

VOLUME 1

**STRESS, CORTISOL AND EXECUTIVE
FUNCTION: A STUDY OF PRE-
ADOLESCENT SCHOOL CHILDREN**

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OVERVIEW

This thesis explores the links between stress, cortisol and executive functioning.

Part one describes the human stress response, and outlines research linking early experiences with the functioning of the stress physiology. The relationship between the stress hormone, cortisol, and cognitive function is then examined, focusing specifically on the development and functioning of executive capacities in early life.

Part two presents the empirical paper. This investigates the link between cortisol, executive function and self-regulatory abilities, in pre-adolescent school children. The procedure used a social stressor, and aimed to examine differences in cortisol responses in relation to executive function. Individual differences in cortisol levels related to some aspects of executive attention and self-regulation.

Part three discusses in more detail the link between early relationships and the development and functioning of the human stress response. It focuses on the links between attachment theory and the neurophysiology of the stress system. Ideas for further exploration are highlighted. The thesis concludes with a reflection on some of the challenges and major learning points encountered throughout the research process. Please note, part two of this project, the empirical paper, was conducted jointly with a colleague (please see thesis entitled “Stress, cortisol and emotional and behavioural problems in pre-adolescent children” by Lucy Partridge). Data collection and aspects of the procedure were shared.

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PART I

REVIEW PAPER

**CORTISOL AND COGNITIVE FUNCTIONING:
THE DEVELOPING HPA SYSTEM AND THE
RELATIONSHIP WITH EXECUTIVE FUNCTION
IN EARLY LIFE**

ABSTRACT

This review aims to explore the relationship between the human stress physiology and cognitive functioning. In particular, it aims to address the question of whether hormones related to stress (specifically cortisol), influence the development of executive function in early life. In order to provide a theoretical and empirical background to this area of investigation, the review firstly outlines literature linking early experiences with the development of the human stress response. Evidence for the relationship between the stress physiology and cognitive function in adults and primates is then summarised. The importance of executive functioning as an aspect of child development is considered, before addressing in detail studies which examine the relationship between executive function and the human stress physiology in early life and childhood. The review ends by proposing that the links between early experiences and later cognitive capacities may be mediated by the development and functioning of the stress physiology.

1.1.0**INTRODUCTION**

This review investigates whether children's cognitive and executive capacities are influenced by the development and functioning of the stress response system. This question will be addressed in the following stages. In section 1.1 the facet of the stress response known as the Hypothalamic Pituitary Adrenocortical system (HPA system) is described, giving an overview of what is known about its function and development. Section 1.2 will provide a brief summary of the literature linking early adversity and early care-giving relationships to the development of the HPA system.

Section 1.3 outlines neurobiological explanations for the impact of the stress hormone cortisol, upon cognitive function. Evidence for the relationship between the stress response system and cognitive function in adults is presented.

Section 1.4 incorporates the main body of the review, and begins by introducing the importance of executive function as an aspect of child development. It assesses in detail the literature exploring the link between the HPA system and cognitive and executive abilities in children aged 3 to 16 years. Finally, section 1.5 will suggest hypotheses explaining the findings, discuss avenues for future exploration within this field, and draw overall conclusions.

1.2.1 THE HYPOTHALAMIC PITUITARY ADRENOCORTICAL SYSTEM

For many years, researchers in the field of developmental psychopathology have highlighted relationships between psychosocial risk factors and later social and emotional problems in children. It is only recently that research has begun to elucidate the missing neurobiological links through which risk factors translate into later difficulties. At the forefront of this field, is the study of the human stress physiology. Of particular significance is the development and functioning of the stress response, and the impact that individual differences in this system may have upon psychological functioning.

Everyday life involves managing stressors of varying degrees of severity, and the body must respond to these environmental stressors with physiological adaptations to maintain homeostasis (Gunnar & Tarullo, 2006). One aspect of the human stress system is the “flight or fight” response. This is conducted by the sympathetic nervous system, and involves the body’s physiological preparation for immediate action by mobilizing energy resources, acting primarily on the cardiovascular system through the release of adrenaline and noradrenaline (Carlson, 1998). The other key component of the human stress response is the Hypothalamic Pituitary Adrenocortical system (HPA system). The HPA system is a central feature of the human neuroendocrine physiology, and has been the focus of much recent research.

Please note the term “HPA system” will be used inter -changeably throughout this review with “ the human stress physiology”, “ the human stress response” and “ the stress response system”. These will all serve as general terms in describing the neurobiological components of the Hypothalamic Pituitary Adrenocortical system

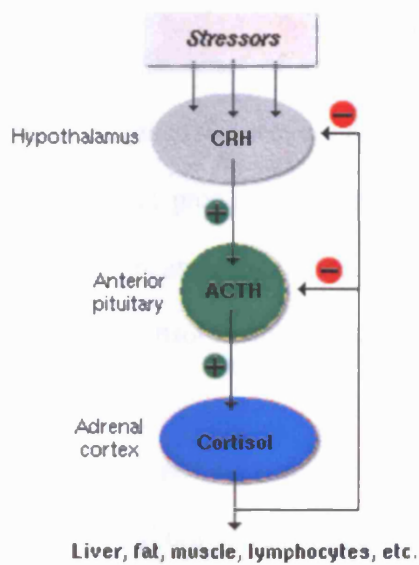
The HPA system consists of a set of responses among glands, hormones and parts of the mid-brain, which facilitate essential biological responses to physiological or psychological stress. Increased activity of this system during states of psychological or physiological challenge conducts an orchestrated set of events resulting in an automatic shift to a state of biological preparedness, aimed at increasing brain responsiveness and behavioural adaptation. This includes increases in heart rate, blood pressure, metabolizing cellular nutrients, and the re-direction of energy to the brain through increased glucose metabolism (Boyce & Ellis, 2005).

The group of hormones produced by the HPA system are known as the glucocorticoids, due to their role in converting protein to glucose for energy. Cortisol is the primary glucocorticoid responsible for the human stress response. It is synthesized and secreted in response to a chain of events beginning in the paraventricular nucleus of the hypothalamus. This produces and secretes two hormones; vasopressin (VP) and corticotrophin releasing hormone (CRH). These act on the pituitary gland, which in turn secretes adrenocorticotrophic hormone (ACTH). ACTH interacts with receptors on the cortex of the adrenal glands, to stimulate and produce the release of cortisol (Keller-Wood & Dallman, 1984).

Physical stressors (e.g. cold, stress, injury) activate the HPA system largely through neurons ascending from the brainstem. Psychological stressors activate the HPA axis through cortico-limbic pathways, including pathways from the central nucleus of the amygdala (Adam, Dougan & Gunnar, 2006). The level of cortisol in the bloodstream is regulated by a feedback mechanism; Glucocorticoids act directly on the pituitary gland to inhibit ACTH secretion, and on the hypothalamus to suppress secretion of CRH (Keller-

Wood et al, 1984). These feedback loops allow a stable level of glucocorticoids at all times, while providing an “emergency override” via the central nervous system, to respond to stressors if needed (Lupien, King, Meaney & McEwen, 2001). This feedback cycle is represented in Figure 1.

Figure 1. Cortisol synthesis and homeostasis



The activity or reactivity of the human stress response is usually established by measurement of its end product, cortisol. This is usually sampled in saliva, as a wide range of studies show high correlations between cortisol levels in blood plasma and cortisol levels in saliva (Kirschbaum & Hellhammer, 1994).

1.2.2 DEVELOPMENTAL CHANGES IN THE HPA SYSTEM

When functioning under normal basal conditions, adults cortisol levels generally follow a circadian rhythm, with highest cortisol levels approximately 30 minutes after waking, followed by a sharp decrease over the next hour or two, and then a more gradual decline over the remaining daytime and evening hours (Gunnar, Kertes & Donzella 2004; Kirschbaum & Hellhammer, 1989).

Following the onset of a stressor, cortisol levels rise rapidly, initiating the catabolic process required to meet the demands of the stressor (Baxter & Tyrell, 1987). Although basal cortisol production eventually follows a circadian rhythm, this daily decline in cortisol is not apparent at birth, but by five to six years, many children show the adult like pattern of cortisol decline throughout the day (Davis, Bruce & Gunnar, 2002).

Research has also focused upon the development of the HPA system's response to stress. Healthy newborn babies exhibit robust increases in cortisol and ACTH in response to aversive stimuli such as inoculations (Gunnar, 1992). However, over the first year of life there is a decrease in cortisol reactivity to potential stressors (although behavioural distress is still apparent) (Gunnar, Broderson, Kruegar & Rigatsu, 1996).

This decline continues into toddlerhood and the pre-school years. For example, when examining salivary cortisol responses in transition to pre-school, on average, levels do not increase when children first start school (De Haan, Gunnar, Tout, Hart & Stansbury, 1997). This period of "hyporesponding" to stress has been compared to a similar period in rats termed "the stress hyporesponsive period" (SHRP) (Gunnar et al, 2006). In rats it is difficult to elevate corticosterone (the rodent equivalent of cortisol) between 4 and 14

days after birth. It is thought the SHRP serves a protective function from the harmful effects of high levels of cortisol on the developing brain (Gunnar et al, 2006). Overall baseline cortisol levels are also lower in childhood, increasing markedly with the onset of puberty, and into adolescence (Lupien, Meaney, King & McEwen, 2006).

It seems the responsiveness of the HPA system to stress may differ in relation to age and gender, although to date, findings remain somewhat contradictory. (Kirschbaum, Kudielka, Gaab, Schommer & Hellhammer, 1999; Kudielka, Kirschbaum, Kirschbaum & Hellhammer, 2004).

1.3 CHILD MALTREATMENT AND THE DEVELOPING HPA SYSTEM

The reactivity and functioning of the HPA system is influenced by a number of complex factors. Evidence shows that early experiences, and in particular the nature, or absence of early care, plays an important role in shaping the child's developing stress response (Gunnar et al, 2006)

1.3.1 INSTITUTIONALISATION, TRAUMA AND ABUSE

The effects of chronic stress on the functioning and reactivity of the HPA system has been documented in a number of high risk and clinical populations. This includes those who have suffered maltreatment, and institutional rearing (Gunner & Donzella, 2002). It appears that infants and toddlers living in highly deprived rearing environments show marked disturbances in cortisol rhythms (Gunnar et al, 2006). Much focus has been placed upon study of Romanian orphans. Some groups show significantly lower cortisol levels than controls (Carlson & Earls, 1997), whilst others have significantly elevated cortisol (Gunnar, Morrison, Chisolm & Schuder, 2001).

Permanently placing such children in supportive adoptive families can lead to re-development of a normal pattern of cortisol regulation, although elevated levels are often apparent many years later, particularly in severe cases of prior deprivation (Gunnar et al, 2006). These findings are supported by studies of children placed in foster care following neglect, whom have been found to show significantly lower morning cortisol than comparison groups (Dozler et al, 2006). Diminished cortisol reactivity to a stressor has also been identified in children subjected to chronic maltreatment (Moss, Vanyukov, Yao and Kirillova, 1999)

When young children experience prolonged exposure to stress, they may undergo maladaptive “wear and tear” of their physiological response systems (Bugental, Martorell & Barazza, 2003). It is thought that chronically high levels of cortisol, due to consistent stress, have an enhanced negative feedback effect on the release of the hormone CRH, explaining the eventual down-regulation and lowering of cortisol in conditions of chronic adversity (Herbert et al, 2006). This is consistent with findings in the animal literature, which show low morning cortisol levels and lack of a diurnal variation in rhesus monkeys exposed to chronic stress and care giving disruptions (McCormack et al, 2003).

Overall, no uniform pattern of cortisol regulation has been identified in maltreated children, although the most severe and chronic deprivation generally relates to a blunting of HPA function (Cicchetti & Rogosch, 2001). There is a group who hyper-secrete cortisol, and a group who appear to manifest hypo-cortisolism. There may be a number of interacting factors influencing the pattern of HPA functioning, and the duration of such alterations following adverse experiences. These may include the severity, nature and

duration of abuse, its developmental timing, and the presence and timing of protective factors, such as adoption to a supportive family (Cicchetti et al, 2001). Overall, findings on children exposed to severe deprivation early in life and those exposed to severe abuse highlight that such conditions increase the risk for long term alterations in HPA functioning (Adam et al, 2006).

1.3.2 THE IMPACT OF EARLY RELATIONSHIPS ON THE DEVELOPING HPA SYSTEM

It appears that more subtle variations in the early care-giving environment may also play a part in shaping the development of the HPA system. Studies of attachment indicate that secure, emotionally responsive relationships act as a strong buffer in the HPA system's response to stress. Evidence shows a decline in cortisol reactivity following the first year and through toddlerhood (Gunnar et al, 1996).

As previously stated, this is thought to serve a protective function for brain development (Gunnar et al, 2006). However, the decline in reactivity to novel situations is dependent on the presence of a secure attachment figure (Ahnert, Gunnar, Erickson & Nachmias, 2004; Nachmias, Gunnar, Mangelsdorf, Hornik Parritz, & Buss, 1996; Van Bakel & Riksen-Walraven, 2004). In addition, toddlers with a history of insensitive, unresponsive care, reflected by a disorganised attachment style, have been found to show particularly high cortisol levels, in comparison with securely attached children in response to Ainsworth's "strange situation" assessment (Hertsgaard, Gunnar, Erickson & Nachmias, 1995).

A wealth of recent research has focused on cortisol levels of children in daycare. A number of studies have highlighted a rise in cortisol throughout the day in such settings

(Dettling, Gunnar, & Donzella 1999; Tout, de Haan, Kipp, & Gunnar, 1998). It appears this rise is related to inadequate stress regulation due to the absence of a consistently available and responsive caregiver within the daycare environment. In group settings the quality of care and responsiveness of the day care provider influences whether cortisol levels rise or fall over the day (Dettling, Parker, Lane, Sebanc & Gunnar, 2000; Gunnar, Larson, Hertsgaard, Harris & Broderson, 1992). For a review of child care literature see Gunnar and Donzella, (2002).

The importance of early relationships in functioning of the HPA system is supported by animal studies. For example, maternal behaviour among rats (extensive licking and grooming) following stressful experiences of pups, serves to decrease the levels of corticosterone released in response to repeated stress (Levine, 2001). The general finding within the animal research is that maternal factors are crucial for the regulation of young animals developing stress response systems, and their adaptive response to stress (Levine ,2001).

1.3.3 SUMMARY

It seems the presence of a sensitive and responsive caregiver is crucial in allowing infants and toddlers to regulate their physiological response to stress. Overall, research linking early relationships and maltreatment to the functioning of the HPA system provides support for the notion that early experiences, particularly the presence and nature of early care, play an integral part in shaping the human stress system, and the ability to physiologically regulate stress.

The following section moves on to explore the link between the stress physiology and cognitive functioning. It begins by describing neurobiological models which attempt to explain the relationship between stress hormones and cognition. It then provides evidence supporting these models from studies carried out with adults.

1.4 THE ROLE OF CORTISOL IN COGNITIVE AND EXECUTIVE FUNCTION

Although the short term effects of glucocorticoids are essential, chronically high or low levels of cortisol and problems in the up or down regulation of cortisol in response to stress are associated with difficulties in cognitive and executive abilities, and behavioural self regulation (Blair, Granger & Razza, 2005). This relationship is particularly pertinent when considering the emerging knowledge on the activity of cortisol at certain sites within the brain.

1.4.1 NEUROBIOLOGICAL MODELS OF CORTISOL AND COGNITION: THE HIPPOCAMPUS

The hippocampus is located within the temporal lobe of the brain. It is widely recognized as playing a crucial role in memory and learning. It has been suggested that the affect of cortisol on cognitive performance is in part related to the impact of cortisol on this neural structure (Jamieson & Dinan, 2001). Understanding how glucocorticoids influence hippocampal functioning may also help explain the impact of cortisol on less studied areas of the brain and other aspects of cognitive function.

Nerve cells (neurons) are the core component of the brain and spinal cord. They transmit information through the firing of electrical impulses (action potentials). Cortisol binds to 2 types of nerve cell receptor: Glucocorticoid receptors (GR) and Mineralocorticoid receptors (MR). MR's are found predominantly in cells within the hippocampus, and

have a higher affinity for cortisol. GR receptors have a lower affinity for cortisol, are less prevalent in the hippocampus, but are widely distributed throughout many other brain regions (McEwen, Gould, & Sakai, 1992).

Hormones, such as the glucocorticoids, affect the communication between neurons by binding to the receptor site of the cell (only neurons which possess the specific receptors for the hormone will bind with cortisol). The hormone then alters the activity of the cell, influencing its excitability, and the rate of firing of its action potential (Carlson, 1998). Stimulation of neurons in this manner can lead to long term changes in the strength and excitability of connections between cells, known as long term potentiation (LTP). It is through this process of strengthening of connections that learning is thought to occur. (Carlson, 1998).

Studies show that the ratio of MR to GR receptors occupied by cortisol impacts on cognitive function, through facilitating or inhibiting LTP. LTP is inhibited when cortisol levels are particularly low, or very high. It is best facilitated when cortisol levels are induced mildly. Therefore the relationship between cortisol and cognition follows an inverted U-shaped curve (Lupien, & McEwen, 1997).

Glucocorticoids may affect hippocampal function in other ways. Cumulative exposure to high levels glucocorticoids, for example in the face of chronic stress, facilitates the death of hippocampal neurons. It seems glucocorticoids do not directly kill hippocampal cells, but increase their vulnerability to other toxic agents, seizures and blood supply restrictions (Sapolsky, 1996). At present it is not clear whether this cell loss is permanent or whether later cell re-generation takes place (Belanoff, Gross, Yager & Schatzberg, 2001).

1.4.2 NEUROBIOLOGICAL MODELS OF CORTISOL AND COGNITION: THE PRE-FRONTAL CORTEX

Aside from the hippocampus, there are a number of other less investigated neural structures possessing GR and MR receptors. These may also be crucial in the influence of the HPA system upon cognitive performance. One such area is the mid pre-frontal cortex, including the anterior cingulate gyrus (ACC). This system is collectively known as the anterior attention system. It has been proposed that the ACC is involved in the modulation of attention and executive function (Posner & Rothbart, 1994). It is involved in controlling or directing attention and action by modulating cognitive and affective processing, and is thought to underlie the ability to effortfully control or regulate behaviour (Posner et al, 1994). While less is known about the impact of glucocorticoids on regions such as the ACC, the same general principles may hold true, that cortisol impacts on the interconnections and communication within these neural circuits, affecting their development and functioning (Gunnar, 1998).

1.4.3 SUPPORT FOR NEUROBIOLOGICAL MODELS: ADULT LITERATURE

Studies have examined the effects of introducing external (exogenous) glucocorticoids, (e.g. hydrocortisone) with primates and adults, then measuring the impact on various areas of cognitive function. This has allowed the effect of both chronic and acute changes in cortisol to be studied. A few studies have concentrated on inducing a stress response, then examining cognitive and executive changes in relation to the stress response.

The (previously described) inverted U-shaped curve relationship between cortisol and cognitive performance is apparent when collectively examining this literature. This supports neurobiological theories described above. For example, moderate increases in

cortisol have been found to positively influence learning and declarative memory (Lupien et al, 2002). While both very high and very low levels of cortisol relate to decrements in cognitive performance. When normal levels of cortisol are suppressed using drug treatment, recent and delayed declarative memory is impaired (Lupien et al, 2002).

Studies measuring the impact of both acute and chronically elevated levels of cortisol in adults have reported negative effects on a range of functions. These include deficits in verbal memory following cortisol infusion, (Newcomer, Craft, Hershey, Askins, & Bardgett, 1999), and acute stress induction using a “social stressor” (Kirschbaum, Wolf, May, Wippich & Hellhammer, 1996). A significant relationship between chronically high levels of exogenous cortisol and decreased visuo-spatial memory and executive function has also been identified (Young, Sahakian, & Robbins, 1999).

In line with the proposed impact of cortisol on pre-frontal areas, Skosnik, Chatterton, Swisher & Park, (2000) found evidence for an influence of cortisol upon executive function and self regulatory abilities. They examined the acute effects of psychological stress (using a computer game technique) on the selective attention of adults. Participants demonstrated a reduction in the ability to inhibit attention and ignore irrelevant stimuli following the stressor. This was correlated with an increase in levels of cortisol. These findings are also supported by studies of chronic cortisol activation. Lyons, Lopez, Yang and Schatzberg, (2000), found that after prolonged exposure to cortisol infusion, monkeys showed significant declines in their ability to effortfully regulate their behaviour and inhibit responses.

A number of factors may help explain variations in the effect of cortisol on cognition across studies. The dose and amount of cortisol infusions, the type of stressor, time of day, as well as the age and gender of the sample may all influence this relationship.

For a full review of cortisol and cognitive function in adults and primates see Belanoff et al, (2001); Herbert et al, (2006).

1.4.4 SUMMARY

There is now compelling evidence that the brain is a major target for glucocorticoid hormones, and that cortisol does significantly influence a range of cognitive functions, particularly when cortisol levels are very high, very low, or chronically activated. The effects of cortisol are not universal, but appear to be selective, impacting on specific areas of cognitive functioning, particularly those involved in conscious learning, attention and regulation of behaviour. This seems to coincide with the areas of the brain known to have most receptors for the glucocorticoid hormones.

The review initially highlighted the relationship between early experiences and the stress physiology. It then underlined the link between cortisol and cognition, using neurobiological theory and evidence from adult literature. The review will now focus upon the link between the stress system and executive and self regulatory capacities in early life and childhood.

1.5 CORTISOL, COGNITIVE FUNCTIONING AND CHILD DEVELOPMENT

The vast majority of research linking human stress physiology with cognitive functioning has focused upon adults. It is not yet clear how individual differences in the stress physiology impact on children's developing cognitive and executive abilities. This section begins by outlining the importance of executive and self regulatory capacities as central aspects of children's social, emotional and academic development. It will then discuss the literature relating cortisol with the development and functioning of these crucial domains of cognitive functioning in early life.

1.5.1 THE RELEVANCE OF EXECUTIVE FUNCTIONING AND SELF REGULATION IN EARLY DEVELOPMENT.

Executive function is the "higher order" cognitive system that controls and co-ordinates mental processes. It is associated with the active maintenance of information in working memory, the appropriate directing and sustaining of attention, and the ability to monitor the outcome of behaviour. It underpins the ability to think creatively and flexibly, and to engage in problem-solving (Miyake, Friedman, Emerson, Witzki & Howerter, 2000). Executive abilities are thought to underlie the effortful control of behaviour (Rothbart & Posner, 2005).

Effortful control has been defined as the ability to inhibit a dominant response in order to perform a sub-dominant response, detect errors and engage in planning. This includes the ability to suppress or control inappropriate behavioural and emotional responses (Rothbart et al, 2005). Differences in effortful control allow children to regulate their behaviour to achieve a goal, and develop more efficient strategies for coping (Rothbart et al, 2005). It is thought to play a critical role in temperament (Rothbart, Ellis, Rueda &

Posner, 2003). It increases with age, and shows trait-like stability by the fourth year of life. The maturation of executive capacities thought to underpin self regulation therefore play an integral part in child development, and the progression from infancy to childhood.

In recent years, there has been burgeoning interest in the study of executive functioning and its relationship to social and emotional development. Research now indicates a link between difficulties in self regulatory components of executive function, and social and emotional problems within childhood (Brophey, Taylor and Hughes, 2002). (For a review see Eisenberg et al, 2004; Kochanska & Knaack, 2003).

It is now apparent that early experiences, particularly those of early care-giving relationships, relate to the development of executive abilities (Mezzacappa, Kindlon & Earls, 2000). It has also emerged that the impact of social adversity upon mental health is in part mediated by executive function. In a recent longitudinal study, children's impulsivity and ability to sustain attention partially accounted for the relationship between the quality of their family environment and school readiness (as measured by language, social and cognitive development), (NICHD, 2003). This underlines the necessity of executive abilities in the processing and consolidation of information that schooling provides, the regulation of behaviour in social interactions, and progress in school and the world in general (Blair, 2002; NICHD 2003).

1.5.2 THE RELATIONSHIP BETWEEN CORTISOL AND EXECUTIVE FUNCTIONING IN EARLY LIFE

As evidenced in the previous section, it is clear that the acquisition of executive capacities is an integral aspect of child development. Infancy and childhood is a time of rapid growth and development of the neural structures thought to underpin executive function and self-regulation. It is also a time of rapid development and shaping of the human stress physiology. It is therefore important that the implications of individual differences in stress physiology for the development of these cognitive capacities are investigated in early life. The following section reviews in more detail those studies which explore the relationship between cortisol and executive function in children.

The following studies were identified using “psychinfo” and “metalib” databases. A variation of search terms around the themes of “cortisol”, “cognitive functioning” and “children” were employed. The identified literature fell into two categories, (i) cortisol and executive/cognitive functioning in childhood, and (ii) cortisol and self regulation in infancy. These areas will be addressed separately. The review will end by presenting further questions, hypotheses and providing an overall summary.

1.5.3 CORTISOL AND EXECUTIVE FUNCTION: STUDIES WITH CHILDREN

Blair, Granger and Razza, (2005) examined the relationship between cortisol reactivity and executive function in 169 four to five year olds from low socio-economic status (SES) backgrounds, attending a “Head Start” scheme. Children were seen for two 45 minute testing sessions, either in the morning or afternoon, and were given; (i) a “peg tapping” task of executive function, requiring inhibition of a natural tendency to mimic the experimenter (ii) An “item selection” task, requiring shifting of attention, identifying

similar objects along one dimension (e.g. shape) then identifying similar objects on another dimension (e.g. colour) and, (iii) A measure of receptive vocabulary and a letter identification task. In addition, teacher self-report measures of behaviour and attention were gathered.

Saliva samples were collected on one of the sessions, over three time points. Due to the normal time lag in cortisol elevations following a stressor, the saliva samples aimed to reflect cortisol levels before testing, on starting the assessment, and cortisol towards the end of the session.

Children from families with lower income to needs ratio (i.e. highest deprivation) showed highest cortisol levels at each of the three time points. The measures of executive function were combined to give one score (fluid cognitive functioning). Regression (controlling for variables such as age, gender, income to needs, and receptive vocabulary) showed that lower cortisol at the first and third time points, and higher cortisol at the second time point was associated with highest fluid cognitive functioning. This indicated that moderate elevations in cortisol when faced with the tasks, followed by efficient down regulation, best facilitated executive function. Lower cortisol at time one and higher levels at time two were also positively associated with teacher reports of self regulation.

The study underlined that greater socio-economic adversity is linked with higher overall cortisol levels in young children and highlighted a relationship between HPA function and self-regulation. In interpreting the findings it should be noted that collection of cortisol took place on only one of two testing sessions (usually the first). It's possible that children giving samples in the second session could have habituated to the procedure,

reducing their reactivity, confounding cortisol levels. In addition, a low income and predominantly white sample was used, raising issues of generalizing findings across all children.

A positive relationship between cortisol and executive attention in children was also identified by Annett, Stansbury, Kelly and Strunk (2005). They examined HPA reactivity and cognitive function in 63 five-to-12 year olds whom had asthma, and were about to take part in a trial of asthma medication. Asthma drugs are known to have a significant effect on HPA function, therefore all children were given a 28 day “wash out” of medication prior to the study. Children’s cortisol reactivity was assessed using infusion of ACTH. Subsequent cortisol production in response to the ACTH was then measured using blood samples at 30 and 60 minutes post infusion.

Four days prior to the ACTH infusion, children completed a neuropsychological battery. This included a broad assessment of memory and learning, and an attention task. The attention assessment comprised of (i) a delay task, whereby children inhibit and self pace responses to gain points, (ii) a distractibility task, in which multiple stimuli are present on a computer screen and the child is required to attend and respond when the correct stimuli appear. (iii) A vigilance task, requiring children to respond when a particular sequence of stimuli occur separately on the screen.

Cortisol reactivity was defined by plotting and joining up the two cortisol readings on a graph, then establishing the area under the curve (AUC) giving an overall measure of cortisol across the two points. Cortisol levels were positively related to both the delay aspect of the attention task, and verbal memory. Using a stepwise multiple regression,

controlling for age and gender, results showed both verbal memory and delay performance significantly predicted variance in cortisol (AUC).

These results appear to fit with the previously cited study, and much of the adult literature. However, despite the medication “washout” period, children with asthma may still have differed in HPA function as a result of long term medication use, and the effect of their condition on the immune system. Although reactivity was elicited using ACTH, its possible this doesn't have the same impact on cortisol response as exposure to an environmental stressor. Therefore, it would be important to replicate this study using non-asthma suffering children in the context of a natural stressor.

Davis, Bruce and Gunnar, (2002) studied the relationship between six-year-old children's performance on tasks of executive attention and cortisol production. 58 children participated in two neuropsychological tasks of inhibitory control, and two delay of gratification tasks. Four saliva samples were collected at 20 minute intervals throughout the hour-long testing session. Parents also collected saliva samples on two normal school days at three points across the day. Parental reports of effortful control were gathered. The two inhibitory control tests consisted of; (i) A Go, No-Go task, in which children respond to target letters on a computer, and were required to inhibit responses to non-target letters. (ii) An attentional control task, involving inhibiting or directing attention to different stimuli.

The delay of gratification tasks consisted of; (i) Asking children to choose a prize from a box (given as a reward for participating). Children were asked to keep their hands in their lap and give their response without touching or pointing. Ability to inhibit this response

was coded on a five-point scale of inhibitory control (ii) Children were told they would get another prize, and were asked to sit with their back facing the experimenter while it was wrapped. The experimenter left the room to “get some ribbon”. Children were asked not to peek. Ability to inhibit this response whilst the experimenter left the room was again coded on a scale.

Interestingly, carrying out the neuropsychological tasks did not raise children’s cortisol levels. In fact, levels decreased over the testing period (testing started at 4pm). This fits with the normal diurnal variation in cortisol, and suggests the demands of the testing situation were not sufficient to elevate levels above the normal decline. It may be that those children who were more reactive to testing simply experienced less of a decline than normal at this time of day.

Greater accuracy on the neuropsychological tasks was correlated with higher mean cortisol concentrations, (both home cortisol and during the testing session). This supports findings that relatively higher levels of cortisol, or moderate elevations (within the normal range) best facilitate cognitive function involving the anterior attention system. This also lends support to the U-shaped curve theory explaining the relationship between cortisol and cognitive function e.g. Lupien et al, (1997).

Neither the delay of gratification tasks or parental reports of effortful control were related to home or laboratory cortisol levels. This may suggest effortful control is not as closely related to the functioning of the anterior attention system and executive abilities as first thought. Alternatively, it could be that individual differences in cortisol relate in a highly subtle manner to aspects of executive attention, which are picked up by

neuropsychological measures of inhibitory control, but not recognized by the broader observational measures examining effortful control. Children within this study showed cortisol levels within the normal range. It may be that such observational measures are less sensitive in distinguishing highly subtle differences in executive abilities.

It is also possible that the delay of gratification tasks measured other abilities apart from behavioural self regulation. For example, it is likely that expressive language capacity would influence children's ability to choose a toy without pointing or touching.

Lupien, King, Meaney and McEwen, (2001) compared cortisol levels and cognitive functioning in 300 children ranging from six to 16 years old, from high and low SES backgrounds. Children were given a neuropsychological battery measuring declarative and non-declarative memory, verbal fluency and selective attention. Declarative memory was measured using free and delayed recall tests. Non-declarative memory was measured using an implicit recall task. The selective attention measure consisted of a visual detection task, in which children stated whether a target was present or absent within a display presented for 50 milliseconds.

Cortisol levels aimed to assess reactivity to the tasks and were taken at the beginning of the neuropsychological session (8am) and at the end of the session, 60 minutes later. The two samples were combined to give mean morning cortisol levels.

Deprivation was positively related to cortisol levels, but only for younger children. Low SES six to 10 year olds had significantly higher mean morning cortisol, than high SES six to 10 year olds. In this age group, high SES children performed significantly better than

low SES children on the task of selective attention. No differences were found on memory and language tasks. Cortisol levels increased with age for both high and low SES groups, with the largest change between the ages of 10 and 12, corresponding with the onset of puberty. Between the ages of 12 and 16 there was no significant difference in cortisol levels between the high and low SES groups, suggesting onset of puberty and transition to secondary education may, through some means, have an equalizing effect on children's HPA function. Between ages 10 to 16, the SES difference in selective attention was not apparent.

The higher cortisol in the low SES group (six to 10 year olds) alongside lower selective attention scores, seems, at first glance to conflict with previous studies. For example, Blair et al (2005), Annett et al (2005), Davis et al 2002, affirm that higher cortisol levels (within the normal range) correspond to higher performance on executive tasks. In addition, the adult literature highlights that a detrimental effect on cognitive performance only occurs when cortisol levels are particularly high. This would suggest that the differences in selective attention between the groups may be due to other factors related to SES, aside from cortisol, e.g. the quality of children's school environment.

It may be that a positive relationship between cortisol reactivity/regulation and executive performance would be apparent if this study had examined the sample as a whole, rather than just comparing SES groups. This relationship may also have been more apparent had the study examined individuals change in cortisol levels across time points, rather than the AUC technique, which gives an overall measure of cortisol across the collection period. This study highlights the difficulties of interpreting such correlational studies, which, of course, do not provide causal relationships.

1.5.4 SUMMARY

Overall, for children aged between four and 10, those who have higher mean cortisol and greater cortisol elevations, perform best on the tasks of executive functioning. This fits with the inverted U-shaped curve theory of cortisol and cognitive functioning (Lupien et al, 1997). Cortisol levels within these studies fall in the normal range, so a detrimental impact on cognitive function would not be expected, even for those with higher reactivity here (as has been shown with very high cortisol levels in the adult literature). Additionally, it appears that those children who show greater cortisol elevations, but are then able to efficiently down-regulate their cortisol have highest levels of executive functioning.

1.5.5 THE RELATIONSHIP BETWEEN CORTISOL AND SELF REGULATION IN PRE-SCHOOL AGE CHILDREN

The relationship between cortisol and behavioural self-regulation has been examined in the context of pre-school children's HPA functioning in day-care environments, as well as studies charting developmental changes in the HPA system. As it is more difficult to carry out neuropsychological assessments of executive function with children at day-care age, studies often use observational measures, assessing capacities thought to be underpinned by executive function and the anterior attention network. This usually comprises observational measures of effortful control.

Gunnar, Tout, de Haan, Pierce and Stansbury, (1996) looked at cortisol levels in 46 pre-school children during the initial weeks of their first school year (group formation), and several weeks later, when children were thought to have acclimatized to their new environment (familiar group period). Morning salivary cortisol was taken over a number

of days during each of the two periods. Higher median cortisol in both group formation and familiar group periods was found to correlate with lower scores of teacher rated effortful control.

Dettling, Gunnar and Donzella, (1999), examined cortisol levels of children in day-care and at home. They gathered salivary cortisol levels of 70 children aged between three and eight years, at mid morning and mid afternoon on two home days and two day-care days. Parents and teachers completed observational measures of effortful control. Eighty per cent of children showed the expected diurnal decline in cortisol when at home. However, younger children were likely to show elevations in cortisol throughout the day at day-care. As age increased, there was a decline in the percentage of children showing a rise in cortisol through the day in childcare.

Self regulation was rated as lower for those children who had higher HPA activation in the day-care environment. When controlling for age, the rise in cortisol at day-care was positively correlated with parent ratings of the impulsivity sub-scale (of effortful control) for boys, and negatively correlated with the inhibitory control sub-scale for girls. Teacher reports did not reveal any such relationships.

Dettling, Parker, Lane, Sebanc and Gunnar, (2000) assessed a sample of 58 children, from three to six years old. Children rated as lower on effortful control were most prone to elevations in cortisol levels from mid-morning to mid-afternoon in childcare.

Watamura, Donzella, Kertes and Gunnar, (2004) studied the development of temperament and HPA function in 77 children ageing from one to three years. Parents

collected children's saliva at home when children awoke, then at 10am and 4pm. When controlling for age, results showed parent reports of effortful control were negatively correlated with cortisol as measured by the AUC. This relationship was not apparent in the children above 30 months of age.

It seems age plays a role in whether a relationship between baseline cortisol levels and self regulation (as rated by behavioural measures), is identified. For example, Davis, Davis, Donzella, Krueger and Gunnar, (1999), used a sample of seven to 12 year old children, starting the first week of a new school year, measuring cortisol at three points during the day. No significant relationship between HPA activity and parental measures of effortful control were found.

Gunnar, Sebanc, Tout, Donzella and Dulmen, (2003) identified both direct and indirect pathways between HPA function and effortful control. In a sample of 82 three to five year olds, they found that children who were rejected by their peers had significantly higher cortisol levels. Teacher ratings of effortful control were related to cortisol. This was mediated by aggression and peer rejection i.e. those children with lower effortful control who were aggressive, were more likely to be rejected by peers and have higher cortisol levels. A direct link between cortisol and effortful control was also obtained, after controlling for this pathway. This study highlights the difficulties in discerning links between effortful control and HPA activity, especially when in peer group contexts. It suggests there may be a number of factors mediating this relationship, and that both direct and indirect links need to be explored.

1.5.6 SUMMARY

Children of pre-school age who show higher mean cortisol levels in relation to their peers are generally rated as showing lower behavioural self-regulation. Lower effortful control appears to become more apparent in such group settings, particularly without the availability of an adult to provide adequate emotional and behavioral regulation for the child.

1.5.7 THE HPA SYSTEM AND COGNITIVE FUNCTIONING IN EARLY LIFE: FURTHER HYPOTHESES AND AVENUES FOR FUTURE RESEARCH.

The link between cortisol and cognitive functioning in childhood is a complex picture. The following section discusses those areas requiring further exploration and clarity, and highlights avenues for further research.

(i) A significant issue within this field is that of causality. It is not known whether the link between cortisol and self-regulation in pre-school children is the result of an already present difficulty in behavioural regulation, which consequently leads such children to place themselves in situations more likely to elevate cortisol (i.e. conflicts and peer rejection). Or whether it is due to an underlying difference in cortisol regulation which impacts on the areas underpinning executive abilities, hence influencing self-regulatory capacities. One hypothesis may be that there is a bi-directional relationship between cortisol and behavioural self-regulation, with HPA function influencing self-regulation, which in turn impacts on the child's behaviour within the day-care environment, further elevating their cortisol levels and HPA functioning.

Cortisol levels are influenced by many variables such as age, gender, time of day, time since eating, and physical activity. Although these are often controlled for, it is possible there are other unknown confounding variables which relate to cortisol, but independently influence cognitive functioning. This makes it even more difficult to speculate on the cause of relationships when they occur. One example is the (previously mentioned) finding that high SES children have lower cortisol levels and higher cognitive function. The relationship between SES and cognitive function may be due to factors unrelated to cortisol, such as quality of schooling. To account for these difficulties, future investigations should incorporate the random allocation of participants to control conditions, in which cortisol levels are manipulated experimentally (by both exogenous and endogenous means).

(ii) Age appears to play an important role in the relationship between cortisol and cognitive functioning. It seems this relationship is dynamic across childhood. In pre-school children, higher mean cortisol levels are related to lower self regulation, while in older children, higher mean levels, higher reactivity, and efficient down regulation, relate to better executive abilities.

For both the pre-school and school aged children, the higher mean cortisol levels found in the research did not exceed the expected range. This would suggest that the dynamic relationship between cortisol and cognitive function is not simply due to the presence of a greater stressor (i.e. the day-care environment), or relatively higher cortisol, in the pre-school children. However, it should be noted that it is difficult to accurately define a “normal” cortisol range in early childhood, as it is a time of rapid development of the

HPA system, meaning that accurately comparing cortisol levels across this developmental period is problematic.

In providing hypotheses regarding the cause of the differing relationship between cortisol and executive function across childhood, there are a number of possible explanations; Firstly, there are notable methodological differences when comparing studies with the pre-school and school aged children. Studies with pre-school children use mean cortisol levels, often sampled over an extended time period, and in conditions of more prolonged adaptation to a social/peer environment. Studies with older children usually sample cortisol over a shorter time period, e.g. at points throughout a neuropsychological testing session. It may be that higher mean cortisol levels over a prolonged period such as adaptation to a new environment are indicative of difficulties in executive function, regardless of age. Similarly, a short-term moderate increase of HPA function in response to a time-limited challenge, may be adaptive regardless of age- In other words, it is possible that the same trend would be revealed with both groups, had these studies used comparable methodologies.

It is also possible that the dynamic relationship between cortisol and cognition may in part be related to developmental changes in the HPA system over this time period. As described previously in this review, development of the HPA system leads to progressively higher baseline levels of cortisol as children grow older (Lupien et al, 2001). Pre-schoolers and toddlers usually have lower baseline levels of cortisol, and lower cortisol reactivity (Gunnar et al, 1996). This is thought to serve as a protective factor against the possible deleterious impact of high cortisol on the developing brain. It may be that moderately higher cortisol in pre-school children (even when within the

normal range) relates to difficulties in self regulation, as the developing frontal areas of the brain (including the ACC) are less mature, and more vulnerable to the negative effects of such hormones during this period. As the HPA system develops and baseline levels become naturally higher, it fits that those areas which have receptors for corticosteroids, such as the ACC, are more able to manage relatively higher levels of cortisol (perhaps in part as there are more developed receptors and connections than in infancy). At this stage better cognitive functioning is linked to how readily the HPA system can elevate cortisol levels in response to a challenge, then down regulate these levels after the challenge has been met (Blair et al, 2005).

In pre-school children and toddlers, individual differences in self regulation and HPA become more apparent in the day-care environment. The day-care environment is a demanding situation for both behavioral and physiological self regulation. As children grow older, they are more able to regulate their behaviour in such situations, without requiring a responsive caregiver. It is therefore likely that individual differences in self regulation become more subtle as children develop. This could help explain why relationships between cortisol and specific executive abilities in older children (using neuropsychological measures) are identified, but links between cortisol and observational measures of effortful control are not (e.g. Blair et al 2005).

As yet, no studies have examined the impact of a more robust stressor on cortisol and cognitive functioning in children (previous investigations have measured cortisol elevations in response to administration of neuropsychological tasks alone). The use of a more challenging situation may elucidate individual differences in cortisol reactivity, allowing the effects of a greater range of cortisol responses to be explored.

Based on the adult literature, it would be expected that particularly high and low cortisol reactivity would relate to lower cognitive performance (in line with Lupien et al, 1997). However, this requires further investigation, as the relationship between cortisol and HPA function could vary across age groups, as is highlighted by the differences between pre-school children and older children. Furthermore, the effects of blunted or unusually high cortisol levels on executive abilities, e.g. in the case of severely deprived or institutionally reared groups is yet to be explored in children.

(iii) It is implausible to assume that self regulatory abilities are influenced by the development and functioning of the HPA system alone. Studies with adults highlight that when cortisol levels change drastically and rapidly, e.g. due to medical conditions, executive functions do not just disappear, but change in a more subtle manner. As children develop, individual differences in cortisol reactivity and regulation appear to relate to specific differences in cognitive functioning. It is therefore likely that individual differences in development of the HPA system through early life have a subtle impact in shaping certain cognitive abilities, and influencing the way in which specific areas of the brain respond to cortisol. Longitudinal studies would be required to track individual development of HPA functioning, and gauge the corresponding development of specific cognitive and executive functions. Such studies would need to control for a whole range of possible confounding variables which may also impact upon the development of cognitive functioning.

1.5.8 OVERALL CONCLUSIONS

This review aimed to investigate the link between the human stress physiology and cognitive function. In addressing this question, it focused initially on the relationship between adverse early experiences and the functioning and reactivity of the HPA system. This clearly highlighted that at the extreme end, severe deprivation is related to long term alterations in cortisol regulation. A complex picture is presented, with no clear pattern of HPA functioning in maltreated children. In early life, more subtle individual differences in reactivity to stress, relate to the responsiveness of early relationships and quality of care available. Causal relationships between maltreatment, early relationships and HPA alterations are difficult to define, and any specific mechanism within the maltreatment which leads to changes in HPA function has not been identified. At present it is also not clear for how long such changes in stress physiology may continue, or whether the HPA system recovers over time. It seems this would depend on a number of factors such as the length, duration and severity of maltreatment and presence of protective factors (e.g. Romanian orphans being adopted at an early age).

The review then focused on the link between the HPA system and cognitive functioning in adults. Neurobiological theories appeared to support evidence relating cortisol and cognition. The importance of cognitive and executive abilities in children's social and emotional development was underlined. Analysis of the child literature followed this pattern, with studies showing higher but moderate reactivity and efficient down regulation best facilitates specific cognitive and executive abilities. However, for pre-school children, higher cortisol related to lower self regulatory abilities. I hypothesized that the influence of age on the relationship between cortisol and self regulation may be

(in part) due to developmental changes in the HPA system and its impact on specific brain areas across early life.

In drawing overall conclusions, it is pertinent to consider how social experiences, neurophysiology and psychological functioning can combine to further our understanding of child development. When examining the evidence presented in this review, it is now plausible to predict that the HPA system could play a mediating role in the link between early experiences and later cognitive functioning. It may be that optimal functioning of the HPA system is indicative of resilience in children growing up in the face of other developmental risk factors. This underlines that the study of human stress physiology is an exciting and critical area of future exploration, combining psychology and neuroscience in advancing our understanding of child development.

1.5.9

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PART II

EMPIRICAL PAPER

STRESS CORTISOL

AND EXECUTIVE FUNCTION:

A STUDY OF PRE-ADOLESCENT

SCHOOL CHILDREN

2.0**ABSTRACT**

This study investigated relationships between cortisol reactivity, executive function and behavioural self-regulation in a sample of nine to 11 year old primary school children (n=77). Participants were allocated to either a stress induction or control condition. This aimed to manipulate cortisol levels, through the presence or absence of a “socially stressful task” (Trier Social Stress Test for Children). Salivary cortisol was collected to measure physiological reactivity to the two conditions. Following the stress induction or control procedure, participants completed a computer task of executive attention (Attention Network Test). This task aimed to capture any impact upon executive function of changes in cortisol. Parents completed reports of children’s self regulatory abilities.

No group differences in cortisol or executive performance were found between children in the stress or control condition. A relationship between higher cortisol and higher ratings of attention focusing was found. A link between higher cortisol and lower ratings of activation control was also identified. Both relationships were influenced by the stress task. Results supported evidence that some aspects of executive function and self regulation are best facilitated by moderately higher cortisol in the face of a challenging task.

2.1 INTRODUCTION

2.1.1 EXECUTIVE FUNCTION AND SELF REGULATION IN CHILDHOOD

The acquisition of executive function is an important marker in the transition from infancy to childhood. Executive function incorporates the active maintenance of information in working memory, the appropriate directing and sustaining of attention, the planning of actions, and monitoring the outcome of behaviour. (Miyake, Friedman, Emerson, Witzki & Howerter, 2000; Mezzacappa, 2001). Over the course of development, children are expected to gain increasing proficiency in their ability to regulate their emotions and behaviour, and adapt to increasing social demands (Mezzacappa, 2004).

These developing cognitive skills, in part, form the basis for self-regulation of behaviour, known as *effortful control* (Rothbart, Ellis, Rueda, & Posner, 2003). Effortful control incorporates execution of goal-directed behaviour, including selection among competing demands and responses, and initiating and maintaining behaviour. Differences in effortful control allow children to suppress their more reactive tendencies, take in information and plan more efficient strategies for coping (Rothbart & Posner, 2005). Effortful control is considered an aspect of temperament (Rothbart, Ellis, Rueda & Posner, 2003), increasing with age, and showing trait-like stability by the fourth year of life (Konchanska & Knaack, 2003). The development of these executive capacities supports advances in social interaction, and promotes the processing and consolidation of the information schooling provides (Blair, 2002; NICHD, 2003). The acquisition of effortful control has also been related to moral development, social competence and externalizing behaviours (Eisenberg et al, 1996).

In the development of the above self-regulatory capacities, theorists see the role of *emotionality* - that is, a dispositional style of reactivity towards an emotion-inducing situation - as a closely related factor. Theory and research on the development of cognition and emotion suggests a link between emotional reactivity and executive functioning (Blair, 2002). An important next step is to examine how individual differences in these characteristics relate to the development of higher order cognition (Blair, 2002).

One way in which emotional reactivity and regulation has been quantified and studied is through examining individual differences in stress physiology and physiological responses to stress. Studies indicate that the human stress response system may provide one link in understanding the relationship between emotional development, and cognitive functioning. Neurophysiological agents associated with stress have been found to relate to the functioning of a number of cognitive capacities (for recent reviews see Belanoff et al, 2001; Blair, Granger & Razza, 2005; Herbert et al, 2006).

Infancy and childhood are periods of rapid growth and plasticity of the neural structures thought to underpin executive abilities. The relationship between the development and functioning of the stress physiology in early life, and the development of executive and self-regulatory abilities, is therefore an area which warrants more detailed focus.

2.1.2 THE HUMAN STRESS RESPONSE

The body's mechanism for adapting to physiological and psychological challenges centers around the stress response. This includes the nervous system's "fight or flight"

reaction, allowing the body to prepare physiologically for immediate action. The other primary component of human stress response is the Hypothalamic Pituitary Adrenocortical system (HPA system). As a central feature of the neuroendocrine physiology, the HPA system consists of a set of responses among glands, hormones and parts of the mid-brain. In response to a stressor, it conducts relatively automatic increases in heart rate, blood pressure, metabolic mobilization of cellular nutrients, and re-direction of energy to the brain through increased glucose metabolism (Boyce & Ellis, 2005).

Cortisol is the primary hormone involved in the HPA system's response mechanism. It is secreted by the adrenal glands and levels in the bloodstream are regulated by a feedback mechanism (Keller-Wood & Dallman, 1984). This triggers an "emergency over-ride" via the central nervous system, responding to stressors with a rise in cortisol if needed (Lupien, King, Meaney & McEwen, 2001). Physiological measurement of stress in humans focuses on examining activity or reactivity of the stress response, by measuring cortisol levels within blood or saliva. When functioning under normal basal conditions, adult cortisol levels generally follow a pattern of diurnal decline, with highest cortisol approximately 30 minutes after waking, a sharp decrease over the next hour or two, then a more gradual decline throughout the day (Gunnar, Kertes & Donzella, 2004; Kirschbaum & Hellhammer, 1989).

Early adversity is known to influence emotional development. It is now becoming increasingly apparent that early experiences, in particular the presence and nature of early care-giving relationships, play a role in shaping the developing stress response (Gunnar & Tarullo, 2006).

2.1.3 THE ROLE OF CORTISOL IN COGNITIVE AND EXECUTIVE FUNCTION

Studies with both adults and primates indicate a link between cortisol and a range of cognitive capacities. This relationship generally follows an inverted U-shaped curve (Lupien & McEwen, 1997). Moderate increases in cortisol in response to a task or stressor have been found to positively influence learning and declarative memory (Lupien et al, 2002), while very high and very low levels of cortisol, (both at baseline and in response to a stressor or drug infusion) relate to decrements in a range of cognitive tasks (see Belanoff et al, 2001; Herbert et al, 2006).

Neurobiological models attempting to explain this relationship focus predominantly on the hippocampus. Cortisol effects the communication between hippocampal neurons, influencing their excitability, and rate of firing (Carlson., 1998). This process can lead to changes in synaptic activity between the neurons, known as long term potentiation (LTP) (Carlson ,1998). This synaptic activity has a direct influence on information processing and learning. LTP is best facilitated with moderate increases in cortisol, and is inhibited when cortisol levels are particularly low, or very high (Lupien et al, 1997). Furthermore, chronically elevated baseline levels of cortisol, such as in the face of chronic stress, may promote the death of hippocampal neurons, by increasing their vulnerability to other toxic agents, seizures and blood supply restrictions (Sapolsky, 1996).

There are a number of other less investigated neural structures possessing receptors for cortisol. One such area is the pre-frontal cortex, the structure thought to underpin executive and self-regulatory functions (Posner & Rothbart, 1994). While less is known about the impact of cortisol on the pre-frontal cortex, the same general principles may

hold true, that cortisol impacts on the interconnections and communication within these neural circuits, affecting their development and functioning (Gunnar, 1998).

2.1.4 CORTISOL AND EXECUTIVE FUNCTION IN EARLY LIFE

A small number of investigations have begun to explore the link between cortisol and the development of executive capacities in early life. This relationship appears to follow a dynamic path through early life and childhood. Studies with pre-school children, using observational measures of effortful control, show that higher baseline cortisol levels, and higher cortisol when in day-care, or during periods of adaptation to day-care, relate to lower self-regulatory abilities (Dettling, Gunnar & Donzella, 1999; Gunnar, Sebanc, Tout, Donzella & Dulmen, 2003; Gunnar, Tout, de Haan, Pierce & Stansbury, 1996; Watamura, Donzella, Kertes & Gunnar, 2004).

Consistent with the inverted U-shaped relationship found in adults, children aged five to 12 years, who have moderately higher mean cortisol levels or moderately higher mean cortisol elevations in response to the assessment session, generally show higher performance on neuropsychological tasks of executive functioning. These tasks have included switching and directing attention, and inhibition of responses (Annett, Stansbury, Kelly & Strunk 2005; Blair et al, 2005; Davis, Bruce & Gunnar, 2002). In addition, older children who are able to efficiently down-regulate their cortisol levels following a moderate elevation, exhibit most advanced performance on such tasks (Blair et al, 2005). This apparently inconsistent relationship between cortisol and executive function through childhood may in part reflect the rapid development of stress physiology and relevant brain areas across this period.

2.1.5 STRESS, CORTISOL AND EXECUTIVE FUNCTION

In everyday life, children encounter a range of challenges and stressors. One such example is in the school environment, whereby self-regulation in accordance with a range of social, academic and emotional challenges relates to school readiness and adjustment (Blair, 2002). Despite growing interest in the relationship between HPA physiology and cognitive functioning, examination of these links in the context of a *stressor* remains at an early stage.

Considering the importance of self-regulation in any potentially demanding situation, and the possibility of wide variability in children's reactivity to stress, it is apparent that individual differences in children's physiological responses to potential stressors, and the cognitive and executive implications of this, have not been fully considered. Stressors which replicate the social and academic challenges on self-regulation that children may typically face in a classroom environment have not been employed.

To date, studies with children have only examined cognitive function in relation to baseline cortisol throughout the day, or during a cognitive assessment session. One methodological difficulty within this field is the influence upon cortisol of many variables, such as age, gender and time of day. Although studies usually control for such factors, it is possible there are other undetected confounding variables relating to cortisol, independently influencing cognitive functioning.

The present study aims to further investigate the relationship between the HPA system and executive function. In an attempt to overcome some of the methodological difficulties faced by previous research, this study will incorporate an experimental

design, using random allocation of participants to a stress induction or control condition, aiming to manipulate cortisol levels via the presence or absence of the stressor. To the authors knowledge, no previous studies have used random allocation of children to a stress condition, with the aim of influencing physiological arousal then examining subsequent cognitive function.

It is hoped that this design will enable investigation of the following elements; Firstly, it will allow investigation of individual differences in cortisol responses to the stressor, and the subsequent relationship with executive functioning. Secondly, it is feasible that the stress task may lead to an overall group difference in cortisol between the conditions. This could allow examination of group changes in cortisol in relation to any overall group difference in executive function. Studies using social stressors in other areas of research with young people, have shown variable results in their efficacy to evoke an elevation in cortisol across a group of participants. Some studies have elicited a marked cortisol response (Buske-Kirschbaum et al, 1997), whilst others have found no overall group increase in cortisol after a stressor, (Gerra et al, 2000). The study will use a well validated method for evoking a stress response (The Trier Social Stress Test; Kirschbaum, Pirke, & Hellhammer, 1993). It is hoped this will provide the optimum opportunity for examining both individual differences and possible group differences in cortisol levels.

The impact of any alteration in HPA function upon subsequent executive functioning will be examined through measuring the efficiency of three separable components of executive attention (Fan et al, 2002). The sample will comprise nine to 11 year olds, as it is at this age that some components of executive attention are thought to reach stability,

(Rothbart et al, 2003). The relationship between cortisol and children's behavioural self-regulation will also be assessed, using parental reports of children's effortful control.

The study will test the following hypotheses;

(a) Children carrying out the stress condition will have higher levels of cortisol at the second time point (following the stress task) in comparison with children carrying out the control condition. This will correspond with a subsequent group difference in executive attention.

(b) Individual differences in HPA responsiveness will predict subsequent executive attention. More specifically, a moderate increase in cortisol in response to the stress task will relate to higher executive performance. Very high or very low levels of cortisol in response to the stressor will relate with decreases in executive performance.

(c) Individual differences in HPA responsiveness will predict parent reports of children's behavioural self-regulation. More specifically, a moderate increase in cortisol in response to the stressor will relate to higher reports of behavioural self-regulation. As before, very high or very low levels of cortisol in response to the stressor will relate with lower reports of behavioral self-regulation.

2.2

METHOD

Please note, some aspects of the procedure for this project were combined with a second thesis entitled "Stress, theory of mind and emotional and behavioural problems in pre-

adolescent children”, by Lucy Partridge. Data collection was carried out jointly. Please see Appendix one for further details regarding joint aspects of the project.

2.2.1 PARTICIPANTS

In total 77 year five children were recruited from primary schools in north and west London. Researchers met with each class of children to introduce the study, then provide and explain child consent forms. In the next phase, parents or guardians of children wishing to participate were sent information and consent letters (please see Appendix three for child, parent and teacher information and consent forms). After providing consent, they were sent a questionnaire pack, which took around 30 minutes to complete. Children with a statement of special educational needs were not included in the study. Table 1 summarises demographics of the sample.

2.2.2 PROCEDURE

Children were randomly assigned to either the stress induction group, or control group. Twenty seven participants took part in the control group (10 male, 17 female). Fifty were assigned to the stress induction group (23 male, 27 female). The aim of an unbalanced group design, with a higher proportion of participants in the stress condition, was to provide the maximum potential for examining the effects of altered cortisol levels, whilst at the same time maintaining a control condition for comparison of any group differences.

Power calculations indicated that in order to achieve an effect size of .4 at 80% power, the study would require 50 to 55 participants. This calculation was based on Skosnik et al (2000) who found an effect size of .47 in a study of cortisol reactivity and cognitive function with adults.

Table 1. Demographic information (n=77)

| | <u>Mean</u> | <u>Range</u> |
|-------------------------------------|-------------|--------------|
| Child age (Years) | 10.1 | 9.4-11.1 |
| Child Gender | <u>N</u> | <u>%</u> |
| Male | 33 | 42.9 |
| Female | 44 | 57.1 |
| Child Ethnicity | | |
| White UK | 19 | 24.7 |
| White other | 5 | 6.5 |
| Black or Black British Caribbean | 6 | 7.8 |
| Black or Black British African | 7 | 9.1 |
| Asian or Asian British, Indian | 1 | 1.3 |
| Asian or Asian British, Bangladeshi | 15 | 19.5 |
| Asian or Asian British, other | 3 | 3.9 |
| Mixed | 7 | 9.1 |
| Other | 2 | 2.6 |
| Missing | 12 | 15.6 |
| Family Income | | |
| £0-10,000 | 19 | 24.7 |
| £10-20,000 | 10 | 13 |
| £30-40,000 | 9 | 11.7 |
| £40-50,000 | 3 | 3.9 |
| £50-60,000 | 3 | 3.9 |
| £70,000 + | 1 | 1.3 |
| Missing | 25 | 32.5 |
| Family Composition | | |
| >1 adult in household | 44 | 57.1 |
| >1 child in household | 52 | 67.6 |
| Missing | 11 | 14.3 |

Due to the unbalanced group design, a 2:1 ratio was adopted in allocating participants to the stress and control groups. For every two children allocated to the stress procedure, one child was allocated to the control group, using a cycle of three blocks, as follows; (i) Both children carrying out the stress task, (ii) one child carrying out the stress task, and one the control task, (iii) one child carrying out the stress task and one the control task. The stress and control procedures were also counterbalanced between researchers.

Children were seen for one, hour-long session in a quiet room within their school. The two researchers worked concurrently, but in separate rooms, seeing one child at a time. Sessions began between 1pm and 1.30pm or 2 and 2.30pm. Cortisol levels are highest in the morning and decline consistently through the day (Kirschbaum & Hellhammer, 1989). Assessing cortisol levels in the afternoon aimed to allow the greatest potential for observing cortisol responsiveness to the stress induction task, as baseline levels would be lower. Two samples of saliva were obtained for cortisol level determination during the session. Following a stressor, a time delay occurs before cortisol levels reach their peak response. In children, the peak of the salivary cortisol response occurs 20 to 30 minutes after induction of a stressor (Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004).

The first saliva sample was taken upon arrival in the testing room, immediately prior to the introduction of the stress or control task. This initial sample therefore reflected cortisol in response to children's level of arousal at 20 to 30 minutes prior to this point in time. The stress or control condition lasted eight to ten minutes. The second saliva sample was

collected 20 minutes after the end of the stress or control procedure, aiming to reflect the peak of children's HPA reactivity in response to the task

After initial briefing and first saliva collection, children completed the stress or control task. Following this, the measure of executive function, and two theory of mind tasks (not reported here) were administered. This aimed to capture the impact on subsequent performance of any alterations in HPA activity. The order of administration was counterbalanced to control for order effects, and the possible effects of differential levels of stress responsiveness upon the measures used during the session. Children were asked to rate their subjective stress levels throughout the session using a visual analogue scale

2.2.3 STRESS INDUCTION TASK

Stress researchers have used a wide variety of specific stressors for controlled studies with adults and children. The most effective (in terms of mean cortisol responses) consist of tasks that have a social-evaluative component and a degree of uncontrollability. More recently, the role of anticipatory cognitive appraisal has been implicated (Gaab, Rohleder, Nater, & Ehlert, 2005). The Trier Social Stress Test (TSST) (Kirschbaum et al, 1993) was developed to induce a reliable psychological and physiological response in adults. A recent meta-analysis of 165 laboratory stress studies showed the TSST produced the most robust physiological stress responses when compared to several other stress tasks (Dickerson & Kemeny, 2002). It includes a combination of social stressors (public speaking) and a cognitive stressor (mental arithmetic). The original TSST has been modified for use with children, (TSST-C; Buske-Kirshbaum et al, 1997), and has been effective in evoking a stress response in seven to 14 year olds, (Buske-Kirshbaum et al, 1997; Buske-Kirshbaum et al, 2003; Kudielka, Buske-Kirshbaum, Hellhammer & Kirshbaum, 2004).

The TSST-C consists of a five-minute story preparation period, five-minute public speaking, and five-minute mental arithmetic task. In the speaking part, children receive the beginning of a story (please see Appendix four for full script), and are asked to finish the story in front of the experimenters, who are seated behind a desk. After five minutes of free speech, they are then asked to complete a mental arithmetic task (counting backwards in sevens from 758), out loud, in front of the experimenters.

The current study made the following adaptations to the TSST-C, (i) Two minutes story preparation, followed by three minutes of public speaking and three minutes mental arithmetic were adopted, predominantly due to time constraints of the testing session. It was also felt that arousal levels would not be altered if the procedure length was slightly reduced. (ii) An anticipatory component was added, aiming to prolong HPA arousal until the end of the session, by reminding children they may be asked to do “more maths” and would be asked “some more about their story at the end”. (iii) One experimenter conducted the procedure with each child, due to the need for both experimenters to test children concurrently in separate rooms.

2.2.4 CONTROL CONDITION

The control task followed the same structure and time frame as the stress induction task. However, it aimed to remove the social-evaluative element. During the story preparation period, children were told they would not be asked to tell anyone about their story, and that it was just “something to think about”. In place of public speaking, children were given three minutes to privately write or draw something from their story, emphasizing that it would not be shared with anyone (including the experimenter). For the mental arithmetic, children were given the same task, but told they would not have to give any answers, and could “think it

through in their head”. Throughout these periods, the experimenter did not watch the child, busying themselves with their “own work”. For standardized script used in the control task, please see Appendix four.

2.2.5 ETHICAL CONSIDERATIONS

The primary ethical issue was that of inducing stress within the participants. The TSST-C has been used in healthy volunteer samples of children in the past, and it was felt the kinds of stressors in the TSST-C are similar to those demands common in school activities (e.g. public speaking in class or assembly, mental arithmetic etc). Great care and effort was taken to ensure that children were not unduly distressed, or stressed after the procedure had finished. Participants subjective stress levels were monitored closely throughout, using a visual scale. On starting the testing session children were briefed, and it was emphasized that they could withdraw at any time, without any consequences. Children left the session with a thorough de-briefing, positive feedback and the opportunity to play a fun game before returning to class. Ethical approval was obtained via the University College London ethics committee on non-NHS human research (please see Appendix two for letter of ethical approval).

2.2.6 MEASURES

Saliva collection and cortisol assay

Children were firstly asked to rinse their mouth out with water, then spit through a polypropylene straw into a small polypropylene vial (Salicap ®). Samples were frozen and stored in a freezer at approximately -20°C until assay. Guidelines for the assay of saliva samples suggest cortisol remains stable when frozen for up to 6 months, and that mailing does not effect cortisol concentrations (IBL, Hamburg). Cortisol assay took place at

Immuno- Biological Laboratories, Hamburg, using “chemoluminescence”. This technique requires low sample volumes and has high sensitivity (0.15ng/ml).

Executive function

Executive function was measured using the Attention Network Test (ANT; Fan, McCandliss, Sommer, Raz, & Posner, 2002). The ANT was originally developed for adults, and provides a computerized assessment of the efficiency of three separable dimensions of attention; orienting, alerting and executive control (Fan et al, 2002). The ANT has been developed on extensive neuro-imaging studies which show separate neural anatomies for these three attention networks (Fan et al, 2002). Alerting attention refers to the capacity to maintain a state of preparedness for effortful processing of information. Orienting attention refers to the capacity to disengage focus of attention from one stimulus, shift, and re-engage attention onto a different stimulus. Executive control (conflict network) describes the ability to resist effectively, the interference of competing demands and inhibit a dominant response (Mezzacappa, 2004). These basic processes are fundamental across many forms of cognitive function, but among individuals and over the course of development, their efficiency and variability of functioning differs (Mezzacappa, 2004).

Efficiency of the three networks is determined by measuring how response times on a computer task are influenced by the presence of cues preceding a target; (i) Alerting cues; alerting to the imminent appearance of a target (alerting effect) (ii) Spatial cues; prompting direction of attention to the location of the forthcoming target (orienting effect) (iii) Flanker cues; providing congruent or incongruent information alongside the target, requiring resolution of conflict, and inhibition of a dominant response (conflict effect).

The ANT gives an immediate read out of separate scores for the networks, and provides measures of overall reaction time (RT) and the percentage of correct responses. Studies show the efficiency of the networks is uncorrelated (Fan et al, 2002). The ANT provides high immediate test-re-test reliability for scores on each attention network, suggesting each can be delineated and measured independently (Fan et al, 2002).

An adapted version of the ANT for children (Rueda et al, 2004) was used here. In this version, the target consists of a yellow-coloured line drawing of a fish, which appears either alone, or flanked by four identical fish. (Stimuli are shown in Figure 2). Flanking fish are either congruent or incongruent to the direction of the central fish. Children respond to whether the central fish is facing left or right by pressing the corresponding keys on the computer keyboard. Each trial begins with the presentation of a central fixation cross. This is followed by one of four warning cue conditions; (i) A center cue, intended to focus attention to the center of the screen, (ii) a double cue (alerting cue), alerting to the arrival of the impending target, (iii) a spatial cue, orienting to the position of the forthcoming fish or, (iv) no cue, acting as a control. In the center cue condition, an asterisk is presented at the location of the fixation cross. In the double cue condition, an asterisk appears above and below the fixation cross. The spatial cue involves a single asterisk presented in the position of the upcoming target. The target is presented after the warning cue condition, and appears either one degree above, or one degree below the fixation cross.

Efficiency of the alerting network is calculated by comparing the median RT for responses to trials using the alerting cue, with the median RT when no cue is present. Larger alerting scores usually arise when a group has difficulty in maintaining alertness without a cue. The orienting effect is calculated by comparing the median RT for spatial cues from the median

RT for central cues. Larger orienting times arise due to more difficulty in disengaging from the center cue. The conflict effect is calculated by comparing the median RT for congruent flanking trials with the median RT for incongruent trials. Greater differences in RT's between these trials indicate more difficulty in responding to trials requiring inhibition of a dominant response.

The ANT consists of three blocks, each of 48 trials, representing the above cue and target conditions randomly and in equal proportions. Children completed the ANT on a laptop, sitting 50 cm from the screen and were given practice trials using picture cards (Appendix five) prior to the start, illustrating the demands and circumstances to be encountered. These could be repeated at the discretion of the researcher until a child demonstrated they clearly understood the task. Participants received verbal feedback for the initial four trials. Visual and auditory feedback was given throughout by the computer (please see appendix four for standardized verbal instructions for ANT procedure). Children were given a 30 second break between each block of trials. The ANT took 17 minutes to complete.

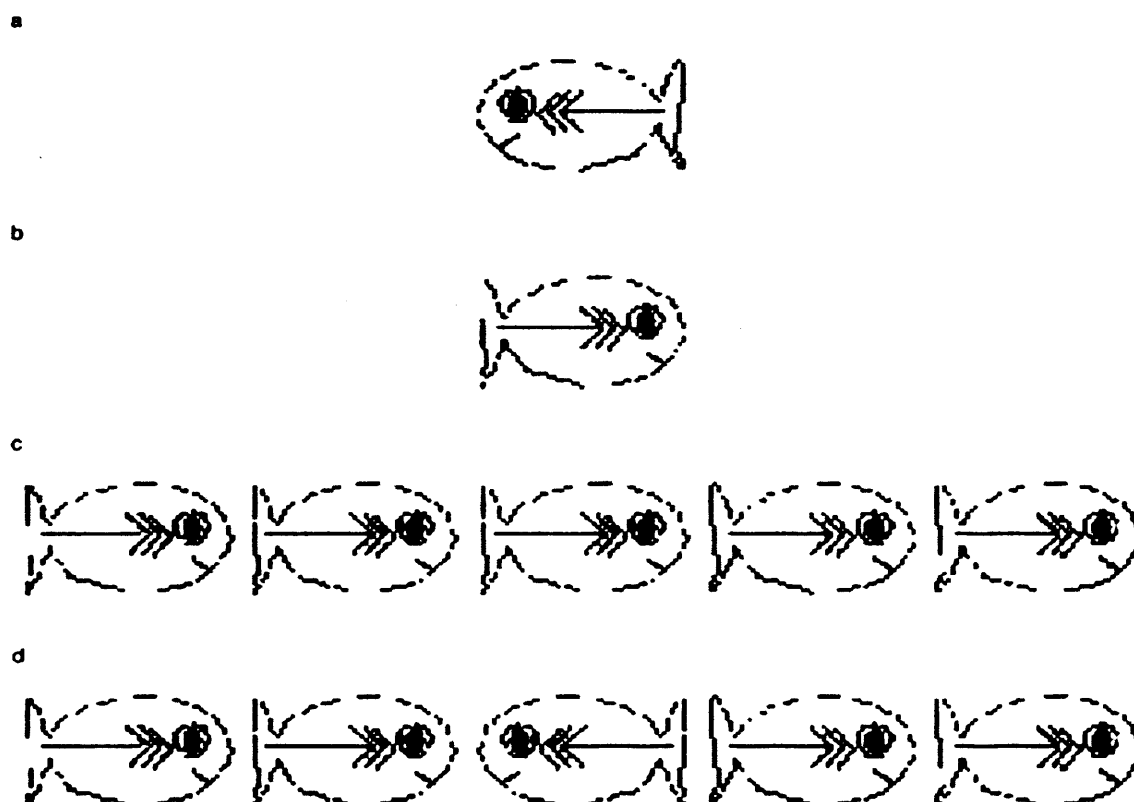


Figure 2. Example of graphics used in the attention network test, including (a) left-oriented target, (b) right oriented target, (c) congruent flankers, (d) incongruent flankers

Parent reports of children's behavioural self-regulation

Parents were asked to complete 30 items taken from the Temperament in Middle Childhood Questionnaire (TMCQ; Simonds & Rothbart, 2006). This is a caregiver report measure, assessing temperament in seven to ten year old children, and is adapted from the Child Behaviour Questionnaire. (CBQ; Rothbart, Ahadi, Hershey, & Fisher, 2001). The full TMCQ includes 157 items measuring 16 primary temperament characteristics. Differences are assessed by asking parents to rate agreement with statements on a five point scale, ranging from, "almost always true", to "almost never true". Factor analysis of the TMCQ shows four broad dimensions of temperament, of which effortful control is one.

Based on personal communication with Jennifer Simonds, June 2006, the following subscales of the TMCQ were selected as measures of children's self regulation, (as their definitions relate directly to the construct of effortful control). (i) Activation control: 15 items assessing the child's ability to perform a task when there is a tendency to avoid, e.g. "can make him/herself do homework even when wants to play" (ii) Inhibitory control: eight items assessing the capacity to plan and suppress inappropriate responses under instruction or in novel situations, e.g., "can stop him/herself when told to stop". (iii) Attention Focusing: seven items measuring the capacity to maintain attention focus when needed, e.g., "is easily distracted when listening to a story".

Scores on each scale were calculated by deriving the mean from items responded to. Please see Appendix six for questionnaire, and breakdown of items in each scale. The internal consistency reliability of the TMCQ scales is 0.81 for Activation Control, 0.80 for Inhibitory Control, and 0.86 for Attention Focusing (Simonds, 2006).

Visual Analogue scale

A ten point visual analogue scale (see Appendix eight) was used to monitor children's subjective experiences throughout, gathering ratings of stress at five time points throughout the session, (i) prior to the stress or control task, (ii) after the stress or control task, (iii) following the second saliva sampling, (iii) after the final task, and (iv) immediately before returning to class. The scale ranged from number one - "very calm and relaxed" - to number ten - "very stressed, not at all relaxed". As well as monitoring children's well-being, this would allow relationships between subjective experiences and physiological arousal to be examined.

Demographics

A brief questionnaire assessing family characteristics, income and ethnicity was included in the questionnaire pack for parents (see Appendix seven).

2.3

RESULTS

Prior to investigating the main aims of this study, preliminary analyses were conducted to check the distribution of the data, and checks were performed for the potential influence of several confounding factors. Next, the effect of the stress task upon salivary cortisol levels was tested, in order to identify whether any overall difference in HPA arousal was present between the two groups. In the following section the analysis focused on the relationship between individual differences in cortisol and executive function. The relationship between executive attentional performance and cortisol was explored using correlations with baseline cortisol. Regressions were adopted in order to predict cortisol at time two (following the stressor) from executive performance. The next stage explored the relationship between parent reports of children's behavioural self-regulation with cortisol, as before, using correlations, followed by regressions.

2.3.1 MISSING DATA

Of the 77 children taking part in the study, in total, 12 parents did not return the TMCQ. Ethnicity data was also not returned for 12 children. In addition, the income data was missing for 25 participants due to omitting of this information, or failing to return the questionnaire. The ANT data was not included for five children, as in these cases the computer program

stopped or malfunctioned at one point through the test. Cortisol samples at time one (baseline, prior to the stress or control task), and time two (post the stress or control task) were present for all participants.

2.3.2 CORTISOL ANALYSIS

Firstly, the cortisol data (measured in $\mu\text{g/dl}$) at time one and two was assessed for normality, separately within the stress and control groups. The distribution of the data was significantly skewed in both groups. Checks for outliers highlighted participant 77 (stress group) as a significant outlier, at both first ($Z=3.71$), and second cortisol samples ($Z= 6.61$). In obtaining normality, all cortisol data required square root transformation, followed by the removal of participant 77. The stress and control group were checked for significant differences in age, gender, income and ethnicity, of which none were found.

The effects of stress

Differences in salivary cortisol between the stress group and the control group were examined using a mixed design analysis of variance, with cortisol at time one versus cortisol at time two as a within-subjects factor, and group (stress group versus control group) as a between subjects factor. It was predicted that children in the stress group (and not the control group) would show an increase in cortisol at time two following the stress task. Notably, there was no significant group x time interaction, $F(1, 74) = 1.12, p = .29$, highlighting that mean cortisol change between time one and two was not significantly different between the stress versus control group. There was however a main effect of time, $F(1, 74) = 6.75, p=.01$, highlighting that cortisol levels in both the stress and control group were significantly lower at time two. Examination of the means for cortisol data in each group showed a slightly lower

(although non-significant) decline in cortisol from time one to time two within the stress group.

Confounding variables

When examining cortisol levels in relation to potential confounding factors (age, hour of starting testing, researcher, income, gender and ethnicity), there were a number of significant effects; a main effect of hour of the testing session was established, $F(1, 74) = 11.82, p = .00$, highlighting that in both groups mean cortisol levels were lower at time one and time two for children starting in the later testing session (2 to 2.30pm start). These descriptive statistics, along with cortisol at time one and time two, and calculation of cortisol change over time (between points one and two) are presented in Table 2.

Table 2. Descriptive statistics for cortisol data (not transformed)

| Mean (SD) cortisol ($\mu\text{g/dl}$) | Time 1 | Time 2 | Change |
|---|---------------|---------------|---------------|
| Stress (n=49) | .24(.13) | .22(.13) | -.01(.13) |
| Control (n=27) | .26(.11) | .21(.09) | -.04(.12) |
| 1-1.30pm start (n=48) | .27(.12) | .25(.12) | - |
| 2-2.30pm start (n=29) | .20(.11) | .16(.08) | - |

A researcher x time interaction was also established, $F(1, 72) = 4.75, p = .03$, indicating that mean cortisol at time two was higher with one researcher. A cortisol x researcher x group interaction was not found. However, the means indicated a trend towards increased cortisol from time one to time two, within the control group, with one researcher.

In addition, age (as a continuous variable) was also examined. A time x age interaction was found, $F(1, 73) = 5.70, p = .01$, with younger children showing higher cortisol than older children at time two. When group was entered into the analysis, a three-way cortisol x age x group interaction was obtained, $F(1, 73) = 6.53, p = .01$, with the younger children showing an increase in cortisol from time one to time two within the stress group, but not in the control group. This trend was not apparent in the older children, suggesting the stress task led to greater HPA activity within the younger sub-group of participants.

The income data was split into two groups using its median value. No main effect or interaction with gender or income on any cortisol data was identified. Ethnicity data was split into four groups; White, Black, Asian, and other. No main effect or interaction of ethnicity upon any cortisol data was found. Due to low numbers in each data cell, ethnicity data next required dividing into two groups; white and non-white. Further analysis showed no relationship between ethnicity and cortisol.

2.3.3 ANALYSIS OF SUBJECTIVE STRESS

Correlations were used to assess whether children's subjective ratings of stress corresponded with cortisol levels. No significant relationships were apparent between either of the cortisol samples and any of the subjective ratings. The second cortisol sample was designed to reflect the arousal experienced at the time of the second V.A.S, 20 to 30 minutes prior to the sample being collected. These however did not correlate. Additionally, no relationship between cortisol change, and change in subjective stress from first to second rating was found.

Change in children's stress ratings were assessed using repeated measures analysis of variance. Children's first and second ratings (pre and post the stress or control task) were entered as a within subject variable. A group x subjective stress interaction was revealed, F

(1, 74) = 19.89, $p = .00$, indicating that ratings of stress were significantly higher at the second V.A.S, but only in those undergoing the stress procedure. Figure 3 shows the relationship between the stress and control group and subjective ratings at points one and two. In both groups, ratings were significantly lower by the end of the procedure, $F(1, 74) = 140.25$, $p < .00$, and the difference in ratings between the groups had diminished, $F(1, 74) = 15.59$, $P < .00$. This is highlighted in Figure 4.

Figure 3. Group difference in subjective stress ratings between first and second V.A.S

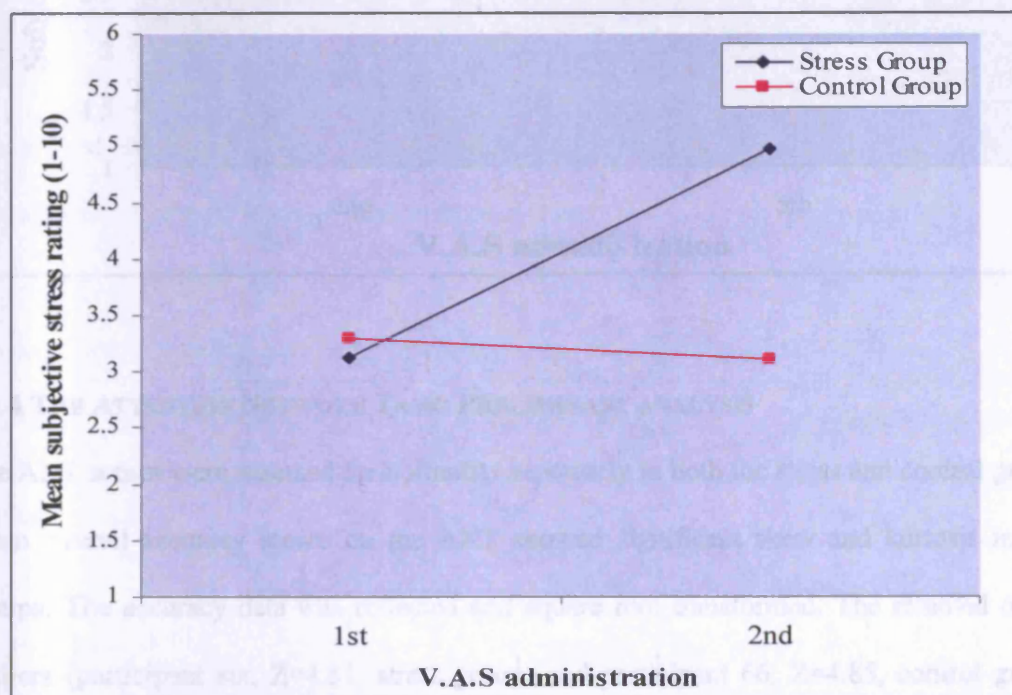
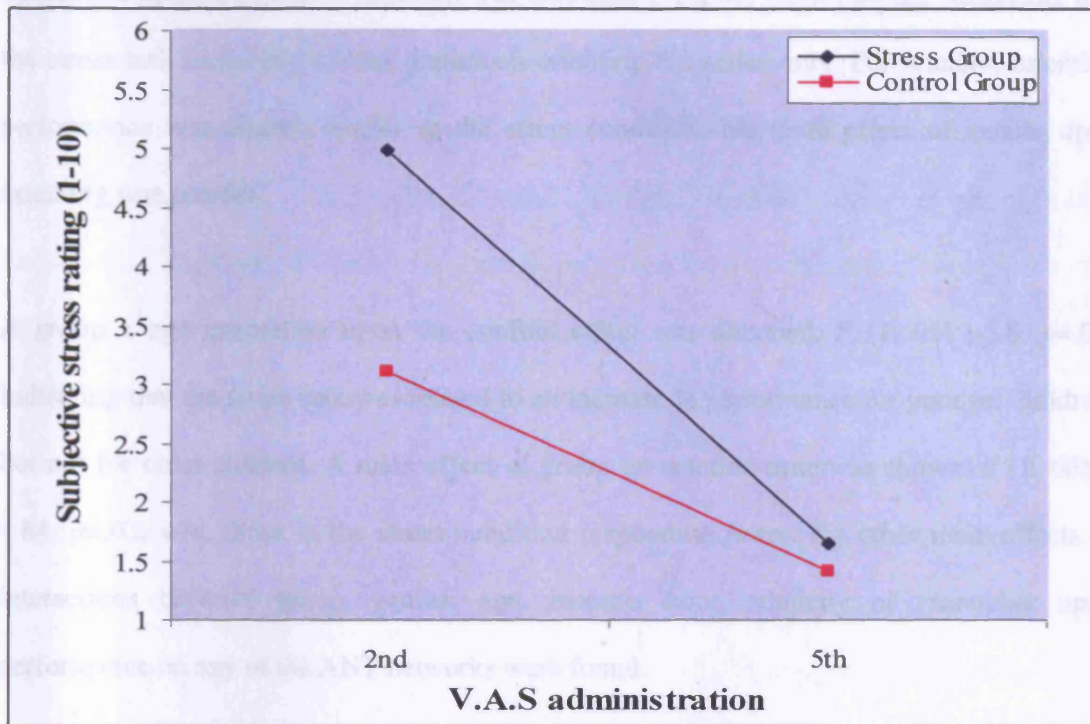


Figure 4. Group difference in subjective stress ratings between second and fifth V.A.S



2.3.4 THE ATTENTION NETWORK TASK: PRELIMINARY ANALYSIS

The ANT scores were assessed for normality separately in both the stress and control groups. Mean overall accuracy scores on the ANT showed significant skew and kurtosis in both groups. The accuracy data was reflected and square root transformed. The removal of two outliers (participant six, $Z=4.81$, stress group, and participant 66, $Z=4.85$, control group), both whom had very low accuracy scores, was also required to obtain normally distributed accuracy data. Table 3 shows descriptive data for the attention network task.

Differences in performance on each of the ANT dimensions, in relation to stress, were examined using univariate ANOVAs. Potential confounding effects of age, gender, ethnicity,

income, and hour of testing were also considered. No overall main effects from the stress group upon any of the ANT networks were established. However, a group x gender interaction upon orienting of attention was obtained, $F(1, 66) = 5.68, p = .02$, suggesting that the stress task had a detrimental impact on orienting for males only. For females, orienting performance was slightly higher in the stress condition. No main effect of gender upon orienting was present.

A group x age interaction upon the conflict effect was obtained, $F(1, 66) = 5.8, p = .01$, indicating that the stress task was related to an increase in performance for younger children, but not for older children. A main effect of group on reaction time was shown, $F(1, 66) = 4.84, p = .03$, with those in the stress condition responding faster. No other main effects or interactions between group, gender, age, income, hour, ethnicity or researcher upon performance on any of the ANT networks were found.

Table 3. Descriptive statistics for the Attention Network Task

| M (SD) ANT scores (ms) (n=68) | Stress Group | Control Group |
|--|---------------------|----------------------|
| Alerting effect | 56.2(54.2) | 40.6(43.6) |
| Orienting effect | 28.1(33.6) | 21.7(55.6) |
| Conflict effect | 91.5(39.3) | 92.4(67.6) |
| Mean overall reaction time | 678.4(91.6) | 730.5(93.8) |
| Mean overall accuracy | 97.7(1.8) | 97.0(2.6) |

2.3.5 THE RELATIONSHIP BETWEEN CORTISOL AND EXECUTIVE FUNCTIONING

In order to investigate the relationships between executive functioning and cortisol, two differing approaches were taken; (i) Cortisol at time one (baseline, prior to the intervention) was examined in relation to executive function using correlations (ii) To assess associations between executive function and the cortisol response, hierarchical regression analyses were used. Cortisol at time two (post-intervention) was the dependent variable, and executive functioning, experimental group, and cortisol at time one, were entered as predictors in the first model. Interactions between executive function and children's experimental group were added to the above predictors in the second model.

Although prior analysis had identified no overall mean difference in cortisol between the groups, it would be important to confirm whether any relationships between cortisol at time 2 and executive function occurred only in the children who had undergone the stress condition, or whether the relationship was apparent across both groups. Therefore, using interactions in the regression aimed to establish whether it was individual differences in cortisol responsiveness *to the stressor* that related with executive function. An independent effect of the interaction (model two) would indicate that the relationship between cortisol and executive performance was not present in both groups.

In the event of a significant effect being identified, it was planned that the regression would be repeated factoring in any additional relevant confounding variables (age, researcher and hour of testing).

Associations between executive functioning and baseline cortisol

No significant relationships between the ANT networks and baseline cortisol were obtained, although a trend towards lower alerting performance being linked to higher initial cortisol levels was indicated, $r(58) = .28, p = 0.19$. Table 4 shows correlations between baseline cortisol and ANT performance.

Table 4. Correlations among ANT performance and cortisol at time one.

| Attention Network (n=65) | Time 1 r |
|-------------------------------------|---------------------|
| Alerting | .28 |
| Orienting | .18 |
| Conflict | .00 |

Associations between executive functioning and cortisol response

Three hierarchical regression analyses were run in order to examine associations between ANT performance and cortisol response (one for each of the three attention networks). There were no significant effects of any ANT score on cortisol response, and no significant group x ANT interactions upon cortisol. The regressions revealed a near significant effect with higher orienting ability predicting higher cortisol at time two, ($\beta = -.21, p = .07$) Results are shown in Table 5.

Table 5. Regression equations predicting cortisol at time two from scores on separate Attention Networks (n=65).

| | B | S.E | β |
|--------------------------|------|-----|---------|
| Alerting network | | | |
| (Model 1 $R^2 = .16$) | | | |
| Cortisol time 1 | .37 | .12 | .37** |
| Group | -.01 | .02 | -.05 |
| Alerting | .00 | .00 | .09 |
| (Model 2 $R^2 = .16$) | | | |
| Cortisol time 1 | .37 | .12 | .36** |
| Group | -.00 | .04 | -.02 |
| Alerting | .00 | .00 | .17 |
| Alerting x Group | .00 | .00 | -.08 |
| Orienting network | | | |
| (Model 1 $R^2 = .19$) | | | |
| Cortisol time 1 | .44 | .11 | .43 |
| Group | -.02 | .02 | -.08*** |
| Orienting | -.00 | .00 | -.21* |
| (Model 2 $R^2 = .23$) | | | |
| Cortisol time 1 | .45 | .11 | .45 |
| Group | .00 | .03 | .02*** |
| Orienting | .00 | .00 | .41 |
| Orienting x Group | -.00 | .00 | -.66* |
| Conflict network | | | |
| (Model 1 $R^2 = .16$) | | | |
| Cortisol time 1 | .39 | .11 | .38 |
| Group | -.01 | .02 | -.06** |
| Conflict | .00 | .00 | .07 |
| (Model 2 $R^2 = .16$) | | | |
| Cortisol time 1 | .39 | .11 | .38** |
| Group | .02 | .05 | .08 |
| Conflict | .00 | .00 | .35 |
| Conflict x Group | .00 | .00 | -.03 |

Note, * $p < .1$, ** $p < .05$, *** $p < .01$

Note. Based on the preliminary data analysis, the following interactions were checked in regard to possible influences on the relationship between the ANT and cortisol: The age x group interaction upon conflict, the gender x group interaction upon orienting, and the age x group interaction upon cortisol at time two. No effects of these interactions were obtained..

2.3.6 PARENTAL REPORTS OF CHILDREN'S SELF-REGULATION: PRELIMINARY ANALYSIS

Parent reports, as measured by the TMCQ, were assessed for normality. The TMCQ showed normally distributed data on all three dimensions. Descriptive data for parental reports are shown in Table 6. Examination of differences in age, gender, income and ethnicity in relation to the TMCQ scores did not reveal any confounding variable upon dimensions of the TMCQ.

Table 6. TMCQ descriptive data

| M (SD) TMCQ scores (n=65) | |
|----------------------------------|-----------|
| Activation control | 3.37(.56) |
| Inhibitory control | 3.35(.57) |
| Attention focusing | 3.35(.79) |

2.3.7 CORTISOL AND PARENT REPORTS OF CHILDREN'S BEHAVIOURAL SELF- REGULATION

Associations between self regulation and baseline cortisol

To examine the relationship between cortisol and the parent report data, as before, correlations were conducted between baseline cortisol, this time with the dimensions of the TMCQ. Correlations are shown in Table 7. No significant relationships between the TMCQ and cortisol at time one were found.

Table 7. Correlations between TMCQ dimensions and cortisol at time 1.

| TMCQ Dimension (n=65) | Time 1 r |
|--------------------------|-------------|
| Activation control | .20 |
| Inhibitory control | .00 |
| Attention focusing | .14 |

Associations between self regulation and cortisol responses

As in the previous analysis, separate hierarchical regressions with each TMCQ dimension were applied, in order to test for associations between TMCQ scores and cortisol response (cortisol at time two). Cortisol at time one and experimental group, were also entered as covariates. As before, the interaction between experimental group and the relevant TMCQ dimension was added to the regression in model 2, to check whether any potential relationship between cortisol responses and the TMCQ occurred only within children who had undergone the stress procedure.

A group x activation control interaction upon cortisol was present ($\beta = -1.77$, $p = .02$). Within the control condition, children who showed higher cortisol levels at time two were rated as lower in activation control, $r(24) = -.48$, $p = .01$. This trend was not apparent within the stress condition $r(41) = .24$, $p = .12$. The regression was repeated, controlling for the effect of age, researcher, and hour of testing session. The interaction remained significant, ($\beta = 1.80$, $p = .01$).

Table 8. Regression equations predicting cortisol at time two from the separate dimensions of the TMCQ (n=64).

| | B | S.E | β |
|----------------------------|------|-----|---------|
| Inhibitory Control | | | |
| (Model 1 $R^2 = .19$) | | | |
| Cortisol time 1 | .46 | .12 | .44*** |
| Group | -.02 | .02 | -.08 |
| Inhibitory control | .00 | .02 | .00 |
| (Model 2 $R^2 = .22$) | | | |
| Cortisol time 1 | .46 | .12 | .44*** |
| Group | .24 | .17 | .97 |
| Inhibitory control | .10 | .07 | .49 |
| Inhibitory control x Group | -.07 | .05 | -1.20 |
| Attention Focusing | | | |
| (Model 1 $R^2 = .25$) | | | |
| Cortisol time 1 | .42 | .11 | .41*** |
| Group | -.03 | .02 | -.13 |
| Attention focusing | .04 | .01 | .25*** |
| (Model 2 $R^2 = .28$) | | | |
| Cortisol time 1 | .43 | .11 | .41*** |
| Group | .16 | .14 | .66 |
| Attention focusing | .11 | .05 | .72** |
| Attention focusing x Group | -.05 | .04 | -1.01 |
| Activation Control | | | |
| (Model 1 $R^2 = .19$) | | | |
| Cortisol time 1 | .47 | .12 | .45*** |
| Group | -.02 | .02 | -.08 |
| Activation control | -.01 | .02 | -.04 |
| (Model 2 $R^2 = .20$) | | | |
| Cortisol time 1 | .43 | .12 | .41*** |
| Group | .38 | .18 | 1.11** |
| Activation control | .14 | .07 | .67 * |
| Activation control x Group | -.11 | .05 | -1.7** |

Note, * $p < .1$, ** $p < .05$, *** $p < .01$

As before regressions were checked for the influence of age x group interactions upon cortisol

Higher attention focusing predicted higher cortisol at time two ($\beta=.25, p=.02$). The effect of the stress condition on this relationship did not reach significance ($\beta=-1.01, p=.15$). However, separate correlations showed the relationship was significant within the stress group, $r(41) = .38, p=.01$, but not the controls, $r(24) = .03, p=.87$. Correlations for cortisol change data within the stress group indicated the same trend, $r(41) = .27, p=.082$. The regression was repeated controlling for age, researcher, and hour of testing session, after which the effect of attention focusing remained ($\beta=.24, p=.03$). Regressions are shown in table 8.

2.4

DISCUSSION

This study focused on the relationship between cortisol and executive function in a sample of nine to 11 year old school children. It aimed to investigate whether individual differences in children's cortisol levels following a psychosocial stressor would predict subsequent performance on a task of executive function, and whether cortisol responses would predict parent reports of their children's behavioural self-regulation. The procedure aimed to manipulate cortisol levels through random allocation of participants to a stress induction or control procedure. Additionally, the study aimed to assess for any potential group differences in cortisol and subsequent executive function.

In re-visiting the hypotheses of this study, the stress induction task did not produce the expected group differences in cortisol or in executive performance. However, it was apparent that for a sub-group of younger children, the stress task did evoke a group difference in cortisol. In regard to individual differences in executive attention, no significant relationships were identified, although a positive relationship between cortisol and orienting of attention

neared significance. Some significant relationships between individual differences in cortisol, and the measures of self-regulation were found. The above findings will be discussed in the following sections.

2.4.1 STRESS AND CORTISOL

There were no overall group differences in cortisol between children in the stress induction group and control group. Within the stress group, it was expected that cortisol would increase from time one to time two (following the stress task). This did not occur. In fact, for both groups, cortisol levels declined at the second time point. The subjective ratings of stress showed a significant increase after the stress induction task, but not after the control task. This confirmed that the stress induction was subjectively “stressful”. There may have been a number of factors which contributed to the disparity between children’s cortisol response and their subjective experiences of stress. These possibilities will be discussed in turn.

Firstly, the finding that physiological reactivity did not correlate with changes in ratings of stress is supported by some other stress induction studies with adults and adolescents. These report a lack of relationship between self reports and cortisol following a social stressor (Garcia et al, 2005; Gold, Zakowski, Valdimarsdottir, Bovbjerg, 2003). Some studies suggest that anticipatory appraisals of the stressor, prior to the task, relate more closely to later cortisol responses than “acute self reports” during the procedure itself (Garcia et al, 2005; Gold et al, 2003). This indicates that it is the build up of anticipation before the task which best facilitates a significant stress response once the task is introduced.

The above finding may help explain why the stressor did not induce an effective physiological response within the current study. Children were aware from the outset that the

task would involve “telling a story and doing mental arithmetic out loud”. However all had been eager to participate, and the context was that of “leaving class for an exciting privilege”. Although children who had returned the required forms knew they would be participating, most did not know it was their turn until a few minutes before. It is doubtful whether prior negative anticipatory appraisals around threat or social evaluation were experienced. The stressor may therefore have been less effective without this significant period of anticipation.

The lower cortisol at the second time point in both groups can also be explained by the normal diurnal decline in cortisol (Gunnar et al 2004; Kirschbaum & Hellhammer, 1989). The impact of diurnal variation was further underlined by the lower cortisol levels in children within the later testing session. Failure to reverse the diurnal variation within the stress group highlighted greater than expected difficulties in altering this pattern.

In line with the present results, some studies have evidenced no change in cortisol following the TSST (Gerra et al, 2000; Gold et al, 2003, Jones, Rollman & Brooke, 1997), while others have produced a marked response (Buske-Kirshbaum et al, 1997; Buske-Kirshbaum et al, 2003; Kudielka, Buske-Kirshbaum, Hellhammer & Kirshbaum, 2004). The reasons for the reported inconsistencies are not readily apparent, but may relate to individual differences in the procedure, such as the degree of anticipatory stress (as discussed above), and time of day. Furthermore, the TSST-C was slightly shortened within the current study. It is possible this reduced the impact of the task. The effect of the researcher upon children’s cortisol levels also underlined how subtle differences, even when the stress procedure is scripted and standardized, may impact on cortisol levels.

2.4.2 STRESS CORTISOL AND AGE

Despite no overall impact of the stressor on cortisol, the stress task related to an increase in cortisol for younger children. This is consistent with theory explaining HPA development. Childhood is a period of rapid development of the stress physiology, with the time leading up to, and including puberty, bringing the greatest change in this system. It fits that across childhood, the relationship between stress and cortisol would be most dynamic. Child-care studies show younger children are most susceptible to elevated cortisol levels in response to the stressors and challenges posed by day-care environments (Dettling et al, 1999). Generally, older children are more able to self-regulate, both physiologically and behaviourally in such settings (Dettling et al, 1999). This is related, in part, to more developed stress physiology, coinciding with advances in emotion regulation. This helps explain why in general, older children within this study became less physiologically aroused by the potentially emotion-inducing task.

It is likely that for some of the younger children the stressor posed greater demands on physiological and emotional reactivity. It may be that older children required a situation posing greater demands on physiological and emotional regulatory capacities to evoke an equivalent stress response.

2.4.3 THE RELATIONSHIP BETWEEN CORTISOL AND EXECUTIVE FUNCTION

Results suggested that children whose HPA system facilitated moderately higher cortisol at the second time point showed most efficient orienting of attention. This relationship did not reach significance. This pattern is consistent with evidence that in children, most efficient ability to switch and direct attention relates to moderate rises in cortisol (Blair et al, 2005). The findings are also consistent with the proposed U-shaped curve relationship between

cortisol and cognitive function (Lupien et al, 1997), with moderate levels of cortisol best facilitating the neurological processes underpinning information processing (LTP). Cortisol levels in this study were within the normal range. Therefore, in line with the above theory, a deficit in cognitive function in relation to changes in cortisol would not be expected.

The relationship between orienting and cortisol was not significantly influenced by children's experimental group. Cortisol at time two varied considerably across both experimental groups, with some children experiencing HPA activation towards the control condition. This may suggest that participation itself, regardless of experimental group, acted as the stressor for some children.

It should be noted that results on the orienting network are best interpreted tentatively. Higher orienting scores indicate more difficulty in disengaging and switching attention towards a spatial cue (Fan et al, 2002). However, it is conceivable that for some children higher orienting scores could indicate higher performance. In such cases, very high levels of effort could have resulted in the spatial cue facilitating an already high level of performance, leading to larger scores.

The findings for orienting tentatively suggest the relationship between orienting and cortisol is a product of cortisol's effect upon the neural networks involving orienting attention, such as the basal forebrain and superior parietal lobe. It would be implausible to expect stress hormones to impact on all neural networks uniformly. It may be that those regions involved in other attention networks are less sensitive to any impact of cortisol. Studies with adults highlight the differential effect of stress hormones. They show that even when cortisol levels change drastically and rapidly, e.g. due to medical conditions or drug infusion, executive

functions do not disappear, but are altered subtly. This would support the finding here, that relatively small changes in cortisol relate to specific and discreet differences in attention.

Links between cortisol and the other attention networks were not found. It may be that the ANT is more sensitive to individual differences in orienting of attention, and that alerting of attention and executive control are less easily differentiated by the computer task. This may relate to the age of the sample, and different developmental stages of each attention network. Voluntary orienting of attention continues developing into adolescence (Rueda et al, 2004). This could explain why orienting is more easily delineated and influenced within the nine and ten year olds.

2.4.4 THE RELATIONSHIP BETWEEN CORTISOL AND PARENT REPORTS OF CHILDREN'S SELF-REGULATION.

Children who showed higher cortisol at the second time point were rated by parents as best able to focus attention. Closer inspection of the two groups suggested this relationship appeared predominantly within the stress condition. In line with the previously described trend (orienting and cortisol) it suggests that children whose stress systems promoted moderately higher cortisol in response to the task, are more able to self regulate in everyday situations, particularly in focusing attention.

To my knowledge, this study is the first to show a link between parent reports of self-regulation and cortisol, in older children. The relationship has been identified with infants in day-care environments (Dettling et al, 1999), although with the reverse pattern, of higher cortisol predicting lower effortful control. The change in the direction of this relationship found here may be attributable to rapid development of the HPA system through childhood.

Infants usually have low baseline levels of cortisol, and lower cortisol reactivity (Gunnar, Broderson, Kruegar & Rigatuso, 1996). This is thought to serve a protective function against the possible impact of high cortisol on the developing brain (Gunnar et al, 1996). Baseline levels increase with HPA development and age. Older children may require higher cortisol to meet the metabolic demands of a stressor, and physiologically, would be more able to manage and regulate higher cortisol, perhaps explaining why moderately higher levels are adaptive.

The relationship between attention focusing and cortisol became most apparent when children were faced with greater demands on self-regulatory capacities (in the stress task). This situation may have provided more opportunity for individual differences in HPA reactivity to manifest themselves. This could explain why relationships between self-report measures and cortisol have not been identified when examining home baseline cortisol levels (Dettling et al, 1999), or levels in older children without using a stressor, (e.g. Davis et al, 2002).

The activation control data suggested children who became most physiologically stressed in the control procedure were rated as being lower on this dimension of the TMCQ. It is difficult to draw firm conclusions regarding the meaning of this result due to the small sample size within the control group. However, the finding would suggest that the control condition brings out individual differences in susceptibility to low-level stressors (i.e. children with low stress thresholds) more clearly. Activation control incorporates the capacity to perform an action when there is a tendency to avoid it, such as carrying out a homework task in the face of competing demands. The finding indicates that being physiologically

reactive to low level stressors links with the ability to engage in behaviour when there is a tendency to avoid.

This does not necessarily suggest a causal relationship. It may be that this result reveals a link between higher reactivity (and perhaps higher anxiety), with more internalizing, avoidant behaviour. It is possible that such children are less easily identified in the more stressful condition, because other sub-groups of children also show reactivity towards the stress task. Hence in the stress group, a number of other individual differences come into effect, masking this relationship.

2.4.5 LIMITATIONS

The failure to evoke a group difference in cortisol was a restricting factor in this study. It meant that examining between group differences in physiological responses, with the aim of relating these to potential group differences in executive function, was not possible. Therefore, any causal link between cortisol and executive functioning cannot be assumed. In addition, a more effective stress induction task may have provided a greater range of stress reactivity across children. This perhaps would have provided more potential for investigating individual differences in relation to executive and self-regulatory abilities.

It is important to consider that the association between cortisol with the parent report measures and the orienting network does not indicate a direction of effect. It may be that children who are better at aspects of orienting, or who have more regulated attention at home, also tend to produce moderate elevations in cortisol in response to challenge.

It is also pertinent to underline that the tentative relationship between orienting and cortisol does not mean that children who do not show moderately higher cortisol after a challenge will be impaired in executive function. Furthermore, this association was relatively weak, and the possibility of type one error should also be considered (as with the other findings).

The relationship between cortisol and orienting may have been revealed more clearly with greater power. Power also proved a limitation when assessing effects within the smaller control group. It was hoped the larger stress group would provide greater opportunity to study the HPA system under conditions of arousal. In hindsight, a balanced group design would have provided more possibility to decipher the between- group differences that did arise. The lack of group differences in cortisol made it difficult to assume that any change in HPA activity was related to the stress or control procedure itself, rather than some other variable or individual difference in diurnal variation.

The procedure was combined with data collection from a second thesis, involving two tasks (not reported here), which together with the ANT, required counterbalancing for order of presentation. This meant the tasks were carried out at varied time points and under different levels of HPA arousal. Therefore, the opportunity to administer the ANT under the full effect of the maximum HPA arousal, examining the subsequent impact, was not always present. The added anticipatory element (e.g. telling children they may be asked to do more maths) aimed to counter this by maintaining arousal throughout the session. The effectiveness of this could not be determined, as the progression of cortisol through the hour could not be followed with further samples.

In line with the above shortcoming, the current study was limited to two cortisol samples per child. Some studies have additionally assessed cortisol “down-regulation” (the dampening down of a response after reactivity) throughout a session, and have found this provides an equally important link with executive function (Blair et al, 2005). It appears that cortisol reactivity and subsequent regulation are relatively separable dimensions, (Ramsey & Lewis, 2003). It may be that various temperament dimensions affecting regulation, such as effortful control, are more strongly related to children’s ability to down-regulate after stress, rather than reactivity (Davis et al, 2002). This could not be captured here.

It is possible that other confounds also influenced cortisol that could not be controlled for. For example, the length of time since children had lunch before testing (known to affect cortisol levels) could not be standardized, due to the demands of the school day. The sample was diverse in relation to both ethnicity and socio-economic factors, potentially allowing the findings to be applied across populations. However, the self-selected nature could have meant those children with more difficulty in effortful regulation of behaviour were less able to fulfill the required steps of speaking to parents and returning consent forms and questionnaires etc, thus de-selecting themselves from the study.

2.4.6 CLINICAL IMPLICATIONS AND FURTHER RESEARCH

Until recently, intelligence has been considered the key predictor of outcome in school. It now appears that self-regulatory skills, such as social responding, or the regulation of attention, are equally powerful predictors of school adjustment (Blair, 2002). Results of surveys suggest teachers are often most concerned with children’s regulatory readiness for school activities, rather than with the more strictly cognitive and academic aspects of readiness. Teachers report that of most importance is the capacity of each child to attend, be

responsive, and be engaged in the classroom (Blair, 2002). The study of emotional and physiological reactivity in relation to these capacities indicates that early compensatory interventions, grounded in understanding of brain-behaviour relations, should be considered. A particularly promising direction for early intervention in high risk populations may be the implementation, in pre-school and early school, of programs combining social and emotional competence with early compensatory education (Blair, 2002).

An important area, not addressed within this study, is the relationship between factors occurring in children's rearing experience, which may shape the development and reactivity of the HPA system. Further research could investigate links between children's care-giving experiences, their HPA activity and cognitive abilities, using both cross sectional and longitudinal designs.

2.4.7 CONCLUSIONS

Some findings within this study are consistent with the theory that moderately higher cortisol levels - following a challenge or stressor - are related to dimensions of self-regulation, and best facilitate aspects of executive attention. Results show examination of children's physiological responses to stress may be an important aspect of future research in child development, aiding understanding of the links between emotional and cognitive development. Further research may address fundamental questions about the interaction between physiology and early experiences in relation to psychological functioning

2.5

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PART III

CRITICAL APPRAISAL

OVERVIEW

3.0.

This critical appraisal is divided into four sections. It begins with an extended discussion considering the links between early experiences and HPA development, with particular focus on integration of attachment theory and development of the stress physiology in children. Next, ideas for further research in this field are proposed in relation to attachment, stress physiology and cognitive function. This section is followed by a reflection on the challenges and learning points throughout the research process, considering some of the views I have formed as a result of carrying out the project. The critical appraisal ends with a brief summary of the thesis as a whole.

3.1 EXTENDED DISCUSSION

3.1.1 EARLY RELATIONSHIPS AND HPA DEVELOPMENT

As discussed in part one of this thesis, early experiences have been found to relate to alterations in the development and functioning of the human stress physiology. Long-term changes of the HPA system, corresponding to both hypo and hyper-cortisolism, have been highlighted in studies documenting the effects of chronic stress and deprivation, (Gunnar, Morrison, Chisolm & Schuder, 2001), and children subjected to multiple forms of abuse (Cicchetti & Rogosch, 2001). More subtle differences in HPA reactivity are apparent when examining differences in attachment security and other aspects of early relationships. (Ahnert, Gunnar, Erickson & Nachmias, 2004; Nachmias, Gunnar, Mangelsdorf, Hornik Parritz, & Buss, 1996; Van Bakel, & Riksen- Walraven, 2004).

Much variation is apparent when examining the HPA functioning of children who have suffered abuse, trauma and other adversity. Some studies have attempted to classify particular adverse experiences, in order to relate them to specific patterns of HPA development. This has been a highly complex and, perhaps, futile task. It means imposing a classification system upon a vast range of individual experiences, while controlling for a multitude of factors known to simultaneously shape HPA development. Despite these difficulties, it seems important to identify commonalities across the spectrum of early experience, which may help us understand the mechanism through which the human stress physiology is shaped.

3.1.2 THE HPA SYSTEM - LINKS WITH ATTACHMENT THEORY

When regarding the literature in its entirety, it is apparent that the central commonality through which experiences relate to HPA development is through the presence and nature of early care-giving relationships. It seems the broad continuum of differences within such relationships is manifested in a diverse spectrum of stress physiology. For example, at one extreme, children who have suffered almost total emotional and physical neglect, show flattened or “burnt out” stress responses (Carlson & Earls, 1997). At the opposite end of the spectrum, children who experience less observable differences in their early care, (corresponding to differences in attachment style) show more discreet differences in reactivity to stressors (Ahnert et al ,2004).

Attachment theory highlights that children are biologically predetermined to seek proximity and comfort from their caregiver when their attachment system is activated under circumstances of perceived threat or stress (Bowlby 1969/1982). In other words,

human babies are born with the expectation of having stress managed for them (Gerhardt, 2004). Within a secure attachment, the caregiver is both tuned-in and responsive to the child's emotions.

Children gradually become more able to take on the capacity to regulate their emotions, behaviour and physiology, through accumulation of experiences in which this is carried out for them, within the context of the attachment relationship. If this relationship is disrupted over time, it seems likely that such children will have more difficulty in developing this capacity (Dozler et al, 2006). Early relationships which provide responsive and consistent regulation of an infant's emotions best promote the development of an HPA system that is not hyper-reactive to stress. Rather, the HPA system is able to respond adaptively when needed, and cortisol levels are more easily down-regulated. Essentially, the stress response is affected by how much early stress it has to deal with, and how well the system is helped to recover by the care-giver's responses (Gerhardt, 2004).

Bowlby (1982) emphasized the importance of internal representations of relationships (internal working models), believed to reflect attachment patterns between an individual and caregiver. These are thought to play a central part in the regulation of emotion, as fundamentally, they shape the meanings we give to our experiences. A "secure" working model, in the context of attachment theory, describes an individual's confidence that a protective figure will be accessible and available. A history of emotionally responsive interactions promotes an internal model of emotions as understandable and manageable entities, and a model of "the self" as valued and competent. In line with the child's

developing physical, social and cognitive competencies, internal representations are dynamic and become “updated” over time (Bretherton & Mullholland, 1999).

Expanding knowledge of the stress physiology, its development and links with attachment, raises the question of how internal representations may coincide with physiological change. One hypothesis would be that the two dimensions are intrinsically linked. It seems likely that the development of an HPA system that facilitates regulation of stress, would not be possible without the simultaneous formation of adaptive representations of emotions, and of “the self”. It is therefore plausible that each aspect influences the other, and that the integral part of both adaptive internal representations and stress physiology is the presence of an emotionally responsive relationship in early life.

Attachment security is now known to contribute to later psychopathology either by increasing risk, or by buffering against the effect of other risk factors (Greenberg, 1999). Given the importance of secure early relationships in the development of the stress physiology, it seems likely that part of the physiological basis underpinning the protective nature of a secure attachment lies with the inherent link to the HPA system. It seems logical that through a sensitive early care-giving relationship, the development of an HPA system able to regulate and manage emotional stress, and facilitate behavioural self-regulation, would serve as a resilience factor. This would promote developmental competence, in the face of risk. For example, a child who possesses adaptive behavioural and emotional self-regulatory skills may be protected against involvement in conduct problems in the face of environmental risks such as a deviant peer group and high level of deprivation.

3.2.1 FURTHER RESEARCH

As previously described in part one of this thesis, it is apparent that early experiences relate to the development of executive functioning (Mezzacappa, Kindlon & Earls, 2000).

In the light of the previous discussion (section 3.1.1), and in extension of the empirical paper in part two of this thesis, it now seems pertinent to investigate whether the HPA system plays a mediating role in the link between early experience and executive function. This question could be investigated using the following strategies.

Measures of early relationships, cortisol (including reactivity) and executive function could be employed. Due to the hypothesized importance of early relationships as playing the central role in the development of the stress physiology, attachment security would be measured. Assessments of attachment in infancy and childhood often comprise observation of children's doll play, or story completion, centering on attachment-related themes (Bretherton, Ridgeway & Cassidy, 1990).

It would also be useful to include other measures of the parent-child relationship, such as parenting style (e.g. Greenberger, & Goldberg 1989), which could plausibly influence HPA development independently of attachment security (e.g. in the case of authoritarian parenting). Measurement of variables known to increase risk for difficulties in cognitive function would also be collected, e.g. levels of deprivation.

It is likely that teacher ratings of children's behavioural self-regulation (within the classroom environment) give an accurate representation of self-regulation in response to challenging situations. These would also be incorporated. Ideally, measurement of

executive function could be supplemented with delay of gratification tasks, providing observable information on children's behavioural self-regulation (e.g. Davis et al, 2002)

Links between early experiences and executive function could then be investigated, assessing whether HPA function acts as a mediating variable. Three plausible hypotheses would be: (i) a combination of early experiences predict executive functioning and self regulation (e.g. attachment, deprivation etc). (ii) This relationship is mediated by the functioning of the HPA system (with highest executive function in those who have moderate increases in cortisol and efficient down-regulation following stress). (iii) Attachment security independently predicts HPA functioning and reactivity, in the presence of other variables.

In the light of the difficulties faced in the empirical paper (part two), it would be useful to gain an assessment of children's mean baseline cortisol at set time points throughout the day, either at home or school. These could be compared to samples taken during the experimental session (at the same time of day), therefore providing more information on cortisol reactivity to the procedure. Induction of a stress response could integrate lessons learned from the empirical paper reported here. A more robust anticipatory cognitive element in the form of an extended prior preparation period could be introduced. For example, children could be given the story earlier in the school day, with similar instructions (i.e. that they would be asked "later on in the day" to stand up and complete the story, and should make it as exciting as possible etc). This may elevate the chances of a robust stress response on starting the stress procedure.

3.3.1 LEARNING POINTS AND CHALLENGES

The data analysis revealed a number of insignificant findings. Where relationships were found, some were relatively weak and did not reach significance, not easily providing conclusions. Furthermore, one key aspect of the procedure (the stress task) did not have the effect we had hoped. I found myself being disappointed by this, later realizing that I had been expecting a wealth of definitive results. Through reflection on the study as a whole, and in light of the research in this area, I have learned that often, physiology-behaviour links are extremely subtle, and particularly difficult to define. It is now clear that the effects of relatively small physiological differences do not manifest themselves in widespread and easily measurable changes. Additionally, I have learned that the effects of stress hormones are only one part of a complex puzzle between social experiences and behaviour.

From a clinical psychology perspective, I now feel the most important aspect within this field is the opportunity to add to the burgeoning evidence linking social experiences with physiology, child development, and subsequent outcomes. Being able to provide evidence that early relationships shape children's neurology - arguing that this in turn significantly impacts on psychological functioning - would be a valuable contribution. Combining psychological theory and physiology in understanding integral factors in mental health provides a more concrete argument when presenting evidence to social policy makers. It provides weight to the argument that children's early relationships are vital, and factors such as high levels of deprivation and stress reduce parents' ability to meet their child's emotional needs, potentially resulting in further social problems.

I found that collecting data within primary schools in London was a particularly engaging experience. Observing the differences in ethos, structure and organization, and way in which this was reflected in the pupils, was fascinating. The recruitment phase was not always straightforward, sometimes involving much negotiation. However, after speaking with 379 children in their classes, 77 were recruited (20%). Whilst the recruitment phases were at times frustrating, and the response rate sometimes appeared low, on reflection a total of 20% is very satisfactory, and perhaps higher than we expected.

I was surprised by the openness and interest of many staff. Speaking to one (or often two) classes together, hoping to capture the attention of as many children as possible underlined how difficult it would be, as a teacher, to engage a class of 10 year olds on a daily basis. One challenging aspect of data collection was the use of the stress induction task. Carrying out the procedure with children who found it to be particularly demanding, while staying neutral and not giving encouragement or support, was not easy. It was important to use clinical skills in assessing children's well-being and giving thorough debriefs.

For me, data collection in schools underlined the importance of links between psychologists and education. Having psychologists based within, or linked closely to every school (as is sometimes, but not always, the case) would provide the optimum opportunity to build relationships with staff. Teachers themselves are in an ideal position to notice potential mental health difficulties in pupils. Therefore, strong relationships with teachers and a recognizable face, provides greater potential for discussing possible concerns. This means the possibility of early intervention is increased. The additional link with families through school, sometimes more difficult to build through CAMHS services

alone, (due to many factors such as stigma of mental health problems and services), could also greatly aid engagement.

3.4 OVERALL SUMMARY OF THE THESIS

The thesis explored the links between stress, cortisol and executive functioning. Part one reviewed literature in relation to the human stress response, its physiology and development. It examined the research indicating links between cortisol and cognitive function, in adults. It then focused upon the relationship between cortisol, executive functioning and self-regulatory abilities in infancy and childhood. The review highlighted the relationships between early experiences and the human stress physiology, and concluded by hypothesizing that the human stress response system may provide a mediating link between social experience and the development of cognitive function.

Part two presented the empirical paper, investigating the link between cortisol, executive function and self-regulatory abilities, in primary school children. Participants were randomly assigned to a stress procedure or control group. This aimed to evoke overall group differences in cortisol and subsequent executive function. No group differences in cortisol or executive function were found. Individual differences in cortisol levels measured after the stress or control task related to aspects of executive attention and self-regulation. Overall, the findings supported evidence that moderate arousal in stress physiology, as indicated by relatively higher cortisol levels in the face of a challenging situation, are related to higher self regulatory abilities and aspects of executive attention. Results support further investigation of the human stress response as providing a possible mediating link in the relationship between social adversity, and psychological functioning.

Part three discussed in more detail the link between the development and functioning of the human stress response, and early relationships. It focused on the theoretical links between attachment theory and the neurophysiology of the stress system. Ideas for further research were proposed, incorporating the investigation of attachment and parent-child factors in extension of the empirical paper reported here. The thesis concludes with a reflection on some of the challenges and major learning points encountered throughout the research process.

3.5

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APPENDICES

APPENDIX 1: JOINT CONTRIBUTIONS TO THE EMPIRICAL PAPER

Aspects of the empirical paper were carried out alongside my colleague, Lucy Partridge. Please see thesis entitled “Stress cortisol and emotional and behavioural problems in pre-adolescent children”

The following tasks were shared equally with my colleague Lucy Partridge:

Designing of the procedure, including transcripts and protocols

Constructing consent forms and information letters for participants, parents and teachers

Making initial contact with schools and teachers

Recruitment in schools i.e. Carrying out presentations to the classes, further meetings with teachers and head-teachers etc

Data collection i.e. the testing sessions with children

Producing, disseminating and collecting questionnaire packs

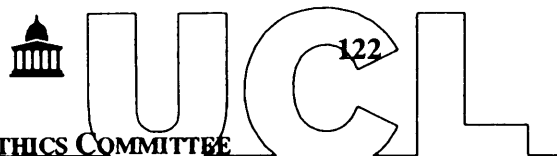
Arranging finances for the cortisol analysis

Liason with laboratory regarding cortisol analysis

Entry of shared aspects of data

Initial analysis of cortisol

Further data analysis and all other aspects of the project were carried out individually. This included the review paper (part one), critical review (part three) and all other sections of the empirical study.



APPENDIX 2: ETHICAL APPROVAL VIA UCL RESEARCH ETHICS COMMITTEE

Dr Pasco Fearon and Dr Steven Butler
Sub-Department of Clinical Health Psychology
UCL
Gower Street
London

24 January 2006

Dear Dr Fearon and Dr Butler

Re: Notification of Ethical Approval

Re: Ethics Application: Social stress: effects on theory of mind and executive function in children

Further to the meeting of the UCL Research Ethics Committee on Thursday 19 January, I am pleased to inform you that the above research has been given ethical approval for the duration of the project.

Dr Vivien Sleight, lay member of the Committee, who reviewed the application, would relish the opportunity of accompanying the researchers to one of the designated primary schools in London to act as an observer. I do hope that this will be possible and I would be grateful if you could liaise with the Secretary of the Committee, Ms Helen Dougal, to discuss this matter further on tel: 020 7679 7844.

Approval is subject to the following conditions:

1. You must seek Chair's approval for proposed amendments to the research for which this approval has been given. Ethical approval is specific to this project and must not be treated as applicable to research of a similar nature. Each research project is reviewed separately and if there are significant changes to the research protocol you should seek confirmation of continued ethical approval by completing the 'Amendment Approval Request Form'.

The form identified above can be accessed by logging on to the ethics website homepage:
<http://www.grad.ucl.ac.uk/ethics/> and clicking on the button marked 'Key Responsibilities of the Researcher Following Approval'.

2. It is your responsibility to report to the Committee any unanticipated problems or adverse events involving risks to participants or others. Both non-serious and serious adverse events must be reported.

Reporting Non-Serious Adverse Events

For non-serious adverse events you will need to inform Ms Helen Dougal, Ethics Committee Administrator (h.dougal@ucl.ac.uk), within ten days of an adverse incident occurring and provide a full written report that should include any amendments to the participant information sheet and study protocol. The Chair or Vice-Chair of the Ethics Committee will confirm that the incident is non-serious and report to the Committee at the next meeting. The final view of the Committee will be communicated to you.

Reporting Serious Adverse Events

The Ethics Committee should be notified of all serious adverse events via the Ethics Committee Administrator immediately the incident occurs. Where the adverse incident is unexpected and serious, the

APPENDIX 3: PARENT, CHILD AND TEACHER INFORMATION AND CONSENT FORMS



Sub-Department of Clinical Health Psychology
UNIVERSITY COLLEGE LONDON
GOWER STREET LONDON WC1E 6BT

INFORMATION SHEET

Dear Parents/Guardians,

Our names are James Fairbairn and Lucy Partridge. We are trainee Clinical Psychologists and are carrying out a study conducted by University College London and your child's school. We are writing to invite you and your child to take part in this study. The study is looking at factors involved in the development of behavioural and emotional difficulties in children and aims to help professionals to help children with these difficulties.

• **What is the purpose of the research?**

Many things affect children's chance of developing behavioural and emotional problems, one important factor is how children perform under pressure. We are hoping to find out more about the link between performance under pressure and children's emotional and behavioural difficulties. We are particularly interested in how children are able to think flexibly, avoid distraction and think about other people's thoughts and feelings, when are faced with a mildly challenging task (like some of those which children experience within the classroom environment). The results of the study will help us understand young people's social understanding, concentration and behaviour better, and may help us to develop strategies to prevent behavioural and emotional problems in children.

• **Why has my child been chosen?**

This school has been chosen because it takes both boys and girls and we have asked your child to be involved only because of their age, not because of anything to do with their behaviour. All children of your child's age within the school are being asked to participate. We hope to collect information from around sixty families who will have a wide range of opinions, all of which will be useful to the research.

• **What will my son / daughter have to do if they take part?**

The children taking part will be randomly allocated into two different groups. In one group the children will be asked to carry out some challenging tasks in which they may feel they are being evaluated, such as giving a short speech and doing some mental arithmetic. In the second group, the children will complete less challenging tasks and will not feel evaluated. None of the tasks in either group will be more demanding than those regularly carried out within the school environment. Children in both groups will then be asked to complete questions about characters in some stories and pictures, and carry out a 10 minute computer task, involving tapping a button in response to changing pictures and positions. Most children enjoy tasks like these. The children will also be asked to give some samples of their saliva (by spitting in a pot) to measure the way their body responds

to the tasks. These samples will be analysed to determine the amount of cortisol in them. Your child's teacher will also be asked to give some information on your child's language comprehension. The things that we would like to do would take about one hour in total, and would be carried out during the school day with the agreement of your child's teacher. Everyone taking part will be entered into a raffle, where they have the chance of winning a family trip to the cinema.

- Will I need to do anything if I agree that my child can take part?

Parents or guardians are asked to sign the attached consent form and return it to the class teacher. We will then send you some questionnaires, which should take about 30 minutes to complete and should also be returned to the class teacher. The questionnaires ask for brief background information about you, and about your child's feelings and behaviour as well as your opinions on being a parent.

- Is the research confidential?

Yes. All information collected will be used for research purposes only and an ID number used to keep answers confidential. Teachers will not see the forms the children and parents complete. Your child will also not see the answers you provide regarding their behaviour. The saliva collected from your child will be anonymous and only identifiable by a number. Once it has been analysed it will be destroyed.

- Are there any risks from taking part?

There is no reason to believe that taking part in this study would be harmful in any way. Your child will be asked to take part in a mildly stressful activity which is similar in many respects to the sorts of challenges that children face at school quite regularly (e.g. reading aloud, doing tests). However we will monitor children's feelings carefully, and if we thought they were finding the tasks unduly stressful, the session would be ceased immediately. All proposals for research with people are reviewed by an ethics committee before they can begin. This proposal has been examined by the UCL Committee on the Ethics of Non-NHS Human Research. If you do have any concerns, you are free to contact us at the address given below.

- Who should I contact if I have any questions?

Please contact James Fairbairn or Lucy Partridge if there is anything that is not clear or if you would like more information.

- Do we have to take part?

You and your child do not have to take place in this study if you do not wish to. You, or your child, may withdraw at any time without having to give a reason. Your child's decision to take part or not, will not affect their schooling or teaching in any way.

- If you do choose to take part...

Thank you very much, for your help. Please sign and return the slip at the bottom of this information sheet and the attached questionnaires.

Thank you for taking the time to read this information sheet.

Yours sincerely

Lucy Partridge and James Fairbairn
Sub-Department of Clinical Health Psychology
University College London,
Gower Street, WC1 6BT
Email: lucy.partridge@ucl.ac.uk, j.fairbairn@ucl.ac.uk

Please note: The researchers have undergone a criminal records check.

Clinical Psychology Investigation of Factors Affecting Behaviour in Children

If, once you have read the information sheet, you would like your son/daughter to be involved in the research please complete this slip. If you do decide that you would like your child to take part in the study you can always change your mind and withdraw him/her from the study without giving a reason. This will not affect your child's schooling in any way.

Please complete this slip and return it, with the completed questionnaires to your child's class teacher or to the box in school.

I have read the information sheet and agree to my child taking part in this study.

Childs Name.....

Signed

Date

Name in capital letters



Sub-Department of Clinical Health Psychology
UNIVERSITY COLLEGE LONDON
 GOWER STREET LONDON WC1E 6BT

INFORMATION SHEET FOR PUPILS

Dear Pupil,

After our talk today we wanted to give you some more information about our project to take home and think about.

- **Why is this research happening?**

The study is about how children in Year 5 understand other people's feelings and actions, and their ability to concentrate when doing a computer task. We're looking at what makes these tasks easier or harder. We are especially interested in how these sorts of tasks can be more difficult when you are under a little bit of pressure or when the task is more challenging.

- **Why have I been chosen?**

You have been chosen because you are in Year 5 and all the children in your class have been invited to take part. We need about 60 children in your school and other schools to take part.

- **What will I have to do?**

There will be two groups of children and there is an equal chance you will be group 1 or group 2. We would like all the children to tell a short story and do some mental arithmetic for a few minutes. In one group these tasks will be more difficult. We would also like you to answer some questions about characters in some stories and pictures, and to do a task on a computer. There are no right or wrong answers.

We will also ask you to spit a small amount of saliva into a little pot so that we can send it off to a laboratory. At the laboratory they will look to see how much of a hormone called 'cortisol' is in it, which tells us how your body responds to challenges. Once we have got this measurement we will not keep the samples you give us (they will be destroyed).

Everyone taking part has the chance of winning tickets for their family to go to the cinema.

- **Do I have to take part?**

It's up to you! If you decide you don't want to we will not mind. You do not have to take part if you do not want to.

- **Will information I give be kept private?**

Yes. Each person will have a number so that your name will not be written on any of the questionnaires, answer sheets or on your saliva sample. We will carefully lock everything away and password protect any information on a computer.

- **What do I do now?**

Talk to your parent/guardian and if you decide that you want to be involved you need them to complete the consent sheet. If you and your parent/ guardian would like you to take part, please return all the forms to the school office. If you have any questions please let your teacher know.

Thank you for your help!

James and Lucy



Sub-Department of Clinical Health Psychology
UNIVERSITY COLLEGE LONDON
GOWER STREET LONDON WC1E 6BT

Dear

Re: Clinical Psychology Investigation of Factors Affecting Behaviour in Children

We are Trainee Clinical Psychologists based at University College London and are currently designing a study that aims to investigate factors which may influence the occurrence of behavioural and emotional problems in children. We are writing in order to outline the research and to ask if it would be possible to conduct the research with your year 5 pupils.

The project will be investigating two specific concepts. The first is a concept known as 'theory of mind' or 'mentalisation'. This describes the ability to think about our own and other people's thoughts and feelings, and also to make sense of other people's behaviour in relation to how they may be thinking and feeling. The second concept is a type of attention (known as effortful control), which is the ability to avoid immediate inappropriate responses and avoid distraction. We are particularly interested in how children are able to think flexibly, avoid distraction and think about other people when they are faced with a mildly challenging task (like some of those which children experience within the classroom environment). The study aims to establish whether being faced by a challenging task may impact on these particular concepts and to investigate how and why this happens. The results of the study will help us understand young people's, concentration, social understanding and behaviour better, and may help us to develop strategies to prevent behavioural and emotional problems in children.

The study will provide valuable information for schools and clinical services regarding the link between performance under pressure, concentration, 'theory of mind' and behavioural and emotional problems in children.

The research will involve us writing to parents with information about the study and seeking their consent. They will be asked to complete sections of three standardised questionnaires assessing perspectives on their child's behaviour, concentration and their opinions of being a parent (which should take around 30 minutes). The children will be randomly allocated to two groups. In one group the children will be asked to carry out some challenging tasks in which they may feel they are being evaluated, such as (i) some mental arithmetic and (ii) a short speech in front of the researchers. In the second group the children will complete less challenging tasks and will not be evaluated. It is felt that all tasks will not be more demanding than those regularly carried out within the school environment.

In order to investigate children's physiological responses to the challenging situation, we will ask for two small samples of saliva during the procedure from all children (by spitting into a pot). Children in both groups will also be asked to complete a 10 minute computer task, involving responding to changing pictures and positions, as well as thinking about scenarios involving others thoughts and feelings. We will work with each child

individually, and the procedure will take a maximum of one hour with each child. Children will be de-briefed and given positive feedback to ensure the experience is both an enjoyable and valuable one.

Finally teachers would be asked to give very brief information about the child's verbal ability and key stage attainment.

We envisage collecting data in the summer and autumn terms if this were convenient with the school.

The research has been approved by the UCL Committee on the Ethics of Non-NHS Human Research and all information (aside from the consent) will be anonymous. Both of us have undergone criminal record checks and we have experience in working with children within the NHS.

We would be very happy to discuss any questions that you may have and you can contact us at the address above or via email: j.fairbairn@ucl.ac.uk, lucy.partridge@ucl.ac.uk or via telephone: James: 07976986608, Lucy: 07855323306.

We hope it will be acceptable for us to contact you shortly about your possible involvement. We would be more than happy to meet with you to discuss any questions that you have before deciding whether or not you would like to take part.

Thank you for your time.

Yours sincerely,

James Fairbairn and Lucy Partridge

Trainee Clinical Psychologists

APPENDIX 4: TRANSCRIPT OF THE PROCEDURE (VERBAL INSTRUCTIONS)

Prior to testing, class teachers will be given a list of the children we will be testing on each day, so they can fore-warn the child in the morning before their testing session. Check children have had an early lunch. First child starting at 1pm.

In class room, both children (stress/non stress) will be picked up by the experimenter carrying out the “non-stressed” condition

Hello are you ready? We’re going to to do the games and activities for our study, you’ll be back in about an hour. Before we go, can you have a sip of water.

First room should contain; Blank and lined paper, pens and pencils, lap-top, 2 chairs, desk and tasks. Once in first room, experimenter who is carrying out the “non- stressed” condition should say to both children.

Thanks for returning all the forms. Today we wanted to do some games and activities with you, as part of the study which we spoke to you and your class about. As part of our study I’ll ask you to spit some saliva into a little pot so that we can measure something in it called cortisol, which changes depending on how you are feeling.

We are seeing lots of children in your class and some from other schools the same age as you. Some of the tasks will be easy, but some you might find harder. Do you still want to carry on? Just to let you know, if you do want to stop at any time, then that is fine, just tell me, I won’t mind at all, and you can stop. Also, if you have any questions, I can answer them all at the end. Is that Ok?

☺ First saliva sample collection, carried out by both experimenters

To start off with, I’d like you to spit some saliva into this pot, using this straw, try and make the pot half full. Thank you.

First visual scale by both experimenters (take children to areas where can’t see each others choice on scale)

This is a scale representing how you are feeling. If this is feeling very happy (point to picture 5), this is ok (point to picture 3) and this is very worried or stressed (point to picture 1), can you point to the scale to show me how you are feeling now?

Thank you for doing that, we’re now going to split up and James/Lucy is going to take X to theroom and we’re going to stay here. You’ll see each other back in class in about an hour.

Stress condition

Throughout stress test, give adequate positive feedback, either facial or verbal.

Ok, firstly I’m going to tell you the beginning of a story, listen carefully because I’m going to ask you to make up the ending. I’m going to ask you to stand up and tell me out loud your ending to the story. You need to listen carefully as I want you to make your story really good.

Yesterday my best friend Robert and I went home from school, suddenly we had the idea to visit Mr Gregg who lived in the big old house located in the dark forest near our town. Mr Gregg was a crazy old man and our parents didn't like the idea that we sometimes went visiting him. There was a rumour in town that there was a mystery about the old house. When we arrived at the house, we were surprised that the door was open. Suddenly we heard a strange noise, and cautiously we entered the dark hall.....

What do you think happens next? I want you to make it up. I'm going to give you 2 minutes to prepare your story. Then I will ask you to stand up and tell me your story. Try and make it as exciting as possible, see if you can make it better than all of the other children's.

Experimenter sits behind the desk and holds clip board, watching child prepare story for 2 minutes.

If child asks you to repeat the story say

Try and remember the best you can. it doesn't matter if you can't remember all of the story I read you, but I want you to make up what may happen next

If child tries to make conversation with the experimenter (unless relating to distress/not wanting to continue), say....

It's important for the study that we don't talk through this bit, so please try to get on with the task quietly.

After 2 minutes of preparation

The 2 minutes is up, can you stand up and tell me the rest of your story now. I would like you to keep telling the story for 3 minutes.

If the child finishes before 3 minutes, use following prompts, until child is not able to continue.

Can you tell me a bit more about that? And what else might happen?

Thank you for telling me your story. Don't forget about your story, as I am going to ask you some more about it at the end.

Mental arithmetic task

Now we are going to do some mental arithmetic. You're going to do this for 3 minutes. I would like you to subtract/take away 7 from 758, then continue take away 7 from each answer. For example 758 take away 7 is 751, then you would take away 7 from 751 and so on.... Try to do it as fast as you can and as accurately as possible. Off you go...

(if failing at any point ask to stop and start again at 758)

Continue for 3 minutes after starting.

If child stops or finds very difficult...

Just keep going and do your best

Answers: 758, 751, 744, 737, 730, 723, 716, 709, 702, 695, 688, 681, 674, 667.

Thankyou

☺ Second visual scale

Have another look at this scale, can you show me how you are feeling at the moment using the scale again.

Tasks

→ AFTER THE 1ST TASK (OR 2ND IF DOING EYES AS ONE OF FIRST TWO: NOTE TIME) IS COMPLETE TAKE SECOND SALIVA SAMPLE AND NOTE EXACT TIME DONE

Stories

Nb counterbalancing – read either physical or ToM stories first.

Read each story to child, record answers verbatim.

We're going to look at some stories now. On each page you will find a short story to read. After you've read and understood the story, there will be a question. I'd like you to tell me the answer to this question. I'll read through the story and question with you.

Show practice question and ask question

Ok, lets carry on

As I said before, don't forget your story from the first task as I will be asking more about it at the end. I might also ask you to do some more maths at the end too.

→ Second saliva sample?

Eyes

Show practice item

In this folder I've got lots of pictures of people's eyes. Each picture has four words round it. I want you to look carefully at the picture and then chose the word that best describes what the person in the picture is thinking or feeling. Let's have a go with this one.

Point to words as they are read. Make sure child picks one of the options and given encouraging feedback without revealing whether they are right are wrong.

Now look at this person. Do you think he is feeling jealous, scared, relaxed or hate?

Ok, let's have a go at the rest of them. You might find some of them quite easy and some of them quite hard, so don't worry if it's not always easy to choose the best word. I'll read all the words for you so you don't need to worry about that. If you really can't choose the best word, you can have a guess.

Proceed with the test items in exactly the same way as the practice item.

(Prompt)

If taking a long time prompt with: *Which word do you think fits best?*

As I said before, don't forget your story as I will be asking more about it at the end. I might also ask you to do some more maths at the end too.

→ Second saliva sample?

Attention Network Task

Task takes approximately 17 minutes.

As I said before, don't forget your story as I will be asking more about it at the end. I might also ask you to do some more maths at the end too.

Make sure child is sitting 50cm's from computer screen

Now we're going to play a game on the computer where your job will be to feed a hungry fish. The way that you feed a fish when it appears on the screen is by pressing the cursor button on the keyboard that matches the way the fishes mouth is pointing. -

Show cue cards of single fish

If you were going to feed this fish, which arrow would you press?

When you are sure they understand say

*Sometimes the hungry fish will be alone and sometimes it will be swimming with some other fish as well. When you see more fish your job is to feed **only** the fish in the middle. So what matters is where the middle fish's mouth is pointing*

Then show them cue cards of the stimuli with flankers (first congruent, then incongruent)

If you were going to feed the fish in this picture, which arrow would you press?

If child doesn't understand explain again, pointing out the middle fish, and its direction

Try and feed it as fast as you can each time without making mistakes. You can use one hand or two, it's up to you.

Try and keep focused on the cross at the centre of the screen and remember you have to feed the fish as fast as you can when it appears, trying not to make any mistakes.

Give visual supervision for initial four trials to make sure they have understood. Give encouragement when getting correct answer at start .e.g. remind to focus on cross and feed fish in middle.

Ask if child would like a rest after each block. Give max of 30 seconds between each block. Give encouragement after each block.

→ Second saliva sample?

→ Second saliva sample

Approx 15 minutes after stress test. **WRITE DOWN EXACT TIME.**

I would like you to spit some more saliva into this pot.

☺ Third visual scale: Immediately after second saliva sample
Can you please tell me how you are feeling at the moment using the scale again.

End of testing

☺ Fourth visual scale: Immediately after last task.
Can you please tell me how you are feeling at the moment using the scale again.

Can you remember the story you made up at the beginning? Is there anything else you would like to add to it?

I'm not going to ask you any more questions about it and I don't need to ask you any more maths questions.

You've done all the tasks that I wanted you to do and you've done really well. I know some of them were quite hard, but you did really well.

Give child one example of something they did well.

Shall we do something fun before you go back to class? Lets play a game of hangman!

(Don't forget to turn over to last page!)

☺ Final visual scale
Can you tell me how you are feeling again, using the scale?

Thank you for all your hard work today, I am very impressed. When we have finished seeing everyone in your class, we will come back and tell your whole class more about why we have asked you to do these games and activities today. You will be able to ask any questions then, but I was wondering if you had any questions you would like to ask me now?

Great, let's go back to class.

Control condition

I'm going to tell you the beginning of a story, listen carefully because I'd like you to make up an ending to the story. I'm not going to ask you tell me your story, but I'd just like you to have a think about it.

Yesterday my best friend Robert and I went home from school, suddenly we had the idea to visit Mr Gregg who lived in the big old house located in the dark forest near our town. Mr Gregg was a crazy old man and our parents didn't like the idea that we sometimes went visiting him. There was a rumour in town that there was a mystery about the old house. When we arrived at the house, we were surprised that the door was open. Suddenly we heard a strange noise, and cautiously we entered the dark hall.....

What do you think happens next? I'm going to give you for 2 minutes to prepare your ending to the story. It can be as exciting as you like. The story is for you to keep to yourself, you won't have to tell it to anyone. I'd just like you to think about it. Whilst you're doing that, I'll sit here and do some reading.

Experimenter stays in room, but does not pay attention to child, gets on with "own work".

If child asks what the point is say

It's just a thinking task/ a task for you to do some thinking

If child asks you to repeat story say

Try and remember the best you can. it doesn't matter if you can't remember all of the story I read you, but think to yourself about what may happen next. Remember, you won't have to tell anyone.

If child tries to start conversation (unless related to distress/ wanting to stop), say.....

Try and keep going through this bit without talking, as it is important for the study. Think of other things to add into your story. Maybe you could think of other people who could be in the story.

After 2 minutes

That's 2 minutes up. Like I said, you don't need to tell me your story, but I'd like you to either write some of your story down or draw a picture of something in your story. I'm not going to ask to see your writing or drawing, the exercise is just for you to do some thinking.

Give 3 minutes.

If child finishes before 3 minutes encourage them to continue.

Can you think of anything else you would want to write/draw about the story?

Mental arithmetic task

Now I would like you to do some arithmetic. It's just going to be in your head – you don't need to tell me the answers. You're going to do this for 3 minutes. I would like you to take away 7 from 758, then continue taking away 7 from each answer. For example 758 take away is 751, then you would take away 7 from 751 and so on.... Off you go...

(time 3 minutes)

How did you find that? It's quite difficult isn't it.

☺ Second visual scale

Have another look at this scale, can you show me how you are feeling at the moment using the scale again.

Great, are you feeling ok to carry on?

Tasks

Tasks were administered the same as in the stress condition.

No reminders about further story or maths tasks were given between tasks as in the stress condition.

→ Second saliva sample

Approx 15 minutes after stress test. WRITE DOWN EXACT TIME.

I would like you to spit some more saliva into this pot.

☺ Third visual scale: Immediately after second saliva sample

Can you please tell me how you are feeling at the moment using the scale again.

End of testing

☺ Fourth visual scale: Immediately after last task.

Can you please tell me how you are feeling at the moment using the scale again.

You've done all the tasks that I wanted you to do and you've done really well. I know some of them were quite hard, but you did really well.

Give child one example of something they did well.

Shall we do something fun before you go back to class? Lets play a game of hangman!

☺ Final visual scale

Can you tell me how you are feeling again, using the scale?

Thank you for all your hard work today, I am very impressed. When we have finished seeing everyone in your class, we will come back and tell your whole class more about why we have asked you to do these games and activities today. You will be able to ask any questions then, but I was wondering if you had any questions you would like to ask me now?

Great, let's go back to class.

APPENDIX 5 : CUE CARDS FOR ATTENTION NETWORK TEST PRACTICE TRIALS

Please note, when used in the study, each picture was presented separately

1.



2.



3.



| No. Trials | 1 | 2 | 3 | 4 | 5 | Time (sec) |
|------------------------------------|---|---|---|---|---|------------|
| 1. Look at the fish with the arrow | | | | | | NA |
| 2. Look at the fish with the arrow | | | | | | NA |
| 3. Look at the fish with the arrow | | | | | | NA |

APPENDIX SIX: PARENT REPORT OF CHILDREN’S SELF REGULATION, (FROM TMCQ)

Temperament in Middle Childhood Questionnaire (Rothbart and Simonds 1994)

Child’s name.....

Id number..... (For researcher use only)

Instructions: Please read carefully before starting

On the page you will see a set of statements that describe children’s reactions to a number of situations. We would like you to tell us what your child’s reaction is likely to be in those situations. There are of course no “correct” ways of reacting; children differ widely in their reactions, and it is these differences we are trying to learn about. Please read each statement and decide whether it is a “true” or “untrue” description of your child’s reaction within the past six months. Use the following scale to indicate how well a statement describes your child.

- Circle If the statement is:
- 1 Almost always untrue of your child
 - 2 Usually untrue of your child
 - 3 Sometimes true, sometimes untrue of your child
 - 4 Usually true of your child
 - 5 Almost always true of your child

If you cannot answer one of the items because you have never seen your child in that situation, for example, if the statement is about the child playing wildly and recklessly, and you have never seen your child play that way, then circle NA (not applicable).

Please be sure to respond by circling a number or NA for every item. If you find an item objectionable or upsetting, you may make an exception to this instruction and skip the item.

| My Child..... | Almost always untrue | Usually untrue | Sometimes true sometimes untrue | Usually true | Almost always true | Does not apply |
|--|----------------------------|-------------------|---------------------------------------|-----------------|--------------------------|----------------------|
| 1.Can stop him/herself when told to stop | 1 | 2 | 3 | 4 | 5 | NA |
| 2.Is easily distracted when listening to a story | 1 | 2 | 3 | 4 | 5 | NA |
| 3.Looks around the room when doing homework | 1 | 2 | 3 | 4 | 5 | NA |
| 4.Can make him/herself do homework, even when s/he wants to play | 1 | 2 | 3 | 4 | 5 | NA |

| 5. Can say hello to a new child in class, even when feeling shy | 1 | 2 | 3 | 4 | 5 | NA |
|--|----------------------|----------------|---------------------------------|--------------|--------------------|----------------|
| My Child..... | Almost always untrue | Usually untrue | Sometimes true sometimes untrue | Usually true | Almost always true | Does Not apply |
| 6. Has a hard time speaking when scared to answer a question | 1 | 2 | 3 | 4 | 5 | NA |
| 7.Can take a band aid off when needed, even if painful | 1 | 2 | 3 | 4 | 5 | NA |
| 8.Can stop him/herself from doing thing too quickly | 1 | 2 | 3 | 4 | 5 | NA |
| 9.Can make him/herself run fast, even when tired | 1 | 2 | 3 | 4 | 5 | NA |
| 10.Has an easy time waiting to open a present | 1 | 2 | 3 | 4 | 5 | NA |
| 11.Has a hard time making him/herself clean their room | 1 | 2 | 3 | 4 | 5 | NA |
| 12.When a child is left out, can ask that child to play | 1 | 2 | 3 | 4 | 5 | NA |
| 14.When working on an activity, has a hard time keeping their mind on it | 1 | 2 | 3 | 4 | 5 | NA |
| 15.Has a hard time waiting his/her turn to talk when excited | 1 | 2 | 3 | 4 | 5 | NA |
| 16.Has a hard time paying attention | 1 | 2 | 3 | 4 | 5 | NA |
| 17.Needs to be told by a teacher to pay attention | 1 | 2 | 3 | 4 | 5 | NA |
| 18. Is very careful and cautious when crossing the street | 1 | 2 | 3 | 4 | 5 | NA |
| 19.Has a hard time working on an assessment s/he finds boring | 1 | 2 | 3 | 4 | 5 | NA |
| 20.Does a fun activity when she is supposed to do homework instead | 1 | 2 | 3 | 4 | 5 | NA |
| 21.Can make him/herself get out of bed, even when tired | 1 | 2 | 3 | 4 | 5 | NA |
| 22.Can apologise or shake hands after a fight | 1 | 2 | 3 | 4 | 5 | NA |
| 23.Needs to be told to pay attention | 1 | 2 | 3 | 4 | 5 | NA |
| 24.Can make him/herself take medicine or eat food that s/he knows tastes bad | 1 | 2 | 3 | 4 | 5 | NA |
| 25.Likes to plan carefully before doing something | 1 | 2 | 3 | 4 | 5 | NA |
| 26.Can make him/herself pick up something dirty to throw it away | 1 | 2 | 3 | 4 | 5 | NA |
| 27.Is able to keep secrets | 1 | 2 | 3 | 4 | 5 | NA |
| 28.Has a hard time slowing down when the rules say to walk | 1 | 2 | 3 | 4 | 5 | NA |
| 29.Gets distracted when trying to pay attention in class | 1 | 2 | 3 | 4 | 5 | NA |
| 30.Has a hard time getting moving when tired | 1 | 2 | 3 | 4 | 5 | NA |
| 31.Can make him/herself smile at someone, even when s/he dislikes them | 1 | 2 | 3 | 4 | 5 | NA |

APPENDIX 6 (CONTINUED) ITEMS FOR INDIVIDUAL SUB-SCALES OF THE TMCQ USED WITHIN THIS STUDY**Activation Control (15 Items)**

- Can make him/herself do homework, even when s/he wants to play.
- Can say hello to a new child in class, even when feeling shy.
- Has a hard time speaking when scared to answer a question.
- Can take a band-aid off when needed, even when painful.
- Can make him/herself run fast, even when tired.
- Has a hard time making him/herself clean own room.
- When a child is left out, can ask that child to play.
- Has a hard time working on an assignment s/he finds boring.
- Does a fun activity when s/he is supposed to do homework instead.
- Can make him/herself get out of bed, even when tired.
- Can apologize or shake hands after a fight.
- Can make him/herself take medicine or eat food that s/he knows tastes bad.
- Can make him/herself pick up something dirty in order to throw it away.
- Has a hard time getting going(moving) when tired.
- Can make him/herself smile at someone, even when s/he dislikes them.

Attention Focusing (7 Items)

- Is easily distracted when listening to a story.
- Looks around the room when doing homework.
- When working on an activity, has a hard time keeping her/his mind on it.
- Has a hard time paying attention.
- Needs to be told by teacher to pay attention.
- Needs to be told to pay attention.
- Gets distracted when trying to pay attention in class.

Inhibitory Control (8 Items)

- Can stop him/herself when s/he is told to stop.
- Can stop him/herself from doing things too quickly.
- Has an easy time waiting to open a present.
- Has a hard time waiting his/her turn to talk when excited
- Is very careful and cautious when crossing the street.
- Likes to plan carefully before doing something.
- Is able to keep secrets.
- Has a hard time slowing down when rules say to walk.

APPENDIX 7: DEMOGRAPHICS QUESTIONNAIRE

Family, Education and Occupation

Name

Date **Id number** (to be filled in by researcher).....

We would be grateful if you would please answer the following questions about your family, education, occupation and the ethnicity of your child.

(1) Please indicate who lives in your household:

- Mother Stepmother Father Stepfather
 Grandmother Grandfather
 Other adults (please list)
 Children (please list ages)

(2) Does your child have contact with other family members? Yes No

If yes, please list

(3) Please indicate your marital status.

- Married Separated Divorced Remarried Never married

(4) Please indicate your relationship to your child.

- Mother Father Stepmother Stepfather Grandmother Grandfather
 Other (describe)

(5) Please indicate the ethnicity of your child.

White UK Other (describe)

Black or Black British Caribbean African Other (describe)

Asian or Asian British ▼ Indian ▼ Pakistani ▼ Bangladeshi
 ▼ Other (describe)

.....

Chinese ▼

Mixed ▼ (describe)

Other ▼ (describe)

(6) Please indicate your total household income using the categories below

- £0-£10,000 í
- £10,000-£20,000 í
- £20,000-£30,000 í
- £30,000-£40,000 í
- £40,000-£50,000 í
- £50,000-£60,000 í
- £60,000-£70,000 í
- £70,000 + í

() If employed, please write the full title of your main job.

.....

Thank you

APPENDIX 8: VISUAL ANALOGUE SCALE

