girls)[c]	-.10	.05	-.15*	.02			
Step 3					.12	4.61*	.05
Suppression * Sex	.13	.04	.31**	.05			
Reappraisal * Sex	-.08	.04	-.20⁺	.02			

[a] Initial model for cortisol reactivity (sqrt): step 1 – age, parental education level (lg10), puberty (sqrt), time of day (lg10); step 2 – suppression, reappraisal, sex; step 3 – suppression * reappraisal, suppression * sex, reappraisal * sex; step 4 – suppression * reappraisal * sex. [b] We also tested a model with cortisol recovery (sqrt) as a dependent variable. This initial model was the same as that for cortisol reactivity, except that in step 1 peak cortisol value (lg10) was also entered in the model. As none of the variables of interest individually explained at least 1% of the variance in the first model, there was no final model for cortisol recovery. [c] Sex was coded as 0 (girl) or 1 (boy).
⁺ $p < .10$, *$p < .05$, ** $p < .01$

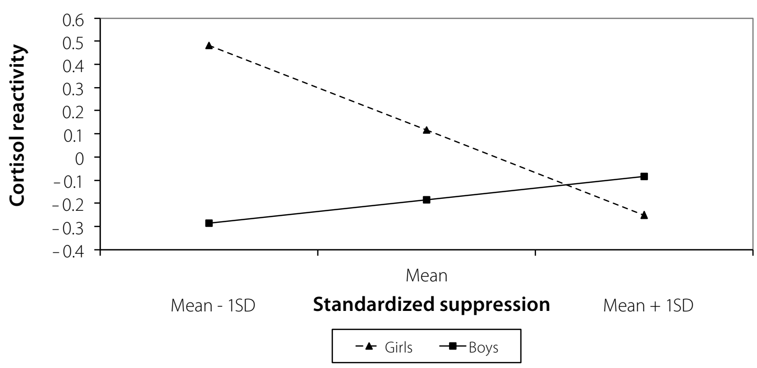

Figure 3 The relation between suppression use and cortisol reactivity separately for girls and boys.

2.3.5. Associations between Cortisol Recovery and Emotion Regulation Strategies

As none of the variables of interest individually explained at least 1% of the variance in the first model, there was no final model for cortisol reactivity. Hence, there was no relation between use of reappraisal and suppression, and cortisol recovery.

2.3.6. Associations between sAA Reactivity and Emotion Regulation Strategies

The final regression model for the prediction of sAA reactivity from emotion regulation strategies was marginally significant, and is summarized in Table 4. There was a significant effect of suppression, such that more suppression was related to lower sAA reactivity.

Table 4 Final regression models for the prediction of sAA reactivity and recovery.

	B	*SE B*	*β*	*Part²*	R^2_{model}	F_{change}	R^2_{change}
sAA reactivity[a]					.03	2.60⁺	
Suppression	-.03	.01	-.19*	.03			
Reappraisal	.01	.01	.09	.01			
sAA recovery[b]							
Step 1					.14	8.61***	
Age	.64	.42	.12	.01			
Puberty	-1.36	.98	-.10	.01			
Peak sAA value	.19	.04	.35***	.12			
Step 2					.21	6.16**	.07
Suppression	-.66[c]	.19	-.26***	.06			
Reappraisal	.33	.18	.14⁺	.02			

[a] Initial model for sAA reactivity (lg10): step 1 – age, parental education level (lg10), puberty (sqrt), time of day (lg10); step 2 – suppression, reappraisal, sex; step 3 – suppression * reappraisal, suppression * sex, reappraisal * sex; step 4 – suppression * reappraisal * sex. [b] Initial model for sAA recovery (sqrt) was the same as that for sAA reactivity, except that in step 1 peak sAA value (sqrt) was also entered in the model. [c] Note that lower values for sAA recovery represent quicker recovery.
⁺ $p < .10$, * $p < .05$, ** $p < .01$, *** $p \leq .001$

2.3.7. Associations between sAA Recovery and Emotion Regulation Strategies

The final regression model for the prediction of sAA recovery from emotion regulation strategies was significant and is summarized in Table 4. There was a significant effect of suppression, such that more suppression was related to quicker sAA recovery.

2.4. Discussion

The current study sought to examine whether 10-year-old children's spontaneous use of the emotion regulation strategies suppression and reappraisal during a psychosocial stress task was related to their cortisol and sAA responses to that task. We found that the stress task led to an overall increase in both cortisol and sAA, indicating that it was effective in inducing stress in the current sample. For cortisol, reactivity was higher in girls than in boys. For the relation between physiological stress reactivity and emotion regulation strategies we found that more use of suppression was related to lower cortisol reactivity in girls, and to lower sAA reactivity in the whole sample. In addition, we found that more use of suppression was also related to quicker sAA recovery. There was no relation between reappraisal and physiological responses to the stress task.

The finding that girls showed higher cortisol reactivity than boys is contrary to results in adults, where men are often found to show higher reactivity to psychosocial stressors than women (see review by Kudielka, et al., 2009). Sex differences in cortisol reactivity to a psychosocial stressor have not been consistently found in middle childhood (e.g. Gunnar, et al., 2009). However, this may have to do with limited power to detect these differences, due to smaller sample sizes. A possible explanation for our findings might lie in psychological differences between boys and girls at this age, as research has shown that girls report more fear of failure and criticism than boys (Gullone, 2000). The socio-evaluative nature of the stress task may therefore have made the task more stressful for the girls than for the boys. Alternatively, as boys have been found to feel more competent at math than girls (e.g. Herbert & Stipek, 2005), it is possible that they perceived the mental arithmetic part of the stress task as less stressful than girls did, leading in turn to less cortisol reactivity in boys.

Contrary to our findings for cortisol, there was no sex difference in sAA reactivity to the stress task. This is consistent with earlier research in adults (see Rohleder & Nater, 2009), and children (Sumter, Bokhorst, Miers, Van Pelt, & Westenberg, 2010). This is possibly due to the fast responding nature of the SNS, which may make this system less susceptible to individual differences in how the stress task is perceived and experienced. Consistent with the fast-responding nature of the SNS, in the current study sAA showed a faster increase in concentration in response to the stress task than cortisol, in addition to an earlier peak and faster recovery to the stress task.

The finding that the use of more suppression was related to lower physiological reactivity and quicker sAA recovery to the stress task is opposite to what we had hypothesized: we had expected to find that more use of suppression would be associated with higher physiological reactivity and slower sAA recovery. One possible explanation for this unexpected finding is that our current study used a state measure of emotion regulation, versus the trait measure used in most other studies. A state measure of emotion regulation is more likely to be influenced by interactions between participant dispositional factors and the situational demands (Egloff, et al., 2006). As such, it is possible that for children who experienced the stress task as more demanding, this experience resulted in higher physiological reactivity, slower sAA recovery, and more difficulty in suppressing the outward signs of their distress.

Another possible explanation for the unexpected finding that the use of more suppression was related to lower physiological reactivity and quicker sAA recovery may lie in the difference in age of the current sample versus those in previous studies (e.g. Lam, et al., 2009). In adults, suppression could be regarded as a less effective emotion regulation strategy, as it may increase physiological arousal (Gross, 1998a) and the experience of negative affect (Gross & John, 2003), as opposed to reducing it. For 10-year-olds, however, suppression could be the most adequate strategy that is currently available. Although around this age emotion regulation shifts from a behaviourally oriented approach to the use of more cognitive strategies (Meerum Terwogt & Stegge, 1995), it could be argued that the efficiency with which these emerging cognitive strategies are used is still limited. As a result, the well-practiced behaviourally oriented strategies like suppression may still work better to reduce physiological responses than the more cognitive strategies like reappraisal.

It should be noted that the negative association between suppression and physiological reactivity and sAA recovery can be interpreted in different ways. It may indicate, for example, that suppression is effective in decreasing physiological responding. But it is also possible that children who were more stressed by the task and experienced more persistent arousal, were less able to use suppression to regulate their emotions. Our current research is unable to differentiate between these two different explanations for the found effects. A next step would be to experimentally manipulate the use of reappraisal and suppression in order to investigate the causal direction of the current findings.

The finding that suppression was related to lower cortisol reactivity in girls but not in boys could mean that girls and boys use suppression differentially, resulting in different effects on cortisol reactivity. Indeed, in middle childhood, girls tend to replace one emotional display with another, whereas boys tend to neutralize their emotional expressions (Zeman, et al., 2006). Perhaps the way girls use suppression is more demanding, thereby providing distraction from the demands of the stress task, which could in turn lead to lower cortisol reactivity. For sAA, this distraction may not influence reactivity because it is a faster responding measure. This would be in line with our first possible explanation, namely that suppression is effective in decreasing physiological responding.

Although we did not find any sex differences in state use of suppression in the current sample, Gullone et al. (2010) found that trait use of suppression was higher in boys than in girls. As a result, boys may be more experienced in using this type of emotion regulation. This could mean boys were able to use suppression regardless of their level of distress, whereas girls may have had more difficulty using suppression as stress levels increased, resulting in lower self-reported suppression scores. This would be in line with our second possible explanation, namely that stressed children are less able to use suppression to regulate their emotions.

A third, and more general, possible explanation is that girls in the current study that were high in their use of suppression, also used other strategies to actively regulate their emotions. It may be that the use of one or a combination of these strategies led to the current results. Therefore, methodological research into the specificity of the ERQ scales when used in children would be interesting, as well as inclusion of other emotion regulation strategies in future studies. For example, the other antecedent focused strategies proposed by Gross (1998b): situation selection, situation modification, and attentional deployment, could be operationalized to investigate their relation to physiological stress responses.

Although we had expected that more use of reappraisal would be associated with lower physiological stress reactivity, we did not find a relation between reappraisal and physiological stress reactivity. For sAA, this is in line with the results of earlier studies investigating the relation between reappraisal and SNS activation (e.g. Egloff, et al., 2006; Gross, 1998a). Perhaps reappraisal, being a very cognitively based emotion regulation strategy, is unable to influence the relatively fast and automatic response of the SNS.

More generally, the lack of an association between reappraisal and cortisol and sAA stress reactivity in the current study may be the result of the way the reappraisal items in our state measure of emotion regulation were formulated. We asked participants to indicate to which extent the items were true for them *during* the stress task, as opposed to *prior* to the stress task. Thus, the answers to the questions may indicate a more response-focused use of reappraisal, versus the antecedent-focused use indicated in the model by Gross (Gross, 1998a). Perhaps the response-focused type of reappraisal is unable to influence physiological reactivity, as it is employed once physiological responses have already been initiated.

Another possible explanation for the lack of a relation between reappraisal and physiological stress responses is that the relation is curvilinear, as is for example the case for the relation between physiological responses and (mal)adaptive functioning (e.g. Charmandari, et al., 2005). However, post-hoc analyses showed no evidence of a curvilinear relation between reappraisal and the physiological stress responses.

There are several strengths and limitations to the current study that provide directions for future research. First, the study measures both HPA axis reactivity and SNS reactivity, thus providing information on both of the major physiological stress systems. This is important, as alterations in both of these systems have been related to psychopathology (e.g. Boyce et al., 2001; Van Goozen, Matthys, Cohen-Kettenis, Buitelaar, & Van Engeland, 2000), and both have a different function and time frame within stress responses.

Second, state measures are rarely used to relate the use of emotion regulation strategies to physiological responding, despite evidence that situational demands, in addition to participant dispositional factors, are important in determining the use of emotion regulation strategies (Egloff, et al., 2006). The current study shows that 10-year-old children are already able to reliably report on their strategy use during a stress task, and indicates that meaningful links between these reports and other important variables can be found. This opens the way for future research employing state measures of emotion regulation, for example more frequent sampling of state emotion regulation use during a stress task as a way to help determine causality of the current results.

Third, studying a large sample of children from a relatively small age range allowed us to reveal pronounced sex differences in the relation between use of suppression and cortisol reactivity. This stresses the importance of sample sizes large enough to allow for between-sex comparisons. However, this also limits the generalizability of our results to other age groups. Future studies are needed to explore the relation between emotion regulation and physiological responding across the lifespan, as it seems plausible that developmental changes influence the relation between emotion regulation and physiological reactivity.

Finally, it is important to note that the amount of variance in cortisol and sAA stress reactivity explained by the use of reappraisal and suppression is small. This indicates that, at least for 10-year-olds, the spontaneous use of these emotion regulation strategies is not very effective in regulating their physiological reactions to psychosocial stress. Future research could focus on whether the use of these strategies is more effective at other ages, and which other factors, for example self-esteem, personality, experienced emotions, and position in the peer group, contribute to physiological stress reactions at this age.

In conclusion, the current study is the first to investigate the relation between the spontaneous use of the emotion regulation strategies suppression and reappraisal and physiological stress responses to a psychosocial stressor in children. Our results indicate that these emotion regulation strategies are used and can be successfully measured even in 10-year-olds. While the effect of suppression on physiological responses was opposite of what could be expected, reappraisal appeared altogether ineffective at down-regulating physiological responses to a psychosocial stressor in this age group. These results stress the importance of a developmental perspective on the relation between the use of specific emotion regulation strategies and physiological responses, as it shows that results from studies in adult populations are not necessarily generalizable to children. Interestingly, we found a sex difference in cortisol reactivity to the psychosocial stressor. We also found a sex difference in the relation between cortisol reactivity and the use of suppression, indicating that at this age sex may be an important moderator variable in research studying stress reactivity and its correlates.

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3

The Relation between Gaze Aversion and Cortisol Reactivity in Middle Childhood[5]

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5 A revised version of this chapter has been accepted for publication in Hormones and Behavior

Summary

The present study sought to investigate the relation between ethological observations of children's gaze aversion during a psychosocial stress task and their cortisol reactivity to the task, and how this relation might be moderated by how stressful the children perceived the stress task to be.

Videos of 140 children (74 girls; M_{age} = 10.60 years) performing a psychosocial stress task in front of a jury were coded for displays of the children's gaze aversion from the jury, and saliva samples were taken to determine their cortisol reactivity. A questionnaire assessed the children's level of perceived stress. Results showed higher cortisol reactivity in children who perceived the task as more stressful. Furthermore, a quadratic relation between gaze aversion and cortisol was found which depended on the level of perceived stress: for children with low levels of perceived stress, cortisol reactivity was *lowest* with intermediate levels of gaze aversion, whereas for children with high levels of perceived stress cortisol reactivity was *highest* at intermediate levels of gaze aversion.

These results indicate a certain degree of coherence between subjective and physiological stress responses in 9- to 11-year-olds, and indicate that gaze may play an important role as a behavioural coping strategy at this age.

3.1. Introduction

Behavioural coping strategies are the earliest available means to voluntarily regulate emotions and deal with stressful situations (Zimmer-Gembeck & Skinner, 2011). One such behavioural strategy is gaze behaviour. When confronted with a stimulus that is threatening or induces stress, a person can adopt a vigilant strategy, by looking more at the stimulus, or an avoidant strategy, by looking away from the stimulus (Wilson & MacLeod, 2003). Being able to voluntarily engage and disengage attention is considered a critical dimension of self-regulation (Posner & Rothbart, 2000), and as such, the development of adequate use of gaze aversion could be considered an important attainment in children's development. However, coping with a stressful situation not only occurs at the behavioural level but at the physiological level as well (McEwen, 2004), for example by the release of the stress hormone cortisol (Gunnar & Quevedo, 2007). Knowing how coping with stress at these different levels is combined during stress regulation is important, as difficulties with stress regulation are related to psychopathology (e.g. McEwen, 2003; Mogg, Philippot, & Bradley, 2004). To date, relatively little is known about the relation between gaze aversion and cortisol reactivity. The current study aims to investigate how the use of gaze aversion, as a behavioural response to a stressor, is related to cortisol reactivity to that stressor in middle childhood.

Gaze aversion is one of the first behaviours for coping with stressful stimuli. Infants already use gaze aversion to diminish stimulation. For example, 6-month-old infants use gaze aversion to regulate their emotions when confronted with a stranger, especially if this stranger is insensitive and controlling (Mangelsdorf, Shapiro, & Marzolf, 1995). Also at older ages gaze aversion seems to be employed as a way of regulating psychosocial stress. For example, 8-year-olds show more gaze aversion during face-to-face questioning than during questioning across a live video link (Doherty-Sneddon & Phelps, 2005). In adults, it has been found that under conditions of social stress, individuals high in social anxiety looked at emotional faces for less time than individuals low in social anxiety, indicating that anxious individuals might use gaze aversion as a strategy to reduce their discomfort (Garner, Mogg, & Bradley, 2006).

Cortisol is a steroid hormone that is released in response to stress by activation of the hypothalamic-pituitary-adrenocorticol (HPA) axis. It takes about 25 min for cortisol to reach peak levels in response to a stressor (Gunnar & Quevedo, 2007). Although cortisol reactions to stress serve to adapt to these challenges and as such are generally beneficial, failure to recover from cortisol elevations after stressor termination, or repeated cortisol increases as a result of repetitive exposure to stress is thought to have adverse effects (McEwen, 2004). The amount of cortisol in saliva can be used as a non-intrusive indicator of HPA axis activation in response to stress tasks used in psychological research (Hellhammer, Wüst, & Kudielka, 2009; Kirschbaum & Hellhammer, 1994).

Few studies have investigated the relation between cortisol reactivity and gaze aversion. Sgoifo et al. (2003) subjected adult participants to a stress task in which they were asked to describe their own personality features in front of a four-person audience and a video camera. Results showed that participants that displayed more gaze aversion from the audience during the task had lower cortisol reactions to the task. However, this result may not generalize to children, as the use of gaze aversion as a coping strategy may change over age, in relation to cognitive development. In addition, cortisol reactivity to stressors also shows developmental changes (Gunnar, Wewerka, Frenn, Long, & Griggs, 2009). Indeed, the relation between gaze aversion and cortisol reactivity seems different in a younger population. In a study with healthy 6-15 year old children, cortisol responses to a social challenge stress test were related to the use of gaze during the test: increased cortisol reactivity was found in children who displayed very high or very low levels of gaze aversion, i.e. either continuous staring or total gaze aversion, while lower reactivity was found in children with "good quality" gazing, who kept eye contact with the jury without staring or avoiding (Hessl, Glaser, Dyer-Friedman, & Reiss, 2006). This indicates that in childhood, the relation between gaze avoidance and cortisol reactivity might well be U-shaped, with intermediate levels of gaze aversion related to lower cortisol reactivity.

The goal of the present study was to relate ethological observations of children's gaze aversion during a psychosocial stress task to their cortisol reactivity to the task. Middle childhood was targeted as an age group as this is considered the earliest age at which children are able to independently participate in a laboratory session. Additionally, for this age there is an age-appropriate stress test that has repeatedly proven to be effective in inducing a physiological stress response (Buske-Kirschbaum et al., 1997). Based on the results by Hessl et al. (2006) we hypothesized that the relation between gaze aversion and cortisol reactivity would be U-shaped, with lowest levels of cortisol at intermediate levels of gaze aversion. In addition, we took into account that gaze aversion as a strategy to cope with a stressful task might depend on how stressful children experience the task. Therefore, we also measured perceived stress and explored whether this moderated the relation between gaze aversion and cortisol reactivity.

3.2. Method

3.2.1. Participants

Children (age 9-11) were recruited through 31 primary schools in Nijmegen and surrounding areas (the Netherlands) to participate in a study on responses to stress and their consequences for cognitive functioning. Exclusion criteria were the use of psychotropic or corticosteroid medication, stuttering, and a diagnosis of a developmental disorder. Recruitment (for details see De Veld, Riksen-Walraven & De Weerth, 2012) resulted in 165 participants. Two children were excluded because during data collection it was discovered they met one of the exclusion criteria. Furthermore, children were excluded from the present analysis for different reasons: five because they did not complete the entire data collection protocol, six because they were too distressed to complete the stress task, and twelve because observational data were missing or incomplete due to technical problems. The final sample for this study therefore consisted of 140 children (74 girls; M_{age} = 10.60 years, *SD* = .53). The majority of the participants was Caucasian (94%), and had at least one parent with a college or university degree (77%).

The study was approved by the ethics committee of the Faculty of Social Sciences of the Radboud University Nijmegen, the Netherlands. All children participated voluntarily, and all parents provided written informed consent prior to their child's participation.

3.2.2. Procedure

A week before the stress test, all children completed questionnaires and memory tasks during a home visit (not relevant for the current study).

Testing for the present study took place after school in the laboratory of the Behavioural Science Institute of the Radboud University Nijmegen. On arrival, children were led to a separate room, where the experimenter explained that they would be asked to do some tasks and fill out several questionnaires. After this introduction, children provided a saliva sample (S1; within 5 min after arrival), filled out several questionnaires, and performed a memory task. This was followed by a 30-min relaxation period during which children listened to relaxing music, and could read a magazine or make puzzles. Right after relaxation they filled in a short questionnaire and provided a second saliva sample (S2). After this, children were taken to an adjacent room where an adapted and extended version of the Trier Social Stress Test for Children (TSST-C; Buske-Kirschbaum, et al., 1997) was initiated to induce stress. The TSST-C consists of a public speaking task and a mental arithmetic task in front of a jury of two confederates in white lab coats. In the present study, children were asked to pick a favourite and least preferred present out of six small items (e.g. an inflatable ball or toilet brush) right before entering the TSST-C room (Jones et al., 2006), and were told that a favourable judgement by the jury would earn them their favourite present, whereas in case of an unfavourable judgement they would get the least preferred present. After the TSST-C, children were seated in front of the TSST-C jury. There they performed a working memory task, supplied a saliva sample (S3), filled out a short questionnaire, and performed an additional memory task. This entire procedure took approximately 34 min. Afterwards, the children went back to the first room. There they provided another saliva sample (S4), completed the perceived stress questionnaire (see Section 3.2.3), another questionnaire, provided a fifth saliva sample (S5), completed another questionnaire, received positive feedback on their performance during the stress task, and completed a short questionnaire. Then, a 25-min post-stress relaxation period was initiated. Ten minutes into this relaxation period, a saliva sample was obtained (S6). After relaxation, children completed several questionnaires, performed a memory task, provided a last saliva sample (S7), completed a last questionnaire, and were debriefed. The entire procedure took approximately 2.5 h. More details on the laboratory session can be found in De Veld et al. (2012).

3.2.3. Instruments and Measures

Behavioural observations

The TSST-C procedure was recorded by a wall-mounted camera placed in between and slightly above the two jury members. Recordings were scored afterwards by two trained assistants and the first author using The Observer 9.0 (The Observer XT). Observers were blind to the other study variables. As studies indicate that people often gaze upwards just prior to answering a mental arithmetic question (e.g. Previc & Murphy, 1997), and this was confirmed by our own observations, the mental arithmetic task was not used for the present study. Observations therefore started at the initiation of the public speaking component of the TSST-C, and ended when the jury indicated the public speaking component of the TSST-C was over. Data were coded using interval coding (Bakeman & Gottman, 1997). For every 2-s interval, the observer coded whether the child's gaze had been towards the jury or averted (see Table 1 – "Gaze"). Children's gazes at the jury and the camera were conflated because it was impossible to determine the exact direction of the participants' gaze. Interobserver reliability calculated over 24% of the recordings was good (Cohen's kappa = .80). The proportion of time the child averted its gaze from the jury was computed by dividing the number of intervals scored with 'Aversion' by the total number of intervals.

We expected the child's gaze behaviour to depend upon who is speaking. For example, little variation in gaze behaviour between children might be expected when the jury speaks to the child. Therefore, we also coded who spoke during every 2-s interval (jury, child, or nobody, see Table 1 – "Who speaks?"; Cohen's kappa = .93).

Cortisol

To obtain reliable saliva samples, participants were asked to only drink water in the 2 h before participation, to limit physical exercise in the hour prior to participation, and to abstain from eating for at least 45 min before participation. The sampling procedure was as follows. Participants swallowed all saliva in order to empty their mouths, and

Table 1 Behaviour categories and definitions.

Behaviour	Definition
A. Gaze	
1. At jury	Child looks at jury or camera at some point within the 2-s interval
2. Aversion	Child looks neither at jury, nor camera for the entire 2-s interval
B. Who speaks?	
1. Child	Child is speaking at some point during the 2-s interval, jury is silent
2. Jury	Jury is speaking at some point during the 2-s interval, regardless of whether the child also speaks
3. Nobody	Nobody is speaking for the entire 2-s interval

collected all subsequently secreted saliva in their mouths for 2 min, after which they used a short straw to spit the saliva into a small tube. This procedure was repeated until at least 0.25 ml of saliva was collected, with a maximum total collection time of 5 min.

Seven saliva samples were obtained, namely at -57 (S1), -2 (S2), 26 (S3), 36 (S4), 42 (S5), 58 (S6), and 80 (S7) min from the onset of the stressor. All samples were kept frozen at -20 °C until their shipment to the analysis lab.

Cortisol concentrations were determined at the Endocrinology Laboratory of the University Medical Center Utrecht, using an in house competitive radio-immunoassay employing a polyclonal anticortisol-antibody (K7348). [1,2-3H(N)]-hydrocortisone (Amersham TRK407) was used as a tracer. The lower limit of detection was 1 nmol/L and inter-assay and intra-assay variations were below 10%.

All cortisol data were screened for outliers, which were defined within each assessment point as values greater than 3 SD above the mean. All outliers (n = 23) were winsorized by replacing their values with the value of 3 SD above the mean (Tukey, 1977).

To compute the strength of children's cortisol response to the public speaking task, we first determined a baseline value by selecting the lower of the two pre-stress values for each participant. Taking into account that cortisol takes about 25 min to reach peak levels (Gunnar & Quevedo, 2007), S4 was selected to represent stress reactivity to the public speaking task. Both variables were lg10 transformed to normalize their distributions.

Perceived stress

Children's perceived stress during the TSST-C was assessed with a translated version of the questions used as a manipulation check used by Buske-Kirschbaum et al. (1997).

Children were asked ten questions on how they experienced the TSST-C procedure and were asked to mark their answers on 10 cm visual analogue scales. Five out of ten items pertained to the public speaking component of the TSST-C. Reliability analysis on these items revealed that the reliability of the perceived stress scale could be improved by omitting two out of five items. (i.e. 'How much did you like the story?' and 'Do you think there was enough time to tell a good story?'). The remaining items were: 1) How difficult was it for you to tell the story? 2) How difficult was it to tell the story in front of other people? and 3) How scary was the idea that the audience would evaluate your story? Cronbach's alpha was .69 for these remaining items. Participants' scores on these items were summed to obtain a perceived stress score, with higher scores reflecting more perceived stress.

Potential confounders

Parental education level was assessed for both parents on an 8-point scale (1 = primary education, 8 = university degree). Values for both parents were averaged to obtain a single score for analysis.

Participants' stage of pubertal development was assessed through self-report on a 5-point scale using Tanner criteria (Marshall & Tanner, 1969, 1970), with higher scores indicating more advanced physical development.

3.2.4. Statistical Analyses

Square root (sqrt) and logarithm (lg10) transformations were applied where necessary to correct skewed data.

To examine the association between children's gaze aversion during the public speaking task and their subsequent cortisol reactivity, we subsequently performed two hierarchical regression analyses with cortisol reactivity as the dependent variable. Variables used in interaction terms were centred prior to their inclusion. In the first regression analysis, all potential confounders (sex, age, parental education, and pubertal stage), predictors (gaze aversion, perceived stress), the quadratic term (gaze aversion * gaze aversion), and interactions (gaze aversion * perceived stress, gaze aversion * gaze aversion * perceived stress) were entered in separate steps. In the second regression analysis, only variables that individually explained at least 1% of variance in the first model (calculated as $(\text{part correlation})^2 \times 100$) were entered to eliminate irrelevant confounders and increase power.

3.3. Results

3.3.1. Preliminary Analyses

Descriptives and correlations of the study variables are presented in Table 2. An overview of cortisol levels throughout the session is presented in Figure 1. Due to a

Table 2 Descriptives and correlations for the study variables.

	Descriptives		Correlations				
	N	*M* (*SD*) or *Md* (*IQR*)	1.	2.	3.	4.	5.
1. Cortisol reactivity	140	-.14 (-.91 – .56)	-				
2. Gaze aversion	140	.52 (.25)	.08	-			
3. Perceived stress	140	21.36 (5.48)	.28**	.11	-		
4. Age	140	10.60 (.53)	-.07	-.14	-.08	-	
5. Pubertal stage	140	2.00 (1.5 – 2.5)	-.11	-.01	-.03	.25**	-
6. Parental education	140	7.00 (5.13 – 7.5)	.05	-.07	-.08	.15	.08
7. Sex	74 girls 66 boys						

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

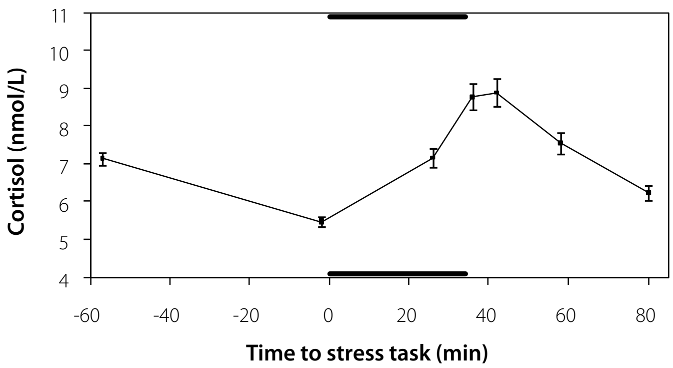

Figure 1 Cortisol reactivity to the stress task. Duration of the stressor is indicated with the dark grey bars.

significant correlation between the baseline and peak cortisol variables ($r = .33$, $p < .001$), reactivity was recalculated by saving the standardized residuals from a regression of the peak reactivity variable on the baseline values (Schuetze, Lopez, Granger, & Eiden, 2008). The resulting peak residualized reactivity variable was used as the dependent variable in all analyses. As the data revealed that children almost always gazed towards the jury when the jury was speaking (*Md* = 1, *IQR* = .90 – 1), gaze aversion was recalculated excluding children's gaze aversion during those intervals.

Table 3 Final regression model for the prediction of cortisol reactivity.[a]

	B	*SE B*	*β*	R^2_{model}	F_{change}	R^2_{change}
Step 1				.11	5.61**	
Gaze aversion	.08	.10	.07			
Sex[b]	-.11	.05	-.18*			
Perceived stress	.02	.01	.41**			
Step 2				.12	.46	.01
Gaze aversion * gaze aversion	.16	.38	.03			
Gaze aversion * perceived stress	.00	.02	-.01			
Step 3				.14	4.35*	.03
Gaze aversion * gaze aversion * perceived stress	-.16	.08	-.25*			

[a] Initial model for cortisol reactivity (sqrt): step 1 – age, parental education level (lg10), puberty (sqrt), time of day (lg10); step 2 – sex, gaze aversion, perceived stress; step 3 – gaze aversion*gaze aversion, gaze aversion*perceived stress; step 4 – gaze aversion*gaze aversion*perceived stress. [b] Girls = 0, boys = 1.
* $p < .05$, ** $p < .01$.

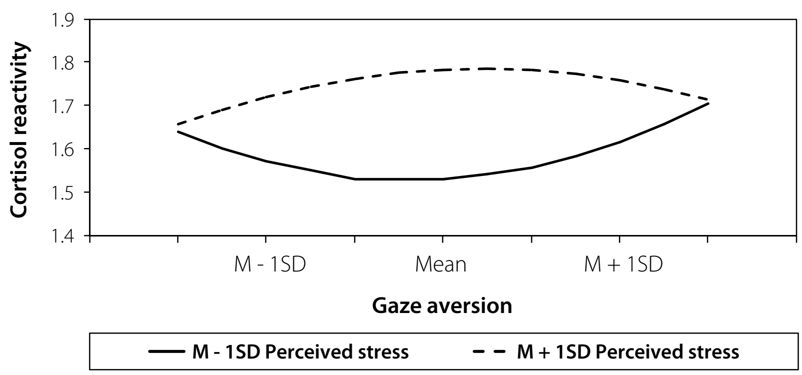

Figure 2 The quadratic relation between gaze aversion and cortisol reactivity, separately for children with high versus low levels of perceived stress.

3.3.2. Association between Gaze Aversion and Cortisol Reactivity

The final regression model for the association between gaze behaviour and cortisol reactivity was significant ($F(6, 133) = 3.74$, $p < .01$), and is summarized in Table 3. There was a significant main effect of sex, indicating that girls showed higher cortisol reactivity than boys, and a significant main effect of perceived stress, such that more perceived stress was related to higher cortisol reactivity. In addition, there was a significant gaze aversion * gaze aversion * perceived stress interaction, indicating that the quadratic relation between gaze aversion and cortisol reactivity depended on the level of perceived stress. To further explore the nature of this effect, the interaction was plotted based on Aiken and West (1991). Figure 2 shows that for participants with low levels of perceived stress, cortisol reactivity was *lowest* with intermediate levels of gaze aversion, whereas for participants with high levels of perceived stress, cortisol reactivity was *highest* at intermediate levels of gaze aversion.

3.4. Discussion

In this study we investigated whether children's gaze aversion during a psychosocial stress task was related to their cortisol reactivity to the task, and explored whether this relation was moderated by perceived stress. Based on the results reported by Hessl et al. (2006) we expected that the relation between gaze aversion and cortisol reactivity would be U-shaped, with lowest levels of cortisol at intermediate levels of gaze aversion. We indeed found the hypothesized U-shaped relation, but only for participants with low levels of perceived stress. For children with high levels of perceived stress, in contrast, cortisol reactivity was highest at intermediate levels of gaze aversion. Finally, we found that more perceived stress was related to higher cortisol reactivity, and that girls showed higher cortisol reactivity than boys.

Although a relation between perceived stress and cortisol reactivity is often assumed, such coherence is not consistently found (e.g. Buske-Kirschbaum, et al., 1997; Buske-Kirschbaum et al., 2003; Cohen et al., 2000; Hjortskov, Garde, Ørbæk, & Hansen, 2004). In the present study we did find that higher levels of perceived stress were related to higher cortisol reactivity. This might well be due to the type of questions we used to operationalize perceived stress. A meta-analysis by Denson et al. (2009) suggested that although ratings of global mood states are unrelated to cortisol reactivity, there are relations for more specific appraisals of challenge, threat, novelty, intensity, and surprise. The current perceived stress measure focused on how difficult the children had found it to tell their story to the jury and how scary the children had found the idea that the jury would evaluate the story. This approach might have tapped the constructs of challenge and threat in a successful way, hence explaining our findings of a positive relation between perceived stress and cortisol reactivity.

The hypothesized U-shaped relation between gaze aversion and cortisol reactivity, indicating the *lowest* cortisol reactions at intermediate levels of gaze aversion, was only confirmed for children with low levels of perceived stress. For children with high levels of perceived stress, this relation was reversed, with the *highest* cortisol reactivity at intermediate levels of gaze aversion. Our hypothesis was based on a control group of unaffected siblings of children with fragile X syndrome (FXS) in a study by Hessl et al. (2006). Interestingly, their results suggested an inverted U-shape relation between gaze aversion and cortisol reactivity in the clinical group. FXS is a heritable cause of developmental disability, characterized not only by cognitive impairment, but also by social anxiety and withdrawal, among other things (Hessl, et al., 2006). Although it is somewhat speculative to assume that the FXS and control groups from Hessl et al. (2006) correspond to high and low perceived stress levels, this does not seem unlikely and therefore interesting to point out.

An important next question is *why* the relation between children's gaze aversion and cortisol reactivity was different according to children's level of perceived stress. It is possible that intermediate levels of gaze aversion in children with low levels of perceived stress indicate, or even facilitate, relaxation, whereas for children with high levels of perceived stress intermediate gaze aversion might indicate the lack of a consistent use of gaze behaviour as a coping strategy. For these children, high levels of gaze aversion might dampen cortisol reactivity by shutting out the stressor, whereas low levels of gaze aversion might dampen reactivity by providing children with a sense of control over the situation. These findings appear to fit the model about dispositional coping modes proposed by Krohne (1989). In this model, two independent dimensions are deemed central to determining a person's coping style: 1) intolerance of uncertainty, and 2) intolerance of emotional arousal. Four potential coping styles are then defined by combining these dimensions: 1) "repressors" who employ an avoidant coping style due to high intolerance of emotional arousal but low intolerance of uncertainty, 2) "sensitizers" who employ vigilance due to low intolerance of emotional arousal but high intolerance of uncertainty, 3) "unsuccessful copers" who switch relatively fast between vigilance and avoidance due to high intolerance for both emotional arousal and uncertainty, and 4) "nondefensives" who neither use vigilance, nor avoidance very often due to low intolerance for both emotional arousal and uncertainty. It would be interesting for future research to further investigate the applicability of this model, for example by using continuous coding of gaze behaviour, as the main distinction in gaze behaviour between unsuccessful copers and nondefensives would be that the first would show faster and more frequent shifts between vigilance and avoidance. Indeed, we did observe some very short-duration shifts in gaze in a subsample of children, indicating that even with intervals as short as 2 s there is some loss of relevant information. Headset eye-tracking devices might be instrumental in this respect but their presence might affect gaze behaviour. Alternatively, self-report questionnaires allowing a classification of each participant into one of the above-mentioned coping style categories could be incorporated.

It must be noted that the present study does not allow any conclusions about the causal direction of the relation between gaze aversion and cortisol reactivity. It is possible that the level of gaze aversion serves as a behavioural coping strategy to such an extent that it dampens cortisol reactivity. However, it is also possible that cortisol levels influence attentional processes that steer gaze aversion. For example, research in adults has shown that cortisol administration can reduce anxiety-driven selective attention to threat (Putman, Hermans, Koppeschaar, van Schijndel, & van Honk, 2007). Finally, it is also possible that gaze aversion and cortisol levels dynamically influence each other. Future research will be needed to determine the exact mechanism responsible for the findings in the present study, for example by experimentally manipulating levels of gaze aversion and of cortisol.

Another aspect of the current study that must be noted is that the current analysis did not control for the potential effects of other behaviours that have been shown to be related to physiological stress responses, like displacement (Mohiyeddini, Bauer, & Semple, 2013; Pico-Alfonso et al., 2007). It is possible that use of displacement behaviour allowed some children to gaze toward the jury without substantial cortisol reactivity. Future research would therefore benefit from the inclusion of additional behavioural categories, like displacement, to develop a more complete understanding of how behaviour during psychosocial stress is related to physiological stress responses.

Another suggestion for future research would be to investigate the relation between SNS activation and gaze aversion. This would complement the present research, as SNS activation is another important feature of the physiological stress response (Gunnar & Quevedo, 2007). As the SNS is a much faster responding system than the HPA axis, it would be expected that the relation between this system and gaze aversion is of a much more dynamic nature. In this regard, momentary measures of SNS activation, like salivary alpha-amylase, would seem unsuited for this purpose. Future research would therefore benefit from using both moment-to-moment measurements of SNS activation, as well as continuous measures of gaze behaviour. In addition, a suggestion for future research would be to recruit enough participants to allow for sex differences in cortisol reactivity to emerge, as the present finding that girls showed higher cortisol reactivity than boys has probably become visible thanks to relatively large power as a result of our large sample size.

The present study makes several important contributions to our understanding of the interrelations between children's emotional, behavioural and physiological stress responses. First, we found that higher levels of perceived stress were related to higher cortisol reactivity, indicating a certain degree of coherence between subjective and physiological stress responses in 9- to 11-year-olds. Second, the finding that the relation between gaze aversion and cortisol differed as a result of how stressful the children found the task underlines the complexity of human stress responses. At the same time it shows that gaze may play an important role as a behavioural coping strategy at this age. And finally, the fact that children who experienced the task as highly stressful showed the highest cortisol reactivity at intermediate levels of gaze aversion might be pointing at an absence of consistent gaze behaviour that is exacerbating the cortisol reaction to stress in these children.

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4

Acute Psychosocial Stress and Children's Memory[6]

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6 This chapter is submitted for publication

Summary

We investigated whether children's performance on working memory (WM) and delayed retrieval (DR) tasks decreased after stress exposure, and how physiological stress responses related to performance under stress.

158 children (83 girls) performed two WM tasks (WM forward and WM backward) and a DR memory task first during a control condition, and one week later during a stress condition. Stress condition salivary alpha-amylase (sAA) and cortisol were assessed. Only WM backward performance declined over conditions. Relations between physiological stress responses and performance within the stress condition were present only for WM forward and DR. For WM forward, higher cortisol responses were related to better performance. For DR, there was an inverted U-shape relation between cortisol responses and performance, as well as a cortisol*sAA interaction, with concurrent high or low responses related to optimal performance.

This emphasizes the importance of including curvilinear and interaction effects when relating physiology to memory.

4.1. Introduction

At school, children are confronted with diverse stressors, for example pressure to perform well, or social intricacies that are part of life at school. It is important to know how such stressful experiences can influence subsequent school performance. Two memory processes that are important to school performance are working memory (WM) and the delayed retrieval (DR) of previously learned materials. Although the effects of stress on these processes have been investigated in adults, research in children is limited, despite the high societal relevance of such knowledge. The current study sought to increase our understanding about the effects of stress on children's memory, focusing on WM and DR.

Research into memory processes often distinguishes declarative long term memory and working memory. Declarative long term memory refers to "the explicit storage of facts and events, which can later be intentionally retrieved" (Wolf, 2007, p. 167). This retrieval can be referred to as DR. WM, on the other hand, refers to the temporary storage and manipulation of information required for task performance (Baddeley, 1992).

Human stress responses involve two physiological systems: the fast responding sympathetic nervous system (SNS) that works through the release of epinephrine and norepinephrine, and the slower responding hypothalamic-pituitary-adrenocorticol (HPA) axis that works through the release of glucocorticoids (Gunnar & Quevedo, 2007). Activation of these systems is thought to affect memory processes through the activation of glucocorticoid and adrenaline receptors in brain areas important for WM and DR, like the prefrontal cortex, hippocampus, and amygdala (e.g. Arnsten, 2009; McGaugh & Roozendaal, 2002; Roozendaal, 2002). Information about activation of these systems can be obtained from saliva, by measuring the amount of salivary alpha-amylase (sAA) as an indicator of SNS activation (Rohleder & Nater, 2009; but see also Bosch, Veerman, de Geus, & Proctor, 2011), and the amount of salivary cortisol as an indicator of HPA axis activity (Hellhammer, Wüst, & Kudielka, 2009; Kirschbaum & Hellhammer, 1994).

With regard to DR, many studies have shown that elevated glucocorticoid levels result in impaired DR (Kuhlmann, Kirschbaum, & Wolf, 2005). However, these results are more consistent for emotional than neutral stimuli (Wolf, 2009), and seem to depend on the testing taking place in an arousing test situation, i.e. when the SNS is also activated. When Kuhlmann and Wolf (2006) changed the experimental setting in which participants performed a DR task such that is was believed to be less arousing, the previously found negative effect of cortisol administration vs. placebo on DR disappeared. Tollenaar, Elzinga, Spinhoven and Everaerd (2008) found that impaired DR of words five weeks after encoding was related to larger cortisol increases to a stress task only when DR was tested during this stress task, when blood pressure and heart rate were highest. These results suggest that negative effects on DR may occur only when the HPA axis and SNS are activated simultaneously.

Results of studies investigating the impact of acute stress on adult WM have been inconsistent. Some studies found that stress exposure decreased WM performance (e.g. Elzinga & Roelofs, 2005; Schoofs, Wolf, & Smeets, 2009), whereas others found no effects (Kuhlmann, Piel, & Wolf, 2005; Smeets, Jelicic, & Merckelbach, 2006), or even an increase in WM performance (Lewis, Nikolova, Chang, & Weekes, 2008). Also, findings differ across WM components: some researchers found effects only for the attentional/ immediate retention component (digit span forward; Elzinga & Roelofs, 2005), while others found effects only for the executive functions component (digit span backward; Schoofs, et al., 2009). As with DR, negative effects of stress on WM seem to be the result of an interaction between HPA axis and SNS activity. Elzinga and Roelofs (2005) only found impaired WM performance in cortisol responders as compared to non-responders when testing took place while participants were aroused, i.e., when participants showed elevated heart rate and blood pressure in addition to high cortisol levels.

All abovementioned studies were conducted with adults. However, these results might not generalize to children, as both physiological stress responses and memory change across development (Gunnar, Wewerka, Frenn, Long, & Griggs, 2009; Schneider & Pressley, 1997). In children, increased arousal at the time of retrieval has been found to impair accuracy on short-answer questions about a staged event (Nathanson & Saywitz, 2003), and to increase errors in answering direct questions about an emotion-inducing film, although only when the interviewer was non-supportive (Quas & Lench, 2007). However, we know of only one study that investigated the effects of acute stress on WM and DR in children. Quesada, Wiemers, Schoofs and Wolf (2012) compared digit span WM and visual-spatial DR performance of 8-10 year-old children that were either exposed to a psychosocial stressor (*n* =22), or a control task (*n* = 22). Four saliva samples were obtained throughout the procedure in both the control and stress condition, which were analyzed for cortisol and sAA. Children in the stress condition performed worse on the DR memory task than children in the control condition. No effect of condition was found for WM. Within the stress condition, higher cortisol reactivity was related to worse DR performance. No relation was found between sAA and DR performance.

The current study is similar to the Quesada et al. (2012) study in the sense that we also sought to investigate the effect of psychosocial stress on WM and DR in middle childhood. However, our study also elaborates on this previous study in several ways. First, the use of a large sample of children (*N* = 158) that all participated first in a control condition and then in a stress condition increases power. Second, WM and DR in the current study were assessed during, as opposed to after, the stress task, such that SNS activity was likely to still be high. Third, we assessed both HPA axis and SNS activity during stress, and assessed the interaction effect of HPA axis and SNS activity on memory performance. And fourth, we tested DR memory for verbal, as opposed to visual-spatial, material, as learning verbal materials is a fundamental aspect of school performance. We hypothesized that WM and DR would be worse in the stress condition as compared to the control condition. In addition, we hypothesized that within the stress condition there would be an interaction effect of HPA axis and SNS activity on WM and DR performance: HPA axis activation would only lead to worse memory performance if SNS activation was high. Because there are indications that the effect of glucocorticoids on memory follows an inverted U-shape (e.g. Lupien & McEwen, 1997; Sandi & Pinelo-Nava, 2007), also known as the Yerkes-Dodson law (Mendl, 1999), we also hypothesized that both children with low and high HPA axis reactivity would show poorer memory performance than children with intermediate HPA axis reactivity.

4.2. Method

4.2.1. Participants

Children (age 9-11) were recruited through 31 general education primary schools in Nijmegen and surrounding areas (the Netherlands) for participation in a study on different aspects of responses to stress and their consequences for cognitive functioning. Exclusion criteria were stuttering, a diagnosis of a developmental disorder, and the use of psychotropic or centrally acting corticosteroid medication. Recruitment (for details see De Veld, Riksen-Walraven & De Weerth, 2012) resulted in 165 participants. Five children were excluded because they did not complete the entire data collection protocol, and two children were excluded because during data collection it was discovered they met one of the exclusion criteria. Thus, the final sample for this study consisted of 158 children (83 girls; M_{age} = 10.61 years, SD = .52). The majority of the participants was Caucasian (94%), and had at least one parent with a college or university degree (79%). Two participants were excluded from the analyses relating physiological stress responses and memory performance within the stress condition due to missing sAA data for baseline (S1 and S2; n =1) or S3 (n =1).

The study was approved by the ethics committee of the Faculty of Social Sciences of the Radboud University Nijmegen, the Netherlands. The children participated in the study voluntarily, and all parents provided written informed consent prior to their child's participation.

4.2.2. Procedure

The study used a within subjects design, with all children performing a WM task and a DR memory task first in a control condition in a mobile lab at home, and approximately

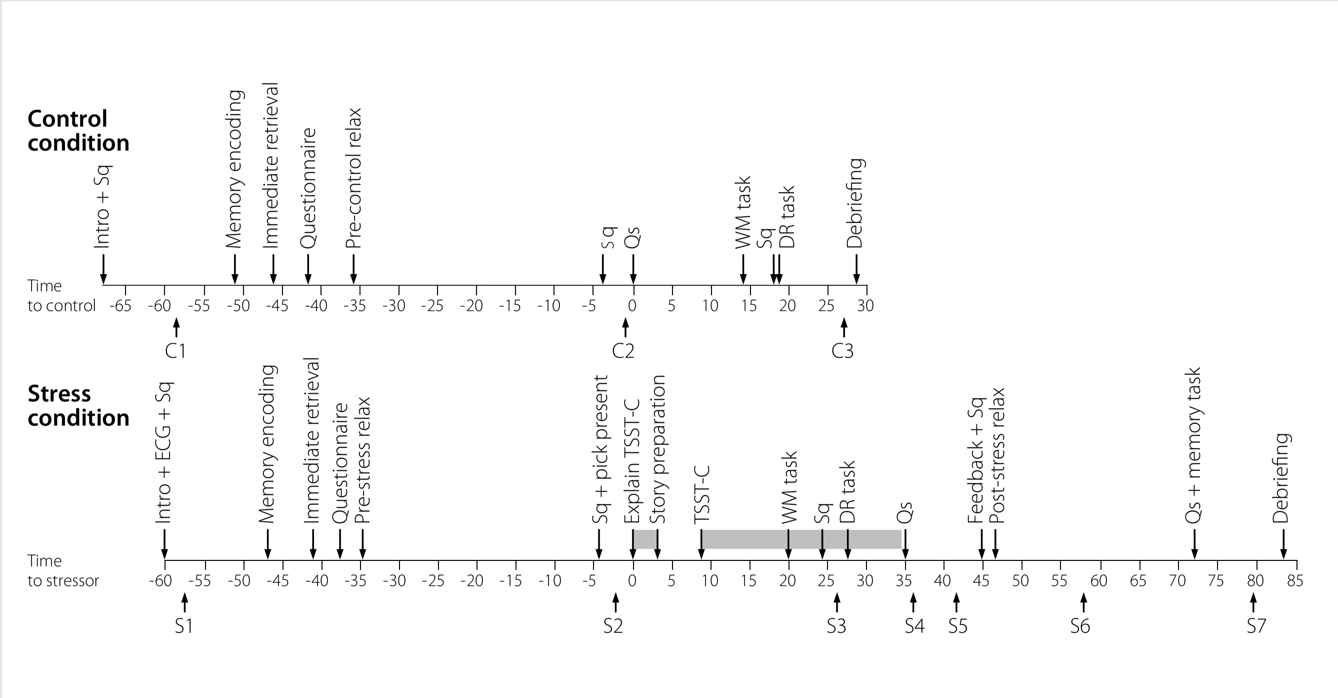

Figure 1 Overview of the procedures for the control condition (top), and the stress condition (bottom). Intro = introduction; Sq = short questionnaire; Qs = questionnaires; C = control condition saliva sample; S = stress condition saliva sample. The shaded areas in the stress condition indicate the presence of the TSST-C jury.

one week later in a stress condition in the laboratory of the Behavioural Science Institute of the Radboud University Nijmegen. For an overview of the procedures in the control and stress condition, see Figure 1. All testing took place after school (*Md* = 15:45h, *IQR* = 14:11h – 16:03h).

Control condition

During the control condition at home, all testing took place in a mobile lab (van parked in front of the home). After a short introduction in which children were told that they would be asked to do some tasks and fill out several questionnaires, children practiced providing a saliva sample (C1), and filled out a short questionnaire. This was followed by the encoding phase of the DR memory task (see Section 4.2.3), immediate retrieval for this task, and a questionnaire. Then, a 30-min relaxation period commenced, during which children could read magazines or make puzzles while listening to relaxing music. After relaxation they filled out a short questionnaire, provided a saliva sample (C2), and filled out two other questionnaires. Then they performed the WM task (see Section 4.2.3), filled out a short questionnaire, and performed delayed retrieval for the DR memory task (see Section 4.2.3). The procedure ended with a last saliva sample (C3). The entire procedure took approximately 1.5 h.

Stress condition

During the lab visit, children first received a short introduction. Thereafter, they provided a saliva sample (S1) and completed a short questionnaire. This was followed by the encoding phase of the DR memory task (see Section 4.2.3), immediate retrieval for this task, and a questionnaire. This was followed by a 30-min relaxation period during which children could read a magazine or make puzzles, while listening to relaxing music. Right after relaxation they filled out a short questionnaire, and provided a second saliva sample (S2). After this, children were led to an adjacent room where a stress task took place (adapted and extended TSST-C; Buske-Kirschbaum et al., 1997). The TSST-C consists of a public speaking task in which children provide the ending to a story, and perform a mental arithmetic task in which they count backwards from 758 to zero by repeatedly subtracting seven from the most recently acquired number. During both tasks, a jury of two confederates in white lab coats watches the child perform. In the present study, before starting the TSST-C children had been asked to pick a favourite and least preferred present out of six small items (e.g. an inflatable ball or toilet brush) right before entering the TSST-C room (Jones et al., 2006), and had been told that a favourable judgment by the jury would earn them their favourite present, whereas in case of an unfavourable judgment they would get the least preferred present. After the TSST-C, children were seated in front of the TSST-C jury, and were joined by the experimenter. The experimenter then conducted the WM task (see Section 4.2.3), asked children to supply a saliva sample (S3) and fill out a short questionnaire, and conducted the DR memory task (see Section 4.2.3). The stress task lasted approximately 34 min. Afterwards, the children were escorted back to the first room, where they provided another saliva sample (S4), and completed two questionnaires. This was followed by a fifth saliva sample (S5), the completion of another questionnaire, positive feedback on their performance during the stress task, and a short questionnaire. Then, a 25-min post-stress relaxation period was initiated. Ten minutes into this relaxation period, a saliva sample was obtained (S6). After relaxation, children completed several questionnaires, performed a memory task, provided a last saliva sample (S7), and completed a last questionnaire. The entire procedure took approximately 2.5 h.

4.2.3. Instruments and Measures

Delayed retrieval memory task

To fit the purpose of the current study, a new DR memory task was devised based on materials from De Deyne et al. (2008b). For the encoding phase of the DR memory task, children were seated in front of the black screen of a laptop and listened to a pre-recorded short story played on the laptop (see Figure 2). Parts of this story contained five word categories, with eight exemplars each. Upon hearing a category in the story (e.g. professions), this category's name appeared in yellow capital letters on the black laptop screen. Upon hearing an exemplar (e.g. pilot, dentist), this exemplar appeared in white lowercase letters underneath the category name (see Figure 2). Exemplars were presented on screen for 4 s each; the category name stayed on screen until all exemplars of that category had been presented. The order in which categories were presented within the task was fixed; the order in which exemplars were presented within each category was randomized across participants.

Right before the encoding phase of the DR memory task, the experimenter had outlined the stimulus presentation to the children, and had instructed them to do their best to remember as many of the presented words as possible. Children had also been told that the experimenter would ask them to name as many of the words of one of the categories as possible later during the procedure. When a child indicated that it had understood the nature of the task, the experimenter started the encoding phase.

To allow for comparison of memory performance over the two conditions, we constructed two versions of the memory task (version A and B). Task order over conditions was counterbalanced across participants. Categories and exemplars were derived from De Deyne et al. (2008a; 2008b). Words in version A and B were matched on typicality, goodness of example of category, exemplar generation frequency, estimated age of acquisition, familiarity, and imageability according to the norms presented in De Deyne et al. (2008a).

DR memory performance was assessed by asking children to name as many exemplars of a randomly selected category as possible in 2 min. If a child indicated

Version A
Today was a busy day at school. First, we spoke about what we would like to be when we grow up. There were a lot of different PROFESSIONS
 actor; lawyer; fire fighter; vet; pilot; butcher; dentist; dustman
After that, we went to the school's garden. On our way there we saw a lot of BIRDS
 eagle; magpie; vulture; tit; falcon; pelican; heron; woodpecker
In the school's garden, we checked up on our VEGETABLES
 eggplant; beet; zucchini; watercress; leek; tomato; chicory; sprouts
Afterwards, we went back to school for gym class. We could choose from different SPORTS
 ballet; boxing; rugby; running; horseback riding; table tennis; gymnastics; volleyball
On our way back from school we saw a lot of different VEHICLES
 jeep; tram; truck; scooter; helicopter; caravan; tractor; moped
We had a fun day. THE END

Version B
Today there was a neighbourhood party. It started with a treasure hunt in which people had dressed up. Some were dressed as INSECTS
 cricket, dragonfly, fruit fly, beetle, cockroach, caterpillar, butterfly, woodlouse
There were also other animals. Some were dressed as FISH
 swordfish; trout; herring; carp; eel; piranha; pike; stickleback
After the treasure hunt we went to eat something, namely FRUIT
 apricot; fig; melon; strawberry; pumpkin; lemon; kiwi; peach
There were also musicians at the party. They all played different MUSICAL INSTRUMENTS
 accordion; banjo; bass guitar; harp; organ; flute; violin; trumpet
And people used different fabrics to make their own CLOTHING
 blouse; shirt; suit; cap; scarf; socks; top; swimsuit
We had a fun day. THE END

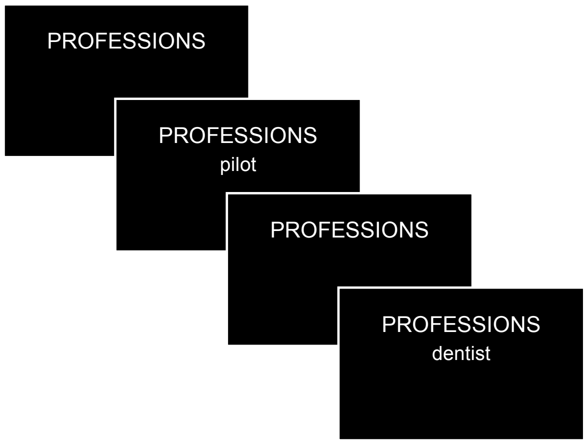

Figure 2 English translations of the Dutch stories used during the memory encoding phase (top), and an impression of the stimulus presentation sequence (bottom).

not to remember any more words within those 2 min, he or she was told that there was still time to think. Memory performance was defined as the number of correctly retrieved exemplars of the tested category. This could result in a score between 0-8.

Working memory task

Working memory was assessed with a digit span test based on that from the Wechsler Intelligence Scale for Children (Wechsler, 1991). Again a version A and B were constructed, the order of which was counterbalanced across participants. In the digit span test, digit sequences of increasing length are presented, with two trials for each sequence length. In the forward condition, indicating passive storage, digits are to be repeated in the order presented. In the backward condition, indicating executive functioning, digits are to be repeated in reversed order. If responses to both trials of a particular sequence length are incorrect, the current condition is terminated. One point is given for each correct answer. Participants' performance in each condition was determined by summing all points received in that condition. This could result in a score between 0-16 in the forward condition, and between 0-14 in the backward condition. Because WM forward and backward have been argued to assess different memory processes (e.g. Reynolds, 1997), and data in adults suggests different underlying neural mechanisms (Sun et al., 2005), the two subtests were analyzed separately.

Cortisol and sAA

To obtain reliable saliva samples, participants were asked to only drink water in the 2 h before participation, to limit physical exercise in the hour prior participation, and to abstain from meals at least 45 min before participation.

The sampling procedure was as follows. Participants swallowed all saliva in order to empty their mouths, and collected all subsequently secreted saliva in their mouths for 2 min, after which they used a short straw to spit the saliva into a small tube. This procedure was repeated until at least 0.25 ml of saliva was collected, with a maximum total collection time of 5 min.

During the control condition, three saliva samples were obtained, namely at –55 (C1), -1 (C2), and 27 (C3) min from the onset of the control task. During the stress condition, seven saliva samples were obtained, namely at -57 (S1), -2 (S2), 26 (S3), 36 (S4), 42 (S5), 58 (S6), and 80 (S7) min from the onset of the stressor. Timing of samples C2 and C3 in the control condition corresponded to the timing of samples S2 and S3 in the stress condition. Due to practical constraints, we analyzed saliva samples during the control condition in a subsample of $n = 53$ participants.

All samples were kept frozen at -20 °C until their shipment to the analysis lab. Cortisol concentrations were determined at the Endocrinology Laboratory of the University Medical Center Utrecht, using an in house competitive radio-immunoassay employing a polyclonal anticortisol-antibody (K7348). [1,2-3H(N)]-hydrocortisone (Amersham TRK407) was used as a tracer. The lower limit of detection was 1 nmol/L and inter-assay and intra-assay variations were below 10%.

sAA concentrations were determined from the same saliva samples that were used to determine cortisol concentrations. Analysis was performed at the Endocrinology Laboratory of the University Medical Center Utrecht. Alpha-amylase was measured on the DxI analyzer (Beckman Coulter Inc., Fullerton, CA, USA). Saliva samples were diluted 500x with 0.2% BSA in 0.01 M Phosphate buffer pH 7.0. Inter-assay variation was <2.2%.

All physiological data were screened for outliers, which were defined within each assessment point as values greater than 3 SD above the mean. On the assessment points relevant to the current study, there were 11 outliers out of a total of 580 data points for cortisol (S1: 3; S2: 3; S3: 5), and 14 outliers out of a total of 576 data points for sAA (C1: 1; C2: 1; S1: 4; S2: 4; S3: 4). All outliers were winsorized[7] by replacing their values with the value of 3 SD above the mean (Tukey, 1977).

For the manipulation check (see Section 4.3.2) C2 and S2 served as pre-task measurement, and C3 and S3 as post-task measurement.

To determine children's physiological responses to the stress task at the time of the WM and DR task in the stress condition, we first determined a baseline value for cortisol and sAA by selecting the lowest pre-stress value for each participant from S1 (n = 17 for cortisol, 27 for sAA) and S2 (n = 139 for cortisol, 129 for sAA). Then, a delta increase was computed by subtracting this baseline value from the value at S3.[8]

Potential confounders

Participant's stage of pubertal development was assessed on a 5-point scale using Tanner criteria (Marshall & Tanner, 1969, 1970), with higher scores indicating more advanced physical development.

Parental education level was assessed for both parents on an 8-point scale (1 = primary education, 8 = university degree). Values for both parents were averaged to obtain a single score for analysis.

4.2.4. Statistical Analyses

Square root (sqrt) and logarithm (lg10) transformations were applied where necessary to correct skewed data.

7 Analyzing the data without participants whose cortisol and/or sAA values had been winsorized yielded comparable results to those presented in the manuscript.

8 It is important to note that although S3 was chosen to compute delta increase, cortisol and sAA levels were still elevated at S4, indicating that cortisol and sAA values were elevated throughout memory testing.

To check whether our control condition was indeed non-stressful, and our stress condition induced a cortisol and sAA response, we conducted a repeated measures MANOVA (n = 53) with Condition (control vs. stress) and Time (pre vs. post control/stressor) as within subject factors and cortisol and sAA values as outcomes.

To examine the effect of stress on memory performance, we performed a repeated measures MANOVA (n = 158) with Condition (control vs. stress) as a within subject factor and WM forward, WM backward and DR as outcome variables.

To examine whether the strength of children's physiological responses in the stress condition was related to their memory performance in the stress condition, we performed two hierarchical regression analyses (n = 156) for each dependent variable (WM forward, WM backward, and DR). Variables used in interaction terms were centred prior to their inclusion. In the first model, all possible confounders (sex, age, parental education, and pubertal stage) and predictors were entered in separate steps. These first models are presented in a footnote to the tables with the final models (see Results). For the second (final) model, only variables that individually explained at least 1% of variance in the first model (calculated as (part correlation)^2 x 100) were retained to eliminate irrelevant confounders and increase power. Significant interactions and quadratic effects were plotted based on Aiken and West (1991).

4.3. Results

4.3.1. Preliminary Analyses

Descriptives of study variables, and correlations between stress condition memory variables, physiological variables, and confounders are presented in Table 1.

A Mann-Whitney U test revealed that the children from whom control condition saliva was analyzed (n = 53) were slightly younger (Md = 10.3) than the others (Md = 10.8), U = 1349.5, p < .01. No significant differences were found for the distribution of boys/girls, parental education level, puberty, stress condition cortisol reactivity, and stress condition sAA reactivity.

A Wilcoxon Signed Rank Test revealed that the time between encoding and DR was shorter in the control condition (Md = 64 min), than the stress condition (Md = 69 min), z = -7.62, p < .001. To test whether this timing difference was related to the DR performance difference between conditions, we calculated the difference in timing between conditions and the difference in DR performance between conditions for each participant separately, and then correlated these difference scores. This correlation was not significant (r = .04, p = .64), indicating that any difference between DR performance between conditions was unrelated to differences in time between encoding and retrieval.

Table 1 Descriptives and correlations for the study variables.

		Descriptives	Correlations									
		M (*SD*) or *Md* (*IQR*)	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.	Delta cortisol	1.10 (-0.10 - 2.70)	-									
2.	Delta sAA[a]	57.5 (11.25 - 154.00)	.13	-								
3.	WM fw control[b]	8.00 (7.00 - 9.00)	-.05	-.20*	-							
4.	WM fw stress[b]	5.00 (4.00 - 6.00)	.14$^+$	-.11	.64**	-						
5.	WM bw control[c]	8.00 (7.00 - 9.00)	.04	-.06	.35**	.36**	-					
6.	WM bw stress[c]	5.00 (4.00 - 6.00)	.02	-.03	.40**	.43**	.50**	-				
7.	DR control	3.51 (1.50)	-.05	.11	.10	.06	.00	.12	-			
8.	DR stress	3.25 (1.49)	.00	.07	.07	-.02	.08	.13	.29**	-		
9.	Age	10.61 (0.52)	-.04	.02	.03	.10	.16*	.17*	-.04	.00	-	
10.	Parental education	7.00 (5.50 - 7.50)	-.07	.00	.00	-.09	-.06	-.29**	-.11	-.10	.10	-
11.	Pubertal stage	2.00 (1.50 - 2.50)	-.04	-.05	.09	.02	.12	-.04	.03	.01	.27**	.09

[a] $N = 156$, for all other variables $N = 158$. [b] fw = forward. [c] bw = backward
$^+$ $p < .10$. * $p < .05$. ** $p \leq .01$.

4

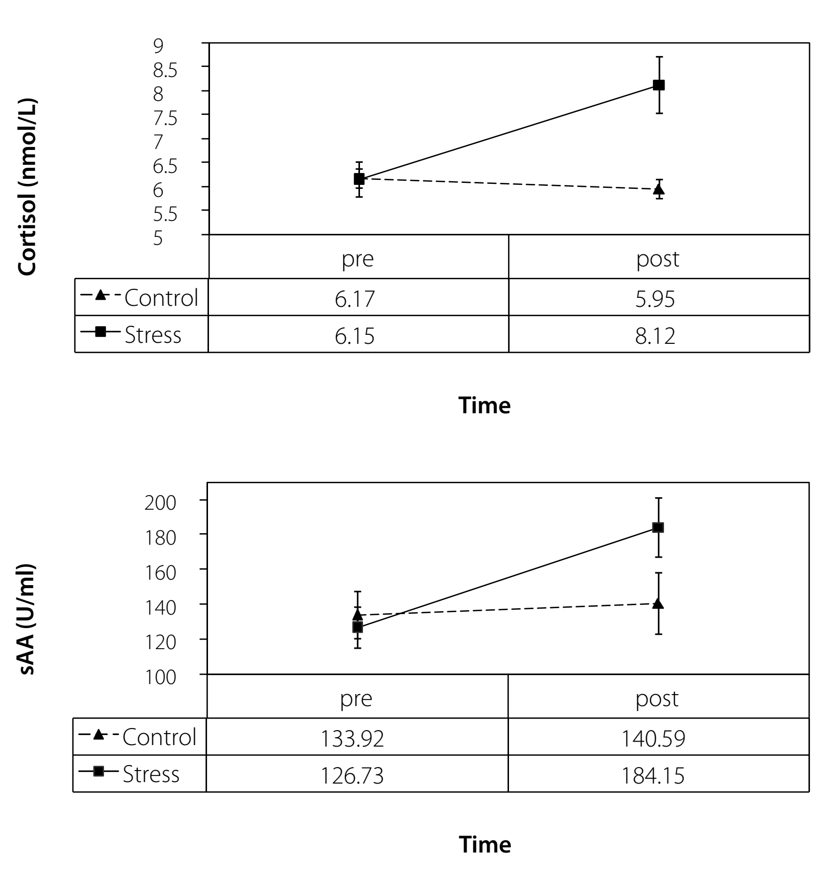

Figure 3 Increases in cortisol (upper panel) and sAA (lower panel) over time for the control and stress conditions. Plots display means and standard errors of raw data, statistical analyses were performed with log-transformed cortisol data and sqrt-transformed sAA data.

4.3.2. Manipulation Check

We first checked whether our control condition was indeed non-stressful, and our stress condition induced a cortisol and sAA response. A repeated measures MANOVA with Condition (control vs. stress) and Time (pre vs. post control/stressor) as within subject factors and cortisol and sAA values as outcomes showed a significant multivariate Condition * Time interaction, Wilks' Lambda = .56, F (2, 51) = 20.21, p < .001, multivariate partial eta squared = .44. Univariate tests showed a significant

Condition * Time interaction for both cortisol, F (1, 52) = 27.70, $p < .001$, partial eta squared = .35, and sAA, F (1, 52) = 21.34, $p < .001$, partial eta squared = .29. Post-hoc Bonferroni-corrected paired-samples t-tests showed that this effect was the result of stable control condition levels for both cortisol, t (52) = 1.49, p = .14, and sAA, t (52) = -.14, p = .89, while in the stress condition there was an increase in both cortisol, t (52) = -4.90, $p < .001$, and sAA t (52) = -5.91, $p < .001$ (Figure 3). This indicates that each condition worked as intended.

4.3.3. Effects of Stress on Memory Performance

Next we examined the effect of stress on memory performance, using repeated measures MANOVA with Condition (control vs. stress) as a within subject factor and WM forward, WM backward and DR as outcome variables. There was a significant multivariate effect of condition, Wilks' Lambda = .94, F (3, 155) = 3.12, $p < .05$, multivariate partial eta squared = .06. Univariate tests showed a significant effect of condition for WM backward, F (1, 157) = 4.93, $p < .05$, partial eta squared = .03. These results were due to lower memory scores in the stress condition vs. the control condition (see Table 1, p. 77). There were no effects of condition for DR, F (1, 157) = 3.51, p = .06, and WM forward, F (1,157) = .67, p = .41.

4.3.4. Relation between Physiological Stress Responses and Memory Performance within the Stress Condition

Next, we used hierarchical regression analyses to examine whether within the stress condition children's physiological stress responses were related to their performance on the different memory tasks.[9] The final regression model for WM forward was significant, and is summarized in Table 2 (p. 80). There was a significant linear effect of cortisol, such that a stronger cortisol response was related to better WM forward performance.

The final regression model for WM backward was also significant, F (7,148) = 11.78, $p < .001$. In this case, however, all coefficients for cortisol and sAA variables were non-significant.

The final regression model for DR was also significant, and is summarized in Table 2 (p. 80). There was a significant quadratic effect of cortisol, which is depicted in Figure 4 (top), indicating that children with relatively small and large cortisol responses had poorer DR performance in the stress condition than children with intermediate cortisol responses. In addition there was a significant sAA * cortisol interaction effect, which is shown in Figure 4 (bottom). When the sAA response to the stress task is small, larger cortisol responses are related to worse DR performance. When the sAA response to the stress task is large, larger cortisol responses are related to better DR performance.

9 Similar analyses performed for the control condition (n = 53) yielded no significant results.

Table 2 Final regression models for the prediction of WM forward and DR in the stress condition.

	B	*SE B*	*β*	R^2_{model}	F_{change}	R^2_{change}
WM forward[a]				.41	109.54**	
Step 1						
WM forward control	.64	.06	.65**			
Step 2				.44	8.29**	.03
Cortisol reactivity	.26	.09	.17**			
Delayed retrieval[b]						
Step 1				.10	8.59**	
DR control	.32	.08	.32**			
Sex[c]	-.39	.23	-.13			
Step 2				.10	.18	.00
sAA reactivity	.01	.03	.03			
Cortisol reactivity	-.03	.63	.00			
Step 3				.15	4.16*	.05
Cortisol reactivity * cortisol reactivity	-3.77	1.87	-.15*			
Cortisol reactivity * sAA reactivity	.37	.17	.17*			

[a] Initial model for WM forward (sqrt): step 1 – WM forward control (sqrt), sex, age, parental education level (lg10), puberty (sqrt); step 2 – sAA reactivity (sqrt), cortisol reactivity (lg10), sAA reactivity * sAA reactivity, cortisol reactivity * cortisol reactivity, cortisol reactivity * sAA reactivity. [b] Initial model for DR: step 1 – DR control, immediate recall, time between encoding and retrieval (lg10), age, parental education level (lg10), puberty (sqrt); step 2 – sAA reactivity (sqrt), cortisol reactivity (lg10); step 3 – sAA reactivity * sAA reactivity, cortisol reactivity * cortisol reactivity, cortisol reactivity * sAA reactivity.
[c] Girls = 0, Boys = 1
[+] $p < .10$. * $p < .05$. ** $p < .01$.

To test whether the quadratic effect of cortisol was further moderated by sAA we performed an additional regression analysis that included the cortisol * cortisol * sAA interaction. Although the model as a whole was significant, F (7,148) = 4.17, $p < .001$, the coefficient for the interaction was not. Thus, the quadratic effect of cortisol was not moderated by sAA.

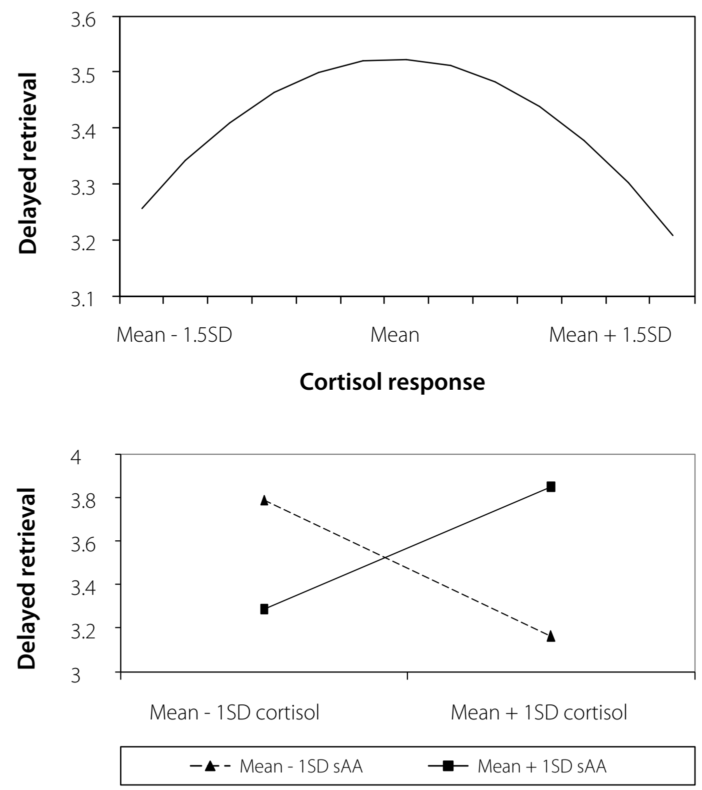

Figure 4 The relation between stress condition cortisol responses and delayed retrieval memory performance. There was a significant quadratic relation (upper panel), as well as a significant interaction between sAA and cortisol (lower panel). Note: average DR memory performance in the control condition was 3.5.

4.4. Discussion

The current study aimed to investigate the effect of acute stress on children's WM and DR for verbal material. A manipulation check indicated that children showed a cortisol and sAA response in the stress condition, whereas they did not in the control condition, indicating that our manipulation was successful. The expected decline in memory performance in the stress vs. the control condition was found only for WM backward, and not for WM forward or DR. However, within the stress condition, performance on the WM backward task was not related to either cortisol or sAA responses, whereas significant relations were found for WM forward and DR. Specifically, for WM forward stronger cortisol responses to the stress task were related to better performance. For DR, we found a significant quadratic effect of cortisol, such that both children with very small and very large cortisol responses remembered fewer words than children with intermediate cortisol responses. In addition, the linear relation between cortisol responses to the stress task and DR was different for children with different sAA responses. That is, for children with a small sAA response, larger cortisol responses were associated with worse DR performance, whereas for children with a large sAA response, larger cortisol responses were associated with better DR performance.

The finding that performance on the WM backward task decreased in the stress condition as compared to the control condition was consistent with our hypothesis. However, within the stress condition we found no relation between the cortisol and sAA responses and WM backward performance. This makes it unlikely that the decrease in WM backward performance from the control to the stress condition was the direct consequence of an increase in activation of the HPA axis and SNS in the stress condition. The question then is what other mechanism can be responsible for the observed decline in performance from control to stress. One possible factor that may be involved is the regulation of emotions that arise in response to the stress task. An emotion regulation strategy that might be particularly important in this respect is cognitive reappraisal, a strategy that involves reinterpreting a situation in such a way that the emotional impact of the situation is changed (Gross, 1998). Emotion regulation in general has been argued to be an aspect of executive functioning (Zelazo & Cunningham, 2007), and adult fMRI studies have shown that reappraisal use activates regions in the brain known to also play a role in working memory and executive functioning (Ochsner & Gross, 2008). As WM backward requires executive functioning for the manipulation of the stored material, it seems possible that as children engaged in reappraisal during the stress task, their WM backward capacity decreased. Because we measured reappraisal use during the stress task in the current sample to answer a different research question (see De Veld, et al., 2012), we were able to perform post-hoc analyses that showed that higher self-reported use of reappraisal during the stress task was related to lower WM backwards scores. As WM forward merely consists of the passive storage of items, this aspect of WM would remain unaffected, as evidenced by the absence of a correlation between reappraisal use and WM forward scores in the post-hoc analysis.

We had expected to also find a decrease in WM forward and DR performance in the stress condition as compared to the control condition, however, we did not find such an effect. Perhaps, this resulted from the fact that the average cortisol response to the stress condition in the current study was relatively small (median increase of 1.1 nmol/L, versus an average increase of approximately 10 nmol/L in Quesada et al., 2012). It is possible that larger increases are necessary for effects of stress vs. control condition performance to emerge.

Although children did not show a significant decrease in WM forward performance across conditions, we did find a significant relation between cortisol reactivity to the stress task and WM forward performance. Contrary to what we had expected, however, this relation was positive: higher reactivity was related to better instead of worse performance. One possible interpretation is that this finding results from a combination of two factors: 1) the Yerkes-Dodson law (Mendl, 1999). This is the idea that optimal performance occurs at some optimal state of stress. Here it would result in an inverted U-shape relation between glucocorticoids and memory in which both low and high levels are associated with worse performance, whereas intermediate levels are associated with optimal performance (e.g. Lupien & McEwen, 1997; Sandi & Pinelo-Nava, 2007), and 2) relatively small increases in cortisol in the current sample. This combination could have led to a pattern of results in which participants' scores all fell on the left side of the inverted U, thus resulting in the appearance of a positive linear relation. This would also imply that in studies where cortisol responses are stronger, findings should shift to a curvilinear or negative linear relation between WM forward and cortisol reactivity.

Our results for DR support the hypothesis of an inverted U-shape relation between glucocorticoids and memory: both children with small and large cortisol responses showed poorer DR performance than children with intermediate cortisol responses. This is consistent with the previously mentioned Yerkes-Dodson law (Mendl, 1999). Interestingly, this law also states that the optimal level of stress decreases when task difficulty increases (Mendl, 1999). This would be in line with the interpretation that for WM forward the participants' scores all fell on the left side of the inverted U, resulting in our finding of a positive linear relation, whereas for the more difficult DR task, scores were scattered around the optimal state, resulting in our finding of an inverted U-shape. Here, as with WM forward, an inverted U-shape relation would imply that in studies in which cortisol responses are stronger, findings should shift, in this case to a negative linear relation between DR and cortisol reactivity. The results found by Quesada et al. (2012) could be taken to confirm this notion, as the results of this study indicated both a pronounced average cortisol response, and a negative relation between cortisol reactivity and DR for visuo-spatial stimuli. The absence of an effect of cortisol on WM forward in the Quesada et al. paper might in turn have resulted from scores being scattered around the optimal state for this measure. Taken together, these results point towards the existence of non-linear relationships between cortisol and memory. Because studies in humans often have not investigated non-linear relationships, these may have remained underreported up till now. The present research hence provides a platform for further investigating possible non-linear relationships between HPA axis activity and memory.

An unexpected, yet very interesting, finding was that for children with a high sAA response, higher cortisol responses were associated with better DR performance, whereas for children with a low sAA response, higher cortisol responses were associated with worse DR performance. In other words: DR performance was highest when there was concurrent activation or deactivation of the SNS and HPA axis. Based on hypotheses that one of the functions of HPA axis reactivity to stress is to suppress stress-induced SNS activation (Sapolsky, Romero, & Munck, 2000), it has been argued that such concurrent (de)activation is indicative of a well-coordinated stress system that prevents allostatic load, resulting in fewer adverse outcomes (Bauer, Quas, & Boyce, 2002). These results could be taken to indicate that allostatic load is indeed lowest when the HPA axis and SNS show a concurrent response, thereby optimizing performance. This would be in line with a study by Quas, Yim, Rush, and Sumaroka (2012), where concurrent HPA axis and SNS activation during encoding were associated with better memory for a stressful event. These results signify the importance of incorporating interactions between HPA axis and SNS responses when investigating effects of stress on memory.

Some limitations to our study should be acknowledged. The manipulation check was performed on a subsample that was slightly younger than the remaining participants. It is therefore possible that the difference in physiological responses to the control vs. stress condition was different for this latter group. This seems unlikely however, as participants in the subsample did not differ from the remaining participants with regard to their cortisol and sAA responses to the stress condition.

Findings regarding the difference in memory performance over conditions are limited by the fact that the order of the conditions was not counterbalanced. This leaves room for alternative interpretations like motivational changes, interference effects, and practice effects. Practice effects potentially contributed to the absence of a difference in WM forward and DR performance between conditions, as familiarity with the tasks might have undone any stress induced decline in performance. Additionally, slight differences between conditions with regard to time between encoding and retrieval, type and duration of the control/stress task, and timing of C3 vs. S3 could have decreased the strength of our stress manipulation. Future research would benefit from a more comparable control and stress condition. It should be stressed, however, that the aforementioned limitations do not apply to our findings regarding the relations between physiological stress responses and memory performance within the stress condition.

The results of this study help uncover how stress relates to different facets of memory in middle childhood. However, lacking the inclusion of multiple age groups or the utilization of a longitudinal design, we cannot draw definitive conclusions about the developmental changes in the effects of stress on memory. Therefore, future research could benefit from a cross-sectional or longitudinal design.

In conclusion, the current study showed that physiological responses to a stress task were related to children's WM forward and DR performance under stress. The decline in WM backward over conditions, without a relation between physiological stress responses and WM backward under stress, inspires further research into factors such as emotion regulation strategies, that might contribute to adverse effects of stress on cognitive functioning. The relations found between physiological responses to stress and WM forward and DR emphasize the importance of including curvilinear and interaction effects in models relating memory and physiology.

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5

Does the Arrival Index Predict Physiological Stress Reactivity in Children?[10]

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10 This chapter is submitted for publication

Summary

Knowledge about children's stress reactivity and its correlates is mostly based on one stress task, making it hard to assess the generalizability of the results. The development of an additional stress paradigm for children, that also limits stress exposure and test time, could greatly advance this field of research. Research in adults may provide a starting point for the development of such an additional stress paradigm, as changes in salivary cortisol and alpha-amylase over a 1 h pre-stress period in the laboratory correlated strongly with subsequent reactivity to stress task (Balodis et al., 2010, *Psychoneuroendocrinology, 35*, 1363-1373). The present study examined whether such strong correlations could be replicated in 9- to 11-year-old children.

Salivary cortisol and alpha-amylase (sAA) samples were collected from 158 children (83 girls) during a 2.5 h visit to the laboratory. This visit included a 1 h pre-stress period in which children performed some non-stressful tasks and relaxed before taking part in a psychosocial stress task (TSST-C). A higher cortisol arrival index was significantly and weakly correlated with a higher cortisol AUCg but unrelated to cortisol reactivity to the stressor. A higher sAA arrival index was significantly and moderately related to lower sAA stress reactivity and to a lower sAA AUCi. Children's personality and emotion regulation variables were unrelated to the cortisol and sAA arrival indices.

The results of this study do not provide a basis for the development of an additional stress paradigm for children. Further replications in children and adults are needed to clarify the potential meaning of an arrival index.

5.1. Introduction

Knowledge about children's physiological stress reactivity and its correlates is important, as previous research has indicated that repeated and long-lasting activation of the stress-system has adverse effects (e.g. Charmandari, Tsigos, & Chrousos, 2005; McEwen, 2008; Sapolsky, 1998). At the moment, most knowledge on this topic is based on one stress task, the Trier Social Stress Test for Children (TSST-C; Buske-Kirschbaum, et al., 1997). This makes it unclear how results generalize to other stressful situations. In addition, research in this area is associated with ethical, and practical considerations regarding the exposure to a stress task. Developing an additional stress task that also reduces the impact of these issues could significantly increase the number of studies in this area, and thereby increase the pace at which important knowledge is gained. Attractive options in this respect would be the limitation of stress exposure, or a shorter test time. Research in adults indicates that both of these might be feasible. Balodis et al. (2010) found that for two physiological stress measures, namely salivary cortisol and alpha-amylase (sAA), the change in concentration over the first hour in the lab, termed the 'arrival index', was strongly correlated with reactivity to a subsequently performed stress task. If these results could be replicated in children, they might be a first step towards an additional stress research paradigm that also alleviates some of the difficulties associated with current stress tasks. Therefore, the primary aim of the current study was to investigate the relation between the arrival index and subsequent stress reactivity in children for both sAA and cortisol.

Psychological research investigating human stress responses often uses sAA as an estimate of activation of the sympathetic nervous system (SNS) (Rohleder & Nater, 2009; but see also Bosch, Veerman, de Geus, & Proctor, 2011), whereas salivary cortisol is used as an estimate of activation of the hypothalamic-pituitary-adrenocorticol (HPA) axis (Hellhammer, Wüst, & Kudielka, 2009; Kirschbaum & Hellhammer, 1994). Research that uses standardized protocols to induce SNS and HPA axis reactivity in children has made a significant contribution to our understanding of how stress reactivity might differ as a function of factors like early adversity (e.g. Gunnar, Frenn, Wewerka, & Van Ryzin, 2009; Mueller et al., 2011), genotype (e.g. Mueller, et al., 2011; Mueller et al., 2012), sleep characteristics (e.g. Pesonen et al., 2012; Räikkönen et al., 2010), medical conditions (e.g. Buske-Kirschbaum et al., 1997; Buske-Kirschbaum et al., 2003; Dockray, Susman, & Dorn, 2009), and psychological problems (e.g. Hipwell, Keenan, & Marsland, 2009; Krämer et al., 2012; Randazzo, Dockray, & Susman, 2008). However, the use of such stress induction protocols is associated with points of concern. First, there are important ethical considerations: To what extent is it justifiable to expose children to a situation that is expressly designed to be stressful? Especially in certain clinical populations purposeful exposure to a stressful situation may not be

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desirable. Second, there are practical considerations in terms of manpower and time needed to execute the stress induction protocols. For example, the execution of the TSST-C (Buske-Kirschbaum, et al., 1997), the most effective protocol to induce a cortisol stress response during middle childhood (Gunnar, Talge, & Herrera, 2009), requires one experimenter and two confederates. In addition, as it is advised to incorporate a 45- to 60-minute pre-stress period to establish a reliable baseline (Gunnar, et al., 2009), and cortisol takes about 20-25 minutes to reach peak levels, the entire procedure of charting a stress response is time consuming. The development of an additional stress research paradigm that also reduces the impact of these factors could greatly advance the field of stress research in children.

The above mentioned paper by Balodis et al. (2010) could provide a starting point for the development of such an additional paradigm. These researchers examined the relation between pre-stress changes in cortisol and sAA levels, and cortisol and sAA reactivity in response to a psychosocial stress task in fifty healthy young adults. Participants were asked to provide a saliva sample upon arrival in the lab, and again one hour later, just before the stressor, to assess the arrival index (i.e. change in cortisol and sAA concentrations). After this first hour, the participants performed the Trier Social Stress Test (TSST; Kirschbaum, Pirke, & Hellhammer, 1993). To assess cortisol and sAA reactivity to the TSST the participants provided additional saliva samples immediately after TSST termination, and 40 minutes thereafter. Correlation analyses between the arrival index and commonly used measures reflecting reactivity to the stressor showed Pearson correlations of up to $r = .76$ for cortisol and $r = .86$ for sAA, leading the authors to underline the importance of the arrival index as an index of stress (Balodis, et al., 2010).

The findings presented above are very interesting, as this first hour in the lab is most often disregarded as being a period that allows biomarker levels to return to baseline prior to the initiation of the stress task. The results of Balodis et al. (2010) imply that the changes in biomarker concentrations during this period share a large amount of variance – 58% for cortisol and 74% for sAA – with reactivity to a subsequently administered stress task. This might indicate that increases in cortisol and sAA from home to arrival in the laboratory are interesting indices of stress reactivity in themselves. As a result, replicating the results presented above in a sample of children could lie at the foundation of the development of an additional stress paradigm for middle childhood that shares important aspects – like unpredictability and uncontrollability – with current procedures, while at the same time being sufficiently different to prevent redundancy.

In the present study we therefore replicated the analyses conducted by Balodis et al. (2010) with data that we had collected using a similar experimental timeline in a sample of 9- to 11-year-old children. Thus, the current study examined the correlation between the arrival index and different measures of physiological stress reactivity for both cortisol and sAA in children. In their discussion of the findings, Balodis et al. (2010) also suggested that individual differences in the arrival index might reflect individual differences in personality and trait mood but the data necessary to investigate this in their sample were not available. As our data included information on the children's personality characteristics and trait use of emotion regulation strategies, we also explored whether these variables were related to the arrival index in the current sample.

5.2. Method

5.2.1. Participants

Parents and children were invited through 31 primary schools in Nijmegen and surrounding areas (the Netherlands) to participate in a study on different aspects of responses to stress and their consequences for cognitive functioning. Exclusion criteria were a diagnosis of a developmental disorder, the use of psychotropic or centrally acting corticosteroid medication, and stuttering. Recruitment (for details see De Veld, Riksen-Walraven & De Weerth, 2012) resulted in 165 participants. Two children were excluded because during data collection it was discovered they met one of the exclusion criteria. Furthermore, five children were excluded from the present analysis because they did not complete the entire data collection protocol. Thus, the sample for the current study consisted of 158 children (83 girls; M_{age} = 10.61 years, *SD* = .52). The majority of the participants was Caucasian (94%), and had at least one parent with a college or university degree (79%).

The study was approved by the ethics committee of the Faculty of Social Sciences of the Radboud University Nijmegen. All parents provided written informed consent prior to their child's participation.

5.2.2. Procedure

A week before the stress test, all children completed questionnaires and memory tasks during a home visit (not relevant for the current study). At the end of the home visit, parents were handed the questionnaire to rate their child's personality (see Section 5.2.3), and were requested to bring the completed questionnaire along to the lab visit one week later.

The lab visit took place after school in the laboratory of the Behavioural Science Institute of the Radboud University Nijmegen. On arrival, children were led to a separate room, where the experimenter explained that they would be asked to do some tasks and fill out several questionnaires. After this introduction, children provided a saliva sample (S1; within 5 min after arrival), filled out a short questionnaire, performed a memory task, and filled out a trait emotion regulation questionnaire (see

Section 5.2.3). This was followed by a 30-min relaxation period during which children listened to relaxing music, and could read a magazine or make puzzles. Right after relaxation they filled in a short questionnaire and provided a second saliva sample (S2). After this, children were taken to an adjacent room where a TSST-C (Buske-Kirschbaum, et al., 1997) took place. This test was slightly modified by extending it with memory tasks and including presents to increase motivation (for details see De Veld, et al., 2012). During this period children supplied a third saliva sample (S3). This entire procedure took approximately 34 min. Afterwards the children went back to the first room. There they provided another saliva sample (S4), filled in several questionnaires, provided a fifth saliva sample (S5), completed another questionnaire, received positive feedback on their performance during the stress task, and completed a short questionnaire. Then, a 25-min post-stress relaxation period was initiated. Ten minutes into this relaxation period, a saliva sample was obtained (S6). After relaxation, children completed several questionnaires, performed a memory task, provided a last saliva sample (S7), completed a last questionnaire, and were debriefed. The entire procedure took approximately 2.5 h.

5.2.3. Instruments and Measures

Cortisol and sAA

To obtain reliable cortisol measures, participants were asked to only drink water in the 2 h before arrival in the lab, to limit physical exercise in the hour prior to arrival, and to abstain from meals at least 45 min before arrival.

Seven saliva samples (S1-S7) were obtained throughout the course of the procedure, at -57, -2, 26, 36, 42, 58, and 80 min from the onset of the stressor. Participants swallowed all saliva in order to empty their mouths, and collected all subsequently secreted saliva in their mouths for 2 min, after which they used a short straw to spit the saliva into a small tube. This procedure was repeated until at least 0.25 ml of saliva was collected, with a maximum total collection time of 5 min. Samples were kept frozen at -20°C until their shipment to the analysis lab.

Cortisol concentrations were determined at the Endocrinology Laboratory of the University Medical Center Utrecht, using an in house competitive radio-immunoassay employing a polyclonal anticortisol-antibody (K7348). [1,2-3H(N)]-hydrocortisone (Amersham TRK407) was used as a tracer. The lower limit of detection was 1 nmol/L and inter-assay and intra-assay variations were below 10%.

sAA concentrations were determined from the same saliva samples as were used to determine cortisol concentrations. The analyses were performed at the Endocrinology Laboratory of the University Medical Center Utrecht. Alpha-amylase was measured on the DxI analyzer (Beckman Coulter Inc., Fullerton, CA, USA). Saliva samples were diluted 500x with 0.2% BSA in 0.01 M Phosphate buffer pH 7.0. Interassay variation was <2.2%.

All physiological data were screened for outliers, which were defined within each assessment point as values greater than 3 SD above the mean. All outliers were winsorized by replacing their values with the value of 3 SD above the mean (Tukey, 1977).

The *arrival index* was defined as the percent change in cortisol and sAA concentrations from S1 to S2, calculated as S2 minus S1 divided by S1 and multiplied by 100. A positive score indicates an increase in biomarker concentration from S1 to S2, with higher scores indicating stronger increases from arrival to pre-stress, whereas a negative score indicates a decrease, with lower scores indicating stronger decreases.

The *stress index* was defined as the percent change in cortisol and sAA concentrations from S2 to S3, calculated as S3 minus S2 divided by S2 and multiplied by 100. A positive score indicates an increase in biomarker concentration from S2 to S3, with higher scores indicating stronger increases in response to the stressor, whereas a negative score indicates a decrease, with lower scores indicating stronger decreases.

The *area under the curve* with respect to ground (AUCg), as a measure for total biomarker concentration, and the area under the curve with respect to increase (AUCi), as an additional measure for reactivity, were calculated for S2-S6 for both cortisol and sAA using the formulas described in Pruessner et al. (2003).

Personality

The personality traits *Extraversion, Agreeableness, Conscientiousness, Emotional stability, and Openness-intellect* were assessed using the Big Five Bipolar Rating Scales (B5BBS-25; Mervielde, 1992). This questionnaire consists of 25 Dutch bipolar markers, five for each personality trait. The two opposite poles are connected by a seven-point rating scale that is used to indicate which of the two poles is most descriptive of the child. The factor structure of this measure has been found to correspond to the Big Five personality traits, and the measure has been validated for use in school-aged children (Mervielde, Buyst, & De Fruyt, 1995). Cronbach's alpha indices of reliability for the five scales ranged from .69-.90 in the current sample. Mean item scores were computed for all five personality traits.

Trait emotion regulation strategy use

Children's trait use of emotion regulation strategies was assessed with an adapted version of the Emotion Regulation Questionnaire (ERQ; Gross & John, 2003). The ERQ is a 10-item questionnaire assessing the use of both suppression and reappraisal. The four-item suppression scale includes items such as "I keep my emotions to myself". The reappraisal scale contains six items such as "I control my emotions by changing the way I think about the situation I am in". Responses are indicated on a seven-point Likert scale (1 = strongly disagree, 7 = strongly agree).

For use in the current study, the Dutch translation of the ERQ (Koole, 2004) was adapted for the use in 10-year-old children by simplifying the formulation of the items, and extending the instructions (see De Veld, et al., 2012). Principal components analysis revealed a two-factor solution, corresponding to the original factor structure reported in adults by Gross and John (2003). Reliability in the current sample was sufficient for both scales (Cronbach's alpha .64 for suppression, and .68 for reappraisal). Mean item scores for each scale were computed as indices for trait use of *reappraisal* and *suppression*.

5.2.4. Statistical Analyses

To assess whether there was a significant increase in cortisol and sAA to the stressor, we used repeated measures ANOVA with Time (S1-S7) as a within subject factor. As both cortisol and sAA data for each assessment point were not normally distributed, these data were normalized with log10 (cortisol) and sqrt (sAA) transformations prior to analysis. In case of a violation of the sphericity assumption, multivariate statistics are reported.

To examine the association between the arrival index and subsequent reactivity measures we performed three correlation analyses. In the first, we correlated the cortisol arrival index to the cortisol stress index, AUCg, and AUCi. In the second, we correlated the sAA arrival index to the sAA stress index, AUCg, and AUCi. In the third and final correlation analysis we correlated the arrival indices for both cortisol and sAA to the emotion regulation strategies and personality traits. As none of the physiological data were normally distributed non-parametric Spearman's rank order correlations were used for all analyses.

5.3. Results

5.3.1. Preliminary Analyses

Descriptives for all relevant variables are presented in Table 1.

A repeated measures ANOVA on the cortisol data with Time as a within subject factor indicated a significant effect of time, Wilks' Lambda = .19, F (6, 152) = 105.02, $p <$ .001, multivariate partial eta squared = .81. A repeated measures ANOVA on the sAA data with Time as a within subject factor also indicated a significant effect of time for this variable, Wilks' Lambda = .41, F (6, 148) = 36.24, $p < .001$, multivariate partial eta squared = .60. For both cortisol and sAA the significant effect of Time was due to an increase in concentration in response to the stressor (see Figure 1).

5.3.2. Cortisol

All correlations between cortisol measures are presented above the diagonal in Table 2 (p. 98). The cortisol arrival index showed a significant positive correlation with the cortisol AUCg ($r_s = .24$, $n = 158$, $p < .01$). Thus, a higher cortisol arrival index was related

Table 1 Descriptives for the study variables.

	N	*Md* (*IQR*) or *M (SD)*
Arrival index cortisol	158	-25.46 (-33.79 – -13.18)
Stress index cortisol	158	18.52 (-3.03 – 51.14)
AUCg cortisol	158	407.23 (318.01 – 528.69)
AUCi cortisol	158	235.40 (92.71 – 344.95)
Arrival index sAA	156	-30.16 (-44.02 – -7.58)
Stress index sAA	156	51.72 (12.81 – 119.07)
AUCg sAA	155	9457.75 (6381.75 – 17072.50)
AUCi sAA	155	2315.50 (592.75 – 5783.50)
Trait suppression	158	3.37 (1.01)
Trait reappraisal	158	4.50 (0.88)
Extraversion	157	4.81 (0.83)
Agreeableness	157	5.21 (0.68)
Conscientiousness	157	4.77 (0.94)
Emotional stability	157	4.87 (0.65)
Intellect/openness	157	5.50 (0.64)

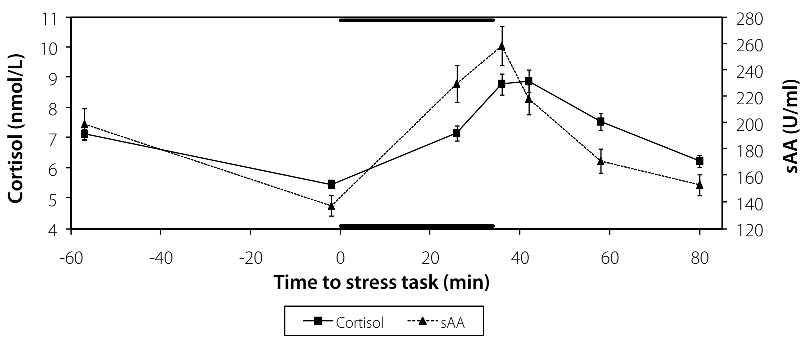

Figure 1 Cortisol and sAA responses to the stress task. Duration of the stressor is indicated with the dark grey bars.

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to a higher cortisol AUCg. The cortisol arrival index was unrelated to the cortisol stress index and cortisol AUCi.

5.3.3. Salivary Alpha-Amylase

All correlations between sAA measures are presented below the diagonal in Table 2. The sAA arrival index showed a significant negative correlation with both the sAA stress index (r_s = -.43, n = 155, p < .001), and sAA AUCi (r_s = -.41, n = 154, p < .001). Thus, a higher sAA arrival index was related to a lower sAA stress index, and a lower sAA AUCi.

Table 2 Correlations between the arrival index, stress index, AUCg, and AUCi, separately for cortisol (above the diagonal) and sAA (below the diagonal).

		1.	2.	3.	4.
1.	Arrival index	-	.02	.24**	.10
2.	Stress index	-.43**	-	.69**	.90**
3.	AUCg	-.10	.27**	-	.85**
4.	AUCi	-.41**	.83**	.59**	-

** $p \leq .01$.

Table 3 Correlations between the cortisol and sAA arrival indices, and personality and emotion regulation variables.

	Cortisol arrival index	sAA arrival index
Trait suppression	-.05	-.10
Trait reappraisal	-.08	.00
Extraversion	.09	-.01
Agreeableness	-.14	.09
Conscientiousness	.03	-.05
Emotional stability	.00	.14
Intellect/openness	.05	.14

5.3.4. Relation between Arrival Indices, Trait Emotion Regulation, and Personality Traits

There were no significant correlations between the cortisol and sAA arrival indices on the one hand, and trait emotion regulation use and personality traits on the other (see Table 3).

5.4. Discussion

In the current study we replicated the analyses conducted by Balodis et al. (2010) in a sample of 9- to 11-year-old children by examining the correlation between the change in salivary biomarkers of stress during the first hour in the laboratory and stress reactivity to a subsequent psychosocial stress task. For cortisol we found that a higher arrival index was unrelated to cortisol reactivity as reflected in the stress index and the AUCi. A higher cortisol arrival index did show a significant but weak correlation with a higher cortisol AUCg, which is not a measure of cortisol reactivity but rather represents total hormone concentration (Pruessner, et al., 2003). This correlation can be partly explained by the fact that both measures include the pre-stress sample S2: the arrival index as an end point, and the AUCg as a starting point. That is, a high arrival index signifies a relatively high pre-stress value at S2, and as this value serves as a baseline in computing the AUCg, a relatively high value at S2 will also result in a relatively high value for the AUCg. Because the arrival index was not related to the AUCi and the stress index, these results would be indicating that in the current sample changes in cortisol over the pre-stress period in the laboratory were not related to cortisol reactivity to the stress task. For sAA, we did find a relation between changes in pre-stress concentrations and subsequent reactivity. For this measure, a higher arrival index was significantly but only moderately correlated with both a lower stress index and a lower AUCi. As the average response pattern showed a decrease in sAA over the first hour in the laboratory, this indicates that for children whose sAA is high on arrival, and whose levels either remain high or even increase, reactivity to the stressor is lower. This is in line with the law of initial values (Wilder, 1962), and could be indicating a ceiling effect. Finally, we also explored whether the arrival indices for sAA and cortisol were related to personality characteristics or trait emotion regulation strategy use. However, this was not the case.

The current results do not correspond to the findings of a strong correlation between the change in salivary biomarkers over the first hour in the laboratory and subsequent stress reactivity to a stress task that were reported by Balodis et al. (2010) in a study with adults. There are several potential explanations for the observed differences. One is that the difference in findings might be due to possible differences in the pre-stress part of the protocols used in the two studies, for example with respect to foreknowledge about the upcoming stress task, or the procedure during the pre-stress period. The children participating in the current study were told that they would come to the lab to participate in tasks that are similar to those performed at school, and had already participated in a relatively stress-free control condition one week before the laboratory session. Hence, the children might have been rather unsuspecting about the upcoming stress task. In addition, they were engaged in non-stressful tasks and a relaxation period during the pre-stress period. Both of these factors might have led to less elevated baseline samples or less anticipatory stress in the current sample (Nicolson, 2008). However, as we neither have information about the foreknowledge that participants in the study by Balodis et al. (2010) had, nor about the content of their pre-stress period, it can not be said with certainty that these factors contributed to the difference in findings.

The difference between the current findings and those reported by Balodis et al. (2010) might also be due to the different ages of the respective samples. Although cortisol responses to psychosocial stress seem similar in middle childhood and adulthood (Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004; Yim, Quas, Cahill, & Hayakawa, 2010), there is some evidence that sAA reactivity may differ across these age groups (Yim, Granger, & Quas, 2010). It is also possible that children and adults differ on psychological factors like appraisals of the laboratory visit, or rumination during the pre-stress period in the laboratory.

In sum, the cortisol arrival index was not related to subsequent cortisol reactivity to stress, and the correlations between the sAA arrival index and measures for subsequent sAA reactivity to stress were much weaker than those found by Balodis et al. (2010). As such, the results of the current study do not provide a basis for the development of an additional stress induction protocol that can be used in middle childhood. Moreover, the arrival indices of neither cortisol nor sAA were related to personality and trait emotion regulation. This indicates that, at least in children this age, inter-individual differences in the arrival index not necessarily represent a child characteristic but might instead be related to differences in biomarker concentrations due to external factors, like mode of transportation to the lab, or specific activities during the pre-stress period.

For now, it seems that a pre-stress period employed in stress research with children can only be used as it was originally intended: to make children feel comfortable in the lab in order to minimize differences in baseline physiological activity resulting from anticipatory elevations, thereby allowing for clear assessment of individual differences in physiological responses to the subsequent stressor (Gunnar, et al., 2009). Nonetheless, given the differences between the results of the current study in children and those found in adults by Balodis et al. (2010) it would be interesting if researchers used the TSST with both children and adults to gain further knowledge on the arrival index. In doing so, it is particularly important to keep the pre-stress procedure consistent between age groups, both with respect to foreknowledge about the upcoming procedure, as well as the content of the pre-stress period. The addition of a saliva sample taken at home could provide information about biomarker levels prior to arrival in the lab. The results of such a study could shed more light on the potential meaning of an arrival index in participants of different ages, and provide valuable knowledge with regard to the development of an additional stress test for children.

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6

Summary, Conclusions, and General Discussion

6.1. Summary of the Research Project

The encounter of a stressor triggers physiological, emotional, and behavioural changes aimed at coping with the stressor (Steptoe, 2007). However, relatively little is known about how stress responses at the physiological, emotional, and behavioural level are interrelated, especially in children. Such knowledge is important if we are to obtain a clear picture of the mechanisms involved in human stress responses. In addition, knowledge about the interrelation between stress responses at these different levels could eventually aid our understanding of the origins and treatment of psychological disorders, as certain patterns of physiological, emotional, and behavioural responses to stress have all been found to be related to psychopathology (e.g. Charmandari, Tsigos, & Chrousos, 2005; Hughes, Gullone, & Watson, 2011; McEwen, 2003; Mogg, Philippot, & Bradley, 2004). Therefore, *the first aim of this thesis was to investigate whether children's physiological stress responses, i.e. their hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system (SNS) activation to a stressor, was related to their emotional responses to the same stressor in terms of emotion regulation strategy use, and to their behavioural responses to the stressor in terms of gaze aversion*.

Stress has also been found to influence cognitive functioning (e.g. Schoofs, Preuss, & Wolf, 2008; Wolf, 2009). Although there is quite an extensive body of literature on the relation between physiological stress responses and memory in adults, less is known about how physiological stress responses are related to memory in children, especially with regard to the interactive effects of the SNS and HPA axis. This is surprising considering the obvious relevance of such knowledge in the context of children's school performance. As such, *the second aim of this thesis was to investigate whether children's physiological stress responses were related to their cognitive functioning during stress in terms of delayed retrieval (DR) of declarative memories and working memory (WM) performance*.

Finally, the present thesis aimed to answer a question that emerged during the course of the project, based on a publication by Balodis, Wynne-Edwards, and Olmstead (2010) that appeared after the present data had been collected. This publication reported strong correlations between adult participants' changes in salivary cortisol and alpha-amylase (sAA) over a one hour pre-stress period in the laboratory ('arrival index'), and their subsequent cortisol and sAA reactivity to a psychosocial stress task. The final study presented in this thesis aimed to replicate these findings in our sample of 9- to 11-year-old children, and investigated whether the arrival index was related to children's trait emotion regulation strategy use, and personality traits.

The thesis consists of four studies that are all based on data that were collected in the same sample of 158 children between 9- and 11-years of age. Five types of measures were used to address the different research questions in this thesis: (1) saliva samples to assess children's physiological responses, i.e., cortisol and sAA levels in response to the stress task; (2) self report questionnaires to assess trait and state use of the emotion regulation strategies reappraisal and suppression; (3) video observations to quantify the children's gaze aversion during the stress task; (4) memory tasks to assess children's DR and WM during a control condition, and during the stress task; and (5) a questionnaire filled out by the children's parents to assess the children's personality traits. In this final chapter, the studies presented in the previous chapters will be summarized, followed by the main conclusions of the thesis and a general discussion.

Study 1. The aim of the first study (Chapter 2) was to investigate whether children's spontaneous use of the emotion regulation strategies reappraisal and suppression during a psychosocial stress task was related to their cortisol and sAA responses to the task. According to classical theories on stress, inter-individual differences in physiological responding may follow from differences in cognitive appraisals of the stressful situation, and emotional responses associated with those appraisals (Frijda, 1986; Lazarus & Folkman, 1984). Therefore, the current study investigated whether the extent to which people try to regulate their appraisals and emotions in the face of a stressor, for instance by using emotion regulation strategies, might also be related to the strength of their physiological stress responses.

To examine this, 158 children participated in a laboratory session that included exposure to a stressful task: the Trier Social Stress Test for Children (TSST-C; Buske-Kirschbaum et al., 1997). Seven saliva samples were obtained throughout the session, which were later analyzed for cortisol and sAA to assess children's physiological stress reactivity and their subsequent recovery. Children filled out an adapted version of the Emotion Regulation Questionnaire (ERQ; Gross & John, 2003; Dutch version by Koole, 2004) to provide information about the extent to which they had used reappraisal and suppression during the TSST-C. Furthermore, participant sex was included as a potential moderator of the relation between emotion regulation and physiological stress responses, and pubertal status was assessed and included as a potential confounder.

The results showed overall increases in cortisol and sAA in response to the stressor, with higher cortisol reactivity in girls than in boys. With regard to emotion regulation, more use of suppression was related to lower cortisol reactivity in girls, and lower sAA reactivity and quicker sAA recovery in all children. The use of reappraisal was not related to the children's cortisol or sAA responses.

In conclusion, reappraisal during stress seems ineffective at down-regulating children's physiological stress responses. Due to the correlational nature of the study, is unclear whether suppression can down-regulate children's physiological stress responses, or whether children with stronger physiological stress responses are less able to use suppression to regulate their emotions.

Study 2. The aim of the second study (Chapter 3) was to investigate the relation between ethological observations of children's gaze aversion during a psychosocial stress task and their cortisol reactivity to the task. Since gaze aversion as a strategy to cope with a stressful task might depend on how stressful children experience the task, we also measured perceived stress and explored whether this moderated the relation between gaze aversion and cortisol reactivity.

Observational data from 140 of the participating children were available for analysis. Data of these participants were coded for gaze aversion using interval coding with 2-second intervals. Perceived stress was assessed with a self-report questionnaire (original manipulation check for the TSST-C; Buske-Kirschbaum, et al., 1997). Cortisol reactivity was assessed from the obtained saliva samples.

Results showed higher cortisol reactivity in children who perceived the task as more stressful. Furthermore, a quadratic relation between gaze aversion and cortisol was found which depended on the level of perceived stress: for children with low levels of perceived stress, cortisol reactivity was *lowest* with intermediate levels of gaze aversion, whereas for children with high levels of perceived stress cortisol reactivity was *highest* at intermediate levels of gaze aversion.

In conclusion, there is a certain degree of coherence between subjective and physiological stress responses in 9- to 11-year-olds. Furthermore, considering that children who perceived the task as relatively stressful showed lower cortisol levels at both low and high levels of gaze aversion, gaze may play an important role as a behavioural coping strategy at this age.

Study 3. The aim of the third study (Chapter 4) was to investigate whether children's performance on WM and DR tasks decreased after stress exposure, and how physiological stress responses were related to performance under stress.

To investigate this, 158 children performed a WM task and a DR memory task first in a control condition in a mobile lab at home, and approximately one week later in a stress condition in the laboratory of the Behavioural Science Institute of the Radboud University Nijmegen. The WM task consisted of both a forward and a backward digit span task based on those from the Wechsler Intelligence Scale for Children (Wechsler, 1991). The DR task was newly developed to fit the needs of the current study. Children were asked to memorize five word lists that corresponded to five different word categories (e.g. professions). Each word list contained eight words. DR memory performance was assessed by asking children to name as many words from a randomly selected category (list) as they could in 2 minutes. Saliva samples were taken to assess cortisol and sAA levels.

When comparing children's performance on the tasks between conditions, only WM backward performance was worse in the stress versus the control condition. Relations between physiological stress responses and performance within the stress condition were present only for WM forward and DR. For WM forward, higher cortisol responses were related to better performance. For DR, there was an inverted U-shape relation between cortisol responses and performance, as well as a cortisol * sAA interaction, with concurrent high or low responses related to optimal performance.

In conclusion, the finding that cortisol reactivity interacted with sAA reactivity in predicting DR memory performance suggests that stress influences children's memory through a complex interaction of HPA axis and SNS activation. The finding of an inverted U-shape relation between cortisol responses and DR performance emphasizes the importance of including curvilinear and interaction effects when relating children's physiological stress responses to memory.

Study 4. The fourth study (Chapter 5) aimed to answer a question that emerged during the course of the project, based on a publication that appeared after the present data had been collected (Balodis, et al., 2010). This fourth study investigated whether the changes in the children's salivary cortisol and alpha-amylase over a 1 h pre-stress period in the laboratory (the 'arrival index') were correlated to their subsequent reactivity to the stress task. Knowledge about children's stress reactivity and its correlates is mostly based on one stress task, making it hard to assess the generalizability of the results. The development of an additional stress paradigm for children, that also limits stress exposure and test time, could greatly advance this field of research. Research in adults may provide a starting point for the development of such an additional stress paradigm, as the arrival index correlated strongly with subsequent reactivity to the stress task (Balodis, et al., 2010). The present study examined whether such strong correlations could be replicated in our sample of 9- to 11-year-old children. In addition, we explored whether children's trait emotion regulation strategy use and personality characteristics were related to the arrival index in the current sample.

Cortisol and sAA samples were collected from 158 children (83 girls) during their visit to the laboratory. This visit included a 1 h pre-stress period in which children performed some non-stressful tasks and relaxed before taking part in a psychosocial stress task (TSST-C; Buske-Kirschbaum, et al., 1997). Children filled out an adapted version of the Emotion Regulation Questionnaire (ERQ; Gross & John, 2003; Dutch version by Koole, 2004) to provide information about their trait use of the emotion regulation strategies reappraisal and suppression. Parents completed the Big Five Bipolar Rating Scales (B5BBS-25; Mervielde, 1992) to provide information about the personality traits Extraversion, Agreeableness, Conscientiousness, Emotional stability, and Openness-intellect of their child.

Results showed that a higher cortisol arrival index was significantly and weakly correlated with a higher cortisol AUCg but unrelated to cortisol reactivity to the stressor. A higher sAA arrival index was significantly and moderately related to lower sAA stress reactivity and to a lower sAA AUCi. Children's personality and emotion regulation variables were unrelated to the cortisol and sAA arrival indices.

In conclusion, the results of this fourth study do not provide a basis for the development of an additional stress paradigm for children. Further replications in children and adults are needed to clarify the potential meaning of an arrival index. Moreover, the arrival indices of neither cortisol nor sAA were related to personality and trait emotion regulation, indicating that, at least in children this age, inter-individual differences in the arrival index do not necessarily represent a child characteristic.

6.2. Conclusions

Taken together, the results of the four studies presented in this thesis can be summarized in the following main conclusions:

- Children's use of reappraisal in face of a psychosocial stressor appears to neither decrease physiological reactivity, nor facilitate recovery.
- It is important to consider sex as a moderator variable in studies investigating the relation between physiological stress responses and emotion regulation in children.
- Perceived difficulty and threat level of the task seem important determinants of cortisol reactivity to the task.
- Gaze aversion may play an important role as a behavioural coping strategy in middle childhood, especially for children that perceive the situation as highly stressful.
- Stress influences children's memory through a complex interaction of HPA axis and SNS activation.
- In contrast to what has been found in adults, the arrival index , or children's recovery from arrival in the laboratory, does not strongly predict children's subsequent reactivity to the stress task.

6.3. General Discussion

6.3.1. The Relation between Emotional and Behavioural Responses to Stress

The present thesis made a significant contribution to the current knowledge about the relation between children's physiological and emotional responses to stress (Chapter 2), and the relation between their physiological and behavioural responses to stress (Chapter 3). We presented the results of these studies in two separate chapters. However, as both emotion regulation strategy use and behavioural coping by means of gaze aversion are aimed at regulating the response to a stressor, they might be interrelated. Therefore, one might argue that emotion regulation and gaze aversion should have been investigated simultaneously. This was not done in the current thesis because the behavioural data were not available at the time the

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emotion regulation data were analyzed. However, to check this possible interrelation between emotion regulation and gaze aversion, we performed two post-hoc analyses. The first was a correlation analysis that investigated the relation between gaze aversion during the stress task and state use of the emotion regulation strategies suppression and reappraisal. The results of this analysis showed that gaze aversion was unrelated to both suppression (r = .05, p = .55), and reappraisal (r = -.06, p = .46). This indicates that children's state use of suppression and reappraisal was unrelated to their gaze aversion during the stress task.

The second post-hoc analysis investigated whether the results of the regression analyses to predict cortisol reactivity as presented in the separate chapters would hold when emotion regulation and gaze aversion were investigated simultaneously. To do this, we performed a regression analysis with cortisol reactivity as a dependent variable, and all confounders and independent variables from the two separate chapters on emotion regulation (Chapter 2) and gaze aversion (Chapter 3) as predictors. There was no change in which variables were entered in the final model. Within the final model, there were no changes in which variables were significant, or in the direction of the effects. Neither were there any major changes in the betas of the overall model, as compared to when the models were run separately. The results of these post-hoc analyses indicate that emotion regulation and gaze aversion both uniquely explain a part of the variance in children's physiological stress reactivity, and can be investigated separately.

6.3.2. The Effects of Stress on Children's Cognitive Functioning

This thesis shed light on the relation between children's physiological stress reactivity and their cognitive functioning (Chapter 4). In particular, we found that the relation between cortisol reactivity and performance differed for the different memory tasks: for WM backward there was no relation with cortisol reactivity, for WM forward higher cortisol responses were related to better performance, and for DR there was an inverted U-shape relation between cortisol responses and performance, as well as a cortisol by sAA interaction. Although there were theoretical grounds on which to expect a nonlinear effect for cortisol and an interaction effect of cortisol and sAA, these had not yet been investigated in children. Our findings support that this curvilinear and interaction effect exist, at least for the relation between cortisol and DR. As such, curvilinear and interaction terms should be incorporated in future research investigating the relation between physiological stress responses and memory.

6.3.3. Stress Induction Protocols for Children

To answer the main questions posed in the present thesis, all participating children were exposed to a stress task: the Trier Social Stress Test for Children (TSST-C; Buske-

Kirschbaum, et al., 1997). This task – together with slightly modified versions of this task, like the Trier Social Stress Test Modified (Yim, Quas, Cahill, & Hayakawa, 2010) – is the most frequently used stress induction procedure in research with 9- to 11-year-old children. This is not surprising, given that it has been shown to be the most effective method currently available for stress induction in this age group (Gunnar, Talge, & Herrera, 2009). However, the limited availability of other effective stress induction procedures for this age group does have implications with respect to both the practicalities involved in data collection, and the generalizability of the findings in the present thesis.

An important practical issue regarding the use of the TSST-C involves the manpower needed to execute this stress induction procedure: the protocol requires one experimenter and two confederates. The study presented in Chapter 5 sought to examine whether the arrival index could provide a starting point for the development of a protocol that resolved this issue. Unfortunately this was not the case, as children's cortisol arrival index was not related to their subsequent cortisol reactivity to stress, and the correlations between the sAA arrival index and measures for subsequent sAA reactivity to stress were only moderate. However, these findings do not necessarily mean that the issue regarding the manpower needed to execute a stress induction protocol can not be solved. The recently developed Leiden Public Speaking Task (Leiden PST; Westenberg et al., 2009) uses a pre-recorded audience consisting of age matched peers and a female teacher to induce stress. The finding that this task nonetheless successfully induces cortisol and sAA responses in 9- to 17-year-old participants indicates that the presence of a 'live' audience of two confederates in not a necessary condition to provoke a physiological stress response in children and adolescents (Sumter, Bokhorst, Miers, Van Pelt, & Westenberg, 2010). Adaptations of this type to the TSST-C protocol, for example by using a pre-recorded jury, might prove a promising way in which the manpower needed to induce stress in children is reduced, without significantly compromising the stressfulness of the task.

Although talking in front of an audience is a very ecologically valid stressor, it is only one out of numerous potentially stressful situations in children's daily lives. Due to the limited availability of effective stress induction protocols in middle childhood, it is hard to say whether research results found with the TSST-C are generalizable to these other stressors. With regard to the present thesis, for example, we do not know whether the relation between emotion regulation strategy use and physiological reactivity, or between physiological reactivity and gaze aversion, is the same under other stressful circumstances. We do know that emotion regulation strategy use arises from both dispositional and situational factors (Egloff, Schmukle, Burns, & Schwerdtfeger, 2006, Study 1), and that 8-year-olds show more gaze aversion during face-to-face questioning than during questioning across a live video link (Doherty-Sneddon & Phelps, 2005). It is conceivable that as the characteristics of the stressor change, this not only changes these separate responses to the stressor but also how they are interrelated, as a different stressor may require a different set of responses that is tailored to the specific characteristics of that situation. Research replicating the current studies using a different, yet ecologically valid, stress induction protocol would thus help us find out how the intercorrelations between different responses to a stressor are moderated by the characteristics of the stressor at hand.

6.4. Limitations and Future Directions

The present thesis investigated physiological, emotional, and behavioural responses to stress. To our knowledge, it is the first study to investigate children's stress responses in such a comprehensive manner. In addition, this thesis shed light on the relation between children's physiological stress responses and cognitive functioning, in particular with respect to the interaction between cortisol and sAA. However, besides these strengths, there are also some limitations.

The first regards DR and WM performance in the stress condition compared to the control condition. To our knowledge, the present thesis was the first to use a within subjects design to study the effects of stress on cognitive functioning in children. All children first completed the control condition, followed by the stress condition approximately one week later. The rationale behind this fixed order of conditions was that counterbalancing could lead to selective attrition when relatively shy or anxious children were exposed to the TSST-C during their first visit, resulting in missing control condition data for this very interesting subgroup. Unfortunately, this also limits our conclusions with regard to the effect of stress exposure on WM and DR performance. Therefore, future research would benefit from a within subjects design that does counterbalance the order of the conditions.

A second limitation is that the design of the studies presented in this thesis did not allow us to draw causal conclusions. Although this approach provides an ecologically valid way to investigate the relation between different responses to a stressor, we can not draw conclusions about the causal direction of the found effects. Future studies using a stricter experimental design are needed to shed more light on causality.

Although differences in findings between children and adults can be considered an indication of developmental changes, they do not provide information about developmental trajectories from childhood to adulthood. Longitudinal studies will prove invaluable in this regard, and as such provide an important direction for future research. Future studies may also add to the repertoire of physiological, behavioural, and emotional responses under investigation – for example by studying blood pressure, displacement behaviour, or rumination – or investigate how patterns of interactions between physiological, behavioural, and emotional responses to stress predict psychological problems in children.

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Samenvatting
Dankwoord
Curriculum Vitae

Samenvatting van het Onderzoeksproject

Blootstelling aan een stressvolle gebeurtenis leidt tot veranderingen in fysiologie, emotie en gedrag die gericht zijn op het succesvol omgaan met deze stressvolle gebeurtenis (Steptoe, 2007). Er is echter relatief weinig bekend over hoe fysiologische, emotionele en gedragsmatige stressreacties aan elkaar gerelateerd zijn. Kennis hierover is belangrijk om een goed beeld te krijgen van de mechanismen die betrokken zijn bij stressreacties bij mensen. Bovendien zijn bepaalde fysiologische, emotionele en gedragsmatige reacties op stress in verband gebracht met psychopathologie (e.g. Charmandari, Tsigos, & Chrousos, 2005; Hughes, Gullone, & Watson, 2011; McEwen, 2003; Mogg, Philippot, & Bradley, 2004), waardoor inzicht in de relaties tussen deze verschillende reactieniveaus zou kunnen bijdragen aan onze kennis over de oorsprong en behandeling van psychologische stoornissen. Daarom was het *eerste doel van dit proefschrift om te onderzoeken of de fysiologische stressreacties van kinderen gerelateerd zijn aan hun emotionele en gedragsmatige reacties op dezelfde stressvolle gebeurtenis*. Fysiologische reacties werden onderzocht door het vaststellen van de concentratie cortisol en alfa-amylase (sAA) in speeksel als indicator voor activatie van respectievelijk de hypothalamus-hypofyse-bijnierschors-as (HPA-as) en het sympathisch zenuwstelsel (SNS). Emotionele reacties werden onderzocht door het meten van het gebruik van twee emotieregulatiestrategieën. Gedragsmatige reacties werden onderzocht door het bestuderen van (weg)kijkgedrag.

Eerder onderzoek heeft uitgewezen dat stress invloed heeft op cognitie (e.g. Schoofs, Preuss, & Wolf, 2008; Wolf, 2009). Er is een ruime hoeveelheid literatuur over de relatie tussen fysiologische stressreacties en geheugenprestaties bij volwassenen, maar bij kinderen is hierover minder bekend. Dit geldt in het bijzonder voor hoe HPA-as- en SNS-activatie in samenhang met elkaar geheugenprestaties beïnvloeden. Dit is verwonderlijk, omdat dergelijke kennis erg belangrijk is als het gaat om bijvoorbeeld schoolprestaties. Daarom was het *tweede doel van dit proefschrift om te onderzoeken of fysiologische stressreacties van kinderen gerelateerd zijn aan hun cognitief functioneren tijdens stress*. Cognitief functioneren werd hierbij onderzocht met behulp van taken die het werkgeheugen en het ophalen van informatie uit het declaratieve geheugen toetsen.

Tot slot onderzocht dit proefschrift een vraag die na de dataverzameling voor dit onderzoeksproject rees naar aanleiding van een publicatie van Balodis, Wynne-Edwards en Olmstead (2010). In deze publicatie werd een sterke correlatie gevonden tussen concentratieveranderingen in cortisol en sAA tijdens het eerste uur dat volwassen proefpersonen doorbrachten in het laboratorium (de 'arrival index') en hun latere cortisol- en sAA-reacties op een stresstaak. De studie die als laatste in dit proefschrift staat beschreven was erop gericht te onderzoeken of bovengenoemde resultaten gerepliceerd konden worden in onze eigen steekproef van kinderen.

Daarnaast werd onderzocht of de arrival index gerelateerd was aan persoonlijkheidskenmerken van de kinderen en aan hoe zij over het algemeen hun emoties reguleren.

Dit proefschrift beschrijft vier studies die allemaal gebruik maken van data die zijn verzameld in een steekproef van 158 kinderen tussen de negen en elf jaar oud. Vijf verschillende methoden werden gebruikt voor het beantwoorden van de in dit proefschrift gestelde onderzoeksvragen: (1) speekselmonsters voor het bepalen van de cortisol- en sAA-niveaus; (2) zelfrapportage om het gebruik van de emotieregulatiestrategieën "reappraisal" (herinterpretatie van de situatie) en "suppressie"(het onderdrukken van de uiting van emoties) te meten; (3) video-observaties om vast te stellen hoe vaak kinderen wegkeken tijdens de stresstaak; (4) geheugentaken om het declaratieve geheugen en het werkgeheugen van de kinderen te toetsen tijdens een controleconditie en de stresstaak; (5) een vragenlijst ingevuld door de ouders om persoonlijkheidskenmerken van de kinderen in kaart te brengen. Hieronder wordt elke studie samengevat, gevolgd door de belangrijkste conclusies van dit proefschrift.

Studie 1. Het doel van Studie 1 (Hoofdstuk 2) was te onderzoeken of de mate waarin kinderen tijdens een stresstaak spontaan gebruik maken van de emotieregulatiestrategieën reappraisal en suppressie gerelateerd was aan hun cortisol- en sAA-reactiviteit op de stresstaak. Verder bekeken we of de relatie tussen emotieregulatie en fysiologische stressreacties verschillend was voor jongens en meisjes.

Om dit te onderzoeken kwamen 158 kinderen (waarvan 83 meisjes) naar het laboratorium voor deelname aan het onderzoek. Een belangrijk onderdeel van dit onderzoek was het uitvoeren van een stressvolle taak voor een jury: de Trier Social Stress Test for Children (TSST-C; Buske-Kirschbaum et al., 1997). Gegevens over de cortisol- en sAA-niveaus van de kinderen werden gebruikt om een maat te berekenen voor hun fysiologische stressreactie en het herstel daarvan. De kinderen vulden een vragenlijst in om vast te stellen in welke mate zij de twee emotieregulatiestrategieën gebruikt hadden tijdens de stresstaak.

In de huidige steekproef was er gemiddeld genomen een toename in cortisol en sAA als reactie op de stresstaak. Meisjes lieten een grotere toename in cortisol zien dan jongens. Meer gebruik van suppressie bleek gerelateerd te zijn aan minder sterke cortisolreacties voor meisjes. Daarnaast was meer gebruik van suppressie voor de gehele steekproef gerelateerd aan minder sterke sAA-reacties en sneller herstel van het sAA-niveau. Het gebruik van reappraisal was niet gerelateerd aan cortisol- en sAA-reacties en aan het herstel daarvan.

Deze resultaten impliceren dat het gebruik van reappraisal tijdens stress bij kinderen niet leidt tot een minder sterke fysiologische stressreactie. Vanwege de correlationele opzet van dit onderzoek is het onduidelijk of suppressie wel fysiologische stressreacties kan onderdrukken, of dat kinderen die een sterkere fysiologische stressreactie laten zien minder goed in staat zijn suppressie te gebruiken.

Studie 2. In de tweede studie (Hoofdstuk 3) bekeken we of er een relatie bestond tussen het kijkgedrag van de kinderen tijdens de stresstaak en hun cortisolreactie op die taak. Het door een kind wegkijken van de jury werd hierbij beschouwd als een manier om de door de stresstaak opgewekte stress te reguleren. Omdat wegkijken waarschijnlijk ook samenhing met de mate waarin kinderen tijdens de stresstaak stress ervoeren, werd ook onderzocht of de relatie tussen het wegkijken door de kinderen en hun cortisolreactie afhankelijk was van de door hen ervaren stress.

Van 140 deelnemende kinderen waren videobeelden beschikbaar voor het observeren van hun kijkgedrag. Wegkijken werd gescoord door voor elk interval van 2 seconden aan te geven of het kind al dan niet wegkeek van de TSST-C jury. Ervaren stress werd bepaald door middel van zelfrapportage door de kinderen. De sterkte van de cortisolreactie werd berekend op basis van de cortisolconcentratie in de genomen speekselmonsters.

Uit de resultaten bleek dat kinderen die meer stress ervoeren tijdens de stresstaak een sterkere cortisolreactie lieten zien. Daarnaast werd er een kwadratisch verband gevonden tussen wegkijken en de cortisolreactie; dit verband was afhankelijk van de mate van ervaren stress. Meer specifiek: bij gemiddeld vaak wegkijken waren de cortisolreacties van kinderen die aangaven weinig stress te hebben ervaren het *laagst*, terwijl de cortisolreacties van kinderen die aangaven veel stress te hebben ervaren dan juist het *hoogst* waren.

Deze studie laat zien dat er een zekere mate van coherentie is tussen ervaren stress en fysiologische stressreacties bij kinderen tussen de negen en elf jaar oud. Daarnaast vonden we dat kinderen die veel stress ervoeren de minst sterke cortisolreactie lieten zien bij heel weinig, of juist bij heel veel wegkijken. Dit geeft aan dat wegkijken op deze leeftijd wellicht een belangrijke rol speelt in het omgaan met stress.

Studie 3. In Studie 3 (Hoofdstuk 4) onderzochten we of de geheugenprestaties van de kinderen tijdens stress minder goed waren dan in een controleconditie zonder stress. Ook onderzochten we de relatie tussen fysiologische stressreacties en geheugenprestaties tijdens stress.

De 158 deelnemende kinderen voerden allemaal twee keer een aantal geheugentaken uit. De eerste keer deden ze dat in een controleconditie in het mobiele laboratorium tijdens een huisbezoek, de tweede keer ongeveer een week later tijdens een stressconditie in het laboratorium van het Behavioural Science Institute van de Radboud Universiteit Nijmegen. Eén van de taken onderzocht het werkgeheugen van de kinderen. Deze taak bestond uit een voorwaartse en een achterwaartse cijferreeksentaak gebaseerd op die in de Wechsler Intelligence Scale for Children (Wechsler, 1991). De andere taak mat het declaratieve geheugen en was speciaal ontwikkeld voor het huidige onderzoek. Hierbij werd kinderen gevraagd om vijf lijsten met woorden te onthouden, waarbij de woorden op elke lijst tot een bepaalde categorie behoorden (bv. Beroepen). Elke lijst bevatte acht woorden. Om prestaties van het declaratieve geheugen te toetsen werd de kinderen gevraagd om in twee minuten zoveel mogelijk woorden van een bepaalde categorie (lijst) op te noemen. Cortisol- en sAA-waarden werden verkregen uit de afgenomen speekselmonsters.

Alleen prestaties op de achterwaartse werkgeheugentaak waren slechter in de stressconditie dan in de controleconditie. Binnen de stressconditie bleken prestaties op de taken die het voorwaartse werkgeheugen en het declaratieve geheugen toetsten gerelateerd te zijn aan fysiologische stressreacties. Sterkere cortisolreacties hingen samen met betere prestaties op de voorwaartse werkgeheugentaak. Met betrekking tot het declaratieve geheugen was er een kwadratische relatie tussen prestatie en cortisolreactie in de vorm van een omgekeerde U. Daarnaast was er sprake van een interactie-effect voor cortisol en sAA, waarbij betere geheugenprestaties werden gevonden wanneer de cortisol en de sAA-reacties ofwel beide hoog, ofwel beide laag waren.

De resultaten van deze studie impliceren dat stress invloed heeft op het declaratieve geheugen van kinderen via een complexe interactie van HPA-as- en SNS-activiteit. In combinatie met de bevinding dat de relatie tussen geheugenprestatie en cortisolreactie de vorm had van een omgekeerde U laten deze resultaten zien dat het in dit onderzoeksveld belangrijk is om kwadratische en interactie-effecten te toetsen.

Studie 4. In de vierde studie (Hoofdstuk 5) probeerden we een vraag te beantwoorden die ontstond naar aanleiding van een publicatie van Balodis et al. (2010) die verscheen nadat onze data al verzameld waren. Bij volwassenen bleek de arrival index (de verandering in cortisol- en sAA-concentratie tijdens het eerste uur in het laboratorium) erg sterk samen te hangen met hun stressreactie op de situatie die even later als "officiële" stresstaak in het onderzoek werd gebruikt (Balodis, et al., 2010). Het bestaan van een dergelijke samenhang bij kinderen zou als startpunt kunnen dienen voor het ontwikkelen van een nieuwe stresstaak voor deze leeftijdsgroep. Tot dusver is kennis over stressreacties bij kinderen vooral gebaseerd op onderzoeken die gebruik maakten van de TSST-C als stresstaak. Het is dan ook de vraag of de resultaten van deze onderzoeken kunnen worden gegeneraliseerd naar andere stressvolle situaties. Het ontwikkelen van een nieuwe stresstaak voor kinderen kan bijdragen aan duidelijkheid hierover. In deze vierde studie onderzochten we daarom of de arrival indices voor cortisol en sAA ook in onze steekproef van 9- tot 11-jarige kinderen gerelateerd waren aan hun latere cortisol- en sAA-reacties op de stresstaak. Daarnaast bekeken we of persoonlijkheidskenmerken van de kinderen en het gebruik van emotieregulatiestrategieën gerelateerd waren aan de arrival indices.

Cortisol- en sAA-waarden tijdens het bezoek aan het laboratorium waren beschikbaar voor 158 kinderen. Het eerste uur van dit bezoek bestond uit het doen van een aantal niet-stressvolle taken en een rustperiode. Vervolgens deden alle kinderen een stresstaak (TSST-C; Buske-Kirschbaum, et al., 1997). De kinderen vulden een vragenlijst in om aan te geven in welke mate ze over het algemeen gebruik maakten van de emotieregulatiestrategieën reappraisal en suppressie. De ouders vulden een vragenlijst in over de persoonlijkheidskenmerken van hun kind.

Uit de resultaten bleek dat een hogere arrival index voor cortisol zwak samenhing met een hogere AUCg (een maat die de totale concentratie in speeksel tijdens het onderzoek weergeeft) voor cortisol, maar niet met de cortisolreactie van de kinderen op de stresstaak. Een hogere arrival index voor sAA was matig gecorreleerd aan een lagere sAA-reactiviteit. Persoonlijkheidskenmerken en emotieregulatiestrategieën waren niet gerelateerd aan de arrival indices.

De bovengenoemde resultaten bieden geen basis voor de ontwikkeling van een nieuwe stresstaak voor kinderen. Replicatie van dit onderzoek is nodig om de betekenis van de arrival index verder te verhelderen. Het ontbreken van een relatie tussen de arrival indices en persoonlijkheid en emotieregulatiestrategieën suggereert dat verschillen in de hoogte van de arrival index op deze leeftijd wellicht eerder het resultaat zijn van omgevingsfactoren (zoals de reismethode naar het laboratorium), in plaats van kindfactoren.

Conclusies

- Het gebruik van de emotieregulatiestrategie reappraisal tijdens een stresstaak lijkt geen invloed te hebben op de fysiologische stressreactie van kinderen of op het herstel daarvan.
- Het is belangrijk om bij het onderzoeken van de relatie tussen fysiologische stressreacties en emotieregulatie bij kinderen rekening te houden met de sekse van de deelnemers
- De mate waarin kinderen een taak als moeilijk en bedreigend ervaren lijkt een belangrijke indicator van hun cortisolreactie op de taak.
- Wegkijken kan een belangrijke rol spelen bij het omgaan met stress op deze leeftijd, vooral voor kinderen die de situatie als erg stressvol ervaren.
- Stress beïnvloedt het declaratieve geheugen van kinderen door middel van een complexe interactie van HPA-as- en SNS-activiteit.
- De mate waarin het cortisol- en sAA-niveau in speeksel omlaag gaat tijdens het eerste uur in het laboratorium is bij kinderen geen goede voorspeller van hun reactie op een daaropvolgende stresstaak. Dit in tegenstelling tot bij volwassenen.

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Dankwoord

Op deze plaats wil ik graag iedereen bedanken die, op wat voor manier dan ook, een bijdrage heeft geleverd aan dit proefschrift.

Allereerst alle kinderen die aan dit onderzoek hebben deelgenomen: heel erg bedankt dat jullie zo stoer waren om mee te doen! Ik vond het ontzettend leuk om jullie (bijna) allemaal te ontmoeten in de bus of het lab en enorm interessant om al die verschillende reacties op de stresstaak met eigen ogen te zien. Natuurlijk ook mijn dank aan alle ouders. Zonder jullie had dit onderzoek niet gedaan kunnen worden.

Marianne, jouw positieve instelling en goede gevoel voor humor zorgden er op moeilijke momenten voor dat ik er weer even tegenaan kon. Heel erg bedankt voor je betrokkenheid en natuurlijk voor alles wat ik de afgelopen jaren van je heb mogen leren.

Carolina, jouw enthousiasme voor het vakgebied van de psychobiologie is erg aanstekelijk, wat het nog leuker maakte om van jouw expertise te mogen leren. Daarnaast was het ontzettend fijn dat ik altijd bij je aan kon kloppen voor advies of om bot reviewercommentaar te relativeren.

Al mijn collega's wil ik bedanken voor hun gezelligheid en betrokkenheid. Of het nu ging om samen lachen om de grootste onzin tijdens de lunchpauze, constructief advies over mijn onderzoek, of meer persoonlijke gesprekken, jullie waren van alle markten thuis.

Een aantal collega's wil ik nog eens extra bedanken. Roseriet, naast dat je een gezellige kamergenoot was tijdens mijn eerste jaren als promovenda heb ik ook nog eens ontzettend veel van je geleerd. Heel erg bedankt voor beiden! Michelle, heel erg bedankt voor je steun en luisterend oor. Maartje, wat ontzettend fijn dat ik altijd een beroep mocht doen op je relativerende nuchterheid. Bill, thanks for answering all my statistics questions. Toon, bedankt voor alle gezellige afdelingsetentjes en –barbecues en natuurlijk voor de mogelijkheid om dit studiejaar als docent aan de slag te gaan.

Alle studenten en studentassistenten die aan het project hebben meegewerkt wil ik ook ontzettend bedanken, ook jullie waren onmisbaar bij het verzamelen van alle data! In het bijzonder wil ik Luuk Geijsen en Anouk Verhappen noemen, die als onderzoeksstagiaires een enorme hoeveelheid werk hebben verzet voor dit project.

Marlies, Barbara en Pauline, heel erg bedankt voor jullie vriendschap en gezelligheid van de afgelopen jaren! Het is altijd leuk om tijdens een lunch of high tea weer even lekker bij te kletsen en onze afspraken geven me elke keer weer nieuwe energie.

Sanne en Rianne, wat bof ik met zulke lieve vriendinnen als jullie! Ondanks dat we niet bepaald bij elkaar om de hoek wonen kan ik altijd bij jullie terecht. Jullie oprechte interesse, goede gevoel voor humor en vermogen om me aan het denken te zetten waardeer ik enorm.

Lieve Bert, Marleen, Jolein en Stef, heel erg bedankt voor jullie betrokkenheid, gezelligheid, goede zorgen en wijze woorden door de jaren heen.

Lieve Jeffrey en Vivian, het is altijd leuk om jullie weer te zien en dan even bij te kunnen kletsen.

Lieve papa en mama, heel erg bedankt dat jullie me altijd de vrijheid hebben gegeven om mijn eigen keuzes te maken. Het is fijn om te weten dat jullie achter me staan en ik altijd bij jullie terecht kan.

Tot slot mijn twee mannen. Lieve Steven, de afgelopen jaren waren vaak hectisch, maar je was er altijd. Als chef-kok tijdens mijn dataverzameling, als superpapa tijdens de eerste weken na Arlens geboorte, of 'gewoon' als steun en toeverlaat op moeilijke momenten. Bedankt voor je relativeringsvermogen en alle lol die we samen hebben.

Lieve Arlen, hoe klein je ook bent, de belangrijkste dingen in het leven leer ik van jou. En niet te vergeten: jij geeft de allerbeste knuffels!

Curriculum Vitae

Daniëlle de Veld was born on June 24, 1985, in Leidschendam, the Netherlands. After completing her secondary education at the Erasmus College in Zoetermeer in 2003, she studied Psychology at Leiden University. During her Bachelor's programme she participated in the Honours Lectures Series, and was a member of the Evaluation Committee. She received her Bachelor's degree in 2006, and was admitted to the Research Master in Psychology at Leiden University that same year. During her Master's programme she worked as a teaching assistant at Leiden University, and did a four-month research internship at the University of California in San Diego. In 2008 she completed her Master's degree (cum laude) with a thesis on emotion regulation and cognitive control. Immediately after this, she started her PhD project on stress in middle childhood at the department of Developmental Psychology/Behavioural Science Institute of the Radboud University Nijmegen, under supervision of prof. dr. Marianne Riksen-Walraven and dr. Carolina de Weerth. This project resulted in the current thesis. At the moment, Daniëlle has a teaching appointment at the department of Developmental Psychology at the Radboud University Nijmegen.